STUDY OF SOME ORAL HYPOGLYCEMIC DRUGS FOR THE PREVENTION OF PROGRESSION OF DIABETIC COMPLICATIONS IN LABORATORY ANIMALS

Thesis submitted for the fulfillment of the degree of

Doctor of Philosophy

In the faculty of Pharmaceutical Sciences,

Bharati Vidyapeeth Deemed University, Pune.

By

Hemant Vinayak Kamble
M. Pharm.

Research Guide

Dr. Subhash L. Bodhankar

Bharati Vidyapeeth Deemed University,
Poona College of Pharmacy,
Erandwane, Pune - 411 038.

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Certificate

This is to certify that the work presented in the thesis entitled **"Study of some oral hypoglycemic drugs for the prevention of progression of diabetic complications in laboratory animals"** for the Degree of **Doctor of philosophy** (Pharmaceutical Sciences) has been carried out by **Mr. Hemant Vinayak Kamble**, in the laboratories of Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune, under the guidance of **Dr. S. L. Bodhankar**, Professor and Head, Department of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune.

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Date:

Place: Pune

Dr. S. L. Bodhankar

Declaration

This is to certify that the research work entitled **"Study of some oral hypoglycemic drugs for the prevention of progression of diabetic complications in laboratory animals"** has not been submitted in parts or full to any other university by me. This is the original work undertaken by me under the supervision of **Dr. S. L. Bodhankar** in the Department of Pharmacology, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune. Such materials that had been obtained for the research work have been duly acknowledged in the thesis.

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Dedicated to
My beloved Guide, Family & Friends

ACKNOWLEGMENTS

There are lot many ingredients for the successful completion of an endeavor. The first and the foremost is torchbearer who enlightens your pathway, gives an accurate directive to accomplish the same. It is a matter of great pleasure and honor to express my heartfelt gratitude to my respected research guide ***Dr. S. L. Bodhankar****, Professor, Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University (BVDU), Pune, India for his keen interest, patience and constant encouragement in smooth conduct of the research work. I am proud to say that it has been a most productive and enjoyable experience to work under his untiring guidance. Of course, it is my life time achievement to work with him.*

"Great teachers can have a profound impact on one's life. A good teacher can not only make a difficult subject more approachable, but also stoke the fires of creativity, instill leadership values and open one's mind to greater possibilities"

I wish to express my sincere and respectful thanks to ***Hon. Dr. Shivajirao S. Kadam****, Vice Chancellor, Bharati Vidyapeeth Deemed University, Pune for enrolling me in this doctoral programme and his constant words of encouragement for quality research.*

I owe special thanks to ***Dr. Kakasaheb R. Mahadik****, Principal, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University (BVDU), Pune, India for being a constant source of inspiration and support in all my endeavors through his skillful management of men and resources required for carrying out my research work. His keen interest and discipline towards research has always remained as a constant source of encouragement for many of us.*

I take this opportunity to express my deep sense of gratitude to the teaching faculty of Poona College of Pharmacy with special reference to the ***Dr. (Mrs.) Arul Mozhi, Dr. (Mrs.) G. Deshpande, Dr. Shiraskar, Dr. S. R. Dhaneshwar, Dr. (Mrs.) V. B. Pokharkar, Dr. A. P. Pawar, Dr. S. H. Bhosale, Dr. V. M. Kulkarni, Dr. (Mrs.) S. S. Dhaneshwar, Dr. L. Sathiyanarayanan, ,Dr. S.Y. Gabhe, Prof. A. P. Purohit, Dr. Abhay Harsulkar, Dr. S. R. Rojatkar, Dr. R. N. Kamble, Dr. P. R. Nalawade, Dr. V. M. Shinde , Asst. Prof. Sandeep Pathare*** *and* ***Asst. Prof. K. Dhawale*** *for their valuable suggestions & guidance throughout the course.*

I find myself absolutely short of words in expressing my feelings towards my colleagues ***Dr. Vishal Mali, Dr. Mahadev Mahadik, Dr. Anand Zanwar, Dr. Pinaki Ghosh, Dr. Arvind Ghule, Dr. Smitta Mohaod, Mr. Amol Muthal, Mr. Parag Kadam, Mrs. Vaishali Mute, Mr. Amit Kandhare, Mr. Gopal Bihani, Mr. Sameer Sawant, Mrs. Bhagyashree Atre, Sumit Deore, Vijaykumar Kale, Ganesh Barate, Suyog Aher, Vitthal Awad, Aishwarya Balap, Madhuri Shelar, Sadhana Nanaware, Dr. Abhijeet Khopde, Dr. Madan Mane, Mr. Sujit Bhansali, Ashwin Kuchekar, Sameer Ketkar, Akhilesh Mhaske, Abhay Kedarkunte , Atul Rathore, Amol Muthal, Pravin Pawar, Abhijeet Khopade, Sachin Potawale, Madan Mane, Ajinkya Nikam, Prashant Bhondave, Ajinkya Sarkate, Kiran Kemkar, Nilesh Desai, Harshad Kapre, Rahul Jain and Praksh Jadhav*** *for their valuable support and best wishes.*

One flower makes no garland. I can never forget that my Ph.D. research work has been accomplished with the constant camaraderie of all my juniors. I am greatly indebted to ***Asjad, Adil, Shiva, Nilkhant, Prachi, Anuchandra, Shiva, Shweta, Yamini, Nikhila, Tasnim, Brindaraj, and Kamlesh, Akansha, Pooja, Javed, Mithun, Annuya, Devyani, Pooja, Prachi, Snehal, Sharau, Vijay, Diksha and Dalvi*** *for their help.*

I am also thankful to my room partners ***Amol sir, Piush sir, Sujit sir, Gaurav, Pranesh*** *and* ***Rishi*** *Tiwari for their constant support.*

I am also thankful to ***Mr. Thorat sir*** *Librarian of our college for providing good library and referencing facilities and store-in-charge* ***Mr. D. J. Joshi*** *and* ***Mr. Bhimrao Kadam. Mr. Mulla.*** *My special thanks to* ***Mr. Pralhad Patil, Mr.Mandke, Mr. Khade, Mr. Patil, Mr. Sutar*** *and* ***Mr. Sande*** *for helping me in all the possible ways. I must not forget to thank all the non-teaching staff especially to* ***Mr. Kisan Patil*** *for their co-operation.*

I owe special thanks to the ***University Grants Commission (UGC),*** *New Delhi, India, for financial assistance to carry out research work under the scheme of Quality Improvement Programme (QIP).*

I am also thankful to ***Mr. Bapusahebji Thite, Dr. Rajendraji Thite, Mr. Padwal, Mr. D.G. Baheti (Principal, Sitabai Thite College of Pharmacy, Shirur), Dr. Shah, Dr. Tare, Mr. Agarwal, Mr. Deshpande, Mrs. Shelke, Mr. Shinde, Mr. Kothawade, Mr. Awari, Mr. Londhe, Mr. Rane, Mr. Bande, Mr.Ware, Mr. Kharade, Mr. Taniji, Mr. Santosh*** *for proving research support.*

I would like to give respectable thanks to my dearest friends ***Dr.Mahadev Mahadik, Dr. Amol Lokhande, Mr. Sachin Potawale, Mr. Pravin Pawar, Mr.Parag Kadam, Mr. Amol Muthal*** *who always support financially during Ph.D. Course work, without their support this could not possible. I would like to give lots of thanks to* ***Rahul, Yuvraj, Ganesh, Hari, Anandkar, Vasim, Ajay, Aniket, Raju, Nikesh, Rohit, Vaibhav*** *and* ***Prashant*** *for their moral support.*

Above all, I would like to thank my ***Anna, Mummy*** *and my beloved* ***sisters Jyoti tai, Swati tai, Nita tai, Mr. Vitthal Bhagatgaji, Mr. Bhupesh Sonawanedaji, Mr. Mahesh Tambedaji*** *and my father and mother in-low* ***Bhau, Aai, Sangitatai, surekhatai, Manisha tai and Mr. Hanumantdada Kamble, Mr. Balasahebdada Udamle, Mr. Vikasdada Satpute*** *who have given me their unequivocal support and great patience throughout, as always at all times for which my mere expression of thanks likewise does not suffice.*

I would like to reserve a special line for my wife ***Mrs. Rashmi Heamant Kamble****, for her unwavering love, understanding and constant support which have made my life a wonderful experience to live, her continuous support encourage me to be the best. She forms backbone and origin of my happiness.*

I would like to give heartiest thanks to my sweet angel ***'TANISHKA'*** *because when I was admitted to Ph.D. course work, she was only three years old; I think this is the miracle age during those days I was not with her.*

I am also thankful to each and every one of them who helped me directly or indirectly during this work.

Above all I thank ***lord Almighty*** *for showering their infinite bounties, clemencies and graces upon me for being my constant companion, the strongest source of motivation and inspiration and my ultimate guardian.*

Date:

Place: Pune ***Mr. Hemant Vinayak Kamble***

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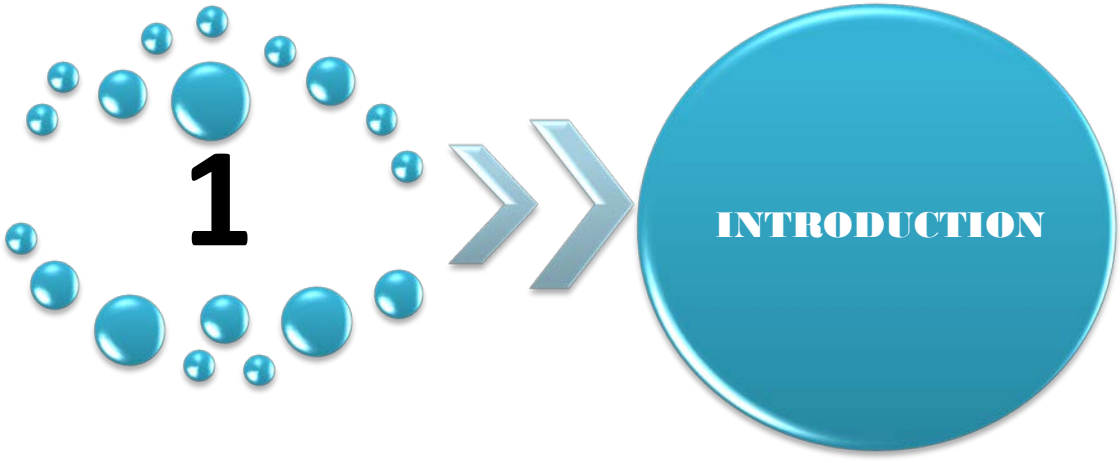
Abbreviations

ACE	:	Angiotensin converting enzyme
ADA	:	American Diabetic Association
ADP	:	Adenosine diphoshphate
AGE	:	Advance Glycation end product
AMP	:	Adenosine monophaosphate
Ang II	:	Angiotensin II
ANOVA	:	Analysis of variance
ANS	:	Autonomic nervous system
AR	:	Aldose reductase
ARBs	:	Angiotensin II receptor blockers
AST	:	Aspartate transaminase
ATR	:	Angiotensin receptor
ATP	:	Adenosine triphospahte
AUC	:	Area under curve
BP	:	Blood pressure
BRS	:	Baroreflex sensitivity
BUN	:	Blood urea nitrogen
Ca^{2+}	:	Calcium
c-AMP	:	Cyclic adenosine monophaosphate
CAD	:	Coronary artery disease
CAN	:	Cardiac autonomic neuropathy
CCB	:	Calcium channel blockers
Cclr	:	Creatinine Clearance
CHF	:	Congestive heart failure
CIRKO	:	Cardiomyocyte- restricted knock out of the insulin receptor
CK	:	Creatine kinase
CK-MB	:	Creatine kinase- MB
CML	:	N^{e}-(carboxymethyl lysine
CNS	:	Central nervous system
CRF	:	Chronic renal failure
CSM	:	Cardiac sympathetic mapping
CTGF	:	Connective tissue growth factor
CPCSEA	:	Committee for the Purpose of control and supervision of Experiment on Animals
d-ATP	:	Deoxyadenosine triphospahte
DbCM	:	Diabetic cardiomyopathy
DCCT	:	Diabetic Control and Complications Trials
DAG	:	Activated by diacylglycerol
DAN	:	Diabetic autonomic neuropathy
DBP	:	Diastolic blood pressure
DC	:	Diabetic Complications
DGK	:	Diacylglycerol kinase
DHBA	:	2,3-didydroxy benzoic acid
DM	:	Diabetes mellitus
DME	:	Diabetic macular edema

DPP	:	Dipeptidyl peptidase
DTNB	:	5, 5-dithiobis (2- Nitrobenzoic acid)
DPN	:	Diabetic peripheral neuropathy
DN	:	Diabetic nephropathy
E-C	:	Excitation- contraction
ECG	:	Electrocardiogram
ECHO	:	Echocardiography
ECM	:	Extracellular matrix
ED	:	Enterodiol
EDP	:	End diastolic blood pressure
EGF	:	Epidermal growth factor
EGFR	:	Epidermal growth factor receptor
EL	:	Enterolactone
EN	:	Entrapment neuropathies
ER	:	Endoplasmic reticulum
ESRD	:	End stage renal disease
ET	:	(endothelian-1)
ETDRS	:	Early Treatment Diabetic Retinopathy Study
ERK	:	Extra cellular signal- regulated protein kinase
FAs	:	Fatty acids
FDA	:	Food and Drug Administration
FFA	:	Free fatty acids
FGF	:	Fibroblast growth factor
GADPH	:	Glyceraldehyde phosphate dehydrogenase
GDM	:	Gestational diabetes mellitus
GFR	:	Glomerular filtration rate
GLP 1	:	Glucagon like peptide 1
GLUT	:	Glucose transporter
GPCR	:	G protein coupled-receptor
GOD/POD	:	Glucose oxidase-peroxidase
GSH	:	Glutathione reductase/ reduced glutathione
H_2O_2	:	Hydrogen peroxidase
HCL	:	Hydrochloric acid
Hb	:	Haemoglobin
HbA1c	:	Glycated haemoglobin
HDL	:	High density lipoprotein
HF	:	Heart failure
HMGA	:	3-hydroxy-3-methylglutaric acid
HR	:	Heart rate
HRV	:	Heart rate variability
HSP	:	Heat shock protein
IHD	:	Ischemic heart disease
i.p	:	Intraperitoneal
i.v.	:	Intravenous
IL	:	Interleukin
IAEC	:	Institutional Animal Ethics Committee
IDDM	:	Insulin dependent diabetes mellitus
IGF	:	Insulin like growth factor
IR	:	Insulin resistance
IRS	:	Insulin receptor substrate

IU	:	International unit
LADA	:	Latent autoimmune diabetes of adults
LAR	:	Lariciresinol
LDH	:	Lactate dehydrogenase
LDL	:	Low density lipoprotein
LVH	:	Left ventricular hypertrophy
LT B4	:	Leucotriene B4
LV	:	Left ventricular
LVEF	:	Left ventricular ejection function
LVSP	:	Left ventricular systolic pressure
Kg	:	Kilogram
M	:	Mortality
MABP	:	Mean arterial blood pressure
MAP	:	Mitogen -activated protein kinases
Max dp/dt	:	Maximum dp/dt
MDA	:	Malondialdehyde
mg/dl	:	Milligram/deciliter
mg/kg	:	Milligram/kilogram
mg	:	Milligram
MI	:	Myocardial infarction
Min	:	Minutes
Min dp/dt	:	Minimum dp/dt
Ml	:	Milliliter
Mm	:	Milimoles
MMP	:	Metalloproteinase
MnSOD	:	Mn^{2+} superoxide dismutase
MRA	:	Magnetic resonance angiogram
MRI	:	Magnetic resonance imaging
MSNA	:	Muscle sympathetic muscle activity
MW	:	Molecular weight
N	:	Normality
NADH	:	Nicotinamide adenine dinucleotide
NADPH oxidase	:	Nicotinamide adenine dinucleotide phosphate-
Na-K ATPase	:	Sodium-Potassium ATPase
NIDDM	:	Non insulin dependent diabetes mellitus
NO	:	Nitric oxide
Nm	:	Nanometer
nM	:	Nanomoles
NPDR	:	Non proliferative diabetic retinopathy
Ns	:	Not significant
OHA	:	Oral Hypoglycemic Agents
p.o.	:	Per oral
PARP	:	Poly ADP ribose polymerase
PDC	:	Pyruvate dehydrogenase complex
PDGF	:	Platelet- derived growth factor
PDN	:	Peripheral diabetic neuropathy
PDR	:	Proliferative diabetic retinopathy
PFK	:	Phosphofructokinase
PI3K	:	Phosphatidylinositol 3-kinaase

PLC	:	Measurement of plasma levels of catecholamines
PMNL	:	Polymorphonuclear leucocytes
PKC	:	Protein kinase C
PPAR-γ	:	Peroxisome proliferator- activated receptor- γ
PNS	:	Peripheral nervous system
r.p.m	:	Revolution per minutes
RAGE	:	Advanced glycation end product receptor
RAS	:	Renin angiotensin system
RBC	:	Red blood cell
ROP	:	Retro orbital puncture
ROS	:	Reactive oxygen species
S.E.M	:	Standard error mean
SBP	:	Systolic blood pressure
SGOT	:	Serum glutamic oxaloacetic transaminase
SDH	:	Sorbitol dehydrogenase
SOD	:	Superoxidase dismutase
SOP	:	Standard operating procedure
SR	:	Sarcoplasmic reticulum
SRF	:	Serum response factor
STZ	:	Streptozotocin
TBA	:	Thiobarbituric acid
T2DM	:	Type 2 diabetes mellitus
TCA	:	Tricarboxylic acid
TEB	:	Terminal end bud
TG	:	Triglyceride
TGF-β1	:	Transforming growth factor β1
TNF-α	:	Tumor necrosis factor-α
TRN	:	Truncal radiculoneuropathy
TZDs	:	Thiazolidinediones
UAE	:	Urinary albumin excretion
UKPDS	:	U.K. Prospective Diabetes Study
VEGF	:	Vascular endothelial growth factor
VTDR	:	Vision-threatening diabetic neuropathy
v/v	:	Volume/Volume
WESTR	:	Wisconsin Endoplasmic Study of Diabetic Retinopathy
WHO	:	World Health Organization
w/v	:	Weight/Volume


1
INTRODUCTION

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin its action or both [Belvis *et al.*, 2009; Smit *et al*., 2013].

1.1 Historical background

Diabetes was first documented in the Egypt, characterized by weight loss and polyurea [Mbanya *et al*., 2010]. However, it was the Greek physician, Aertaeus who coined the term 'diabetes' the word mellitus (honey sweet) was added by Thomas Willis (Britain) in 1776 that Dobson (Britain) firstly confirmed the presence of excess sugar in blood and urine as a cause of their sweetness [Eknoyan, 2009]. According to "The National Medical Journal of India", ancient Indians knew diabetes which they called "sweet urine disease" by determining if ants were attracted to a person's urine! [Pathak *et al*., 2013].

In modern time, the history of diabetes coincided with the emergence of experimental medicine [Gale, 2001]. An important milestone in the history of diabetes is the establishment of the role of the liver in glycogenesis and the concept that diabetes is due to excess glucose production by Claude Bernard (France) in 1857. The role of the pancreas in pathogenesis of diabetes was discovered by Mering and Minkowski (Austria) in 1889. Later, this discovery constituted the basis of insulin isolation and clinical use by Banting and Best (Canada) in 1921[Ahmad, 2002]. "World Diabetes day'' was introduced in 1991 and is celebrated every year on 14th November. The day was chosen to honor Frederick Banting (14th November, which is also his birthday), who along with Charles Best, first conceived the idea that lead to the discovery of insulin in 1921 [Claydon *et al*., 2013].

1.1.2 Current status

Currently 366 million people worldwide 8.3 % of the global population have diabetes and an additional 280 million people have impaired glucose tolerance. Although the prevalence of diabetes varies greatly between ethnic groups and geographical regions, it disproportionately affects people aged 65 years and older [Therasa *et al*., 2010]. The public health toll of diabetes is on an upward trajectory with its prevalence estimated to increase to more than 552 million people worldwide by 2030 [Therasa *et al*., 2010].

1.1.3 Classification of diabetes

Classification of diabetes includes both aetiological types and different clinical stages of hyperglycaemia. Four main aetiological categories of diabetes have been identified as diabetes type 1, type 2, gestational diabetes and other specific types. Sustain hyperglycemia for longer period of time causes acute complications like ketoacidoses (DKA), non-ketotic hyper-osmolar state (NKHS) and chronic complications which are :1) Vascular 2) nonvascular complications. The vascular complications are further subdivided into i) microvascular (neuropathy, nephropathy, retinopathy) and ii) macrovascular complications (coronary artery disease, peripheral vascular disease, and cerebrovascular disease). Nonvascular complications include problems such as gastroporesis, sexual dysfunction and skin changes [Brajendra *et al.,* 2006]. The brief classification is given below-

1.1.3.1 Type 1 diabetes (previously known as insulin-dependent diabetes)

Type 1 diabetes is an autoimmune disease. In diabetes, the immune system attacks and destroys the insulin producing β-cells in the pancreas. The pancreas then produces little or no insulin. At present, scientists do not know exactly what causes the body's immune system to attack the beta cells but they believe that autoimmune, genetic and environmental factors, possibly viruses are involved. Type 1 diabetes accounts for about 5-10 % diagnosed diabetes in the United States. It develops most often in children and young adults [Achenbach *et al*., 2005].

- **Symptoms of type 1 diabetes**

Child- Hunger, weight loss, fatigue, irritability or unusual behavior, blurred vision, yeast infection.

Adult- Thirsty, hungry, tired all the time, blurry vision, numbness or tingling in feet and weight loss.

- **Diagnosis for type 1 diabetes**

  - Fasting blood glucose level- Diabetes is diagnosed if it is higher than 126 mg/dl two times.
  - Random (non-fasting) blood glucose level- People may have diabetes if it is higher than 200 mg/dl and might have symptoms such as increased thirst, urination and fatigue (this must be confirmed with a fasting test).

- Oral glucose tolerance test - Glucose level is higher than 200 mg/dl 2 hours after glucose consumption.
- HbA1c test - 6.5 % or higher [Belle *et al*., 2011].

1.1.3.2 Type 2 diabetes (previously known as non-insulin dependent diabetes or adult-onset diabetes)

The vast majority of diabetic patients have type 2 diabetes. Type 2 diabetes is far more common than type 1, accounting for about 90% of all cases of DM. In most cases, the onset of type 2 diabetes occurs after age 30, often between the ages of 50 and 60 years and the disease develops gradually. This form of diabetes is characterized by insulin resistance and at least initially, a relative lack of insulin secretion. Most individuals with type 2 diabetes exhibit abdominal obesity which itself causes insulin resistance hypertension and dyslipidemia. This clustering of abnormalities is referred to as the insulin resistance syndrome or the metabolic syndrome. Because of these abnormalities, patients with type 2 DM are at increased risk of developing complications [Olokoba *et al*., 2012].

- **Symptoms of type 2 diabetes:** Increased thirst and hunger, frequent urination, weight loss, fatigue, blurred vision, slow-healing sores, darkened skin.
- **Diagnosis for type 2 diabetes**
  - Fasting blood glucose level- More than 120 mg/dl.
  - Random (non-fasting) blood glucose level- More than 200 mg/dl.
  - Oral glucose tolerance test- More than 200 mg/dl, 2h after glucose consumption.
  - HbA1c Test - 6.5% or higher [Olokoba *et al*., 2012].

1.1.3.3 Gestational Diabetes (GDM)

Some women develop gestational diabetes late in pregnancy. Although this form of diabetes usually disappears after the birth, women who have had gestational diabetes have a 40 to 60% chance of developing type 2 DM within 5 to 10 years. Maintaining a reasonable body weight and being physically active may help prevent development of type 2 diabetes. About 3 to 8 % pregnant women in the United States develop gestational diabetes. As with type 2 diabetes, gestational diabetes occurs more often in some ethnic groups and among women with a family history of diabetes. Gestational diabetes is caused by the hormones of pregnancy or a shortage of insulin [Cheung and Wong, 2011].

- **Risk factors**

Risk factors for GDM are separated into two groups such as unmodifiable and modifiable. Known unmodifiable risk factors include age, genetic background, number of previous pregnancies. Modifiable risk factors include obesity, lack of exercise, dietary fat and smoking [Menato and Signorile, 2008; Cheung, 2009].

- **Symptoms for gestational diabetes**

In most of cases gestational diabetes doesn't cause noticeable signs or symptoms. Rarely, gestational diabetes may cause excessive thirst or increased urination [Ryan *et al*., 2011].

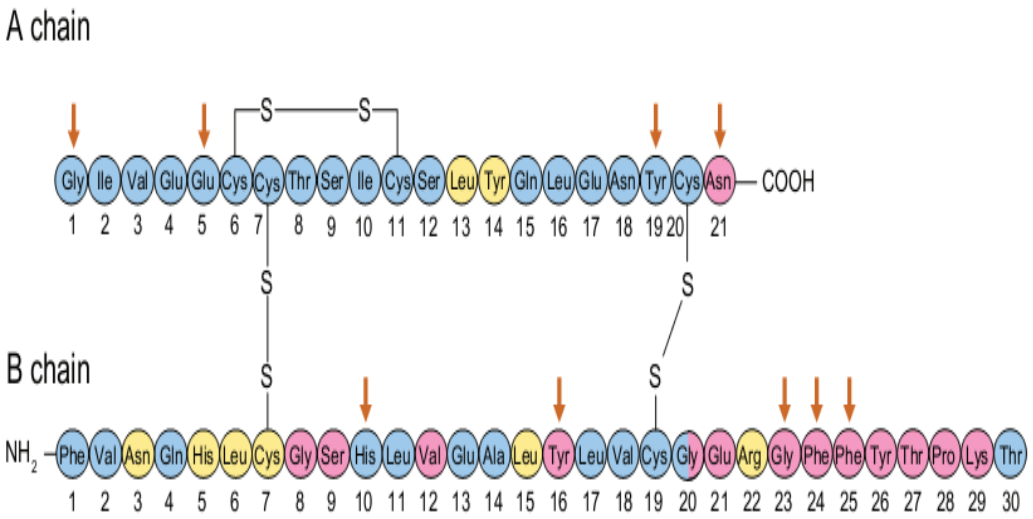
1.1.3.4 Other specific types of diabetes

- Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM.
- Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 diabetes based on age rather than etiology.
- Some cases of diabetes are caused by the body's tissue receptors not responding to insulin.
- Genetic mutations (autosomal or mitochondrial) can lead to defects in β-cell function. Any disease that causes extensive damage to the pancreas may lead to diabetes chronic pancreatitis and cystic fibrosis.
- Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes.
- Excess amounts of certain hormones resulting from some medical conditions such as cortisol in Cushing's syndrome that works against the action of insulin.
- Medications that reduce insulin action, such as glucocorticoids, chemicals that destroy beta cells [Bastaki *et al*., 2005].

1.1.4 Treatment

1.1.4.1 Treatment for type 1 diabetes

Insulin was discovered in 1921 by Banting and Best who demonstrated the hypoglycemic action of an extract of pancreas prepared after degeneration of exocrine part due to ligation of pancreatic duct. It was first obtained in pure crystalline form in 1926 and the chemical structure was fully worked out in 1956 by Sanger [Singh *et al*., 2010].

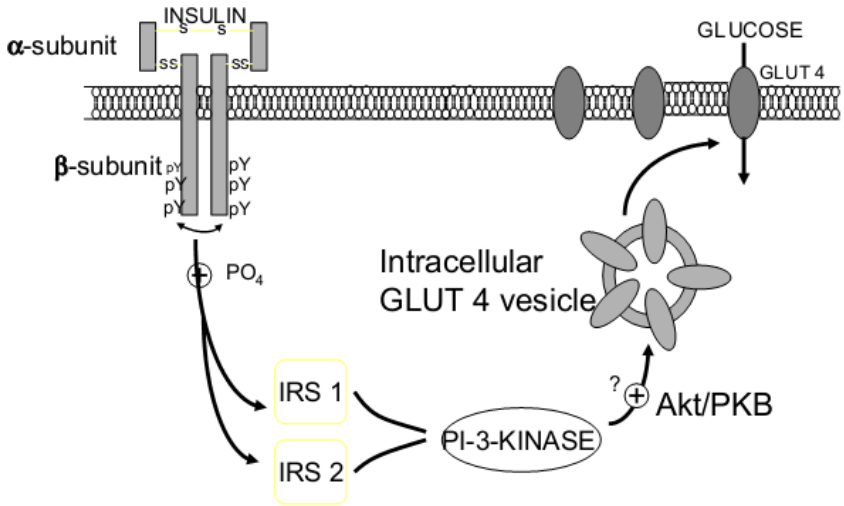


[Adapted from Yamada, 2009]

Figure1.1.4.1 Structure of insulin

1.1.4.1.1 Mechanism of action

Insulin acts on specific receptors located on the cell membrane of every cell, but their density depends on the cell type: liver and fat cells are very rich. The insulin receptor is a receptor tyrosine kinese (RTK) which is heterotetrameric glycoprotein consisting of 2 extracellular α and 2 transmembrane β-subunits linked together by disulfide bonds. It is oriented across the cell membrane as a heterodimer.



[Adapted from Bhattacharya *et al*., 2007]

Figure1.1.4.1.1 Insulin signaling pathway showing binding of insulin with the IR leading to activation of Glut-4 which imports glucose into the cell

The α-subunits have tyrosine protein kinese activity. Binding of insulin to α-subunits induces aggregation and internalization of receptor along with the bound insulin molecules. This activates tyrosine kinase activity of the β-subunits. Pairs of β-subunits phosphorylate tyrosine kinase residues on each other to expose the catalytic site to phosphorylate tyrosine residues of Insulin Receptor Substrate Protein (IRS 1, IRS 2). In turn, a cascade of phosphorylation and dephosphorylation reactions is set into motion which amplifies the signal and results in stimulation on inhibition of enzymes involved in the rapid metabolic actions of insulin [White and Khan, 1988].

Today, diabetics receive insulin, a recombinant protein produced in bacteria (Escherichia coli). Human insulin produced by recombinant technology has replaced bovine and porcine insulin preparations. The commonly available human insulin's represent groups of short, intermediate and long-acting insulin and biphasic mixtures. All groups have different characteristics with respect to the onset and duration of action. DNA recombination technologies provide the opportunity for the creation of insulin analogs with improved function [Duttaroy *et al*., 2005]. One group represents rapid-acting analogs known also as 'rapid-onset' and 'ultra-short-acting' insulin and includes insulin aspart and insulin lispro [Joshi *et al*., 2009]. Both recombinants contain modifications in the amino acid sequence of the insulin chain-B. The advantage of using these analogs is a faster response in comparison with conventional short-acting insulin; therefore, they can be injected immediately before meals or even after eating and are useful for young patients [Lutz *et al*., 2012]. Conventional short-acting insulin; therefore, they can be injected immediately before meals or even after eating and are useful for young patients [Schmid, 2007].

Table 1.1.4.1 Types of insulin

Types of Insulin	**Brand name**	**Generic name**	**Onset**	**Peak**	**Duration**
Rapid acting	Novo Log	Insulin aspart	15 minutes	30 to 90 min	3 to 5 hours
	Apidra	Insulin glulisine	15 minutes	30 to 90 min	3 to 5 hours
	Humalog	Insulin lispro	15 minutes	30 to 90 min	3 to 5 hours
Short acting	Humulin R	Regular (R)	30-60min	2 to 4 hours	5 to 8 hours
	Novolin R				
Intermediate acting	Humulin N	NPH (N)	1 to 3 hours	8 hours	12 to 16 hours
	Novolin N				

Long acting	Levemir	Insulin detemir	1 hour	Peak less	20 to 26 hours
	Lantus	Insulin glargine			
Pre-mixed NPH	Humulin 70/30 Novolin 70/30	70% NPH and 30% regular	30 to 60 minutes	Varies	10 to 16 hours
	Humulin 50/50	50% NPH and 50% regular	30 to 60 minutes	Varies	10 to 16 hours
Pre-mixed insulin lispro protamine suspension (intermediate-acting) and insulin lispro (rapid-acting	Humalog Mix 75/25	75% insulin lispro protamine and 25% insulin lispro	10 to 15 minutes	Varies	10 to 16 hours
	Humalog Mix 50/50	50% insulin lispro protamine and 50% insulin lispro	10 to 15 minutes	Varies	10 to 16 hours
Pre-mixed insulin aspart protamine suspension (intermediate-acting) and insulin aspart (rapid-acting)	Novo Log Mix 70/30	70% insulin aspart protamine and 30% insulin aspart	5 to 15 minutes	Varies	10 to 16 hours

[Adapted from Arshag *et al*., 2006]

1.1.4.1.2 Challenges to oral insulin delivery

Oral delivery of insulin as a non-invasive therapy for DM is still a challenge to the drug delivery technology. Insulin cannot be administered via the oral route due to rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen and poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity. The oral bioavailability of most peptides and proteins therefore is less than 1%. The challenge here is to improve the bioavailability to anywhere between 30-50% [Singh *et al*., 2010].

1.1.4.2 Treatment for type 2 diabetes

1.1.4.2.1 Current therapeutic agents for treatment of type 2 diabetes

Currently available oral agents for the treatment of type 2 DM include a variety of compounds from 6 different pharmacologic classes [Richard *et al.,* 2001].

Table 1.1.4.2.1 Classification of oral therapeutics for type 2 diabetes

Sr. No	Classification	Examples
1	**Sulphonylureas** **a) First line agents** **b) Second line agents**	 e.g. Chlorpropamide, Tolbutamide e.g. Glibenclamide, Glimepiride, Gliclazide
2	**Biguanide**	e.g. Phenformin, Metformin
3	**Thiazolidinediones**	e.g. Roziglitazone, Pioglitazone
4	**Meglitinides analogue**	e.g. Repaglinide
5	**α- glycosidase inhibitors**	e.g. Acarbose, Guar-gum
6	**DPP-4 inhibitors**	e.g. Vildagliptin, Sitagliptin

1] Sulphonylureas

e.g. Chlorpropamide

Formula: $C_{10}H_{13}ClN_2O_3S$; Molecular mass: 276.74 g/mol; FDA approval: 1959, Dose: 500 mg/day; route: oral; metabolism: hepatic, half life: 9h; excretion: renal 33% and fecal: 67%.

Mode of action

Chlorpropamide acts to increase the secretion of insulin, so it is only effective in patients who have some pancreatic β-cell function. It can cause relatively long episodes of hypoglycemia; this is one reason why shorter-acting sulfonylureas such as gliclazide or tolbutamide are used instead. The risk of hypoglycemia makes this drug a poor choice for the elderly and patients with mild to moderate hepatic and renal impairment.

Side effects: Skin rashes, nausea, vomiting and diarrhea.

Contraindication: Obese patients.

2] Biguanide

e.g. a. Phenformin

Formula: $C_{10}H_{15}N_5$; Molecular mass: 205.26 g/mol; Dose: 200-400 mg twice a day; route: oral; metabolism: hepatic; half life: 10-15h; excretion: renal. Phenformin was discovered in 1957 by Ungar, Freedman and Seymour Shapiro,

working for the US Vitamin Corporation. It was marketed by Ciba-Geigy but it was withdrawn from markets in the late 1970s due to a high risk of lactic acidosis.

3] Thiazolidinediones

e.g. Rosiglitazone

Formula: $C_{18}H_{19}N_3S$; Molecular mass: 357.42 g/mol; FDA approval: 1999; Dose: 8 mg/day; route: oral; metabolism: hepatic; half life: 3-4 h; excretion: renal.

Mode of action

Rosiglitazone acts as a highly selective and potent agonist at peroxisome proliferator activated receptors (PPAR-γ) in target tissues for insulin action such as adipose tissue, skeletal muscle and liver. Activation of PPAR-γ receptors regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport and utilization. In this way rosiglitazone enhances tissue sensitivity to insulin.

Side effects: Short breath, weight gain, chest pain, pain spreading to the arm or shoulder, sweating, nausea, stomach pain, low fever, loss of appetite, dark urine, clay-colored stools.

Contraindication: Contraindicated for renal impairment, liver disease and for lactating mother.

4] Meglitinides analogue

e.g. Repaglinide

Formula: $C_{27}H_{36}N_2O_4$; Molecular mass: 452.58 g/mol; FDA approval: 1992, Dose: 0.5-4 mg/day; route: oral; metabolism: hepatic, half life: 1h, excretion: renal and hepatic.

Mode of action

Repaglinide lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the β-cells. This depolarizes the β-cells, opening the cells calcium channels and resulting in calcium influx which induces insulin secretion.

Side effects: Seizure, back pain, nausea and vomiting, fast heart rate, pale or yellowed skin, dark colored urine, fever, confusion or weakness, sore throat, headache and red skin rash.

Contraindications: Insulin dependent diabetes; diabetic ketoacidosis with or without coma; hypersensitivity due to repaglinide or its ingredients.

5] α- glycosidase inhibitors

e.g. Acarbose

Formula: $C_{25}H_{43}NO_{18;}$ Molecular mass: 645.605g/mol; FDA approval: 1995; Dose: 25 mg; 3 times /day; route: oral; metabolism: gastrointestinal tract; half life: 2h, excretion: renal.

Mode of action

In contrast to sulfonylureas, acarbose does not enhance insulin secretion. The antihyperglycemic action of acarbose results from a competitive, reversible inhibition of pancreatic α-amylase and membrane-bound intestinal α-glucoside hydrolase enzymes. Pancreatic α-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, while the membrane-bound intestinal α-glucosidases hydrolyze oligosaccharides, trisaccharides and disaccharides to glucose and other monosaccharides in the brush border of the small intestine. In diabetic patients, this enzyme inhibition results in a delayed glucose absorption and a lowering of postprandial hyperglycemia.

Side effects: Flatulence, diarrhoea.

Contraindications: Hypersensitive as well as diabetic ketoacidosis, inflammatory bowel disease, colonic ulceration.

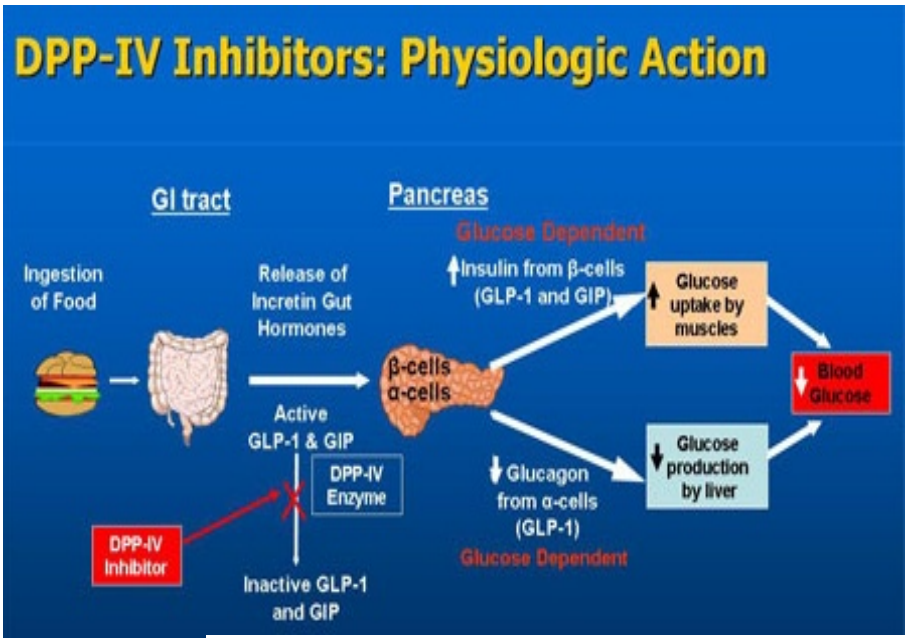
6] DPP-IV inhibitors

e.g. Vildagliptin

Formula: $C_{17}H_{25}N_3O_{2;}$ Molecular mass: 303.39g/mol; FDA approval: Vildagliptin has not been approved by the FDA but is marketed as Galvus in Europe; Dose: 50 mg; 3times/day; route: oral; metabolism: mainly hydrolysis to inactive metabolite; half life: 2-3h; excretion: renal.

Mode of action

In response to a meal, active glucagon-like peptide-1 (GLP-1) is secreted by the L cells of the intestine. Without the presence of vildagliptin, GLP-1 is rapidly inactivated and degraded by the enzyme dipeptidyl peptidase IV (DPP-IV); when vildagliptin is present, vildagliptin binds to DPP-IV allowing GLP-1 to remain active. Active GLP-1 causes the pancreas to increase insulin release and decrease glucagon release.

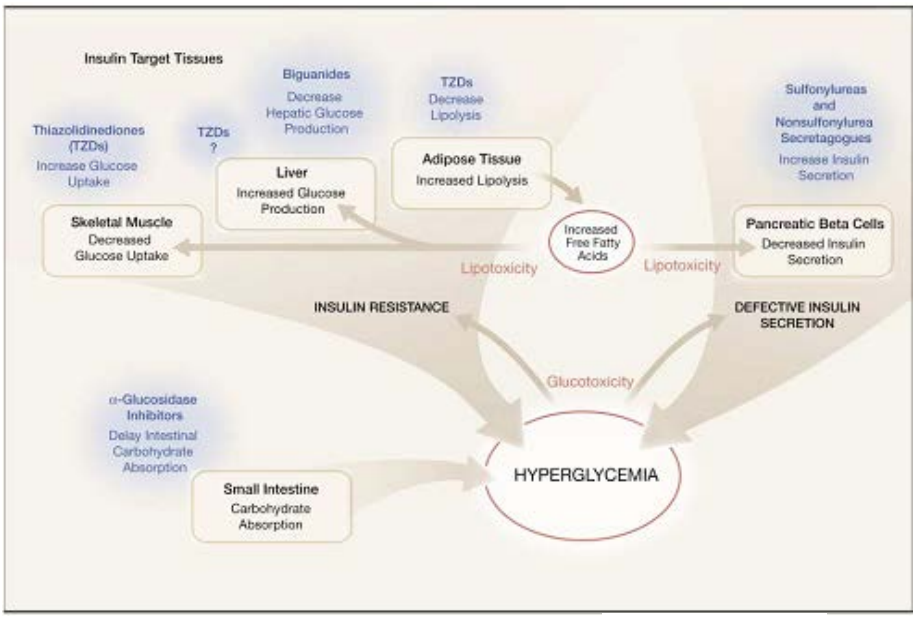


[Adapted from Krentz and Baily, 2005]

Figure1.1.4.1.2 Mechanism of action of DPP-4 inhibitor Vildagliptin

Side effects: In combination therapy with metformin shows feeling dizzy, headaches, sweating and nausea.

Contraindications: Hepatic disorders, dialysis dependent severe renal disorders and diabetic ketoacidosis.



[Adapted from Inzucchi, 2002]

Figure1.1.4.1.3 Side effects DPP-4 inhibitor Vildagliptin

1.1.5 Complications of diabetes

1.1.5.1 Acute complications

Acute complications include;

- **Diabetic ketoacidoses (DKA)**
- **Non-ketotic hyperosmolar state (NKHS)**

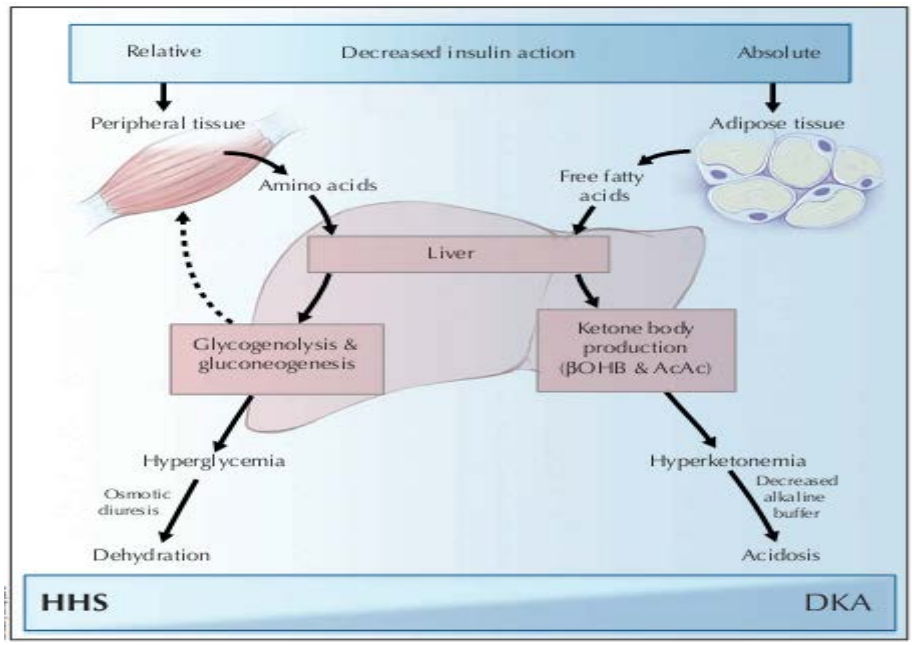
1.1.5.1.1 Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) is a serious life-threatening metabolic complication of diabetes mellitus which is most common in type 1 diabetes, rarely seen in patients with type 2 diabetes mellitus [Lin *et al*., 2010]. Epidemiologically DKA occurs in 4.6-8.0 per 1000 people with type 1 diabetes annually. In the United States, 135,000 hospital admissions occur annually as a result of DKA, at an estimated cost of $2.4 billion or a quarter to half of the total cost of caring for people with type 1 diabetes. It has been documented increasing trend to hospital admissions. The risk is increased in those with an ongoing risk factor such as an eating disorder and those who cannot afford insulin. About 30% of children with type 1 diabetes receive their diagnosis after an episode of DKA [Maldonado *et al*., 2004].

The decreased ratio of insulin to glucagon pramotes gluconeogenesis, glycogenolysis and ketone body formation in the liver and also increases free fatty acid and amino acid delivery from fat and muscle to the liver. Ketosis results from a marked increase in free fatty acid release from adiposities due to increased lipolysis [Cavlan *et al*., 2013].

- **Laboratory criteria for diagnosis of diabetic kitoacidosis**
  - Plasma glucose level > 250 mg/dl or greater
  - Arterial pH < 7.3
  - Bicarbonate (serum) < 15 mEq/dl
  - Blood urea nitrogen (serum) < 25 mEq/dl
  - Osmolality < 320 mosm/kg
  - Urine ketones > +3
  - Plasma ketones positive > 1.2 dilution [Yehia *et al*., 2008]
- **Symptoms of ketoacidosis:** Fatigue, frequent urination or thirst for 1 day or more, muscle stiffness or aching, shortness of breath, abdominal pain, decreased appetite and consciousness, headache, mental stupor that may progress to coma [Hardern and Quinn, 2003].

- **Pathogenesis of diabetic kitoacidosis**



[Adapted from Chiasson *et al*., 2003]

Figure1.1.4.1.4 Pathogenesis of diabetic kitoacidosis

1.1.5.1.2 Non-ketotic hyperosmolar state (NKHS)

Non-ketotic hyperosmolar state is associated is most commonly seen in elders in type 2 diabetes high risk of complications, coma and death. In adults NKHS occurs at a frequency of 17.5 cases/100,000 persons/year. Insulin deficiency and inadequate fluid intake are the underlying causes of NKHS. Insulin deficiency leads to hyperglycemia, which induces an osmotic diuresis leading to profound intravascular volume depletion [Perilli and Saraceni, 2013].

- **Laboratory criteria for diagnosis of Non-ketotic hyperosmolar state**

Plasma glucose level	>600 mg/dL
Effective serum osmolality of	320 moms/kg or greater
Profound dehydration, up to an average of	9L
Serum pH greater than	7.30
Bicarbonate concentration greater than	15 mEq/L
Blood urea nitrogen (serum)	< 30 mEq/dL
Urine ketones	nil
Anion gap	variable

- **Symptoms of Non-ketotic hyperosmolar state:** Mental confusion, seizures, frequent urination, nausea or vomiting, dry mouth, thirst, warm, dry skin

without sweating, high fever, sleepiness, vision loss, hallucinations, weakness or strange movements on one side of the body with or without seizures [Venkatraman and Singhi, 2006].

1.1.5.2 Chronic complication of diabetes

1.1.5.2.1Microvascular (Vascular) complications

- **Vascular complications are**

1] Neuropathy; 2] Nephropathy; 3] Retinopathy

1.1.5.2.1.1 Neuropathy

Diabetic neuropathies are a family of nerve disorders caused by diabetes. Diabetic neuropathies are a heterogeneous disorder that involves different somatic and autonomic nervous systems. Diabetic neuropathy is most frequent microvascular complication of diabetes mellitus [Kouroumichakis *et al*., 2012]. Predominance of diabetic neuropathy is rising with the global burden of type 2 diabetes **[**Flower, 2008; Abbott, 2011**]**. Diabetic neuropathy is damage to the nerves that allow you to feel sensations such as pain. There are a number of ways that diabetes damages the nerves, but they all seem related to blood sugar being too high for a long period of time. People with diabetes can develop nerve problems at any time, but risk rises with age and longer duration of diabetes. The highest rates of neuropathy are among people who have had diabetes for at least 25 years [Boulton *et al*., 2005; Zhang *et al*., 2008]. Neuropathy was diagnosed by history of numbness, paraesthesias, tingling sensations, burning sensation and confirmed by touch sensation using 10 gm monofilament, vibration sense by tuning fork (128 Hz) and ankle reflex. Painful peripheral neuropathy was diagnosed by history of pain worsening at night. Autonomic neuropathy was diagnosed by history of constipation or diarrhoea, gastroparesis and postural hypotension confirmed by blood pressure recording in lying down and standing positions [Ali *et al*., 2013]. Diabetic neuropathy commonly classified as peripheral, autonomic, proximal, focal and multifocal or mixed [Hung *et al*., 2009].

1.1.5.2.1.2 Classification of diabetic neuropathy

1.1.5.2.1.2.1 Diabetic peripheral neuropathy (PN)

Peripheral neuropathy is a major factor in the occurrence of foot ulcers in patients with diabetes. The relationship between diabetes complications and PN is well established in clinic, hospital and population based prevalence studies in many

different populations in developed nations. The prevalence and pattern of DPN vary from country to country, from as low as 1.5% to as high as 100% in patients with type 2 diabetes depending on the differences in screening approaches, diagnostic criteria and the study population. The neuropathy may be silent and undetected. Up to 7.5% of patients with type 2 diabetes have clinical neuropathy at the time of diagnosis. This rate increases to 50% among patients with diabetes who have had diabetes for 25 years. However, epidemiological studies of the impact of peripheral neuropathy on type 2 diabetes in developing countries are scarce [Janghorbani *et al*., 2006].

- **Symptoms: For sensory nerve**- Numbness in the feet, pain or discomfort in the feet, sharp pain or burning feet. **For motor nerve**- Muscle weakness and loss of muscle tone in the feet and lower legs, loss of balance, changes in foot shape that can lead to areas of increased pressure.

- **Pathogenesis of peripheral neuropathy**

The pathogenesis of diabetic peripheral neuropathy involves the progression of diabetes and chronic hyperglycemia to endothelial dysfunction via increased activity of four possible pathways; protein kinase C pathway via diacylglycerol, advanced glycation end product formation, the Polyol pathway and oxidative stress [Tesfaye *et al*., 2004].

- **Treatment for diabetic peripheral neuropathy**

1] Tricyclic antidepressants

Tricyclic antidepressants (TCAs) inhibit the reuptake of nor-adrenaline and/or serotonin, which modulates pain transmission within the central nervous system. Amitriptyline, imipramine and desipramine are considered the first-line treatment for DPN. DPN that is treated with TCAs achieved a 50% reduction in neuropathic pain [Parmindar *et al*., 2012].

2] Serotonin norepinephrine reuptake inhibitors

Duloxetine (Cymbalta) is a serotonin and nor-epinephrine reuptake inhibitor that relieves pain by increasing synaptic availability of serotonin and nor-epinephrine in the descending inhibitory pathway. Duloxetine is the only antidepressant approved by the FDA for the treatment of diabetic peripheral neuropathy [Raskin *et al*., 2005].

3] Selective serotonin reuptake inhibitors

Selective serotonin reuptake inhibitors increase how much serotonin. They selective serotonin reuptake inhibitors block serotonin reuptake so that serotonin level is increased. If you have more serotonin, you have less pain perception. Drug is paroxetine 40 mg/day and citalopram 10-25 mg/day [Huizinga *et al*., 2007].

4] Anticonvulsants

Anticonvulsants were originally developed to prevent seizures and because neuronal hyperexcitability is associated with neuropathic pain, it is believed that they may be used for treating this debilitating symptom of DPN. Anticonvulsants regulate neuronal hyperexcitability by blocking calcium and/or sodium channels, through the enhancement of inhibitory GABAergic neurotransmission and inhibition of glutamatergic neurotransmission [Jensen, 2002].

5] Topical medication

In addition to capsaicin cream, which is available without a prescription, another topical medication is a lidocaine patch e.g. Lidoderm [Huizinga *et al*., 2007].

1.1.5.2.1.2.2 Diabetic autonomic neuropathy (DAN)

DAN results in significant morbidity and may lead to mortality in some patients with diabetes. Autonomic neuropathy (AN) is a condition that results from damage to nerves that assist in organ and organ system functioning. This nerve damage disturbs signal processing between the autonomic nervous system and the brain [Vinik and Erbas, 2001]. Major clinical manifestations of DAN include resting tachycardia, exercise intolerance, orthostatic hypotension, constipation, gastroparesis, erectile dysfunction, sudomotor dysfunction and impaired neurovascular function. Cardiac autonomic neuropathy is the most prominent focus of autonomic dysfunction because of the life threatening consequences of this complication and the availability of direct tests of cardiovascular autonomic function [Boulton *et al*., 2005]. This kind of nerve damage, diagnose by physical examinations and special tests. For example, an ultrasound test uses sound waves to check on your bladder. Stomach problems can be found using x-rays.

- **Pathogenesis autonomic diabetic neuropathy**

The exact pathogenesis of autonomic diabetic neuropathy is complex and remains unclear. Most of the proposed mechanisms of neuronal injury are based on models of somatic rather than autonomic neuropathy. Although many of these mechanisms

might be shared between autonomic and somatic neuropathies [Dimitropoulos *et al*., 2014]. Hyperglycemia plays the key role in the activation of various biochemical pathways related to the metabolic and/or redox state of the cell which in concert with impaired nerve perfusion, contribute to the development and progression of diabetic neuropathies. Experimental data implicate a number of pathogenic pathways that may impact autonomic neuronal function in diabetes including formation of advanced glycation end products, increased oxidative stress with increased free radical production, activation of the polyol and protein kinase C pathways and activation of genes involved in neuronal damage [Pacher *et al*., 2002; Rodica, 2010].

➢ Symptoms and types of autonomic neuropathy

- **Cardiovascular:** Fatigue, resting tachycardia, syncope, dizziness, balance problems, lightheadedness, orthostatic hypotension, painless myocardial infarction, sudden death.
- **Gastrointestinal:** Dysphagia, bloating, nausea and vomiting, diarrhea, loss of bowel control, constipation, faecal incontinence.
- **Genitourinary:** Loss of bladder control, urinary tract infection, urinary frequency or dribbling, dysuria, urgency, nocturia, erectile dysfunction, loss of libido, dyspareunia, vaginal dryness, anorgasmia.
- **Sudomotor:** Gustatory sweating, pruritus, dry skin, limb hair loss, calluses, reddened areas, nail dystrophies, hyperhidrosis and heat intolerance in the upper torso or anhidrosis in the lower extremities, foot ulcers and edema.
- **Endocrine:** Hypoglycemic unawareness.
- **Pupillary:** Miosis, disturbances of dilatation, Argyll Robertson pupil.
- **Miscellaneous:** Difficulty driving at night, depression, anxiety, sleep disorders, cognitive changes [Kastenbauer *et al*., 2004, Kaur, 2013**]**.

➢ Drug treatment for diabetic autonomic neuropathy

Early identification of cardiovascular autonomic neuropathy permits timely initiation of therapy with the antioxidant α-lipoic acid, which appears to slow or reverse progression of neuropathies in some studies. β-Blockers that are cardioselective or lipophilic might modulate the effects of autonomic dysfunction in diabetes either centrally or peripherally by opposing the sympathetic stimulus and thereby restore the parasympathetic-sympathetic balance [Ziegler *et al*., 1999].

1.1.5.2.1.2.3 Focal and multifocal diabetic neuropathy

Diabetic focal neuropathy (mononeuropathy) affects a single nerve. It occurs mostly in older people with diabetes. Focal neuropathies usually occur suddenly and sometimes improve on their own within 6 to 8 weeks. Focal neuropathy may cause pain in a single, limited area of the body, such as a wrist or foot. When focal neuropathy causes nerve entrapment, soreness and pain may develop gradually over several weeks or months. Pain in and around one of the eyes, trouble moving the eyes and double vision. This occurs when one of the cranial nerves is affected. Pain that occurs in a band-shaped area around the chest or abdomen. Weakness and pain in the lower back, often extending to the thigh (femoral neuropathy), sometimes causing paralysis [Kaur, 2013].

1.1.5.2.1.2.4 Cranial nerve palsy

Cranial neuropathy of III, IV and VI as well as Bell's palsy in patients with diabetes occurs due to a microvascular infarct which in the majority, resolves spontaneously over several months. III (3.3%) and VI (3.3%) cranial nerve palsies seem to be equal and greater in prevalence than fourth (2.1%) nerve palsy. Cranial nerve III involvement results in ophthalmoplegia, ptosis and diplopia with sparing of pupillary function. In patients with third nerve palsy, it is advisable to perform a brain Magnetic Resonance Imaging (MRI) scan and a Magnetic Resonance Angiogram (MRA) to exclude other causes of Oculomotor nerve palsy [Kaur, 2013]. NSAIDs commonly are used to treat the pain in ischemic third cranial nerve palsy.

1.1.5.2.1.2.5 Entrapment neuropathy (EN)

EN is characterized by spontaneous and/or paroxysmal pain felt in the cutaneous or deep distribution of an involved sensory or mixed nerve or corresponding to the anatomical course of the nerve trunk or its branches. Upto one third of patients with diabetes may have a nerve entrapment. Common nerves involved are the ulnar, median, peroneal and medial plantar nerves. Electrophysiological studies are the most helpful in identifying blocks in conduction at the entrapment sites [Kaur, 2013]. The symptoms of EN depend of the kind of nerve damaged. Small, superficial, purely sensory nerves will present with burning pain in a typical area served by this nerve. Mixed nerves may give paresthesias and loss of muscle function, sometimes with long-lasting muscle atrophy [Zbigniew, 2010].

1.1.5.2.1.2.6 Truncal radiculoneuropathy (TRN)

It involves pain over a focal area on the chest and/or abdomen, which is usually unilateral. Weight loss in some cases may be profound. However, good prognosis with spontaneous recovery over several months has been reported [Kaur, 2013]. Antidepressant, anticonvulsant, antiarrhythmic, neural blockade agents used in treatment.

1.1.5.2.1.2.7 Lumbosacral radiculoplexus neuropathy (LRN)

LRN also known as Bruns-Garland syndrome. It is most common in older patients with type 2 diabetes and is rarely encountered in those with type 1 diabetes. Males are more frequently affected than females. Severe burning and aching pain which is worse at night affects lower back, buttocks or anterior thighs. Weakness follows pain within a matter of a few days to several weeks and usually unilateral at onset. Later may be bilateral but always asymmetrical in nature. It mainly involves proximal muscles but not uncommon for distal group to be involved. It may slowly progress over several weeks. Weight loss may be dramatic (>10–20 kg). Recovery is heralded by stabilization of body weight and resolution of pain with reasonable prognosis. Muscle strength improves slowly over many months but a number of patients never regain normal lower limb strength [Kaur, 2013]. Corticosteroids are used in the treatment [Thaisetthawatkul and Dyck, 2010].

1.1.5.2.1.2.8 Proximal diabetic neuropathy (PDN)

The term proximal neuropathy refers to nerve damage that specifically affects the thighs, hips or buttocks. It is particular case of the peripheral neuropathy that mostly occurs in senior people and is considered a common complication of diabetes. It is also called lumbosacral plexus neuropathy, femoral neuropathy or diabetic amyotrophy.

- **Diagnosis of PDN**

Motor and sensory nerve conduction studies and electromyographic examination of muscles are the basic techniques used for diagnosing the different types of diabetic neuropathies. Both nerve conduction studies and electromyography study the large diameter myelinated fibers.

- **Symptoms of PDN**

Symptoms of proximal neuropathy may vary depending on which nerves are affected. Pain in the buttocks, hips and thigh of legs. The pain is usually felt on one side of the

body and can either start gradually, or come on all at once. This is followed by weakness in the proximal muscles of the lower limbs sometimes making a patient unable to stand up from sitting position without assistance.

- **Treatment:** Antidepressants, opiates or opiate like drugs and anticonvulsants are commonly used.

1.1.5.2.2.1 Diabetic nephropathy

Diabetic nephropathy (DN) is the leading cause of chronic renal failure and end-stage renal disease worldwide [Mianzhi *et al*., 2012]. Previous reports suggest that 43% of the chronic renal failure (CRF) patients on dialysis have diabetic nephropathy, 60% death cases of diabetes mellitus patients are due to diabetic nephropathy, death cases of diabetes mellitus patients due to renal failure are 17 times more as compared to non-diabetes mellitus patients [Lin *et al*., 2010; Freedman *et al*., 2010]. Metabolic and haemodynamic factors interactions are mainly causative to diabetic nephropathy which activates common pathways for renal damage [Cooper, 2001]. Metabolic derangement, glomerular hypertension, oxidative stress and advanced glycation end products are responsible for progression of diabetic nephropathy [Somani *et al*., 2012]. It includes clinical irregularities of kidney which consists of increased creatinine level and also elevation in urea, albuminuria, arterial blood pressure and retention of fluid [Kafle *et al*., 2012].

- **Pathology of diabetic nephropathy**

Pathologically diabetic kidney shows thickening of glomerular basement membrane and mesangial expansion which leads to microalbuminurea, hyperfiltration, intertubular fibrosis along with increase in extracellular matrix. In post stage of the disease, due to glomerulosclerosis; urine albumin increases to a level which can be detected by normal urine analysis [Korish and Sallam, 2007]. Therefore prevention of the occurrence and the development of DN has become a very urgent issue. Diabetic nephropathy is a chronic complication of type 1 diabetes causes β-cell destruction absolute lack of insulin and type 2 diabetes causes insulin resistance and/or decreased secretion of insulin [Ayoola, 2008**]**. There are five stages in the development of diabetic nephropathy.

Stage I:

Hypertrophic hyperfiltration, in this stage, glomerular filtration rate (GFR) is either normal or increased. Stage I lasts approximately five years from the onset of the

disease. The size of the kidneys is increased by approximately 20% and renal plasma flow is increased by 10%-15% while albuminuria and blood pressure remain within the normal range.

Stage II:

This stage starts approximately two years after the onset of the disease and is characterized by kidney damage with basement membrane thickening and mesangial proliferation. There are still no clinical signs of the disease. GFR returns to normal values. Many patients remain in this stage until the end of their life.

Stage III:

The microalbuminuria stage (albumin 30-300 mg/dU) or initial nephropathy. This is the first clinically detectable sign of glomerular damage. It usually occurs five to ten years after the onset of the disease. Blood pressure may be increased or normal. Approximately 40% of patients reach this stage.

Stage IV:

Chronic renal failure (CRF) is the irreversible stage. Proteinuria develops (albumin > 300 mg/dU), GFR decreases below 60 mL/min/1.73 m^2 and blood pressure increases above normal values.

Stage V:

Terminal kidney failure (TKF) (GFR < 15 mL/min/1.73 m^2). Approximately 50% of the patients with TKF require kidney replacement therapy peritoneal dialysis, kidney transplantation [Mogasen *et al*., 1983].

- **Factors important in the pathogenesis of diabetic nephropathy (DN)**

1. Genetic predisposition

Genetic predisposition to DN is suggested by the observation that the diabetic sibling of a patient with DN has a three-fold greater risk of developing nephropathy than the diabetic sibling of a diabetic without nephropathy. Reported that 83% of type-2 diabetic siblings of probands with DN has evidence of renal disease compared with only 17% of sibling so probands without nephropathy [Olugbenga and Salako, 2004**]**.

In patients with type-2 diabetes, angiotensin converting enzyme (ACE) genotype has been associated with an increased risk for the development of DN, more severe

proteinuria, a greater likelihood of progressive renal failure and an enhanced mortality on dialysis [Wright and Hutchison, 2009; Olugbenga and Salako, 2004].

2. Elevated blood pressure

The presence of hypertension in the diabetic population is two to three times higher than in a nondiabetic. An association between subsequent development of nephropathy and higher systemic pressures, particularly if in the hypertensive range has been observed in prospective studies [Earle and Viberti, 1994; Ismail *et al*., 2008]. Abnormal blood pressure is strongly correlated with the levels of albuminuria. Increased level albuminuria is associated with an increased risk of cardiovascular and renal disease in patients with diabetes and hypertension [Yuyun *et al*., 2003; Jerums and Maclsaac, 2002]. It is also one of the important markers which are routinely used to monitor diabetic nephropathy [Kiran *et al*., 2012].

3. Increased blood sugar level

Increased level of glucose in DN is more likely to develop in patients with lesser degrees of glycemic control. This is supported by previous literature, which showed that the risk of development and progression of albuminuria could be substantially reduced by improving glycemic control [Hostetter, 2003; Rohilla *et al*., 2011].

4. Smoking

Smoking increases fibrinogen concentrations and carboxyhemoglobin concentrations, all of which may result in tissue hypoxia and contribute to vascular damage. In addition smoking may acutely raise blood pressure and thereby affect kidney function. Smoking and metabolic control were found to be independent factor for development and progression of borderline to increased albuminuria level in diabetic patient [Sawicki *et al*., 1994; Pedersen *et al*., 2010].

5. Male gender

Male gender has been associated with the development of nephropathy in diabetes in many studies. It was previously reported that males had a 2.6 time greater risk of developing incipient or overt nephropathy [Westergaard *et al*., 1997].

6. Dyslipidemia

Many observational studies suggested that lipid may play a role in the development and progression of glomerular injury. The level of cholesterol both onset and after a five year follow-up period was positively related with the subsequent increase in urinary albumin excretion in macroalbuminuric patients with type 2 diabetes [Ravid *et*

al., 1995]. Further serum cholesterol was significantly associated with development of abnormally increased urinary albumin excretion [Westergaard *et al*., 1997]. Higher serum cholesterol level and HDL-cholesterol were associated with incidence of renal insufficiency [Trevisan *et al*., 2006; Wanner, 2000].

7. Age

Clinical nephropathy secondary to glomerular disease usually manifests 15-25 years after diagnosis of diabetes and affects 25-35% of patients under the age of 30 years [Appel, 2011].

1.1.5.2.2.2 Metabolic pathways

Three major pathways showing abnormality of intracellular metabolism have been identified in the development of diabetic nephropathy: (i) the activation of polyol and PKC (protein kinase-C) pathways; (ii) the formation of advanced glycation end-products; (iii) intraglomerular hypertension induced by glomerular hyperfiltration. Upstream of these three major pathways, hyperglycaemia is the major driving force of the progression to end stage of renal disease (ESRD) from diabetic nephropathy [Wada and Makino, 2013].

1.1.5.2.2.3 Treatment for diabetic nephropathy

Diabetes mellitus and hypertension are leading causes of end stage renal disease in the United States. Drug therapy that focuses on tight glycemic control and blood pressure control reduces the progression of nephropathy and cardiovascular complications. Angiotensin-converting enzyme (ACE) inhibitors have been shown to reduce the progression of renal disease in patients with diabetes. The angiotensin II receptor blockers (ARBs) losartan and irbesartan have also been shown to reduce microalbuminuria compared with placebo. The non-dihydropyridine calcium channel blockers (CCBs) verapamil and diltiazem have been shown to be as effective as an ACE inhibitor in reducing urinary albumin excretion.

I] Angiotensin-converting enzyme (ACE) inhibitors

Angiotensin-converting enzyme (ACE) inhibitors have demonstrated great utility in the treatment of diabetic renal disease. Until recently, ACE inhibitors were considered first-line therapy for treatment of all diabetic nephropathy. ACE inhibitors can be divided into three groups based on their molecular structure;

1. **Sulfhydryl-containing agents:** e.g. Captopril, Zofenopril
2. **Dicarboxylate-containing agents:** e.g. Enalapril, Ramipril, Quinapril, Perindopril
3. **Phosphonate-containing agents:** e.g. Fosinopril

1. Sulfhydryl-containing agents:

e.g. Captopril

Formula: $C_9H_{15}NO_3S$; Molecular mass: 217.29 g/mol; FDA approval: 1981; Dose: 75-100 mg/day; route: oral; metabolism: hepatic; half life: 1.9 h; excretion: renal.

Mode of action

The mechanism of action of captopril has not yet been fully elucidated. Its beneficial effects in hypertension and heart failure appear to result primarily from suppression of the renin-angiotensin-aldosterone system. Captopril prevents the conversion of angiotensin I to angiotensin II by inhibition of ACE, a peptidyldipeptide carboxy hydrolase. This inhibition has been demonstrated in both healthy human subjects and in animals by showing that the elevation of blood pressure caused by exogenously administered angiotensin I was attenuated or abolished by captopril.

Side effects: Itching, headache, tachycardia, chest pain, palpitations, weakness

Contraindications: Contraindicated for hypersensitive patients.

2. Dicarboxylate-containing agents

e.g. Enalapril

Formula: $C_{20}H_{28}N_2O_5$; Molecular mass: 376.447 g/mol; FDA approval: 2008, Dose: 2.5 to 5 mg/kg; route: oral; metabolism: hepatic, half life: 11 h, excretion: renal.

Mode of action

Enalapril belongs to a class of medications called angiotensin converting enzyme inhibitors. Normally angiotensin I is converted to angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II constricts blood vessels, increasing blood pressure. By inhibiting ACE, enalapril decreases levels of angiotensin II leading to less vasoconstriction and decreased blood pressure.

Side effects: Dizziness, low blood pressure, syncope, dry cough, swelling of face and lips.

Contraindications: Contraindicated in pregnancy, angioneurotic edema and bilateral renal artery stenosis.

3 Phosphonate-containing agents:

e.g. Fosinopril

Formula: $C_{30}H_{46}NO_7P$; Molecular mass: 563. 66 g/mol; FDA approval: 2002; Dose: 10 mg/day; route: oral; metabolism: hepatic; half life: 12h; excretion: renal.

Mode of action

There are two isoforms of ACE: the somatic isoform, which exists as a glycoprotein and the testicular isoform, which has a lower molecular mass and is thought to play a role in sperm maturation and binding of sperm to the oviduct epithelium. Somatic ACE has two functionally active domains N and C which arise from tandem gene duplication. Although the two domains have high sequence similarity, they play distinct physiological roles. The C-domain is predominantly involved in blood pressure regulation while the N-domain plays a role in hematopoietic stem cell differentiation and proliferation. ACE inhibitors bind to and inhibit the activity of both domains, but have much greater affinity for and inhibitory activity against the C-domain. Fosinoprilat, the active metabolite of fosinopril, competes with ATI for binding to ACE and inhibits and enzymatic proteolysis of ATI to ATII. Decreasing ATII levels in the body decreases blood pressure by inhibiting the pressor effects of ATII. Fosinoprilat also causes an increase in plasma renin activity likely due to a loss of feedback inhibition mediated by ATII on the release of renin and/or stimulation of reflex mechanisms via baroreceptors.

Side effects: Feeling light-headed, less urination, fever, chills, body aches, flu symptoms, slow heart rate, weak pulse, muscle weakness, tingly feeling and chest pain.

Contraindications: Hypertensive patients.

II]. Angiotensin II receptor blockers (ARBs)

e.g. Losartan

Formula: $C_{22}H_{23}ClN_6O$; Mol. Mass: 422.91g/mol; FDA approval: 1995, Dose: 100 mg/day; route: oral; metabolism: hepatic, half life: 1.5-2 h, excretion: renal 13-25% and biliary 50-60%.

Mode of action

The renin-angiotensin system, specifically angiotensin II, is implicated in the pathogenesis of essential hypertension, renovascular hypertension, congestive heart

failure and renal diseases associated with albuminuria. The ARBs' mechanism of action, selective inhibition of angiotensin II by competitive antagonism of the angiotensin II receptors has been speculated to reduce adverse effects and possibly improve clinical efficacy. ARBs displace angiotensin II from the angiotensin I receptor and produce their blood pressure lowering effects by antagonizing angiotensin II induced vasoconstriction, aldosterone release, catecholamine release, arginine vasopressin release, water intake and hypertrophic response [Barreras *et al*., 2003].

Side effect: Upper respiratory infections or stuffy nose, dizziness, back pain, diarrhoea, fatigue, low blood pressure, low blood sugar, elevated potassium and chest pain; more serious side effects include low blood pressure and allergic reaction.

Contraindication: Using losartan while pregnant could result in fetal injury or death.

III] Non-dihydropyridine calcium channel blockers (CCBs)

e.g. Verapamil

Formula: $C_{27}H_{38}N_2O_4$; Mol. mass: 454.602 g/mol; FDA approval: 1982, Dose: 5-10 mg/day; route: oral, i.v; metabolism: hepatic, half life: 2.8-7h, excretion: renal.

Mode of action

Verapamil inhibits voltage-dependent calcium channels. Specifically, its effect on L-type calcium channels in the heart causes a reduction in ionotropy and chronotropy thus it reduces heart rate and blood pressure. Calcium channels are also present in the smooth muscle that lines blood vessels. By relaxing the tone of this smooth muscle, calcium-channel blockers dilate the blood vessels. This has lead to their use in treating hypertension, angina pectoris and renal hypertension.

Side effects: Headaches, facial flushing, dizziness, lightheadedness, swelling, increased urination, fatigue, nausea, ecchymosis, galactorrhea, constipation and gingival hyperplasia.

Contraindications: Bradycardia, second and third degree atrioventricular block, heart failure, Wolff-Parkinson-White syndrome.

1.1.5.2.3.1 Diabetic retinopathy

It is a microvascular complication of diabetes. Diabetic retinopathy is an eye disease occurring in persons with diabetes. It involves damage to the small blood vessels in

the peripheral retina or macula or both that leads to impairment of retinal blood flow and capillary blockage can result in hypoxia which can eventually lead to visual disability and blindness in patient [Cade, 2008].

Diabetic macular changes in the form of yellowish spots and extravasations that permeated part or the whole thickness of the retina were observed for the first time by Eduard Jaeger in 1856. This was only possible as a result of the newly developed direct ophthalmoscope that was first described in 1855. Jaeger's findings were controversial at the time and Albrecht Von Graefe openly claimed that there was no proof of a causal relationship between diabetes and retinal complications in 1872. In 1876, Wilhelm Manz described the proliferative changes occurring in diabetic retinopathy and the importance of tractional retinal detachments and vitreous haemorrhages [Wolfensberger and Hamilton, 2001].

However, it was not until 1943 that the work of Arthur James Ballantyne provided evidence that diabetic retinopathy represents a unique form of vascular disease. In the early years of the 20th century, the debate continued whether macular changes were directly related to diabetes or whether they were due to hypertension and arteriosclerosis. A number of multicentre clinical trials during the last ten years have contributed substantially to the understanding of the natural history of diabetic retinopathy and have established the value of intensive glycaemic control in reducing both the risk of onset and the progression of diabetic retinopathy [Kalantzis *et al*., 2006].

The alarming rise in diabetes prevalence is a global public health and economic problem. Diabetic retinopathy is the leading cause of blindness among working-aged adults around the world [Sivaprasad *et al*., 2012]. Despite the significance of this problem and the rising prevalence of diabetes remarkably in emerging Asian countries such as India and China [Cockram, 2000].

Prevalence of diabetic retinopathy related to the two sight-threatening end points proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) based on a pooled individual participant analysis of more than 20,000 participants from 35 studies around the world. 35% of people with diabetes had some form of diabetic retinopathy and 7% had PDR, 7% had DME and 10% were affected by these vision-threatening stages. In 2010, approximately 93 million peoples were affected by diabetic retinopathy and 28 million by vision-threatening diabetic retinopathy (VTDR). This suggests that diabetic retinopathy has the potential to be the leading

cause of visual impairment and blindness worldwide. The importance and impact of three major modifiable risk factors hyperglycemia, hypertension and dyslipidemia on the risk of all diabetic retinopathy end points, including for the first time, PDR and DME. More research is required and study methodologies must be better standardized [Yau *et al*., 2012**]**.

1.1.5.2.3.1.1 Diagnosis of diabetic retinopathy

- **Visual acuity test:** This test uses an eye chart to measure how well a person sees at various distances.
- **Pupil Dilation:** The eye care professional places drops into the eye to widen the pupil. This allows him or her to see more of the retina and look for signs of diabetic retinopathy. After the examination, close-up vision may remain blurred for several hours.
- **Ophthalmoscopy or Fundus Photography:** Ophthalmoscopy is an examination of the retina in which the eye care professional looks through a slit lamp biomicroscope with a special magnifying lens that provides a narrow view of the retina or wearing a headset (indirect ophthalmoscope) with a bright light, looks through a special magnifying glass and gains a wide view of the retina. Hand-held ophthalmoscopy is insufficient to rule out significant and treatable diabetic retinopathy. Fundus photography generally recreates considerably larger areas of the fundus and has the advantage of photo documentation for future reference as well as availing the image to be examined by a specialist at another location and/or time.
- **Fundus Fluorescein Angiography (FFA):** This is an imaging technique which relies on the circulation of Fluorescein dye to show staining, leakage or non-perfusion of the retinal and choroidal vasculature.
- **Optical Coherence Tomography (OCT):** This is an optical imaging modality based upon interference and analogous to ultrasound. It produces cross-sectional images of the retina which can be used to measure the thickness of the retina and to resolve its major layers, allowing the observation of swelling.
- **Digital Retinal Screening Programs:** Systematic programs for the early detection of eye disease including diabetic retinopathy are becoming more common, such as in the UK, where all people with diabetes mellitus are offered

retinal screening at least annually. This involves digital image capture and transmission of the images to a digital reading center for evaluation and treatment referral.

- **Computer Vision Approach:** It is a system developed by researchers of IIT Kharagpur in collaboration with IBM India. It uses data analytics capabilities to automatically compare and analyse retina images of the patient. It can tell if the patient has diabetic retinopathy and also provides risk categorisation ranging from low to medium and high.
- **Slit Lamp Biomicroscopy Retinal Screening Programs:** Systematic programs for the early detection of diabetic retinopathy using slit-lamp biomicroscopy. These exist either as a standalone scheme or as part of the digital program where the digital photograph was considered to lack enough clarity for detection and/or diagnosis of any retinal abnormality.

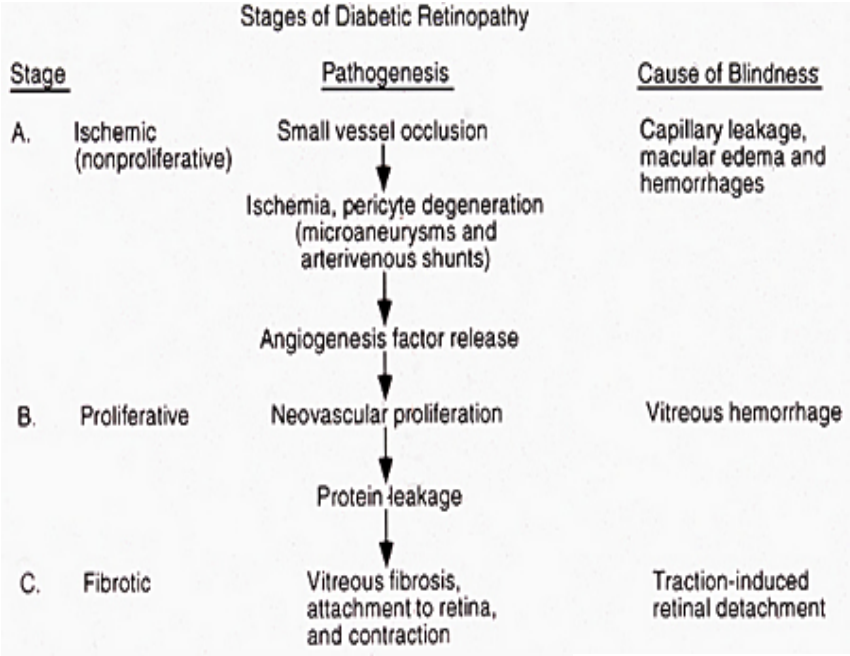
1.1.5.2.3.1.2 Pathogenesis of diabetic retinopathy:

The exact etiology and pathogenesis of diabetic retinopathy has yet to be defined. A number of potential contributing factors have been identified but their relative importance and sequence of interaction are unclear. It has been proposed that the development of a hypoxic retina is the primary factor leading to diabetic retinopathy. A variety of metabolic factors may contribute to hypoxia. In vitro studies have demonstrated lactic acid accumulation in diabetic retinas particularly in the presence of high ambient glucose concentrations.

Decreased erythrocyte 2, 3-diphosphoglyceric acid (2,3-DPG) levels are seen in poorly controlled diabetes as are increased concentrations of advanced glycated hemoglobin (HbA1c). Both of these changes result in increased hemoglobin affinity for oxygen, which could produce tissue hypoxia although this mechanism is responsible for the development of hypoxia. Nevertheless, most investigators accept that hypoxia ultimately does develop and plays a central role in the further pathogenesis of diabetic retinopathy [Blankenship and Skyler, 1978].

- Despite of that diabetic retinopathy is a microangiopathy affecting the retinal precapillary arterioles, capillaries and venules. Retinopathy has features of both microvascular occlusions, microvascular leakage.

1.1.5.2.3.1.3 Stages of diabetic retinopathy



[Adapted Blankenship *et al*, 1978]

Figure1.1.4.1.5 Stages of diabetic retinopathy

1.1.5.2.3.1.4 Systamic factor of diabetic retinopathy

1. Glycaemic control

The Diabetes Control and Complications Trial (DCCT) investigated the effect of hyperglycemia in type 1 diabetic patients, as well as the incidence of diabetic retinopathy, nephropathy and neuropathy. The protective effect of glycemic control has also been for confirmed patients with type 2diabetes [Fong *et al*., 2004]. The U.K. Prospective Diabetes Study (UKPDS) demonstrated that improved blood glucose control reduced the risk of developing retinopathy, nephropathy [King *et al*., 1999**]**.

2. Hypertension

The DCCT and UKPDS have shown that poor control of diabetes cause development and progression of retinopathy [Gillow *et al*., 1999; King *et al*., 1999]. The metabolic and haemodynamic factors tend to interact in the evolution of diabetic retinopathy. Increased blood pressure has been hypothesized through the effects of increased sheer stress of blood flow to damage the retinal capillary endothelial cells in eyes of people with diabetes. The possible mechanisms by which hypertension may affect diabetic retinopathy are haemodynamic (impaired auto regulation and hyperperfusion) and through VEGF (vascular endothelial growth factor). This hypothesis has been

supported by observations from clinical studies which showed an association between hypertension and the presence and severity of retinopathy in people with diabetes [Reema and Pradeep, 2007].

3. Renal disease

A link between renal and retinal angiopathy in diabetes has been long recognized an effect that may be mediated through an increase in blood pressure, fibrinogen levels and lipoproteins [Cho *et al*., 2008; Dedov *et al*., 2009]. Cross-sectional and longitudinal studies reported relationship between microalbuminuria, proteinuria and retinopathy [Ramana *et al*., 2011]. Previously it has been reported that proteinuria were significant determinants of progression of renal disease in type 2 diabetic patients with retinopathy [Trevaisn *et al*., 2002].

4. Elevated serum lipids

Elevated lipid levels in systemic circulation constitute a risk factor for diabetic retinopathy [Lim and Wong, 2012]. Individuals with elevated total serum cholesterol, low-density lipoprotein (LDL) cholesterol or triglyceride levels are more likely to have or develop retinal hard exudates, which can be associated with risk of vision loss, independent of the extent of macular oedema [Ferries *et al*., 1996; Indiculla *et al*., 2012]. The Early Treatment Diabetic Retinopathy Study (ETDRS) and the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) group found a statistically significant association between elevated serum total cholesterol and LDL cholesterol and the severity of retinal hard exudation in patients with diabetic retinopathy (DR) [Klein *et al*., 1991; Ferries *et al*., 1996; Miljanovic *et al*., 2004].

5. Pregnancy

It is recognized that DR can progress rapidly during pregnancy due to hormonal changes. Reported that women with the poorest glycaemic control at baseline in the first trimester were at increased risk of DR progression **[**Best and Chakravarthy, 1997**]**. Retinal and chorioretinal disorders that can arise during pregnancy include central serious chorioretinopathy and occlusive vasculopathy such as retinal artery occlusion and retinal vein occlusion [Errera *et al*., 2012].

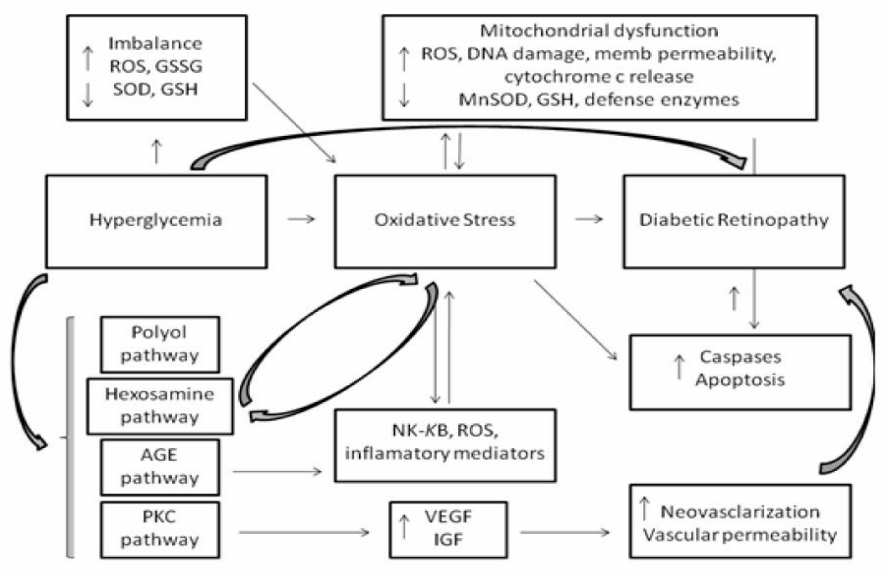
6. Alcohol

A few studies have examined the effect of alcohol consumption on DR. Heavy alcohol consumption to be a risk factor for development of DR in patients without retinopathy at baseline [Akari *et al*., 1993].

1.1.5.2.3.1.5 Symptoms for diabetic retinopathy

Early in diabetic retinopathy there may be no symptoms at all. As the disease progresses symptoms include; blurred vision, fluctuating vision, seeing floating spots, blind spots, changes in color perception, sudden loss of vision, double vision and eye pain in advanced cases.

1.1.5.2.3.1.6 Metabolic pathways responsible for diabetic retinopathy



[Adapted Silva *et al*., 2010]

Figure1.1.4.1.6 Metabolic pathways responsible for diabetic retinopathy

1.1.5.2.3.1.7 Treatment and drugs for diabetic retinopathy

In mild cases, treatment for diabetic retinopathy is not necessary. Regular eye exams are critical for monitoring progression of the disease. Strict control of blood glucose and blood pressure levels can greatly reduce or prevent diabetic retinopathy. In more advanced cases, treatment is recommended to stop the damage of diabetic retinopathy, prevent vision loss and potentially restore vision.

1.1.5.2.3.1.8 Treatment options include

1. Anti-VEGF therapy

Anti-VEGF therapy involves the injection of the medication into the back of your eye. The medication is an antibody designed to bind to and remove the excess VEGF (vascular endothelial growth factor) present in the eye that is causing the disease state. The FDA has approved Lucentis for macular edema and additional treatment options include Avastin and Eylea.

2. Intraocular steroid injection

Intraocular steroid injection is a treatment for diabetic macular edema. This therapy helps reduce the amount of fluid leaking into retina resulting in visual improvement. Due to the chronic nature of diabetic eye disease this treatment may need to be repeated or combined with laser therapy to obtain maximum or lasting effect.

3. Laser surgery

Laser surgery is often helpful in treating diabetic retinopathy. To reduce macular edema, a laser is focused on the damaged retina to seal leaking retinal vessels. For abnormal blood vessel growth (neovascularization), the laser treatments are delivered over the peripheral retina. The small laser scars that result will reduce abnormal blood vessel growth and help bond the retina to the back of your eye thus preventing retinal detachment. Laser surgery may be performed in your ophthalmologist's office or in an outpatient clinic. Laser surgery can greatly reduce the chance of severe visual impairment.

4. Vitrectomy

Vitrectomy may be recommended in advanced proliferative diabetic retinopathy. During this microsurgical procedure that is performed in the operating room, the blood-filled vitreous is removed and replaced with a clear solution. Your ophthalmologist may wait several months to a year to see if the blood will clear on its own before going ahead with surgery. In addition to a vitrectomy, retinal repair may be necessary if scar tissue has detached the retina from the back of your eye. Severe loss of vision or even blindness can result if surgery is not performed to reattach the retina.

1.1.5.2.2 Macrovascular (Non-vascular) complications of diabetes

Type 2 diabetes is a consequence of chronic sustained hyperglycemia which leads to provoke certain macrovascular complications [Ceriello and Kilpatrick, 2013; Aryangat and Gerich, 2010]. The mortality and morbidity of cardiovascular disease (CVD) are markedly increased in diabetic individuals compared to the nondiabetic population [Howard *et al*., 2006; Marzilli *et al*., 2012].

It has been reported that diabetes affects the heart in 3 ways: coronary artery disease (CAD) due to accelerated atherosclerosis; cardiac autonomic neuropathy (CAN) and diabetic cardiomyopathy (DbCM) [Joseph *et al*., 2013].

1.1.5.2.2.1 Coronary artery disease (CAD)

Coronary artery disease (CAD) also known as atherosclerotic heart disease, coronary heart disease, or ischemic heart disease (IHD), is the most common type of heart disease and cause of heart attacks **[**Akinboboye *et al*., 2003; Aronson, 2011**]**. Coronary heart disease is generally caused by atherosclerosis when plaque (cholesterol substances) accumulates on the artery walls, causing them to narrow, resulting in less blood flow to the heart [Efimov *et al*., 2001**]**. It causes angina pectoris (chest pain), shortness of breath and heart attack (myocardial infarction) [Zellweger *et al*., 2004].

1.1.5.2.2.2 Cardiac autonomic neuropathy (CAN)

Cardiac autonomic neuropathy (CAN) is the most serious common form diabetic complication [Debono, 2007**]**. It causes high risk of mortality in diabetic patients [Pop-Busui, 2010]. Commonly it has been called as a silent killer, because very few patients realize that they are suffering from it and yet its effect can be lethal [Khandoker *et al*., 2008**]**.

The impairment of sympathetic and parasympathetic divisions of the autonomic nervous system (ANS) leads to diabetic autonomic neuropathy (DAN); it causes functional changes in many systems including the cardiovascular, urogenital gastrointestinal systems [Shotton *et al*., 2003, Rolim *et al*., 2013**]**. CAN is associated with a high risk of unexpected and sudden death, possibly related to silent myocardial ischemia/infarction, cardiac arrhythmias and hypoxia [Kempler, 2003**]**. Clinical studies found that prevalence of cardiac autonomic neuropathy higher in type 2 diabetic patients [Fleischer *et al*., 2014**]**. Several epidemiological studies among individuals diagnosed with diabetes, it was shown that the 5-year mortality rate from this serious complication is five times higher for individuals with CAN than for individuals without cardiovascular autonomic involvement [Vinik *et al*., 2001**]**.

The diagnosis of CAN is divided into two categories such as clinical and subclinical. Clinical diagnosis based on sign or symptoms such as erectile dysfunction, dizziness, intermittent visual impairment, postprandial hypotension, resting tachycardia or exercise intolerance and subclinical diagnosis perform by conducting five most sensitive and specific methods such as :(1) Study of heart rate variability (HRV) using the ratio of the RR intervals of the electrocardiogram (ECG); (2) Baroreflex sensitivity (BRS); (3) Muscle sympathetic nerve activity (MSNA); (4) Measurement

of plasma levels of catecholamines (PLC); (4) Cardiac sympathetic mapping (CSM) [Gulichsen *et al*., 2012; Rolim *et al*., 2013].

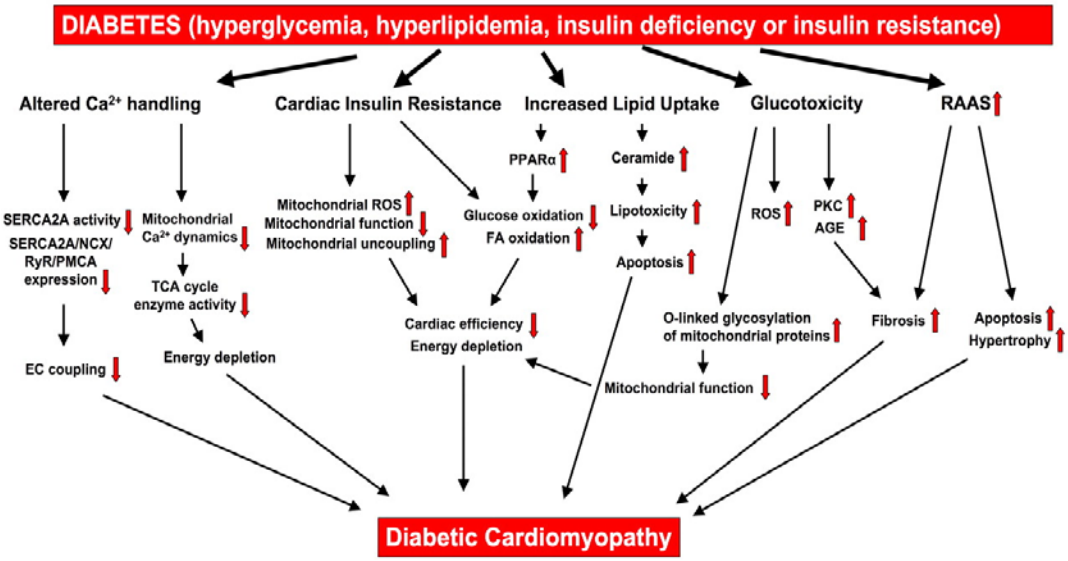
Both vascular and metabolic factors have been invoked in the pathogenesis of diabetic CAN, but their inter-relationships are poorly understood. Vascular factor like nerve ischemia that reduced nerve conduction velocity. Metabolic changes include polyol pathway hyperactivity, oxidative stress, increased advanced glycation and impaired essential fatty acid metabolism. Above mentioned metabolic factors may decrease synthesis of nitric oxide in either the vascular endothelium or in the sympathetic ganglia leading to decreased nerve blood flow that may responsible for development of CAN [Kempler, 2003**]**.

1.1.5.2.2.3 Diabetic cardiomyopathy

Diabetic cardiomyopathy was first clamed around four decades ago [Bell, 2003]. In 1972 Rubler *et al.* frame the term 'diabetic cardiomyopathy' to describe this form of disease of heart muscle in diabetic patients [Battiprolu, 2010; Dokken, 2008]. It has been described as diabetic cardiomyopathy is a distinct primary disease characterized by the presence of abnormal myocardial performance or structure in the absence of coronary artery disease, hypertension and significant vascular disease in patients with diabetes [Kiencke *et al*., 2010]. Incidence and prevalence of diabetic cardiomyopathy are growing worldwide, about 65-70% of diabetic people have been died due to cardiac dysfunction, so diabetes emerges as the one of the leading consequence of cardiovascular disease. Therefore cardiac protection is very perceptive issue in diabetes [Manoria *et al*., 2013].

The etiology of diabetic cardiomyopathy is still poorly understood. The etiology of diabetic cardiomyopathy is multifactorial and incompletely characterized but a picture of the process is beginning to emerge. Two major precipitating factors have been identified. The first is hyperglycemia, a major mediator of many diabetic complications. The second relates to the effect of diabetes on cardiac metabolism. Whereas the normal heart derives its energy from carbohydrates (20% to 40%) and fatty acid metabolism (60% to 80%), the diabetic heart relies almost exclusively on fatty acids as an energy source [Sharma and McNeill, 2006**]**. In addition to hyperglycemia, type 2 diabetic patients encounter the complications associated with hyperinsulinemia and dyslipidemia. The concert of these pathogenetic changes results in a particular sequence of events that include haemodynamic alterations and

structural changes such as extracellular matrix (ECM) protein deposition, fibrosis, myocyte hypertrophy, and necrosis [Farhangkhoee *et al*., 2006].



[Adapted from Bugger and Abel, 2009**]**

Figure1.1.4.1.7 Pathophysiology of diabetic cardiomyopathy: Five major mechanisms and their downstream processing

1.1.5.2.2.3.1 Factors are responsible for development of diabetic cardiomyopathy

- Abnormalities in intracellular Ca2+ homeostasis
- Activation of the renin-angiotensin System
- Hyperlipidemia and lipotoxicity
- Diabetic vasculopathy and microangiopathy
- Disordered copper metabolism
- Hypoxia inducible factor-1 and vascular endothelial growth factor
- Protein kinase C
- Poly-(ADP-ribose) polymerase

[Bugger and Abel, 2009; Mikki *et al*., 2013]

1.1.5.2.2.3.2 Treatment of Diabetic Cardiomyopathy

1. Glycemic Control

Studies have shown that for each 1% elevation in glycosylated hemoglobin, the risk of developing heart failure increases by 8%. [Iribarren *et al*., 2001]. Evidence suggests that good glycemic control is beneficial, at least in early stages of diabetic cardiomyopathy. Good glycemic control remains instrumental to the overall management of diabetic cardiomyopathy. Glucagon-like peptide-1 is an incretin hormone which stimulates postprandial insulin secretion and improves insulin

sensitivity. A GLP-1 analogue in patients shows improvement in LVEF [Nikolaidis *et al*., 2004]. Dipeptidyl peptidase (DPP)-4 inhibitors, or gliptin, are a group of drugs which increase incretin levels by inhibiting the enzyme DPP-4. Thiazolidinediones are primarily insulin sensitizing agents, but in addition to their antihyperglycemic action these drugs also exert beneficial effects on the myocardium, vascular endothelium, myocardium and lipid profile [McGuire and Inzucchi, 2008]. In general, the choice of antidiabetic agents in diabetic cardiomyopathy should be based on clinical characteristics, risk of hypoglycemia, age, volume status and multiple drug therapy [Muraka and Mohaved, 2010].

2. Angiotensin converting enzyme (ACE) inhibitors

Angiotensin converting enzyme (ACE) inhibitors have widespread effect on micro and macrovascular complications in diabetes and may affect myocardial fibrosis through effects on angiotensin II. Meta-analyses of the major ACE inhibitor trials showed that diabetic patients achieve similar reductions in mortality as non diabetic patients with LV systolic dysfunction [Shekelle *et al*., 2003]. Evidence also suggests a beneficial effect of aldosterone antagonism in diastolic heart failure by virtue of their beneficial effects on cardiac hypertrophy and fibrosis**.** Angiotensin II is considered to be a major player in the development of cardiac dysfunction. Angiotensin receptor Blockers like candesartan and telmisartan reduced the risk of developing Type II diabetes**.** Telmisartan has got some PPAR-ϒ inhibition activity. These findings underscore the critical importance of inhibiting the RAAS in diabetic patients, especially when diastolic dysfunction is present and the process is potentially reversible [Muraka and Mohaved, 2010; Hayat *et al*., 2004].

e.g. Candesartan

Formula: $C_{24}H_{20}N_6O_3$; Molecular mass: 440.45 g/mol; FDA approval: 2005, Dose: 4-32 mg/day; route: oral; metabolism: hepatic; half life: 9h; excretion: renal 33% and fecal 67%.

Mode of action

Angiotensin II is formed from angiotensin I in reaction catalyzed by angiotensin converting enzyme (ACE kinase II) angiotensin II is the preclinical pressor agent of renin angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Candesartan blocks the vasoconstrictor and aldosterone secreting effects of

angiotensin II by selectively blocking the binding of angiotensin II to the angiotensin I receptor in many tissues, such as vascular smooth muscle and adrenal gland. Its action is therefore, independent of the pathways for angiotensin II synthesis.

Side effects: Feeling light-headed or dizzy at standing position, headache and chest infection.

Contraindications: Contraindicated for hypertensive patient.

3. β-Blockers

β-Blockers are now well defined in the treatment of heart failure. Concerns in diabetic patients regarding blood sugar, insulin resistance and dyslipidemia meant that diabetic patients with heart failure were less likely to be on β-blockers. However, with the recent advances in the understanding of heart failure and the realization of the importance of the sympathetic nervous system in the release of vasoactive substances, they have become essential in the treatment of heart failure. In one study, carvedilol was shown to have has better effects on glycemic control and insulin resistance compared with metoprolol in the presence of RAAS blockade or in the absence of insulin sensitizers [Bakris *et al*., 2004]. β-Blockers should be given to all diabetic patients with any evidence of heart failure, unless specifically contraindicated. The effect may not be as pronounced as in non diabetics, but it will result in relative risk reduction of mortality [Muraka *et al*., 2010; Hayat *et al*., 2004].

e.g. Carvedilol

Formula: $C_{24}H_{26}N_2O_4$; Molecular mass: 406.474 g/mol; FDA approval: 1995, Dose: 6.25- 25 mg/day; route: oral, i.v; metabolism: liver; half life: 7-10h; excretion: renal 16% and fecal 60%.

Mode of action

Carvedilol is used for treating high blood pressure and congestive heart failure. Carvedilol blocks receptors of the adrenergic nervous system, the system of nerves in which epinephrine (adrenalin) is active. Nerves from the adrenergic system enter the heart and release an adrenergic chemical (norepinephrine) that attaches to receptors on the heart's muscle and stimulates the muscle to beat more rapidly and forcefully. By blocking the receptors, carvedilol reduces the heart's rate and force of contraction and thereby reduces the work of the heart. Carvedilol also blocks adrenergic receptors on arteries and causes the arteries to relax and the blood pressure to fall. The drop in

blood pressure further reduces the work of the heart since it is easier to pump the blood against a lower pressure.

Side effects: Diarrhoea, nausea, vomiting, weakness, headache, cough, vision changes and paresthesias.

Contraindications: Patients with a history of serious hypersensitivity reaction e.g. Stevens-Johnson syndrome, anaphylactic reaction, angioedema.

4. Ca^{2+} channel antagonists

However, nifedipine, diltiazem and verapamil have shown a detrimental effect in heart failure. A short term improvement in ejection fraction was observed with felodipine, but this was not sustained at long-term follow up. With amlodipine there was a reduction of combined fatal and nonfatal events and decreased risk of death in the non-ischaemic subgroup [Hayat *et al*., 2004].

e.g. Nifedipine

Formula: $C_{17}H_{18}N_2O_6$; Molecular mass: 346.335 g/mol; FDA approval: 1989, Dose: 10-20 mg/day; route: oral; metabolism: hepatic; half life: 2h; excretion: renal 50 to 65% and biliary 5-15%.

Mode of action

Nifedipine is a peripheral arterial vasodilator which acts directly on vascular smooth muscle. The binding of nifedipine to voltage-dependent and possibly receptor-operated channels in vascular smooth muscle results in an inhibition of calcium influx through these channels. Stores of intracellular calcium in vascular smooth muscle are limited and thus dependent upon the influx of extracellular calcium for contraction to occur. The reduction in calcium influx by nifedipine causes arterial vasodilatation and decreased peripheral vascular resistance which results in reduced arterial blood pressure.

Side effects: Bloating or swelling of the face, cough, difficult or labored breathing, dizziness, fast, irregular, pounding or racing heartbeat or pulse, feeling of warmth, headache, muscle cramps, rapid weight gain.

Contraindications: Patients with a history of serious hypersensitivity reaction.

5. PARP inhibitors

PARP inhibitors such as 3-aminobenzamide, isoquinolines and napthalimide are commonly used drugs in case of heart failure. PARP-1 is a member of the PARP

enzyme family and is one of the most abundant nuclear proteins which functions as a DNA-nick-sensor enzyme. Recently in endothelial cells, hyperglycaemia induced overproduction of mitochondrial superoxide has been shown to cause DNA strand breaks, leading to an activation of PARP which inhibits GAPDH (glyceraldehyde-3-phosphate dehydrogenase). This leads to the accumulation of glucose and other glycolytic intermediates prior to their entry into the Krebs cycle. These intermediaries activate a number of major transducers of hyperglycemic damage (polyol pathway, AGE formation and PKCβ activation)**.** In addition to the direct cytotoxic pathway regulated by DNA injury and PARP activation, PARP also modulates the course of cardiovascular inflammation and injury by regulating the activation of NF-κB and inducing overexpression of ET (endothelin)-1 and ET receptors [Hayat *et al*, 2004].

6. Thiazolodinediones

TZDs are a new class of compounds for treating patients with Type II diabetes mellitus, which act by increasing insulin sensitivity in skeletal muscle and adipose tissue through binding and activation of PPAR-δ, a nuclear receptor that has a regulatory role in differentiation of cells. Additionally they also act on PPAR-α and increase serum HDL (high-density lipoprotein)-cholesterol, decrease serum triglycerides. To generate sufficient energy to sustain cardiac contractility, myocardial metabolism utilizes a range of substrates including NEFAs (non-esterified fatty acids), glucose and lactate. However, in Type 2 diabetes, as a consequence of insulin resistance, glucose is underutilized and NEFA metabolism is increased impairing contractility. The TZDs, apart from insulin-sensitizing fat and skeletal muscle, increase the expression and function of glucose transporters in the heart, leading to improved glucose metabolism and reduce NEFA utilization by the myocardium. The consequences of this are that they protect against myocardial injury associated with ischaemia and improve recovery of function following ischaemia. Telmisartan is newly reported PPAR-γ activator [Hayat *et al*., 2004; McGuire and Inzucchi, 2008].

e.g. Telmisartan

Formula: $C_{33}H_{30}N_4O_{2;}$ Molecular mass: 514.617 g/mol; FDA approval: 1998, Dose: 40 to 80mg/day; route: oral; metabolism: hepatic; half life: 24h; excretion: renal.

Mode of action

Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin-converting enzyme (ACE, kininase II). Angiotensin II is the principal pressor agent of

the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Telmisartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis. There is also an AT II receptor found in many tissues, but AT II is not known to be associated with cardiovascular homeostasis. Telmisartan has much greater affinity (>3,000 fold) for the AT I receptor than for the AT II receptor. Blockade of the renin-angiotensin system with ACE inhibitors, which inhibit the biosynthesis of AT II from AT I is widely used in the treatment of hypertension. ACE inhibitors also inhibit the degradation of bradykinin, a reaction also catalyzed by ACE. Because telmisartan does not inhibit ACE (kininase II), it does not affect the response to bradykinin. Whether this difference has clinical relevance is not yet known. Telmisartan does not bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and angiotensin II circulating levels do not overcome the effect of telmisartan on blood pressure [Burnier, 2001; Symeonides *et al*., 2007].

Side effects: Tachycardia, bradycardia, hypotension, swelling of arms, legs, lips, tongue, or throat, the latter leading to breathing problems.

Contraindication: Telmisartan can cause serious harm to an unborn baby if used during pregnancy [Battershill and Scott, 2006].

1.1.6 Need of alternative therapy for diabetes

Regardless of the type of diabetes, patients are required to control their blood glucose with medication and/or by adhering to an exercise program and a dietary plan. Due to modernization of lifestyle type 2 diabetes mellitus is becoming a major health problem in developing countries. Patient with type 2 diabetes mellitus are usually placed on a restricted diet and are instructed to exercise the purpose of which primarily is weight control. If diet and exercise fail to control blood glucose at a desired level, pharmacological treatment is prescribed [Pandey *et al*., 2011].

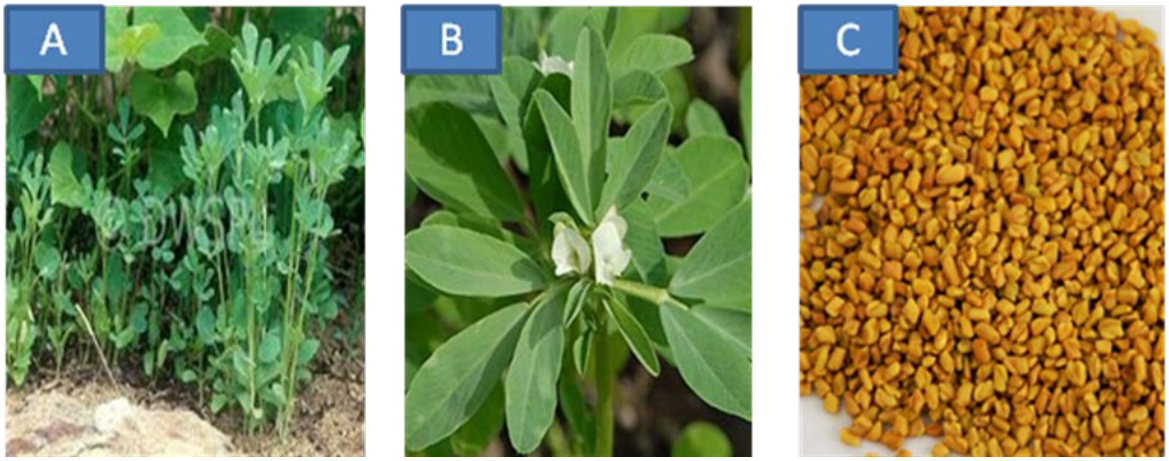
It is essential that in order to prevent diabetic complications, it is very necessary to have a tight control over blood glucose level. The clinicians use criteria for blood

glucose level during fasting which is <100 mg/dl and during post prandial which is < 140 mg/dl, but it is observed that such a tight control is not achieved with single drug [Nyenwe *et al*., 2011]. As a result of that fluctuation in blood glucose level occur which leads to complications of diabetes. Now a day's there is, increased use of bioactive constituents isolated from plants in communities. Biologically active constituent such as alkaloid when administered with prescription medications may show positive health outcome. Currently researchers, scientists and physicians are much inclined towards the concomitant or multiple drug therapy for diabetic complications [Kumar *et al*., 2012]. Therefore study of best dose regimen with multiple drugs is essential.


2
REVIEW
OF
LITERATURE

2. REVIEW OF LITERATURE

2.1 *Trigonella foenum graecum* Linn



[Images adapted from Tripathi and Chandra, 2009].

Figure 2.1 *Trigonella foenum-graecum* A] Herbs B] Flowers C] Seeds

2.1.1 Taxonomy of fenugreek

Taxonomy of the plant is as follows:

Domain: Eukarya

Kingdom: Plantae

Division: Mangoliophyta

Class: Mangoliopsida

Order: Fabales

Tribe: Trifolieae

Genus: Trigonella Botanical

Family: Leguminoseae

Genus: Trigonella

Species: *foenum- graecum*

2.1.2 Synonyms

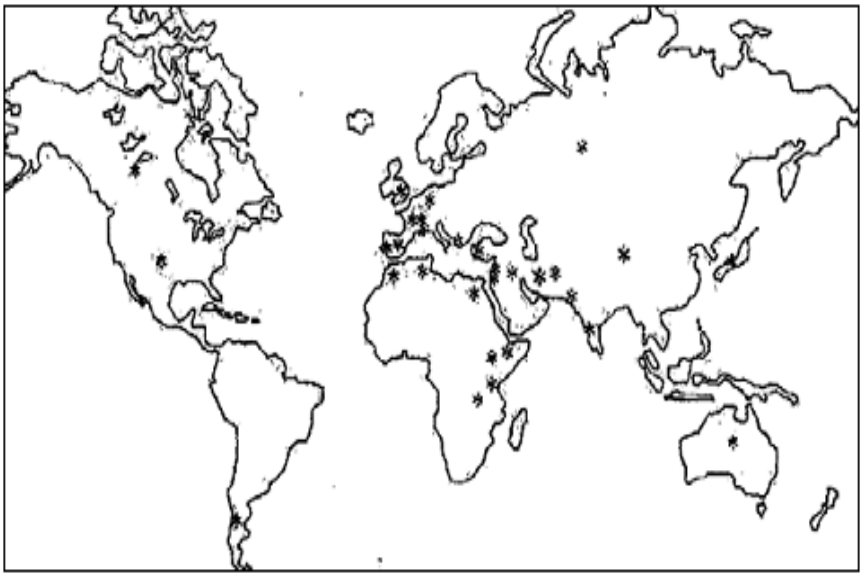
Fenugreek, Methi, Chilbe, Greek hay seed, Trigonella

2.1.3 Vernacular names

Marathi and Gujarati language: Methi, **Punjabi**: Metthi, **Bengali**: Menthiyam, **Hindi**: Methi, **Tamil**: Venthayam, **Kannada**: Menthya, **Malayalam**: Uluwa, **Telugu**: Menthulu.

2.1.4 Distribution

Trigonella foenum-graecum L. is an ancient and annual legume crop mainly grown for multiple uses in many parts of the world. Landraces and species of Trigonella have been found on the continents of Asia, Europe, Africa and Australia. Fenugreek was also cultivated in parts of Europe, northern Africa, west and south Asia, North and South America and Australia (Figure 2.1.4). Total 260 species are distributed throughout the world [Poole *et al*., 2010; Steele *et al*., 2010].



[Adapted from Mehrafarin *et al*., 2011]

Figure 2.1.4 Distribution of fenugreek throughout the World

2.1.5 Parts used: Whole plant, leaves and seeds.

2.1.6 Cultivation and collection

Fenugreek is a dry-land crop which responds even to minimal levels of irrigation. Interest in cultivating fenugreek in temperate climates has increased because of its rain-fed adaptation.

- **Land preparation:** Land should be ploughed 2-3 times followed by planking, to bring the soil to fine tilth. Soil clods should be broken and stubbles of previous crop should be removed.
- **Sowing and seed rate:** Sowing may be done from second fortnight of October to first fortnight of November but first fortnight of November is the best sowing time. 10-15 kg of seeds per ha are required for sowing. Seeds should be soaked in water

for 6 to 8 hrs and dried in shade before sowing to hasten germination. Complete germination of seeds takes about 6-8 days.

- **Spacing:** A spacing of 30 cm between lines and 8-10 cm plant to plant should be maintained while sowing of seeds or broadcasting.
- **Interculture:** In broadcasted crop, plants should be thinned to maintain the plant distance about 8-10 cm. 2 to 3 weeding are required to fenugreek crop. One weeding and hoeing should be done about 20-25 days after sowing. Intercultural operation during the early stage of plant growth minimizes weed competition. Second weeding should be done 45-50 days after sowing**.**
- **Irrigation:** 4-6 irrigations depending on soil type and climate should be given to fenugreek crop. Pre-sowing irrigation should also be given, if moisture level of the soil is not optimum for seed germination. First irrigation should be given at the time of thinning and subsequent irrigation at an interval of 20-25 days**.**
- **Harvesting:** Crop becomes ready for harvest in about 120-150 days. At the time of ripening/maturity, leaves and pods become yellowish and leaves start falling. Timely harvesting is very important for this crop as late harvest leads to seed losses due to pod bursting, whereas in early harvest, the grains remain immature and small. Harvesting should be done early in the morning. After harvest, plants should be dried in threshing yard and threshed by trampling under the feet of bullocks. Seeds should be separated and cleaned by winnowing [Mullaicharam *et al*., 2013].

2.1.7 Morphological characteristics of fenugreek

Table 2.1.7 Morphological characteristics of fenugreek

Morphological characteristics	Description, color and texture	Dimensions
Plant habit	Erect or prostrate, straight or profusely branched	20-130 cm in length
Stem	Circular to slightly quadrangular, greenish, often characterized by pinkish color due to anthocyanin accumulation under field condition	0.5-1 cm in diameter
Leaf	Simple and trifoliate, distinctly petiolate, Stipulate; leaf lamina oval or orbicular with an entire margin. The petioles and leaf lamina varies form greenish to pinkish in the field	1.5-4.5 cm × 0.8-1.5 cm

Petiole	Pale green, pubescent, often anthocyanin tinged	Very small; 0.5-1.1 mm
Flower	Yellow when young and white on maturity	1.6-2.2 cm
Calyx	Campanulate, pale green, pubesce	6-8 mm
Individual sepal	Pale green	13-19 mm
Corolla	Papilionaceous, white, papery	1.5-1.9 mm
Standard/Vexillum/Banner	White, papery	1.5-1.8 cm
Anther lobes	Bright yellow, rectangular	1 -1.5 mm × 0.4-0.5 mm
Filament	Hyaline, tubular	1.7 to 1.9 mm
Ovary	Deep green	1.8-2.5 mm
Stigma	Pale green	1.5-2.1 mm
Style	Pale green	0.2-0.5 mm
Pollen grain	Oval (70-90 %) to circular, orbicular, pink or red when treated with 0.5% acetocarmine	0.032- 0.042 mm × 0.025 -0.027 mm
Ratio of terminal to axillary flowers	All flowers yellow when immature and white when matured	Extremely rare, however the ratio varies as 1:8/1:10/ 1:11/1:13 2-8/plant
Number of pods per plant and pod dimensions	Pods brownish or yellowish brown with mucronate tips	9.5-18.6 cm × 0.2-0.4 cm
Seed	Rectangular to oval in shape with deep grooves between the radical and cotyledon varies in color form pale brown to golden yellow	10-20/pod 3-5 mm × 2-3 mm

[Adapted from Mehrafarin *et al*., 2011]

2.1.8 Chemical constituents

Fenugreek contents alkaloids, amino acids, saponins, steroidal saponins, fibers and other minor constituents also present they are given in detail in Table 2.1.8

Table 2.1.8 Chemical constituents of fenugreek

Sr no	Name of constituents	Types
1	**Alkaloids**	Trimethylamine, neurin, trigonelline, gentianine, carpaine and betain
2	**Amino acids**	Isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine, l-tryptophan, arginine
3	**Saponins**	Graecunins, fenugrin B, fenugreekine, trigofoenosides A-G

4	**Steroidal sapinogens**	Yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccagenin
5	**Fiber**	Galactomannan, neutral detergent
6	**Other**	Coumarin, lipids, vitamins (choline) , minerals (phosphorus and sulphur)

[Adapted from Khalil *et al*., 2004]

Diosgenin, a steroid sapogenin found in fenugreek is the starting compound for over 60% of the total steroid production by the pharmaceutical industry. Other sapogenins found in fenugreek seed include yamogenin, gitogenin, tigogenin and neotigogens. Fenugreek seeds contain 34% alkaloids, including trigonelline 4.8%, gentianine and carpaine compounds [Jayadev *et al*., 2004].

Seed endosperm is rich in protein such as globulin, histidine, albumin and lecithin and 100g endosperm is found to contain protein of 43.8g. However, 100g of fenugreek seed contains 25.4g protein [Mathur *et al.*, 2009]. Other constituent such as fixed oil and volatile oil in the small quantities, it is also rich source of vitamin such as choline [Meghwal *et al*., 2012]. Fenugreek does not contain so many minerals but it has some of them such as it has good amount of phosphorus and sulphur [Meghwal *et al*., 2012].

2.1.9 Dose

Dosing of herbal preparations is highly dependent on a variety of factors, such as growing and harvesting conditions, plant parts and extraction method used and the dosage form chosen by the manufacturer. Standardization by the single constituent markers has proven unreliable. Since no official standard have been established to date to regulate the production of herbal medicine in the United States.

2.1.10 Medicinal uses of fenugreek

Previously it was reported that fenugreek seeds possesses anticarcinogenic activities, hypocholesterolemic activities, hypoglycemic activities, antioxidant, antineoplastic agent, influence on enzymatic activities such as key enzymes in carbohydrate and protein metabolism, immunomodulatory effect, antifertility effect, diabetes management, antiulcer, antihelmintic activity, it influences the digestive key enzymes. In pharmaceutical industry commonly it is used as food stabilizer, food adhesive, and food emulsifier and gum [Meghwal *et al*., 2012; Olaiya *et al*., 2014].

- **For hyperglycemia and hyperlipidemia:**

Suggested oral dose is 25 to 100g whole or defatted *Trigonella foenum graceum* seed powder [Al-Habori and Raman, 1998].The alternative to defatted powdered *Trigonella foenum graceum* seed (100g) divided in two equal doses in type-1 diabetes. *Trigonella foenum graceum* seed powder in capsule form (2.5g twice daily for three month) to treat type-2 diabetes [Smith *et al*., 2003].

- **Inflammation**:

The topical application of aqueous suspension of 50 g seeds in 250 ml of water is used as anti-inflammatory preparation. For oral dose 1-3 g of seeds mixed with food or water taken at meal time [Shapiro and Gong, 2002].

- **Anticarcinogenic activities and complementary cancer therapy:**

Fenugreek is a promising protective medicinal herb for complementary therapy in cancer patients under chemotherapeutic interventions because fenugreek extract shows a protective effect by modifying the cyclophosphamide induced apoptosis and free radical-mediated lipid peroxidation in the urinary bladder of mice [Bhatia *et al*., 2006; Chaterjee *et al*., 2003]. Diosgenin is a crystalline steroid sapogenin found in fenugreek and used as a starting material for the synthesis of steroid hormones such as cortisone and progesterone. It has been found to be potentially important in treatment of cancer [Olaiya *et al*., 2014].

- **Antilipidemic activity:**

Fenugreek seeds have been shown to exhibit hypocholesterolemic effects, lowered serum cholesterol, triglyceride and low-density lipoprotein in hypercholesterolemia suffering patients and experimental models [Moosa *et al*., 2006].

- **Antioxidant activity:**

Fenugreek contains phenolic and flavonoid compounds which help to enhance its antioxidant capacity [Boaz *et al*., 2011].

- **Immunomodulatory effect:**

An agent that intensifies or diminishes the immune responses is known as an immunomodulator and such effect is called as immunomodulatory effect. Immunomodulatory substance interferes with three basic areas of the immune responses directly or indirectly; the mucosal barrier function, the cellular defense function and the local or systemic inflammatory response [Olaiya *et al*., 2014].

2.1.11 Historical uses of fenugreek

Fenugreek is one of the oldest known medicinal plants that have been documented in ancient herbal publications, religious scriptures, travel records and anecdotes dating back in human history. Seeds of the fenugreek plant were found in the tomb of the Egyptian Pharaoh, Tutankhamen (1333 BC–1324 BC) and leaves of the fenugreek plant were used as one of the components of holy smoke that the Egyptians used in fumigation and embalming rites. During the ancient Greek period, fenugreek was cultivated as a forage crop. In ancient Rome, it was used as an aid to induce labor during childbirth and delivery. Utilization of fenugreek in Chinese medicine was first introduced during the Song Dynasty (AD 1057). It was used in traditional Chinese medicine as a tonic and treatment for weakness and edema (tissue swelling due to excess lymph fluid) of the legs. Fenugreek was later introduced to central Europe at the beginning of the 9^{th} century but it was not until the 16^{th} century when cultivation of the plant in England was recorded [Petropoulos, 2002; Basch *et al*., 2003].

2.1.12 Modern uses of fenugreek

In India it has been used as part of traditional medicine practices. Fenugreek contains a myriad of phytochemicals such as steroids, flavonoids and alkaloids, which have been identified, isolated and extracted by the pharmaceutical industry to serve as raw materials for the manufacture of hormonal and therapeutic drugs. Polysaccharides form the mucilage (galactomannan) present in the plant and are finding wider applications in the food, pharmaceutical, cosmetics, paints and paper industries. [Petropoulos, 2002; Basch, 2003].

2.1.13 Fenugreek in World market

Estimates show that the annually cultivated area of fenugreek is about 57000 hectare, with seed production at 68000 tons. Currently, fenugreek represents an important cash crop in India, Morocco, China, Pakistan, Turkey, Egypt and Ethiopia. India claims to produce 70-80 % of the world's exported fenugreek, followed by Morocco. Other exporting countries include Spain, which supplies major market in Italy, Tunisia, Turkey, Lebanon and Israel. The major export market for Indian fenugreek appears to be European Union, Japan, United Arab Emirates, Yemen and South Africa [Fotopoulos, 2002].

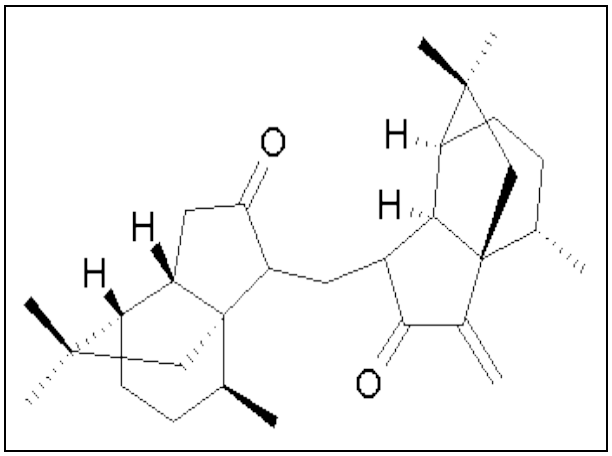
2.1.14 Side effects

At low dose side effects are common such as diarrhea, indigestion, heartburn, gas, bloating and urine odor. At high dose of fenugreek seeds may cause gastro-intestinal disturbance and nausea. Fenugreek may stimulate uterus, pregnant women should avoid fenugreek [Poole *et al*., 2010].

2.1.15 Use with cautions

- **Peanut or chickpea allergy:** Fenugreek is in the same family with peanuts and chick peas and may cause an allergic reaction in female population who are allergic to nuts. Two cases of fenugreek allergy have been reported in the literature [Vinje *et al*., 2012; Faeste *et al*., 2010].
- **Asthma:** Fenugreek is often cited as a natural remedy for asthma. However, inhalation of the powder can cause asthma and allergic symptoms [Patil *et al*., 1997].
- **Migraines:** Fenugreek is often cited as a natural remedy for migraines. However, studies indicate that it may trigger a migraine and/or contribute to the duration and severity of a migraine [Zulfiqar *et al*., 2013].
- **Pregnancy/Lactation:** Fenugreek has documented uterine stimulant effects and has been used in traditional medicine to induce childbirth and hasten delivery by promoting uterine contractions. Avoid use in pregnancy [Shinde *et al*., 2012].

2.2 Trigonelline:



[Structure adapted from Hiroshi, 2006]

Figure 2.2 Structure of Trigonelline

2.2.1 IUPAC name: 1-Methylpyridinium-3-carboxylate

2.2.2 Other names: Nicotinic acid N-methylbetaine, Coffearine, Caffearine,Gynseine

2.2.3 Molecular Formula: $C_7H_7NO_2$

2.2.4 Molecular weight: 137.14

2.2.5 Melting Point: 230-233 ^{0}C

2.2.6 Other names:

- Pyridinium, 3-carboxy-1-methyl-, inner salt (9CI)
- Pyridinium, 3-carboxy-1-methyl-, hydroxide, inner salt (8CI)
- Betain nicotinate
- Betaine nicotinate
- Caffearine
- 3-Carboxy-1-methylpyridinium betaine
- 3-Carboxy-1-methylpyridinium hydroxide inner salt
- 3-Carboxy-1-methylpyridinium inner salt
- Coffearin
- Coffearine
- Gynesis
- N-Methylnicotinate

- N-Methylnicotinic acid
- N' -Methylnicotinic acid
- N-Methylnicotinic acid betaine
- Nicotinic acid, N-methyl
- Nicotinic acid N-methylbetaine
- Trigenolline

Trigonelline, N-methyl nicotinic acid, was first noted in the seeds of *Trigonella foenum-graecum* [Joshi and Handler, 1960]. Trigonelline is a strongly polar hydrophilic compound [Shah *et al.*, 2006]. It is a plant alkaloid comprises about (0.2 - 0.38%) in seeds [Aswar *et al*, 2008]. Trigonelline, the major isolated component of seeds of *Trigonella foenum-graceum* L., is traditionally used to treat diabetes in China [Zhou *et al*, 2013].

2.2.7 Distribution of trigonelline

Trigonelline is also found in jellyfish, sea urchins [Hiroshi, 2006], muscle of Crustacea [Hammer *et al*., 2012] and in the marine sponges Calyx nicaensis [Avila *et al*., 2008]. Trigonelline has also been found in mammals [Zheng *et al*., 2005]. Other common food sources containing trigonelline include coffee, barley, corn, cantaloupe, onions, soybeans, peas and tomatoes [Sandhu and Fraser, 1981].

Table 2.2.7 Other plants containing trigonelline

Common name	Scientific name	Plant part	Refrance
Barley	*Hordeum vulgare*	Seed	Beckstrom S and Duke JA., 1997
Cantaloupe	*Coffea arabica*	Seed	Beckstrom S and Duke JA., 1997
	C.canephora var. *robusta*	Bean	
	C. liberica	Bean	
Corn	*Zea mays*	Seed	Beckstrom S and Duke JA., 1997
Hemp (marijuana)	*Cannabis sativa*	not provided	Budavari S., 1996
Pea	*Pisum sativum*	Seed, Fruit, Sprout Seedling,Leaf,Root, Shoot, Stem	Beckstrom S and Duke JA., 1997
Soybean	*Glycine max*	Seed, Fruit, Sprout Seedling, Leaf, Root, Shoot, Stem	Beckstrom S and Duke JA., 1997
Tomato	*Lycopersicon esculentum*	Root	Li CP., 1974

2.2.8 Pharmacological study

- Allred *et al*., 2009; Sridevi and Giridher, 2013 summarized trigonelline has hypoglycemic, hypolipidemic, neuroprotective, cardioprotective, sedative, memory improving, antimigraine, antibacterial, antitumor, antiviral activities in their review of literature.
- **Hypoglycemic activity of trigonelline**

  In 1967, Mishkinsky and his coworkers firstly reported that trigonelline has hypoglycemic effect. Trigonelline is reported to have a hypoglycemic effect in normal and in alloxan induced diabetes in mice [Shah *et al*., 2006]. Previously it was reported that trigonelline produces hypoglycemic effect in diabetic rats which was last for 24h [Akram, 2013; Narender *et al*., 2011]. However, earlier report have shown the presence of trigonelline inhibit glucose intestinal glucose uptake in-vitro [Al-Habori and Raman, 1998].
- **Hypolipidemic activity**

  Previously it has been reported that hypoglycemic and hypolipidemic activity of trigonelline and ethanolic extract of Iraqi fenugreek seeds in alloxan induced diabetic rabbits [Hamadi, 2012]. Fenugreek seeds reduced serum total cholesterol, LDL and VLDL cholesterol and triglycerides and unchanged HDL cholesterol fraction. These results indicate the usefulness of fenugreek seeds in the management of diabetes in human [Abor *et al*., 2014].
- **Neuroprotective effect and memory improvement**

  Trigonelline, a constituent of coffee beans, demonstrated the regeneration of dendrites and axons, in addition to correct memory impairment [Tohda *et al*., 1999]. In rat cortical neurons, trigonelline showed dendritic and axonal regeneration [Houghton *et al*., 2005]. In 2012, Morani et al., reported trigonelline was effective in painful diabetic neuropathic rat model.
- **Antibacterial activity**

  More recently it has been considered an antibacterial compound against *Streptococcus mutans*, a cariogenic bacterium [Antonio *et al*., 2010]. Trigonelline showed 50% of the antimicrobial effect against S.enterica, which is relevant to human safety [Almeida *et al*., 2006].

- **Anticarcinogenic effect**

  Considering potential bioactivity, trigonelline has inhibited the invasiveness of cancer cells in vitro [Hirakawa *et al*., 2005]. Trigonelline alters the actions of estradiol, using proliferation of estrogen-dependent human breast cancer cells (MCF 7) as a model system [Allred *et al*., 2009].

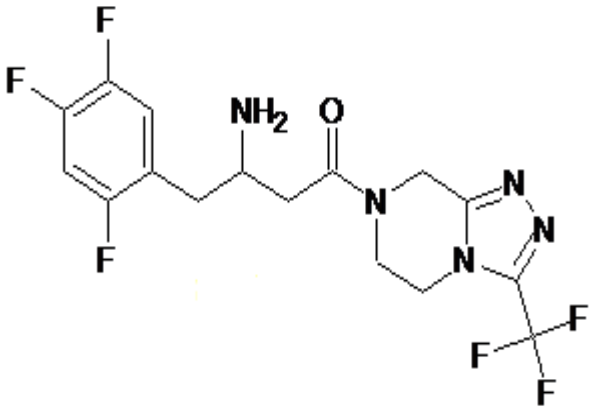
- **Renoprotective effect**

  Xue *et al*., 2011 reported *Trigonella foenum graecum* seed extract protects Kidney function in diabetic rats via its antioxidant activity. Arora *et al*., 2012 reported renoprotective effects of reconstructed composition of *Trigonella foenum-graecum* seeds in animal model of diabetic nephropathy with and without renal ischemia reperfusion in rats. Trigonelline ameliorated diabetic hypertensive nephropathy by suppression of oxidative stress in kidney in streptozotocin induced neonatal diabetic rats [Ghule *et al*., 2012]. Hamden *et al*., 2013 reported inhibition of key digestive enzymes related to diabetes and hyperlipidemia and protection of liver-kidney functions by trigonelline in diabetic rats.

- **Cardioprotective effect**

  Up to date very few evidences are available on cardioprotective effect of trigonelline in diabetes. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats [Murugesan *et al*., 2011].

2.3 Sitagliptin



[Adapted from John *et al*., 2011**]**

Figure 2.3 Structure of Sitagliptin

2.3.1 IUPAC name: 4-Oxo-4-(3-(trifluoromethyl)-5,6-dihydro(1,2,4) triazolo [4,3-a] pyrazin-7(8H)- yl)-1-(2,4,5-trifluorophenyl)butan-2-amine phosphate

2.3.2 Molecular Formula: $C_{16}H_{15}F_6N_5O{\cdot}H_3PO_4$

2.3.3 Molecular Weight: 505.31 [John *et al*., 2011].

2.3.4 Chemical nature

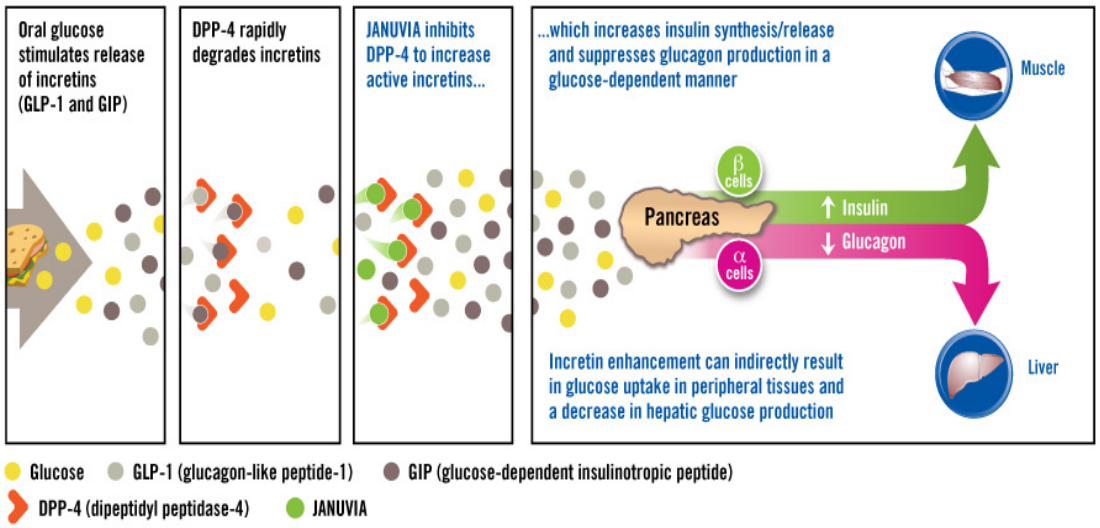
Sitagliptin phosphate monohydrate is a white to off-white, crystalline, non-hygroscopic powder. It is soluble in water and N, N-dimethyl formamide; slightly soluble in methanol; very slightly soluble in ethanol, acetone and acetonitrile and insoluble in isopropanol and isopropyl acetate.

2.3.5 Physiological role of incretins

In 1932, the term "incretin" was used for the first time to refer to a substance derived from the gut presumably a hormone that regulates insulin secretion after meal. Thereafter in 1971, the first incretin GIP and in 1985, second incretin GLP -1 was described. GIP and GLP -1 are secreted by enteroendocrine K-cells in the proximal gut and L-cells in the distal gut respectively. Both GIP and GLP-1 are secreted into the circulation as active hormones within minutes in response to food consumption and are rapidly inactivated by the enzyme DPP -4, a ubiquitous serine protease. Both GIP and GLP-1 bind to specific G-protein coupled receptors present on β-cells and other target tissues. Activation of the incretin receptors on β-cells acutely enhances glucose dependent exocytosis of insulin and long term effects like stimulation of

insulin synthesis, enhancement of β- cell proliferation and promotion of resistance to apoptosis. GLP -1 also lowers plasma glucose levels through inhibition of glucagon secretion, deceleration of gastric emptying and inhibition of food intake [Garg *et al*., 2013].

2.3.6 Mechanism of action



[Adapted from Waget *et al*., 2011]

Figure 2.3.6 Sitagliptin mechanism of action

Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor represents a new class of antidiabetic agents reflecting their ability to extend the biological effects of incretin hormones. Incretins are peptide hormones that play an important role in the physiological control of blood glucose. The initiation of a meal stimulates release of two key incretins, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), from endocrine cells in the gut. Both of these peptides stimulate glucose-dependent release of insulin by pancreatic β-cells and GLP-1 has the additional action of suppressing glucagon release. Under physiological conditions, these hormones are rapidly inactivated by the enzyme dipeptidyl peptidase –IV [Green *et al*., 2006; Gallwitz, 2007].

2.3.7 Pharmakokinetics

Bioavailability of sitagliptin is approximately 87%. Half-life is between 8-14 hours. It is 38% bound to plasma proteins. In vitro studies indicated that the primary enzyme responsible for the limited metabolism of sitagliptin was CYP3A4, with contribution from CYP2C8. It undergoes limited metabolism via enzyme Cytochrome P4503A4

(CYP3A4) and Cytochrome P4502C8 (CYP2C8). Approximately 79% of sitagliptin is excreted unchanged in the urine [Gallwitz, 2007; Herman *et al*., 2005; Beconi, 2006; Neumiller, 2009].

2.3.8 Adverse effects

Upper respiratory tract infection (4.5-6.3%)

Nasopharyngitis (5.2-6.3%)

Urinary tract infection (3.2%)

Headache (1.1-5.9%)

Arthralgias (3%)

Other side effects include**:** Sore throat, cough, fatigue, dizziness, edema, nausea and diarrhea.

2.3.9 Dosage and administration

Monotherapy: 100 mg once daily with or without food twice daily.

Concomitant therapy: sitagliptin (50 mg p.o.) + metformin (1000 mg p.o.) twice daily or sitagliptin (50mg p.o.) + metformin (500mg p.o.) twice daily.

2.3.10 Marketed products

Januvia® (Containing Sitagliptin Phosphate)

Janumet® (Containing Metformin Hydrochloride and Sitagliptin Phosphate)

2.3.11 Manufacturing companies

Dissymmetricx Pvt. Ltd.

Merck Pharmaceutical Pvt. Ltd.

ABS Life Sciences Ltd.

2.3.12 Clinical use of sitagliptin

In October 2006, the U.S. Food and Drug Administration (FDA) approved sitagliptin as monotherapy and as add-on therapy to either of two other types of oral diabetes medications, metformin or thiazolidinediones to improve blood glucose control in patients with type 2 diabetes when diet and exercise are not enough [Rosenstock *et al*., 2008; Daniel *et al*., 2007]. In March, 2007 it was approved in European Union. Sitagliptin is currently approved in 42 countries [Unger, 2010]. In March, 2007 it was approved in European Union. Sitagliptin is currently approved in 42 countries [Pathak and Bridgeman, 2010]. The recommended dose of sitagliptin is 100 mg once daily. It may be taken with or without food. In April, 2007 FDA approved the combination

product of sitagliptin and metformin for type 2 diabetes [Bhinge *et al*., 2013]. Various clinical studies showed sitagliptin decreased postprandial glucose excursion, fasting plasma glucose and hemoglobin levels with neutral weight effect and a low chance of hypoglycemia and gastrointestinal adverse effect [Tremblay *et al*., 2011; Johnson and Schurr, 2011; Yanai *et al*., 2012]. In clinical trials of 1-year duration, sitagliptin improved glycaemic control by reducing both fasting and postprandial glucose concentrations leading to clinically meaningful reductions in glycosylated haemoglobin levels. Monotherapy with sitagliptin 100mg daily decreases mean HbA1c by 0.6-0.79% (mean difference from placebo). When used in combination with metformin or pioglitazone, the mean reduction is HbA1c is 0.7% and 0.9% respectively. Sitagliptin is considered to be weight neutral and lipid neutral [Gallwitz, 2007]. The effects (if any) of DPP-4 inhibitors on diabetes complications and mortality are currently being investigated in ongoing trials such as the Sitagliptin Cardiovascular Outcome Study [Pastromas, 2014].

2.3.13 Precautions and drug interactions

Due to a lack of safety and efficacy data, Sitagliptin is not recommended for use in children under 18 years of age and caution is advised in patients > 75 years old. Sitagliptin has shown reproductive toxicity at high doses and has been detected in high amounts in the milk of lactating animals. Because of a lack of human data this drug should not be used during pregnancy or breast feeding [Campbell and Day, 2007].

Sitagliptin have shown to have a few drug-drug interactions. Sitagliptin is metabolised by CYP3A4 but it does not appear to induce or inhibit cytochrome P450 isoenzymes and does not show interactions with inducers or inhibitors of cytochromes. Clinically important CYP3A4 inhibitors mainly include macrolide antibiotics (e.g. clarithromycin and erythromycin), anti-HIV agents (e.g., ritonavir and delavirdine), antidepressants (e.g. fluoxetine and fluvoxamine), calcium channel blockers (e.g. verapamil and diltiazem), steroids and their modulators (e.g. gestodene and mifepristone) and several herbal and dietary components. A small number of drugs such as rifampin, phenytoin and ritonavir are identified as inducers of CYP3A4. In phase (I) drug interaction studies, sitagliptin did not meaningfully alter the pharmacokinetics of other oral hypoglycemic agents, including metformin,

rosiglitazone or glyburide. Sitagliptin concomitant administration with digoxin (0.25 mg) for ten days increased plasma digoxin concentration; therefore monitoring is advisable to avoid digoxin toxicity but dose adjustment is not recommended [Herman *et al*., 2005; Zhou, 2008; Garg *et al*., 2013].

2.3.14 Contraindications

It is a pregnancy category B drug. Because there are no adequate, well controlled studies of sitagliptin in pregnant women, it should be used during pregnancy only if clearly needed. Caution should be exercised with use of sitagliptin in nursing women. Sitagliptin can pass into breast milk and may harm a nursing baby. In children, safety and efficacy not established. Dosage adjustments are needed in patients with moderate or severe renal function impairment. In moderate renal function impairment (Ccr 30 to less than 50 mL/min) dose should be reduced to 50 mg once daily. In severe renal function impairment (Ccr less than 30 ml/min) dose should be reduced to 25 mg once daily. Sitagliptin is also contraindicated in diabetic ketoacidosis [Drucker *et al*., 2007; Cheng and George, 2005; Herman *et al*., 2005; Klatt *et al*., 2011].

2.3.15 Pharmacological study

Sitagliptin in rodent models have shown reduced glycemia in insulinopenic model such as streptozotocin treated rats or mice [Green *et al*., 2006]. In 2011, Yeom *et al* have been reported that reported sitagliptin preserve the β-cell proportion in the islets in both non-obese and obese diabetic mice. In addition others have shown that mice deficient in DPP-4 and have elevated levels of circulating GLP-I and GIP and are resistant to STZ-induced β-cell destruction [Mu *et al*., 2006]. DPP-IV inhibitor (linagliptin) combined with telmisartan was associated with marked reduction in albumin urea, an early marker for DN and also reduction in tumor necrosis factor alpha (TNF-α) an early indicator for systemic inflammation, which is found during hyperfiltration stage in diabetic nephropathy [Alter *et al*., 2012]. SITA protects renal ischemia reperfusion induced renal damage in diabetes [Vaghasiya *et al*., 2011]. Chronic administration of SITA treatment ameliorated all lesions glomerular, tubulointerstitial and vascular of kidney [Cristina *et al*., 2011]. Sitagliptin showed cardioprotective effect in mice through increasing cardiovascular event [McCormick *et al*., 2014; Apaijai *et al*., 2013]. In preclinical study it has been reported that sitagliptin improved endothelial dysfunction and lipids levels, reduced oxidative

stress, decreased inflammation and platelet aggregation [Scheen, 2013]. Trigonelline is plant alkaloid, is commonly used to treat diabetes in China [Zhou *et al*., 2012]. It showed antihyperglycemic effect through regeneration of pancreatic β-cells [Zhou *et al*., 2013]. Up to date very few evidences are available on cardioprotective effect of trigonelline in diabetes [Murugesan *et al*., 2011; Hamden *et al*., 2013].

2.3.16 Comparison of sitagliptin with other DPP-4 inhibitors

Such as vildagliptin has been approved for use in the European Union and is under regulatory review in United States. The FDA has granted an approval letter for vildagliptin but require additional safety data prior to final approval [Neumiller, 2009]. Saxagliptin (Onglyza-BMS and Astra Zeneca) is approved and recently marketed in India for the treatment of type 2 diabetes. Clinical trials have demonstrated that saxagliptin (doses are 2.5, 5, 10, 20, 40 mg once daily) is non inferior to sitagliptin in monotherapy and in combination therapy with metformin. It has also been observed that when saxagliptin is used as a combination therapy with thiazolidinedione, the incidence of peripheral oedema is increased [Neumiller, 2010]. Alogliptin, a highly selective DPP-4 inhibitor, being developed by Takeda Pharmaceutical Company is currently in phase three clinical trials. Alogliptin in dose range of 25-400 mg causes significant reduction in HbA1c, when used alone or in combination with other oral agents in patients with type 2 diabetes similar to sitagliptin [Neumiller, 2010].

2.3.17 Sitagliptin versus other oral hypoglycemic agents

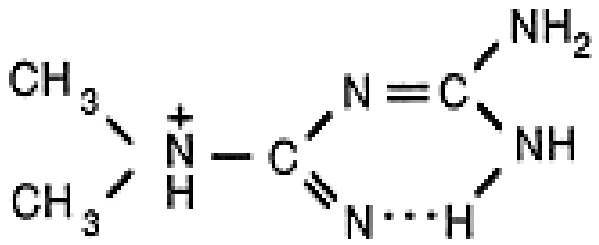
Metformin, a sulphonylurea is widely viewed as the initial drug of choice for treatment of type- 2 diabetes owing to its 30 year track record, efficacy, safety and low cost. There are now at least seven different classes of agents that can be used in combination with metformin including sulphonylureas, glitinides, α-glucosidase inhibitors, thiazolidinediones, insulin (injected/inhaled), exenatide and sitagliptin is an example of DPP-4. Sulphonylureas and glitinides are inexpensive but are associated with weight gain and hypoglycemia. The α-glycosidase inhibitors are effective and safe but associated with gastrointestinal side effects that limit their tolerability. Thiazolidinediones improve insulin action with low risk of hypoglycemia and but side effects like fluid retention and weight are associated with it. The past few years have witnessed considerable progress in pharmacotherapy of type 2 diabetes and ease of

use; favorable adverse event profile and lack of hypoglycemia or weight gain are attractive features for the new class of DPP-4 inhibitors e.g sitagliptin, though long term safety of prolonged DPP-4 inhibition in patients with type 2 diabetes is unknown. There is also no long term data available to inform the patients and physicians about sitagliptin therapy to prevent progression of type 2 diabetes. Additional studies are needed to define the long term efficacy, safety and to determine the relative merit compared with growing number of options now available for treatment of type 2 diabetes [Drucker *et al*., 2007].

2.3.18 Sitagliptin in the World market

Sitagliptin (Merck) was approved by FDA in October 2006. Worldwide sale of around US$ 3.9 billion in 2013 with 75% of sale from the United States. Sitagliptin and metformin combination product marketed by Merck (Janumet) was approved by FDA in October 2007 and worldwide sales of around US$ 128.4 million in the year 2013.

2.4 Metformin



[Adapted from Klip and Leiter, 1990]

Figure 2.4 Structure of metformin

2.4.1 IUPAC name: N, N-Dimethylimidodicarbonimidic diamide

2.4.2 Molecular formula: $C_4H_{11}N_5$

2.4.3 Molecular weight: 129.16

2.4.4 Chemical nature

Metformin HCl is a white crystalline powder. Metformin HCl is soluble in water and in 95% ethyl alcohol. It is practically insoluble in ether and in chloroform. Melting point: 218-220°C [Olusola *et al*., 2012].

2.4.5 History

Metformin is an oral antidiabetic agent representing biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes. The first synthesis of metformin (dimethyl biguanide) is attributed to Werner and Bell from Trinity College, Dublin, Ireland, in 1922 and was a basis for further experimental and clinical studies on the potential therapeutic application of biguanides, particularly metformin [Bell and Hadden, 1997]. The work of Dr. Jean Sterne, a French clinician and his colleagues leads to the discovery of metformin as an oral antidiabetic agent in the 1950s in Paris [Sterne, 1959]. As of 2010, metformin is one of only two oral antidiabetic drugs in the World Health Organization Model List of Essential Medicines the other one being glibenclamide [Maric, 2010].

2.4.6 Mechanism of action

Metformin is effective only in the presence of insulin and its major effect is to decrease hepatic glucose output [Bailey, 1992; Baily, 1996]. In addition, metformin increases insulin-mediated glucose utilization in peripheral tissues (such as muscle and liver), particularly after meals and has an antilipolytic effect that lowers serum free fatty acid concentrations, thereby reducing substrate availability for gluconeogenesis [Bailey, 1992; Stumvoll *et al*., 1995]. Metformin also increases intestinal glucose utilization via nonoxidative metabolism, at least in experimental animals [Baily, 1996]. The lactate produced by this process is largely metabolized in the liver as a substrate for gluconeogenesis [Bailey, 1992]. The latter effect could protect against hypoglycemia. The molecular mechanisms of metformin action are not fully known. Activation of the enzyme AMP-activated protein kinase (AMPK) appears to be the mechanism by which metformin lowers serum lipid and blood glucose concentrations [Prentki and Nolan, 2006]. AMPK-dependent inhibitory phosphorylation of acetyl-coA carboxylases Acc1 and Acc2 then suppresses lipogenesis and lowers cellular fatty acid synthesis in liver and muscle, which in turn improves insulin sensitivity and reduces blood glucose levels [Rojas *et al*., 2013].

2.4.7 Pharmacokinetics

Metformin has an oral bioavailability of 50-60% under fasting conditions, and is absorbed slowly [Padwal *et al*., 2011]. Peak plasma concentrations (Cmax) are reached within one to three hours of taking immediate-release metformin and four to eight hours with extended-release formulations [Idkaidek *et al*., 2011]. The plasma protein binding of metformin is negligible, as reflected by its very high apparent volume of distribution (300-1000 liter after a single dose). Steady state is usually reached in one or two days. Metformin has acid dissociation constant values (pKa) of 2.8 and 11.5 and therefore, exists very largely as the hydrophilic cationic species at physiological pH values. The metformin acid dissociation constant values (pKa) make metformin a stronger base than most other basic drugs with less than 0.01% unionized in blood. Metformin is not metabolized. It is cleared from the body by tubular secretion and excreted unchanged in the urine; metformin is undetectable in blood plasma within 24 hours of a single oral dose. The average elimination half-life in plasma is 6.2 hours. Metformin is distributed to (and appears to accumulate in) red

blood cells, with a much longer elimination half-life: 17.6 h reported as ranging from 18.5 to 31.5 h in a single-dose study of non-diabetic people [Scheen, 1996; Robert *et al*., 2003].

2.4.8 Adverse effect

The most common adverse effect of metformin is gastrointestinal discomfort including diarrhea, cramps nausea, vomiting and increased flatulence; most serious potential side effect of metformin is lactic acidosis incidence of which is approximately 9 per 100000/year in the metformin users; this complication is very rare and vast majority of these cases seem to be releated to comorbid condition such as impaired liver or kidney function rather than to the metformin itself [Fowler., 2007].

2.4.9 Contraindications

The most common contraindications to metformin use in people with type 2 diabetes are renal insufficiency, congestive heart failure over the age of 80 years [McCormack *et al*., 2005].

2.4.10 Dosage and administration

Monotherapy: Initial 500 mg once daily with or without food twice daily. Later 1000 mg twice daily. Combination therapy: sitagliptin (50 mg p.o.) + metformin (1000 mg p.o.) twice daily or sitagliptin (50mg p.o.) + metformin (500mg p.o.) twice daily.

2.4.11 Marketed products

Glucophage® (Containing metformin hydrochloride)

Janumet® (Containing Metformin Hydrochloride and Sitagliptin Phosphate)

2.4.12 Manufacturing companies

Cipla Pvt. Ltd.

Mylan Pvt. Ltd.

Wockhard Pvt Ltd.

2.4.13 Metformin in monotherapy and combination therapy

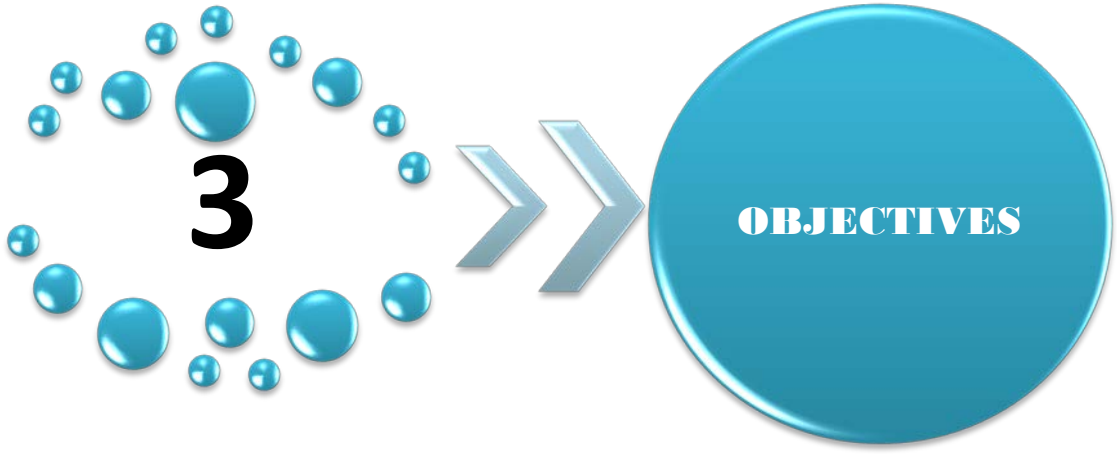
Metformin, a commonly used oral antihyperglycemic agent, both in monotherapy and combination with other agents reduces elevated blood glucose levels by reducing hepatic glucose output and also by improving insulin resistance [Srivastava *et al*., 2012]. Previous findings suggest that metformin monotherapy lowered HbA1c levels

by approximately 1.5%, without causing hypoglycemia. In combination with sulfonylureas, HbA1c was decreased by 1.25% with glibenclamide, 0.75% with glipizide and 0.7% with glimepiride. Glitazones added to metformin decreased HbA1c from 8.1% to 6.8%. When acarbose was added to metformin, HbA1c was reduced by 0.8%-1.0% [Maric, 2010]. Metformin and sitagliptin (Janumet) combination decreased HbA1c level by 1.03% after 18 weeks in patients.

2.4.14 Pharmacological study

Metformin has been shown to significantly reduce basal hepatic glucose production in non insulin dependent diabetes mellitus [Stumvoll *et al.*, 1995]. It inhibits hepatic glucose production by 9% to 30% [Boyle and Freeman., 2007]. In isolated hepatocytes, therapeutic concentrations of metformin enhance the suppression of gluconeogenesis by insulin and reduce glucagon-stimulated gluconeogenesis [Bailey, 1992]. Even if the glucose-lowering effect of metformin is attributed to decreased hepatic glucose production and increased peripheral glucose utilization, other factors could contribute. Indeed, metformin therapy has been associated with a reduction in free fatty acid levels due to decreased adipose tissue release [Abbasi *et al.*, 1998]. Free fatty acids has been implicated in the pathogenesis of insulin resistance because of their effect in increasing hepatic gluconeogenesis and inhibiting glucose uptake and oxidation in skeletal muscle [Matthaei *et al.*, 2000]. Some studies found a small but significant decrease in body weight, which seems due to reduced calorie intake [DeFronzo and Goodman, 1995]. Metformin could reduce hepatic glucose production and fatty acid levels through genetic mechanism. Indeed, when hepatocytes were cultured in the presence of metformin, expression of genes for regulatory proteins of fatty acid oxidation and gluconeogenesis decreased, whereas expression of genes encoding proteins involved in glycolysis increased [Fulgencio *et al.*, 2001]. A significant reduction in insulin and proinsulin levels in lean and overweight patients with NIDDM has been reported [Landin *et al.*, 1994]. In most studies, insulin resistance has been evaluated by oral glucose tolerance test (OGTT) [Jakubowicz *et al.*, 2001]. Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats [Erejuwa *et al.*, 2011]. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through modulation of oxidative stress genes expression

[Alhaider *et al*., 2011]. In 2011, Nagilla *et al*., reported that metformin has neuroprotective and antinociceptive effect in diabetic neuropathy in rats. In 2012, Cittadini *et al*., reported metformin prevents the development of chronic heart failure in the SHHF rat model. Metformin inhibits vascular calcification in female rat aortic smooth muscle cells via the AMPK-eNOS-NO pathway [Cao *et al*., 2013].


3
OBJECTIVES

3. OBJECTIVES

The objectives of study were to evaluate effect of administration two drugs trigonelline + sitagliptin and three drugs trigonelline + sitagliptin + metformin for the prevention of progression of diabetic complications like nephropathy, neuropathy and cardiomyopathy in nicotinamide-streptozotocin induced diabetic Wistar rats.

The objectives of present investigation are-

1] To study the effect of trigonelline (TRIG) and sitagliptin (SITA) in nicotinamide – streptozotocin induced diabetes in Wistar rats.

2] To study the effect trigonelline, sitagliptin alone and concomitant therapy of two drugs trigonelline + sitagliptin in nicotinamide-streptozotocin induced diabetic nephropathy, neuropathy and cardiomyopathy in Wistar rats.

3] To study the effect of metformin alone and triple drug therapy using trigonelline + sitagliptin + metformin in nicotinamide-streptozotocin induced diabetic nephropathy, neuropathy and cardiomyopathy in Wistar rats.


4
MATERIAL
AND
METHODS

4. MATERIAL AND METHODS

4.1 Materials

4.1.1 Animals

Male Wistar rats (200-250g) with either sex were purchased from National Toxicology Center, Pune.

4.1.2 Housing of Animals

Male Wistar rats (200-250 g) were purchased from National Toxicology Center, Pune. During the experiment, rats were housed in standard housing conditions like temperature of 25± 1ºC, relative humidity of 45%-55% and 12 h light: 12 h dark cycle. Rats had free access to food pellets (Navmaharashtra Chakan Oil Mills Ltd., Sangli, India) and tap water *ad ibitum* during the experiment.

4.1.3 Chemicals/ reagents

All the chemicals used in study were of analytical grade.

Table 4.1.3 List of various reagents/chemicals used

Sr. No.	Reagent / Chemical	Manufacturers
1	Streptozotocin	Sigma Aldrich, Mo.USA
2	Sodium citrate	Research Lab. Fine Chem Industries, Mumbai.
3	Nicotinamide	Sigma Aldrich, USA
4	Anesthetic ether	Narson Pharma, Mumbai
5	Urethane	HiMedia Laboratories, Mumbai, India
6	Ketamine	Chandra Bhagat Pharma Pvt. Ltd., Matunga, Mumbai
7	Ethanol	Changshu Yangshu chemicals, china
8	Thiobarbituric acid	Merck specialities Pvt. Ltd.,
9	EDTA	SISCO research laboratories Pvt. Ltd., Mumbai, India
10	Sodium phosphate dibasic	SISCO research laboratories Pvt. Ltd., Mumbai, India
11	Heparin	Gland Pharma Ltd., Mumbai
12	Sodium carbonate	Loba chemicals Pvt. Ltd., Mumbai
13	Sodium potassium tartarate	Loba chemicals Pvt. Ltd., Mumbai
14	Epinephrine bitartarate	Research Lab. Fine Chem Industries, Mumbai.

15	Trichlocroacetic acid	Merck specialities Pvt. Ltd., Mumbai
16	Formaldehyde	Loba chemicals Pvt. Ltd., Mumbai
17	Sodium hydroxide	Merck specialities Pvt. Ltd.
18	Sodium chloride	Merck specialities Pvt. Ltd.
19	(5,5'-dithiobis-2 nitrobenzoic acid) dtnb	Sigma Aldrich, USA
20	Folin phenol reagent	Merck specialities Pvt. Ltd
21	Potassium dihydrogen phosphate	Merck specialities Pvt. Ltd.
22	Hydrochloric acid	Merck specialities Pvt. Ltd.
23	Citric acid monohydrate	Research Lab. Fine Chem Industries, Mumbai.
24	1,1,3,3-tetramethoxypropane (tmop)	Loba chemicals Pvt. Ltd., Mumbai
25	GSH standard in tris- HCL	Loba chemicals Pvt. Ltd., Mumbai
26	MDA standard: 1,1,3,3-tetramethoxy propane (tmop) in tris-HCl	Loba chemicals Pvt. Ltd., Mumbai
27	Tris hydrochloride	Loba chemicals Pvt. Ltd., Mumbai
28	Sucrose	Merck specialities Pvt. Ltd.
29	2-nitro benzoic acid	Merck specialities Pvt. Ltd.
30	Sodium carbonate	Merck specialities Pvt. Ltd.
31	Copper sulphate	Merck specialities Pvt. Ltd.
32	Physiological saline	Alkem Laboratories Limited, Mumbai
33	Disodium ethylenediamine tetra acetic acid	Alkem Laboratories Limited, Mumbai
34	Picric acid	Loba chemicals Pvt. Ltd., Mumbai
35	Biphasic isophane insulin injection	Human Mixtard, Torrent Pharmaceuticals LTD, India

All chemicals were purchased from local vendors. Chemicals used were of analytical grade.

4.1.4 Diagnostic kits

Table 4.1.4 List of diagnostic kits with manufacturers

Sr. No.	Kit	Manufacturers
1	Glucose estimation kit (GOD/POD method)	Accurex Biomedical Pvt. Ltd., Mumbai
2	Insulin immunoassay kit	Mercodia AB (Sylveniusgatan 8A, Sweden)
3	Blood urea nitrogen estimation kit	Accurex Biomedical Pvt. Ltd., Mumbai

4	Uric acid kit	Accurex Biomedical Pvt. Ltd., Mumbai
5	Creatinine kit	Accurex Biomedical Pvt. Ltd., Mumbai
6	CK-MB estimation kit	Accurex Biomedical Pvt. Ltd., Mumbai
7	LDH kit	Accurex Biomedical Pvt. Ltd., Mumbai
8	AST kit	Accurex Biomedical Pvt. Ltd., Mumbai
9	Triglycerides kit	Accurex Biomedical Pvt. Ltd., Mumbai
10	Cholesterol kit	Accurex Biomedical Pvt. Ltd., Mumbai
11	HDL kit	Accurex Biomedical Pvt. Ltd., Mumbai

All Diagnostic kits were purchased from local vendors.

4.1.5 Instruments used

Table 4.1.5 List of instrument used with manufacturer

Sr. No.	Instrument	Manufacturer
1	UV-visible spectrophotometer	Jasco V 530, Japan.
2	Eppendorff's Cryocentrifuge machine	Eppendorff centrifuge 5804 R.
3	Inverted microscope	Olympus.
4	Von Frey Hair equipment, Almemo-2390-5	Trio 3 in 1, IITC life Sciences, Woodland Hills USA.
5	Planter test equipment, 370360	UGO Basile SRL Biological Research apparatus, Comerio, Verse, Italy
6	Randall Sellito equipment 7200	UGO Basile SRL Biological Research Apparatus, Comerio, Verse, Italy
7	Deep freeze	ESCY® Enterprises, Pune
8	Student power lab 8-channel recording system	AD Instrument Pvt. Ltd., Lexington drive, Bella, Australia.
9	Millar	AD Instrument Pvt. Ltd., Lexington drive, Bella, Australia.
10	Tissue homogenizer	Remi motors Ltd, Mumbai-53, India
11	Animal weighing electronic balance	Scale-Tec, Model- CTG 600
12	Metabolic cages	Tecniplast, Italy
13	Animal weighing balance	Contech Instruments, Mumbai, India
14	Chemical weighing balance	Metlar Tolledo, AB-204-S, Classic made in Switzerland
15	Animal Housing cages	Polypropylene cages

4.1.6 Drugs

Trigonelline hydrochloride (Sigma Aldrich, Mo.USA) and Sitagliptin (Hangzhou longshine Bio-Tech Co., LTD, China) were purchased; Metformin (Cipla Pvt. Ltd., Kurkumbh MIDC, India) was obtained as gift sample.

4.1.7 Preparation of drug solutions

Trigonelline and sitagliptin solutions were prepared in distilled water. The volumes of drug solutions were calculated based upon the body weight of the animal.

4.1.8 Storage of drug solution

Drugs were stored in a dessicator. Fresh drug solution was prepared for each day's work. The solution was stored in an airtight amber colored bottle at room temperature until ready for use.

4.1.9 Volume of drug solution

The volume of drug solution was calculated based upon the body weight of animal. In case of oral administration the volume administered did not exceed 2 ml.

4.1.10 Route of administration

The drug solution was administered per orally.

4.1.11 Research protocol approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of control and Supervision on Experimental Animals (CPCSEA), India.

4.2 Methods

4.2.1 Induction of type 2 diabetes by nicotinamide (NICO) – streptozotocin (STZ)

For experimental induction of non insulin dependent diabetes mellitus (NIDDM) in Wistar rats, we followed the protocol reported by [Masiello *et al*., 1998; Kakkar *et al*., 1998; Joshi *et al*., 2004]. Overnight fasted rat were injected with nicotinamide (110 mg/kg, *i.p*.) and Streptozotocin (65 mg/kg, *i.p.*) were injected 15 min after nicotinamide injection in all the groups except group I which was non-diabetic.

Rats having serum glucose more than 250 mg /dl were called 'diabetic' and selected for the study.

4.2.2 Collection of blood and determination of serum glucose

Blood from the experimental rats was withdrawn by retro-orbital plexus technique using capillary glass tubes. The collected blood was placed in Eppendorff tubes (1.5 ml). The serum was separated by centrifugation using Eppendorff Cryocentrifuge (Model no 5810, Germany), maintained at 4 ^{0}C and run at speed of 7000 r.p.m. for 15 min. 10 µl of serum and 1 ml of working reagent (GOD/POD) were mixed and incubated for 15 min at 37 ^{0}C. The UV spectrophotometer (Jasco V-530, Japan) reading was adjusted to 0 by measuring the absorbance of blank (distilled water). The absorbance of sample (As) and standard (Astd) provided by manufacturer (Acuurex Biomedical Pvt Ltd., Mumbai, India) were measured against blank at 505 nm by instrument. Glucose was estimated using formula: Glucose (mg/dl) = As / Astd *100, Where, As = Sample reading; Astd = Standard reading.

4.2.3 Experimental design

4.2.3.1.1 Effect of trigonelline (TRIG) and sitagliptin (SITA) in nicotinamide - streptozotocin induced diabetes in Wistar rats

4.2.3.1.1.1 Acute study of test drugs on serum glucose level in diabetic rats

A group of non diabetic rats was included in the study. The rats were divided into following groups;

Group 1: Non-diabetic;

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*);

Group 3: Trigonelline (TRIG 25 mg/kg, p.o.);

Group 4: Trigonelline (TRIG 50 mg/kg, p.o.);

Group 5: Trigonelline (TRIG 100 mg/kg, p.o.);

Group 6: Sitagliptin (SITA 2.5 mg/kg, p.o.);

Group 7: Sitagliptin (SITA 5 mg/kg, p.o.);

Group 8: Sitagliptin (SITA 10 mg/kg, p.o.);

Group 9: Trigonelline + Sitagliptin (70%+30%) viz; (TRIG 70mg/kg p.o. + SITA 3mg/kg p.o.);

Group10: Trigonelline + Sitagliptin (50%+50%) viz; (TRIG 50mg/kg,p.o.+ SITA5mg/kg p.o.);

Group11: Trigonelline + Sitagliptin (30%+70%) viz; (TRIG 30mg/kg p.o. + SITA 7mg/kg p.o.).

In acute study blood samples were withdrawn by retro orbital puncture method and serum glucose determined at 0, 2^{nd}, 4^{th}, 6^{th} and 24^{th} h respectively after test drugs administration.

4.2.3.1.1.2 Subacute study of test drugs on serum glucose level in diabetic rats

In subacute study (28 days), a group of non diabetic rats was included in the study. rats were divided into following groups;

Group 1: Non-diabetic;

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*);

Group 3: Trigonelline (TRIG, 50mg/kg, p.o.);

Group 4: Sitagliptin (SITA, 5mg/kg, p.o.);

Group 5**:** Trigonelline + Sitagliptin (50%+50%) viz; (TRIG 50 mg/kg, p.o. + SITA 5mg/kg, p.o.).

Subacute study involved daily administration of test drugs in prescribed manner for 28 days (once a day) at predetermined time. During the treatment, blood samples were withdrawn at 6h after test drugs administration on day 7^{th}, 14^{th}, 21^{st} and 28^{th} respectively by retro orbital puncture and serum glucose was determined by GOD/POD method.

4.2.3.1.1.3 Effect on body weight

During the study period of 28^{th} days, the rats were weighed daily using electronic balance.

4.2.3.1.1.4 Estimation of serum insulin

Serum insulin was assayed by Mercodia AB (Sylveniusgatan 8A, Sweden) rat insulin immunoassay kit.

4.2.3.1.1.5 Estimation of serum HbA1c

HbA_{1c} level was measured using (High performance liquid chromatography (HPLC) method, D-10, BIO-RAD, France).

4.2.3.1.1.6 Histological examination of pancreas

At the end of twenty eight day period, the rats were killed under deep anesthesia and pancreas was carefully removed. Isolated pancreas was terminated into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3-5µm in thickness were cut by microtome and stained by hematoxyline-eosin stain. The photographs of each section were observed using cell imaging software for life sciences microscopy (Olympus soft imaging solution Gmbh, Munster, Germany).

4.2.3.1.1.7 Statistical analysis

Data obtained for serum glucose levels (acute and subacute administration) and body weight (28 days) were expressed as mean ± SEM and analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data on serum insulin and HbA_{1c} levels on day 28 by one-way ANOVA followed by Dunnet's test for significance.

4.2.3.2.1 Effect of TRIG, SITA alone and concomitant therapy of two drugs (TRIG+SITA) in diabetic nephropathic rats

For experimental induction of diabetic nephropathy in Wistar rats, we followed the protocol reported by [Qi *et al*., 2011; Kakadiya *et al*., 2010]. A group of non diabetic rats was included in the study.

The rats were divided into following groups;

Group 1: Non-diabetic control;

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*);

Group 3: Trigonelline (TRIG, 50 mg/kg p.o);

Group 4: Sitagliptin (SITA, 5 mg/kg p.o);

Group 5: Trigonelline (TRIG, 50 mg/kg p.o) + Sitagliptin (SITA, 5 mg/kg p.o) respectively.

The rats were allowed to develop diabetic nephropathy for next four weeks. Test drugs were administered for next 4 weeks starting from 5th week to 8th week. After 8 weeks, rats were killed by deep anesthesia and both kidneys were immediately isolated.

4.2.3.2.2 Effect of test drugs on serum glucose level

During the study period of 8 weeks, serum glucose was determined at the interval of 7 days using GOD/POD method.

4.2.3.2.3 Effect of test drugs on body weight

During the study period of 8 weeks, the body weight of rat was recorded daily using electronic balance.

4.2.3.2.4 Effect of test drugs on renal function tests

Levels of serum creatinine, urine creatinine, blood urea nitrogen (BUN) and uric acid were measured using commercial diagnostic kits.

4.2.3.2.5 Effect of test drugs on kidney weight in diabetic nephropathic rats

At the end day of treatment (8th week), kidney weight was recorded using electronic weighing balance.

4.2.3.2.6 Effect of test drugs on enzymatic biomarkers of oxidative stress in diabetic nephropathic rats kidney

At the end of the treatment (last day of 8 week), kidney of individual rat were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris – HCl buffer (pH7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid per-oxidation (MDA).SOD activity was determined by the method of Misera and Fridocich, 1972. GSH assay was carried out by method of Morgon et al., 1979. Malondialdehyde assay was performed by method of Slater and Sawyer, 1971. From obtained data mean change in GSH, MDA, SOD and S.E.M were calculated.

4.2.3.2.7 Histopathological examination of isolated renal tissue of diabetic nephropathic rats

Isolated left kidney was cut into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3-5µm in thickness by microtome and stained by hematoxyline-eosin (H&E) stain and renal fiber staining by Masson's trichome (MT) stain. The stained samples were observed under microscope and analysed by cell imaging software for life sciences microscopy (Olympus soft imaging solution, Munster, Germany).

4.2.3.2.8 Statistical analysis

All of the data are expressed as mean ± S.E.M. Data obtained for serum glucose, body weight and biochemical estimation were analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data on kidney weight, SOD, GSH, MDA levels on last day of study period (on week 8^{th}) by one-way ANOVA followed by Dunnet's test for significance.

4.2.3.3.1 Effect of TRIG, SITA alone and concomitant therapy of two drugs (TRIG+SITA) in diabetic neuropathic rats

Diabetic neuropathy was induced as per reported methods [Visnagri *et al*., 2012; Sharma *et al*., 2012]. A group of non diabetic rats was included in the study. The rats were divided into following groups;

Group 1: Non-diabetic control;

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*);

Group 3: Trigonelline (TRIG, 50 mg/kg p.o);

Group 4: Sitagliptin (SITA, 5 mg/kg p.o);

Group 5: Trigonelline (TRIG, 50 mg/kg p.o) + Sitagliptin (SITA, 5 mg/kg p.o).

The rats were allowed to develop diabetic neuropathy for four weeks. Treatment with test drugs for next 4 weeks started from 5^{th} week to 8^{th} week. After 8 weeks, rats were killed under deep anesthesia and sciatic nerves were immediately isolate.

4.2.3.3.2 Effect of test drugs on serum glucose

During the study period of eight weeks serum glucose level was determined at every week by using GOD/POD method.

4.2.3.3.3 Effect of test drugs on body weight

Effect on body weight was recorded daily using electronic balance.

4.2.3.3.4 Effect of test drug on behavioral tests

4.2.3.3.4.1 Thermal hyperalgesia (Radiant heat test)

Radiant heat hyperalgesia of the left hind paw was assessed using the radiant heat lamp source as per reported method by Fraser *et al*., 2000 for assessing the reactivity to noxious thermal stimuli. The intensity of the radiant heat stimulus was maintained at 55 ± 0.1 ^{0}C. Response of left hind paw withdrawal threshold was noted. Cut-off time of 10 sec was maintained.

4.2.3.3.4.2 Mechanical hyperalgesia (Randall–Selitto paw pressure test)

The nociceptive flexion reflex was quantified using the Randall-Sellito paw pressure device (UGO Basile SRL Biological Research Apparatus, Italy) as per the method available in the literature [Chaplan *et al*., 1994] which applies a linearly increasing mechanical force in (g) to the dorsum of the rat hind paw. Nociceptive threshold, expressed in (g), were applied by increasing pressure to the hind paw until squeak

(vocalization threshold) was entitled. The paw of the rat was placed under the tip and the progressive pressure applied until the rat vocalized. The nociceptive threshold was measured three or four times in order to obtain two consecutive values that differed more than 10% and respecting an interval of at least 10 min between two measures.

4.2.3.3.4.3 Mechano-tactile allodynia (Von-Frey hair test)

Mechanical allodynia was assessed using Von-Frey hair apparatus as per published method described by Fuch *et al*., 2010. Rats were placed individually on an elevated mesh in a clear plastic cage and adapted to the testing environment for at least 15 min. Von-Frey hairs (IITC, Woodland Hills, USA) with calibrated bending forces in (g) of different intensities were used to deliver mechanical stimuli of varying intensity. Starting with the lowest filament force, Von-Frey hairs were applied from below the mesh floor to the planter surface of the hind paw, with sufficient force to cause slight bending against the paw and held for 1sec. Each stimulation was applied five times with and simultaneous interval of 4-5 sec. Care was taken to stimulate random locations on the planter surface. A positive response was noted if the paw was robustly and immediately withdrawn.

4.2.3.3.4.4 Effect of test drugs on motor nerve conduction velocity

Electrophysiological study [Morani *et al*., 2012; Kandhare *et al*., 2012] rats were anesthetized using ketamine (50 mg/kg i.p.). To minimize effects of differences in body temperature on motor nerve conduction velocity (MNCV), subjects were allowed to acclimatize under a 40 W light bulb for 15 min before procedure. The left leg of rat was shaved and cleaned. MNCV was recorded by stimulating the sciatic and tibial nerves at sciatic and tibial notch, respectively by a 200 µs square wave pulse delivered through a pair of mono-polar needle electrodes through stimulator. Recordings were obtained byusing Student Power Lab 8 channel data acquisition system (AD Instrument Pvt. Ltd., Lab Chart 7.3, Australia).

4.2.3.3.5 Effect of test drugs on enzymatic biomarkers of oxidative stress in isolated sciatic nerve tissue of diabetic neuropathic rats

On the last day (8^{th} week) of study period, single sciatic nerve of individual rat were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris –HCl buffer (pH 7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD) and lipid per-oxidation malondialdehyde (MDA). SOD activity was

determined by the method of Misera and Fridocich, 1972. MDA assay was performed by method of Slater and Sawyer, 1971.

4.2.3.3.6 Histopathological examination of isolated sciatic nerve tissue of diabetic neuropathic rats

At the end of 8th week, rats were killing under deep anesthesia and sciatic nerves were carefully removed. Isolated nerves were kept in fixative solution (10%) formalin. It was then cut in section of 3-5µm in thickness by microtome and stained by hematoxyline-eosin (H&E) stain. H&E staining was performed to analyze nerve section quantitatively under light microscope for histopathological alterations such as necrosis, swelling and congestion.

4.2.3.3.7 Stastical analysis

All of the data are expressed as mean ± S.E.M. Data obtained for serum glucose, body weight, thermal hyperalgesia, mechanical hyperalgesia, mechano tactile allodynia and motor nerve conduction velocity were analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data on oxidative stress by one-way ANOVA followed by Dunnet's test for significance.

4.2.3.4.1 Effect of TRIG, SITA alone and concomitant therapy of two drugs (TRIG+SITA) in diabetic cardiomyopathic rats

For the induction of diabetic cardiomyopathy in Wistar rats we followed previously mentioned protocol [Soetikno *et al*., 2012]. A group of non diabetic rats was included in the study. The rats were divided into following groups;

Group 1: Non-diabetic control;

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*);

Group 3: Trigonelline (TRIG, 50 mg/kg p.o);

Group 4: Sitagliptin (SITA, 5 mg/kg p.o);

Group 5: Trigonelline (TRIG, 50 mg/kg p.o) + Sitagliptin (SITA, 5 mg/kg p.o) respectively.

The rats were allowed to develop diabetic cardiomyopathy for 3 weeks. The treatment was started from beginning of 4^{th} week and continued till the end of 11^{th} week. After 11 weeks, rats were killed under deep anesthesia and heart was immediately isolated.

4.2.3.4.2 Effect of test drugs on serum glucose level

During the study period of eleven weeks serum glucose level was determined at every week by using GOD/POD method.

4.2.3.4.3 Effect of test drugs on body weight

Effect on body weight was recorded daily using electronic balance.

4.2.3.4.4 Effect of test drugs on cardiac markers

Cardiac damage markers viz; creatine kinase (CK-MB), lactate dehydrogenase (LDH), aspartate transaminase (AST) were estimated by the commercially available kits. (Accurex Pvt Ltd., Mumbai, India).

4.2.3.4.5 Effect of test drugs on lipid levels

The serum was analyzed for the lipid levels viz. for serum cholesterol, triglycerides, high-density lipoprotein (HDL) using kits procured from Accurex Pvt Ltd, Mumbai, India.

4.2.3.4.6 Effect of test drugs on electrocardiographic and heamodynamic parameters

On last day of study, animals were anaesthetized by urethane (1.25 gm/kg, i.p.); Electrocardiogram (ECG) was recorded using 8 channels recording Power Lab System (AD Instruments, LABCHART 7.3 software, Australia).Heamodynamic

changes were recorded by a polyethylene cannula (PE 50) filled with heparinised saline (100 IU/ml) inserted into the right carotid artery. The cannula was connected to a transducer and the signal was amplified. Left ventricular systolic pressure was measured by means of a Millar mikro-tip transducer catheter (Model SRP-320, Millar instrument, INC 320-7051, Houston, Texas 77023-5417) inserted into the left ventricle via the right carotid artery. The left ventricular functions like dP/dt max, dP/dt min and left ventricular end diastolic pressure signals were obtained from primary signals (left ventricular systolic pressure and blood pressure) by means of an acquisition data system (AD Instruments Pvt Ltd with software (LabChart 7.3; AD Instrument Pvt. Ltd).

4.2.3.4.7 Histopathological examination of isolated cardiac tissue

At the end of study period (last day of 11^{th} week), the rats were killed under deep anesthesia and heart was isolated from all rats. The isolated heart tissue was trimmed into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3-5 µm in thickness by microtome and stained by hematoxyline-eosin (H&E stain). The photomicrographs of each tissue section were observed using cell imaging software for Life Science microscopy (Olympus soft imaging solution GmbH, Munster, Germany).

4.2.3.4.8 Statistical analysis

All of the data were expressed as mean ± S.E.M. Data obtained for serum glucose, body weight were analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data of hemodynamic and biochemical parameters by one-way ANOVA followed by Dunnet's test for significance.

4.2.3.5.1Effect of MET alone and triple drug therapy using trigonelline (TRIG) + sitagliptin (SITA) + metformin (MET) in diabetic nephropathic rats

For experimental induction of diabetic nephropathy in Wistar rats, we followed the protocol reported by [Qi *et al*., 2011; Kakadiya *et al*., 2010]. A group of non diabetic rats was included in the study.

The rats were divided into following groups;

Group 1: Non-diabetic

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*)

Group 3: Metformin (MET 300mg/kg p.o.)

Group 4: Trigonelline (TRIG 50 mg/kg p.o.) + Sitagliptin (SITA 5 mg/kg p.o.) + Metformin (MET 300 mg/kg p.o.).

The rats were allowed to develop diabetic nephropathy for next four weeks. Test drugs were administered for next 4 weeks starting from 5^{th} week to 8^{th} week. After 8 weeks, rats were killed by deep anesthesia and both kidneys were immediately isolated.

4.2.3.5.2 Effect of test drugs on serum glucose level

During the study period of 8 weeks, serum glucose was determined at the interval of 7 days using GOD/POD method.

4.2.3.5.3 Effect of test drugs on body weight

During the study period of 8 weeks, the body weight of rat was recorded daily using electronic balance.

4.2.3.5.4 Effect of test drugs on renal function tests

Levels of serum creatinine, urine creatinine, blood urea nitrogen (BUN) and uric acid were measured using commercial diagnostic kits.

4.2.3.5.5 Effect of test drugs on kidney weight in diabetic nephropathic rats

At the end day of treatment (8^{th} week), kidney weight was recorded using electronic weighing balance.

4.2.3.5.6 Effect of test drugs on enzymatic biomarkers of oxidative stress in diabetic nephropathic rats kidney

At the end of the treatment (last day of 8 week), kidney of individual rat were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris – HCl buffer (pH7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid per-oxidation (MDA).SOD activity was determined by the method of Misera and Fridocich, 1972. GSH assay was carried out by method of Moron *et al*., 1979. Malondialdehyde assay was performed by method of Slater and Sawyer, 1971. From obtained data mean change in GSH, MDA, SOD and S.E.M were calculated.

4.2.3.5.7 Histopathological examination of isolated renal tissue of diabetic nephropathic rats

Isolated left kidney was cut into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3-5µm in thickness by microtome and stained by hematoxyline-eosin (H&E) stain and renal fiber staining by Masson's trichome (MT) stain. The stained samples were observed under microscope and analysed by cell imaging software for life sciences microscopy (Olympus soft imaging solution, Munster, Germany).

4.2.3.5.8 Statistical analysis

All of the data are expressed as mean ± S.E.M. Data obtained for serum glucose, body weight and biochemical estimation were analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data on kidney weight, SOD, GSH, MDA levels on last day of study period (on week 8^{th}) by one-way ANOVA followed by Dunnet's test for significance.

4.2.3.6.1 Effect of MET alone and triple drug therapy using trigonelline (TRIG) + sitagliptin (SITA) + metformin (MET) in diabetic neuropathic rats

Diabetic neuropathy was induced as per reported methods [Visnagri *et al*., 2012; Sharma *et al*., 2012]. A group of non diabetic rats was included in the study.

The rats were divided into following groups;

Group 1: Non-diabetic

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*)

Group 3: Metformin (MET 300 mg/kg p.o.)

Group 4: Trigonelline (TRIG 50 mg/kg p.o.) + Sitagliptin (SITA 5 mg/kg p.o.) + Metformin (MET 300 mg/kg p.o.).

The rats were allowed to develop diabetic neuropathy for four weeks. Treatment with test drugs for next 4 weeks started from 5th week to 8th week. After 8 weeks, rats were killed under deep anesthesia and sciatic nerves were immediately isolate.

4.2.3.6.2 Effect of test drugs on serum glucose

During the study period of eight weeks serum glucose level was determined at every week by using GOD/POD method.

4.2.3.6.3 Effect of test drugs on body weight

Effect on body weight was recorded daily using electronic balance.

4.2.3.6.4 Effect of test drug on behavioral tests

4.2.3.6.4.1 Thermal hyperalgesia (Radiant heat test)

Radiant heat hyperalgesia of the left hind paw was assessed using the radiant heat lamp source as per reported method by Fraser *et al*., 2000 for assessing the reactivity to noxious thermal stimuli. The intensity of the radiant heat stimulus was maintained at 55 ± 0.1 ◦ C. Response of left hind paw withdrawal threshold was noted. Cut-off time of 10 s was maintained.

4.2.3.6.4.2 Mechanical hyperalgesia (Randall–Selitto paw pressure test)

Mechanical hyperalgesia (Randall–Selitto paw pressure test)

The nociceptive flexion reflex was quantified using the Randall-Sellito paw pressure device (UGO Basile SRL Biological Research Apparatus, Italy) as per the method available in the literature [Chaplan *et al*., 1994] which applies a linearly increasing

mechanical force in (g) to the dorsum of the rat hind paw. Nociceptive threshold, expressed in (g), were applied by increasing pressure to the hind paw until squeak (vocalization threshold) was entitled. The paw of the rat was placed under the tip and

4.2.3.6.4.3 Mechano-tactile allodynia (Von-Frey hair test)

Mechanical allodynia was assessed using Von-Frey hair apparatus as per published method described by Fuch *et al*., 2010. Rats were placed individually on an elevated mesh in a clear plastic cage and adapted to the testing environment for at least 15 min. Von-Frey hairs (IITC, Woodland Hills, USA) with calibrated bending forces in (g) of different intensities were used to deliver mechanical stimuli of varying intensity. Starting with the lowest filament force, Von-Frey hairs were applied from below the mesh floor to the planter surface of the hind paw, with sufficient force to cause slight bending against the paw and held for 1sec. Each stimulation was applied five times with and simultaneous interval of 4-5 sec. Care was taken to stimulate random locations on the planter surface. A positive response was noted if the paw was robustly and immediately withdrawn.

4.2.3.6.4.4 Effect of test drugs on motor nerve conduction velocity

Electrophysiological study [Morani *et al*., 2012; Kandhare *et al*., 2012] rats were anesthetized using ketamine (50 mg/kg i.p.). To minimize effects of differences in body temperature on motor nerve conduction velocity (MNCV), subjects were allowed to acclimatize under a 40 W light bulb for 15 min before procedure. The left leg of rat was shaved and cleaned. MNCV was recorded by stimulating the sciatic and tibial nerves at sciatic and tibial notch, respectively by a 200 µs square wave pulse delivered through a pair of mono-polar needle electrodes through stimulator. Recordings were obtained using Student Power Lab 8 channel data acquisition system (AD Instrument Pvt. Ltd., Lab Chart 7.3, Australia).

4.2.3.6.5 Effect of test drugs on enzymatic biomarkers of oxidative stress in isolated sciatic nerve tissue of diabetic neuropathic rats

On the last day (8^{th} week) of study period, single sciatic nerve of individual rat were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris –HCl buffer (pH 7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD) and lipid per-oxidation malondialdehyde (MDA). SOD activity was determined by the method of Misera and Fridocich, 1972. MDA assay was performed by method of Slater and Sawyer, 1971.

4.2.3.6.6 Histopathological examination of isolated sciatic nerve tissue of diabetic neuropathic rats

At the end of 8th week, rats were killing under deep anesthesia and sciatic nerves were carefully removed. Isolated nerves were kept in fixative solution (10%) formalin. It was then cut in section of 3-5µm in thickness by microtome and stained by hematoxyline-eosin (H&E) stain. H&E staining was performed to analyze nerve section quantitatively under light microscope for histopathological alterations such as necrosis, swelling and congestion.

4.2.3.6.7 Stastical analysis

All of the data are expressed as mean ± S.E.M. Data obtained for serum glucose, body weight, thermal hyperalgesia, mechanical hyperalgesia, mechano tactile allodynia and motor nerve conduction velocity were analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data on oxidative stress by one-way ANOVA followed by Dunnet's test for significance.

4.2.3.7.1 Effect of MET and triple drug therapy using trigonelline (TRIG) + sitagliptin (SITA) + metformin (MET) in diabetic cardiomyopathic rats

For the induction of diabetic cardiomyopathy in Wistar rats we followed previously mentioned protocol [Soetikno *et al*., 2012]. A group of non diabetic rats was included in the study.

The rats were divided into following groups;

Group 1: Non-diabetic

Group 2: Diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*)

Group 3: Metformin (MET 300mg/kg p.o.)

Group 4: Trigonelline (TRIG 50mg/kg p.o.) + Sitagliptin (SITA 5 mg/kg p.o.) + Metformin (MET 300mg/kg p.o.).

The rats were allowed to develop diabetic cardiomyopathy for three weeks. The treatment with MET alone and triple therapy using TRIG+SITA+MET was started from beginning of 4^{th} week and continued till the end of 11^{th} week. The animals from normal and diabetic group received only vehicle (distilled water). Blood withdrawal and biochemical estimation were carried out first than histological examination of isolated hearts were carried out.

4.2.3.7.2 Effect of test drugs on serum glucose level

During the study period of eleven weeks serum glucose level was determined at every week by using GOD/POD method.

4.2.3.7.3 Effect of test drugs on body weight

Effect on body weight was recorded daily using electronic balance.

4.2.3.7.4 Effect of test drugs on cardiac markers

Cardiac damage markers viz; creatine kinase (CK-MB), lactate dehydrogenase (LDH), aspartate transaminase (AST) were estimated by the commercially available kits. (Accurex Pvt Ltd. Mumbai, India).

4.2.3.7.5 Effect of test drugs on lipid levels

The serum was analyzed for the lipid levels viz; for serum cholesterol, triglycerides, high-density lipoprotein (HDL) using kits procured from Accurex Pvt Ltd, Mumbai, India.

4.2.3.7.6 Effect of test drugs on electrocardiographic and heamodynamic parameters

On last day of study, animals were anaesthetized by urethane (1.25 gm/kg, i.p.); Electrocardiogram (ECG) was recorded using 8 channel recording Power Lab System (AD Instruments, LABCHART 7.3 software, Australia). Heamodynamic changes were recorded by a polyethylene cannula (PE 50) filled with heparinised saline (100 IU/ml) inserted into the right carotid artery. The cannula was connected to a transducer and the signal was amplified. Left ventricular systolic pressure was measured by means of a Millar mikro-tip transducer catheter (Model SRP-320, Millar instrument, INC 320-7051, Houston, Texas 77023-5417) inserted into the left ventricle via the right carotid artery. The left ventricular functions like dP/dt max, dP/dt min and left ventricular end diastolic pressure signals were obtained from primary signals (left ventricular systolic pressure and blood pressure) by means of an acquisition data system (AD Instruments Pvt Ltd with software (LabChart 7.3; AD Instrument Pvt. Ltd).

4.2.3.7.7 Histopathological examination of isolated cardiac tissue

At the end of study period (last day of 11^{th} week), the rats were killed under deep anesthesia and heart was isolated from all rats. The isolated heart tissue was trimmed into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3-5 µm in thickness by microtome and stained by hematoxyline-eosin (H&E stain). The photomicrographs of each tissue section were observed using cell imaging software for Life Science microscopy (Olympus soft imaging solution GmbH, Munster, Germany).

4.2.3.7.8 Statistical analysis

All of the data were expressed as mean ± S.E.M. Data obtained for serum glucose, body weight were analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data of hemodynamic and biochemical parameters by one-way ANOVA followed by Dunnet's test for significance.

4.2.3.8.1 Assay of lipid Peroxidation (MDA content)

Reagents

- **Thiobarbituric acid (0.67% w/v)**

  Thiobarbituric acid 0.67 gm was dissolved in 50 ml of distilled water and the final volume was made up to 100 ml with hot distilled water.

- **Trichloroacetic acid (10% w/v)**

  Trichloroacetic acid 10 gm was dissolved in 60 ml of distilled water and the final volume was made up to 100 ml with distilled water.

- **Standard Malondialdehyde stock solution (50mM)**

  A standard malondialdehyde stock solution was prepared by mixing 25µ of 1,1,3,3-tetraethoxypropane up to 100 ml with distilled water. 1.0 ml of this stock solution was diluted up to 10 ml to get solution containing 23µ of malondialdehyde/ml. One ml of this stock solution was diluted up to 100 ml to get a working standard solution containing 23ng of malondialdehyde/ml.

Procedure

Tissue homogenate (supernatant) 2.0 ml was added to 2.0 ml of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation and 2.0 ml of clear supernatant solution was mixed with 2.0 ml of freshly prepared thiobarbituric acid (TBA). The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532nm against reagent blank by U.V spectrophotometer. Different concentrations (0-23nM) of standard malondialdehyde were processed as above for obtaining standard graph. The values were expressed as nM of MDA/mg protein [Slater and Sawyer, 1971].

4.2.3.8.2. Assay of endogenous antioxidant (reduced glutathione i.e. GSH)

Reagents

- **Trichloroacetic acid (20% w/v)**

  Trichloroacetic acid 20 gm was dissolved in sufficient quantity of distilled water and the final volume was made up to 100 ml with distilled water.

- **Phosphate Buffer (0.2M, pH 8.0)**

  Sodium phosphate 0.2 M was prepared by dissolving 30.2 gm sodium phosphate in 600 ml of distilled water, the pH was adjusted to 8.0 with 0.2M sodium hydroxide solution and the final volume was adjusted up to 1000 ml with distilled water.

- **5,5-dithiobis-2-nitrobenzoic acid (DTNB) reagent (0.6mM)**

  DTNB reagent 60 mg was dissolved in 50 ml of buffer and the final volume was adjusted to 100 ml with buffer.

- **Standard glutathione (100 µg/ml)**

  10 mg of glutathione was dissolved in 60 ml of distilled water and the final volume was made up to 100 ml with distilled water.

Procedure

Equal volumes of tissue homogenate (supernatant) and 20% TCA were mixed. The precipitated fraction was centrifuged at 2500 rpm at 4°C for 15 min and 2.0 ml of DTNB reagent was added to 0.25 ml of supernatant. The final volume was made up to 3.0 ml with phosphate buffer. The colour developed was read at 412 nm against reagent blank. Different concentrations (10-50 µg) of standard glutathione were prepared and processed as above for standard graph. The amount of reduced glutathione is expressed as µg of GSH/mg protein [Morgon *et al*., 1979].

4.2.3.8.3 Assay of Superoxide Dismutase (SOD)

Reagents

- **Carbonate buffer (0.05 M, pH 10.2)**

  Sodium bicarbonate 16.8 gm and 22 gm of sodium carbonate were dissolved in 500 ml of distilled water and the volume was made up to 1000 ml with distilled water.

- **Ethylene diamine tetra acetic acid (EDTA) solution (0.4 M)**

  EDTA 1.82 gm was dissolved in 200 ml of distilled water and the volume was made up to 1000 ml with distilled water.

- **Hydrochloric acid (0.1 N)**

  Concentrated hydrochloric acid 8.5 ml was mixed with 500 ml of distilled water and the volume was made up to 1000 ml with distilled water.

- **Epinephrine solution (3mM)**

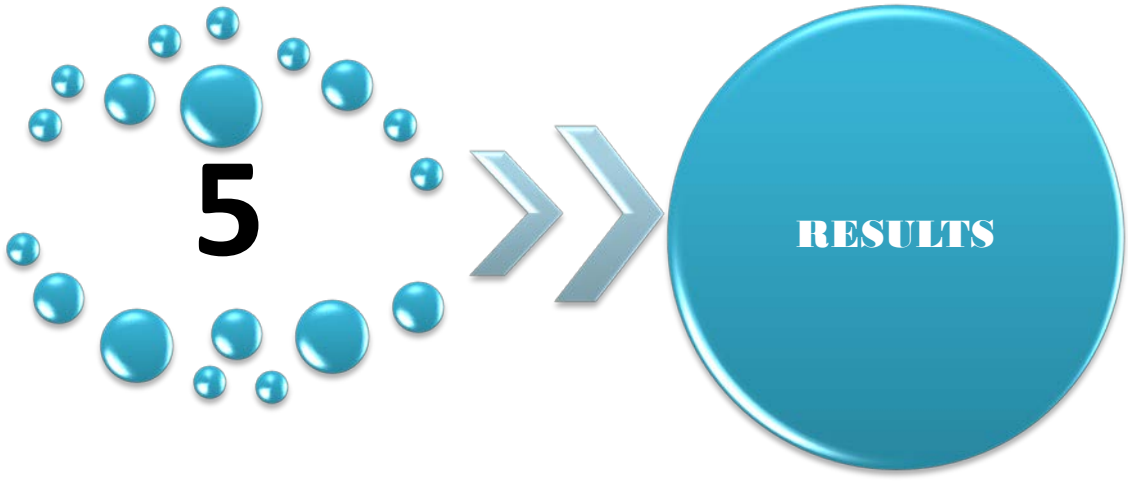
  Epinephrine bitartarate 0.99 gm was dissolved in 100 ml of 0.1N hydrochloric acid and the volume was adjusted to 1000 ml with 0.1N hydrochloric acid.

- **Superoxide dismutase standard (10 U/ml)**

  SOD 1 mg (1000 U / mg) from bovine liver was dissolved in 100 ml of carbonate buffer.

Procedure

Liver tissue homogenate (0.5 ml) was diluted with (0.5 ml) distilled water, to which 0.25 ml of ice-cold ethanol and 0.15 ml of ice-cold chloroform, were added. The mixture was mixed well using cyclo mixer and centrifuged at 2500 rpm at 4°C for 15 min. To 0.5 ml of supernatant, 1.5 ml of carbonate buffer and 0.5 ml of EDTA solution were added. The reaction was initiated by the addition of 0.4 ml of epinephrine and the change in optical density/min was measured at 480 nm against reagent blank. Calibration curve was prepared by using 10-125 units of SOD. Change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme unit. SOD activity is expressed as units/mg protein [Misra and Fridovich, 1972].


5
RESULTS

5. RESULTS

Model 5.1 Effect of trigonelline (TRIG) and sitagliptin (SITA) in nicotinamide (NICO) - streptozotocin (STZ) induced diabetes in Wistar rats

5.1.1 Effect of TRIG, SITA alone and their concomitant TRIG+SITA on serum glucose in diabetic rats (acute study)

5.1.1.1 Effect of TRIG (25, 50 and 100 mg/kg p.o.) on serum glucose level in diabetic rats

Before administration of TRIG in diabetic animals the serum glucose was higher in the range of 423 to 447 mg/dl. TRIG administration showed dose dependant reduction of serum glucose level in rats. Treatment with 50 mg/kg p.o. and 100 mg/kg p.o. of TRIG showed significant reduction in serum glucose at 6h. The criteria used to label the antihyperglycemic activity were that test compound should be able to reduce serum glucose more than 20%. In view of this threshold TRIG 50 mg/kg p.o. and 100 mg/kg p.o. satisfied the criteria. TRIG 100 mg/kg p.o. was most effective because the maximum decrease in serum glucose at 6h was 123.01 mg/dl which was calculated as 27.48%. The results also indicated that the onset of action was 2h, maximum at 6h and declined at 24h (Figure 5.1.1 (a).

5.1.1.2 Effect of SITA (2.5, 5 and 10 mg/kg p.o.) on serum glucose level in diabetic rats

SITA also showed dose dependant reduction in serum glucose level but differed from TRIG in showing more reduction in serum glucose at 6h with dose 10 mg/kg p.o (Figure 5.1.1(a).

5.1.1.3 Effect of concomitant administrations of TRIG + SITA (70% + 30%; 50% + 50%; 30% + 70%) on serum glucose level in diabetic rats

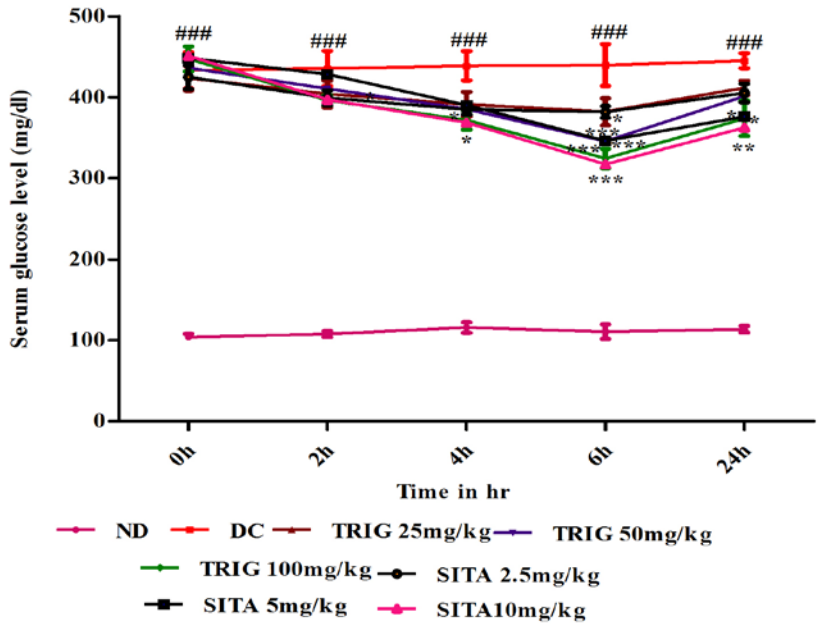
Combinations of various doses of TRIG and SITA showed additive antihyperglycemic action. Combination TRIG 50% + SITA 50% was most effective in reducing the serum glucose than other two combinations resulted in less reduction of serum glucose compared to TRIG 50% + SITA 50% concomitant administration. The results also indicated that increasing SITA dose to 70% and TRIG dose to 30%. Results thus indicated that concomitant administration of test drugs was more effective than administration with single compound. Therefore concomitant

administration of TRIG (50 mg/kg p.o.) and SITA (5 mg/kg p.o.) i.e. 50%+50% was selected for subacute administration for 28 days (Figure 5.1.1. (b).

Table 5.1.1 Effect of TRIG, SITA alone and their concomitant TRIG+SITA on serum glucose in diabetic rats (acute study)

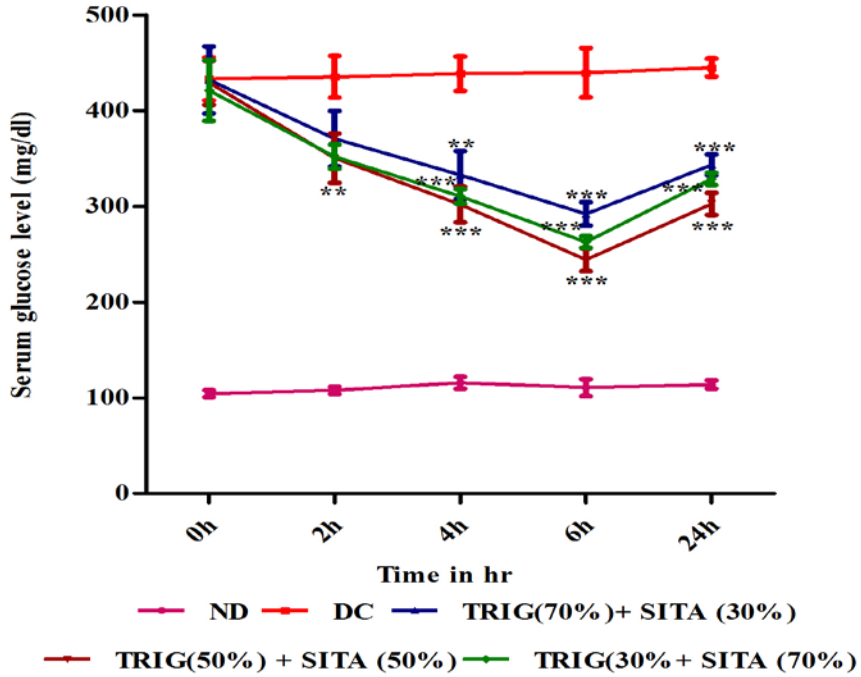
AVG of serum glucose level (mg/dl) in acute study (24 hrs)					
Time in hr / Groups	**0hr**	**2hr**	**4hr**	**6hr**	**24hr**
ND	104.17± 3.52	107.84± 3.73	115.79± 6.25	110.63± 8.92	113.84±4.11
DC	433.29± 22.33$^{###}$	435.41± 21.10$^{###}$	438.75± 17.90$^{###}$	439.63±25.76$^{###}$	445.04± 9.22$^{###}$
TRIG (25mg/kg p.o.)	423.56± 15.08	403.91± 16.52	391.37±15.16	382.08±16.79	411.43± 8.73
TRIG (50mg/kg p.o.)	436.07± 5.07	410.68± 7.29	385.17± 11.07	345.62± 6.15***	401.16± 10.80
TRIG (100mg/kg p.o.)	447.52± 15.27	396.21± 6.15	371.85± 11.76*	324.51± 11.86***	373.90±29.73*
SITA (2.5mg/kg p.o.)	405.90± 14.54	399.34±9.11	384.99±6.35	381.80± 7.18*	405.15± 11.43
SITA (5mg/kg p.o.)	448.33± 14.96	428.51±14.30	390.26±20.43	346.25±6.71***	375.79±3.49*
SITA (10 mg/kg p.o.)	451.22±27.02	397.29±22.70	368.88±22.87*	317.40±21.11***	362.41±16.01**
TRIG + SITA (70% + 30%) viz; (70 mg/kg + 3 mg/kg, p.o.)	432.26± 35.04	370.82± 28.98*	332.75±25.21***	298.15±12.14***	343.46± 10.99***
TRIG + SITA (50% + 50%) viz; (50 mg/kg + 5 mg/kg, p.o.)	429.32± 23.04	350.49± 25.74**	301.96± 18.76***	244.38± 12.07***	302.65± 11.32***
TRIG + SITA (30% + 70%) viz; (30 mg/kg + 7 mg/kg, p.o.)	421.12± 31.90	351.94±12.66**	310.48±7.41***	262.82±6.20***	328.70±6.50***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.1.1 (a) Effect of TRIG, SITA alone and their concomitant TRIG+SITA on serum glucose in diabetic rats (acute study)



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.1.1 (b) Effect of TRIG, SITA alone and their concomitant TRIG+SITA on serum glucose in diabetic rats (acute study)

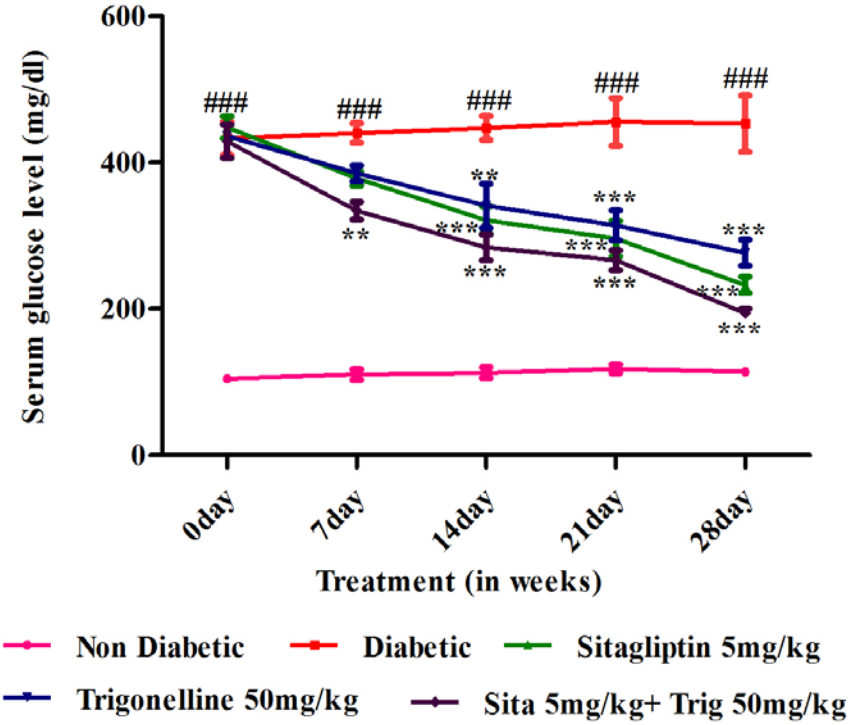
5.1.2 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on serum glucose level in diabetic rats (sub-acute study)

Serum glucose in diabetic rats increased gradually over the observation period of 28 days and this increase was regarded as due to induction of diabetes. On the other hand the concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) for four weeks in diabetic rats was more effective in reducing serum glucose level (54.72%) observed in diabetic rats on day 28. The decrease in serum glucose in concomitantly administered compound group was more than that of alone test compound (TRIG 36.48%; SITA 48.05%) treated rats (Figure 5.1.2).

Table 5.1.2 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on serum glucose level in diabetic rats (sub-acute study)

AVG of serum glucose level (mg/dl) in sub-acute study (28 days)					
Time in day / Groups	Day '0'	Day '7'	Day '14'	Day'21'	Day '21'
ND	104.96± 3.50	110.61± 7.37	112.86±7.21	118.36± 5.93	114.29±1.29
DC	433.29±22.33###	440.42±13.28###	447.36±16.35###	455.80±32.57###	453.11±38.44###
TRIG (50mg/kg p.o.)	436.07± 5.70	385.53±10.27	341.13±30.21**	314.16±20.52***	276.97±17.71***
SITA (5mg/kg p.o.)	448.33±14.96	378.52±9.78	321.46±18.75***	296.06±24.42***	232.88±11.35***
TRIG (50 mg/kg p.o.)+ SITA (5 mg/kg p.o.)	429.32± 23.04	334.29±12.11**	284.20±17.30***	266.74±13.73***	194.38±5.83***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.1.2 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on serum glucose level in diabetic rats (sub-acute study)

5.1.3 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on body weight in diabetic rats (sub-acute study)

5.1.3.1 Determination of body weight in diabetic rats

The body weight of non-diabetic rats increased during study period of 28 days. On other hand the body weight of diabetic rats decrease gradually over the observation period of 28 days and this decrease was regarded to be due to induction of diabetes (Figure 5.1.3).

5.1.3.2 Effect of TRIG (50 mg/kg p.o.) on body weight in diabetic rats

Administration of TRIG (50 mg/kg p.o.) for four weeks in diabetic rats arrested the loss of body weight (Figure 5.1.3).

5.1.3.3 Effect of SITA (5 mg/kg p.o.) on body weight in diabetic rats

Administration of SITA (5 mg/kg p.o.) for 4 weeks in diabetic rats also arrested the loss of body weight observed in diabetic rats. The body weight of SITA (50 mg/kg p.o.) treated rats was more than that of TRIG (50 mg/kg p.o.) treated rats (Figure 5.1.3).

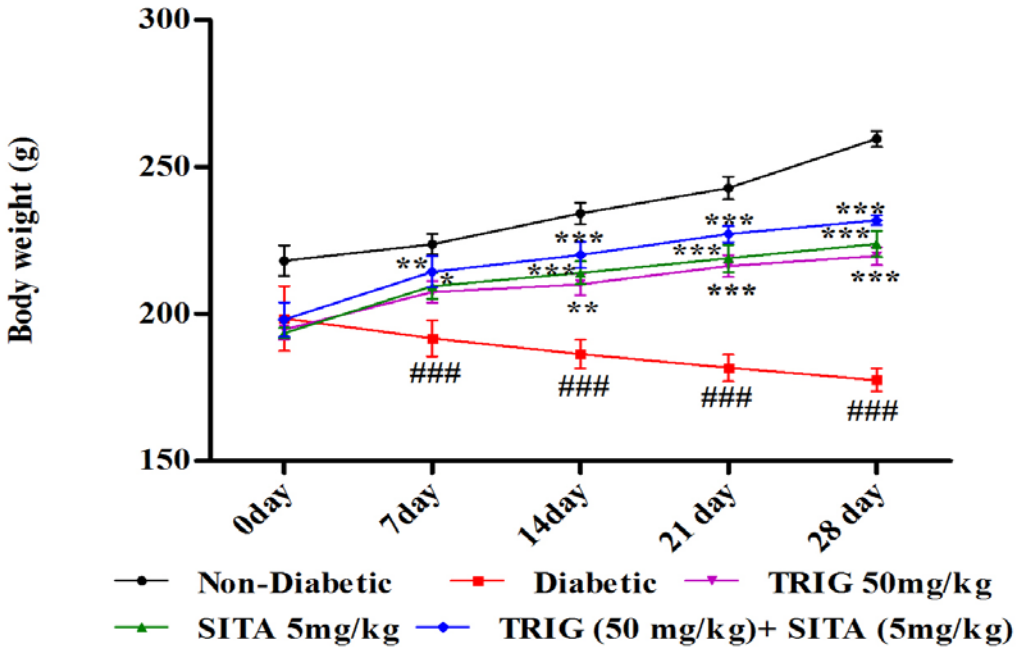
5.1.3.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on body weight in diabetic rats

Concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) for four weeks in diabetic rats was more effective in arresting the loss of body weight observed in diabetic rats. The gain in body weight in concomitantly administered compounds group was more than that of alone compound treated rats. The results thus indicated that test compound administered alone or concomitantly prevent loss of body weight due to diabetes and help to restore normal body weight (Figure 5.1.3).

Table 5.1.3 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on body weight in diabetic rats (sub-acute study)

AVG of body weight (g) in sub-acute study (28 days)					
Time in day / Groups	Day '0'	Day '7'	Day '14'	Day'21'	Day '28'
ND	218.09± 05.1	223.66± 03.5	234.18± 03.5	242.82± 03.9	259.50± 02.7
DC	198.43± 10.9$^{###}$	191.71± 06.11$^{###}$	186.29± 04.8$^{###}$	181.65± 04.5$^{###}$	177.47± 03.9$^{###}$
TRIG (50mg/kg p.o.)	194.63± 03.3	207.49± 03.6	210.01± 03.6**	216.27± 03.7***	219.64± 03.0***
SITA (5mg/kg p.o.)	193.46± 01.7	209.45± 04.4*	213.92± 03.8***	218.88± 04.6***	223.79± 04.4***
TRIG (50 mg/kg p.o.)+ SITA (5 mg/kg p.o.)	198.07± 05.8	214.32± 05.2**	220.03± 04.4***	227.16± 02.8***	231.84± 01.5***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.1.3 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on body weight in diabetic rats (sub-acute study)

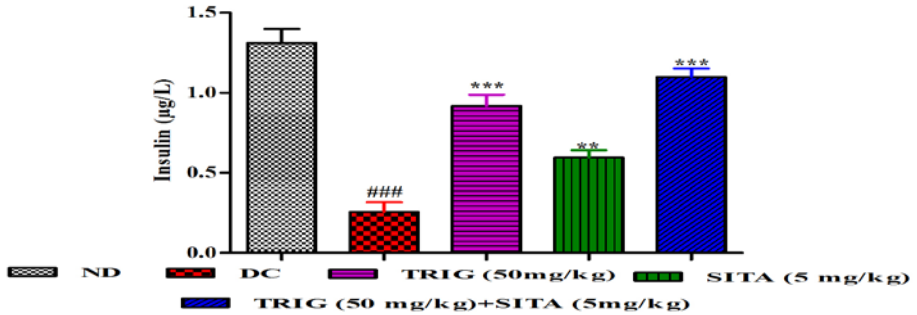
5.1.4 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on serum insulin in diabetic rats

Mean serum insulin level in non diabetic rat was 1.312±0.08 µg/l on day 28. On other hand serum insulin level of diabetic rat was 0.255±0.06 µg/l which showed significant (*P< 0.001*) decrease in mean level of serum insulin. Significant increase in serum insulin level was recorded on day 28, TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., (0.917±0.07, *P< 0.001*; 0.595±0.04, *P< 0.01* and 1.098±0.05 µg/l, *P< 0.001*) treated groups as compared to diabetic rats. TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) showed maximum (1.098±0.05 µg/l) increase in serum insulin level than alone. The results thus indicated that increase in the serum insulin level due to test compounds may be due to release of insulin from pancreas (Figure 5.1.4).

Table 5.1.4 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on serum insulin in diabetic rats

AVG of serum insulin (µg/l) level	
Parameter / Groups	Serum insulin level (µg/l)
ND	1.312±0.08
DC	0.255±0.06$^{###}$
TRIG (50mg/kg p.o.)	0.917±0.07***
SITA (5mg/kg p.o.)	0.595±0.04**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	1.098±0.05***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.1.4 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on serum insulin in diabetic rats

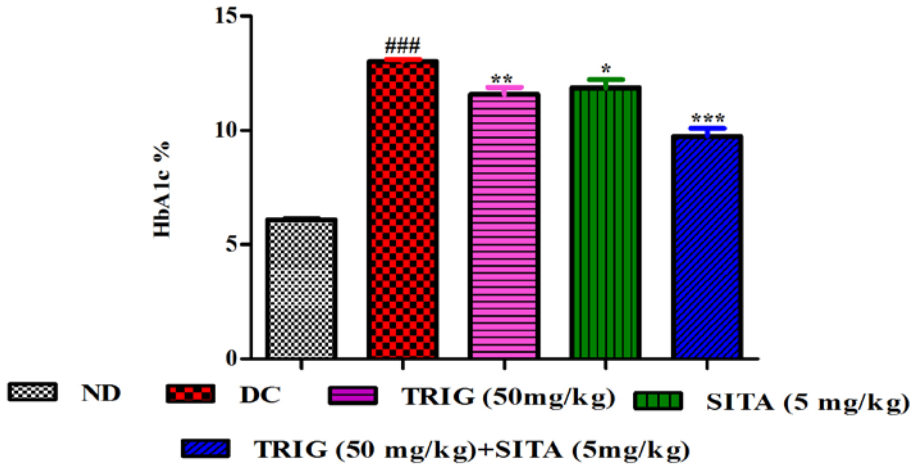
5.1.5 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on glycated hemoglobin (HbA1c) in diabetic rats

Mean HbA1c level in non diabetic rat was 6.10±0.04% on day 28. On other hand HbA1c level of diabetic rat was 13.00±0.10% which showed significant (*P< 0.001*) increase in level of HbA1c. Significant decrease in HbA1c levels was recorded on day 28, TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., (11.57±0.30 %, *P< 0.01*; 11.85±0.36 %, *P< 0.05* and 9.74±0.33 %, *P< 0.001*) as compared to diabetic rats. TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) showed maximum (9.74±0.33%) decrease in HbA1c level than alone. Concomitant administration of TRIG+SITA produced significant reduction in glycated hemoglobin indicated better control over hyperglycemia. However the treatment was ineffective in restoring the normal levels of glycated hemoglobin indicating a weak effect of drug treatment on this parameter (Figure 5.1.5).

Table 5.1.5 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on glycated hemoglobin (HbA1c) in diabetic rats

AVG of serum HbA1c level	
Parameter / Groups	HbA1c level (%)
ND	6.10±0.04
DC	13.00±0.10$^{###}$
TRIG (50mg/kg p.o.)	11.57±0.30**
SITA (5mg/kg p.o.)	11.85±0.36*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	9.74±0.33***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



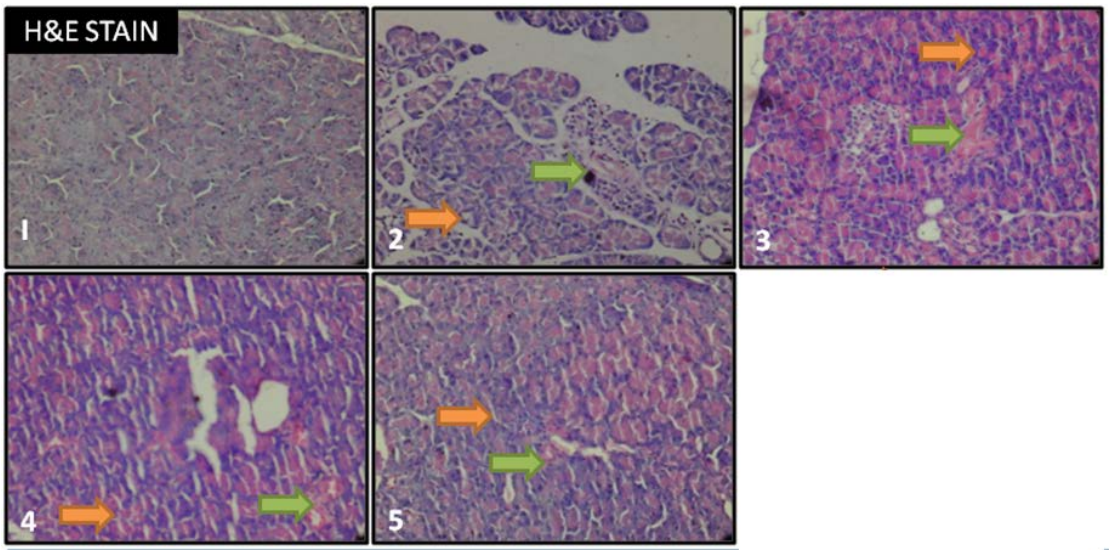
Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.1.5 Effect of TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant administration TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) on glycated hemoglobin (HbA1c) in diabetic rats

5.1.6 Histological examination of isolated rat pancreas of diabetic rats

Histological examinations of the rat pancreas sections under light microscopy with Hematoxyline and eosin (H&E) stain were carried out on last day of study period.

Isolated pancreas of non diabetic pancreas showed (Grade -) normal architecture. Diabetic rats showed (Grade ++++) destruction and size of islets. TRIG treated rats showed (Grade +++) destruction and size of islets. SITA treated rats showed (Grade +++) destruction and size of islets. TRIG+SITA showed (Grade ++) destruction and size of islets. The scoring of grades was as per Dr. Prachi Bhagwat (M.D), Specialty Center for Surgical Pathology and Diabetology, Joshi Laboratory, Pune. Where (Grade -) normal; (Grade ++++) severe; (Grade +++) moderate; (Grade ++) mild (figure 5.1.6).



Where, 1] Non diabetic group; 2] Diabetic group; 3] TRIG (50mg/kg p.o.); 4] SITA (5mg/kg p.o.) and 5] TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.). Orange arrow: Destruction; Green arrow: Size of Islets [Magnification 40x].

Figure 5.1.6 Histological examination of isolated rat pancreas of diabetic rats (H&E stain)

Model 5.2.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) in NICO-STZ induced diabetic nephropathy in Wistar rats

5.2.1.1 Serum glucose level in non-diabetic rats

The results indicated non-significant fluctuation in serum glucose level compared to week '0' reading (Figure 5.2.1).

5.2.1.2 Serum glucose level in diabetic nephropathic rats

Serum glucose level before administration of NICO-STZ in this group was (96.49±5.14 mg/dl). After administration of NICO-STZ the serum glucose level increasing trend and at the end of 4 week after induction of diabetes the serum glucose (422.06±11.80 mg/dl). The vehicle used for dissolving the test compound was double distilled water which could not prevent the rise of serum glucose and at the end 4 weeks of treatment period the serum glucose level was 436.00±18.24 mg/dl (Figure 5.2.1).

5.2.1.3 Effect of TRIG (50mg/kg p.o) on serum glucose level in diabetic nephropathic rats

In the TRIG (50 mg/kg p.o.) treated group the initial serum glucose level was (415.46±16.52 mg/dl) which was reduced to (256.06± 14.43 mg/dl, $P< 0.001$) indicating a decrease of 159.4 mg/dl or 38.36%. The onset of antihyperglycemic effect was evident after 3 week of TRIG (50 mg/kg p.o.) treatment and continuation of treatment showed a trend toward decrease in serum glucose (Figure 5.2.1).

5.2.1.4 Effect of SITA (5 mg/kg, p.o) on serum glucose level in diabetic nephropathic rats

In case of SITA (5 mg/kg p.o.) treated diabetic animals the serum glucose level before treatment (initial) was (402.38±9.33mg/dl). After treatment with SITA (5 mg/kg p.o.) the serum glucose level at the 4^{th} week was (229.26±8.07mg/dl) this reduction of 173.12 mg/dl or 43.02 % indicated significant ($P< 0.001$) antihyperglycemic effect. SITA (5 mg/kg p.o.) appears to be more effective than TRIG (50 mg/kg p.o.) in reducing the serum glucose (Figure 5.2.1).

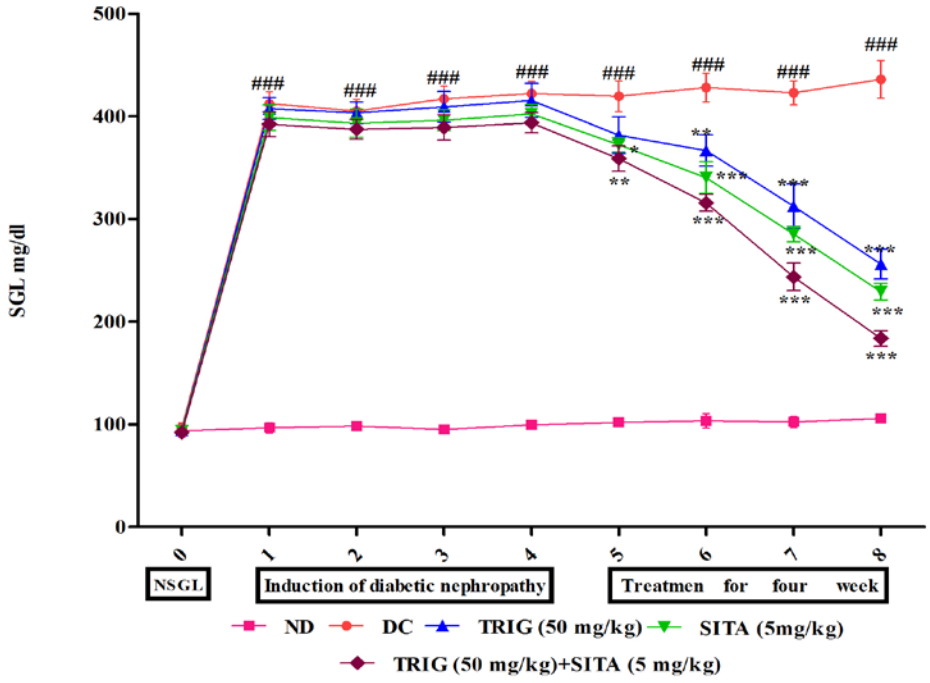
5.2.1.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg, p.o.) on serum glucose level in diabetic nephropathic rats

In the concomitant treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treated group the initial serum glucose level was (393.69±9.91 mg/dl) which was reduced to (183.61±7.59 mg/dl). This reduction of 210.08 mg/dl (53.36 %) was more than the reduction in serum glucose by the individual drug. Early onset of serum glucose was observed at 2^{nd} week (19.81%) compared of 3^{rd} week of onset in individual treatment group. The results thus indicated synergistic effect of concomitant treatment with TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. (Figure 5.2.1).

Table 5.2.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum glucose level in diabetic nephropathic rats

AVG of serum glucose level in diabetic nephropathy (8 weeks model)						
Groups	**Normal SG level**	**After induction of diabetes**	**Treatment in weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	93.80±6.24	99.58±3.37	101.83±3.03	103.49±7.07	102.40±5.43	105.91±1.87
DC	96.49±5.14	422.06±1.80 ###	419.59±15.10 ###	428.02±14.08 ###	422.82±1.60 ###	436.00±18.24 ###
TRIG (50mg/kg p.o.)	94.18±3.48	415.46±16.52	381.71±17.72	366.72±15.27 **	312.39±21.65 ***	256.06±14.43 ***
SITA (5mg/kg p.o.)	93.63±3.70	402.38±9.33	372.26±7.17 *	340.23±15.40 ***	285.26±7.41 ***	229.26±8.07 ***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	92.01±3.10	393.69±9.91	358.67±12.44 **	315.69±8.12 ***	243.63±13.36 ***	183.61±7.59 ***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum glucose level in diabetic nephropathic rats

5.2.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in diabetic nephropathic rats

5.2.2.1 Body weight in non-diabetic rats

The results indicated increase in body weight compare to week '0' reading (Figure 5.2.2).

5.2.2.2 Body weight in diabetic nephropathic rats

Before administration of NICO-STZ the body weight of the rat was 211.85±0.97g. Administration of NICO-STZ resulted in reduction body weight to 170.22±3.21. This reduction of 41.63 g (19.65%) was due to the induction of diabetes. The animals appeared weak and polyuria (not recorded) was evident (Figure 5.2.2).

5.2.2.3 Effect of TRIG (50mg/kg p.o) on body weight in diabetic nephropathic rats

Before administration of TRIG (50 mg/kg p.o.) in diabetic rats the body weight was 186.81±0.98 g. After treatment with TRIG (50 mg/kg p.o.) the body weight was 198.59±1.82 g. The results indicated that TRIG treatment arrested the loss of body weight in the diabetic rats. The observed effect appears to be due to control of the hyperglycemia. The rats showed increase in the body weight from the 1st week after TRIG (50 mg/kg p.o.) treatment and after 3rd and 4th week significant $P< 0.001$) gain in body weight than the initial body weight was observed (Figure 5.2.2).

5.2.2.4 Effect of SITA (5 mg/kg, p.o) on body weight in diabetic nephropathic rats

The initial body weight of diabetic rats was (190.56±1.65g). After treatment with SITA (5 mg/kg p.o.) body weight increase to (206.12±2.23g; $P< 0.001$). SITA (5 mg/kg p.o.) appear to be more effective in arresting the loss of body weight compared to that of TRIG 50 mg/kg p.o. (Figure 5.2.2).

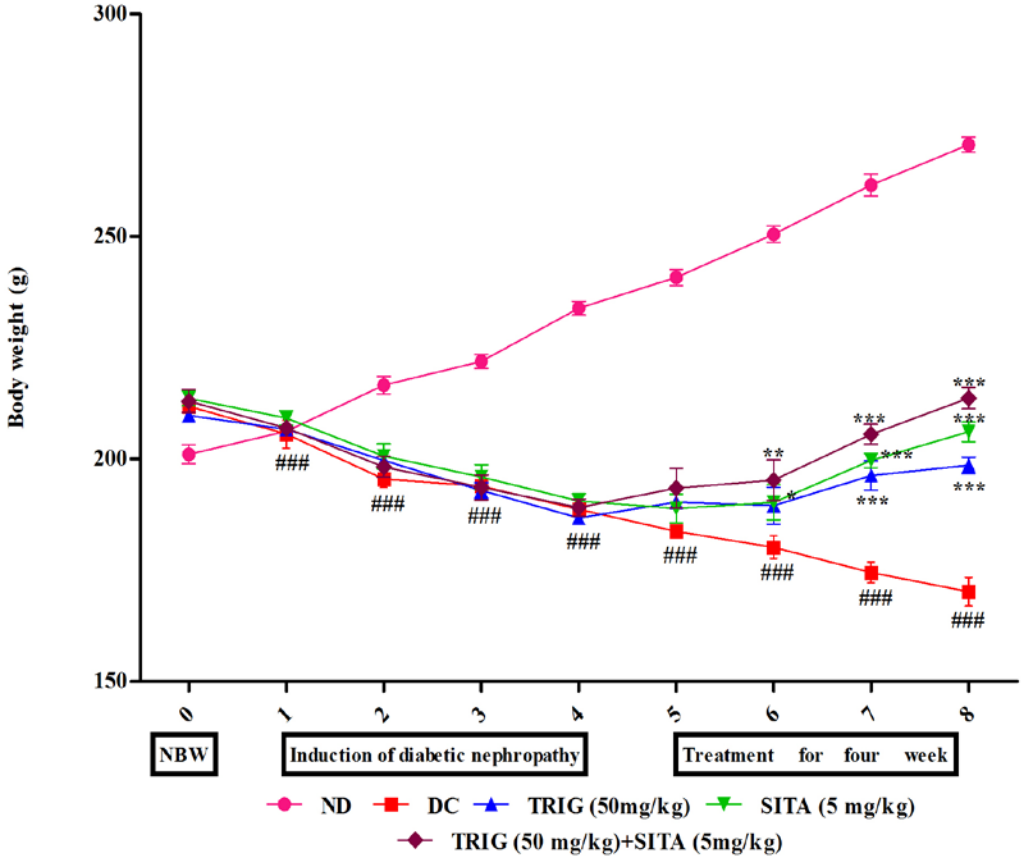
5.2.2.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg, *p.o*) on body weight in diabetic nephropathic rats

Concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was more effective than the individual compound because the gain in body weight after the treatment was more compared to that of individual drug. The treated diabetic animals showed improvement in health compared to diabetic rats (Figure 5.2.2).

Table 5.2.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in diabetic nephropathic rats

AVG of body weight in diabetic nephropathic rat (8 weeks model)						
Groups	**Normal Body weight**	**After induction of diabetes**	**Treatment in weeks**			
	0	**4**	**5**	**6**	**7**	**8**
ND	201.08±2.14	233.92±1.54	240.81±1.84	250.57±1.81	261.63±2.46	270.66±1.67
DC	211.85±0.97	188.70±1.62$^{###}$	183.75±1.38$^{###}$	180.20±2.51$^{###}$	174.55±2.35$^{###}$	170.22±3.21$^{###}$
TRIG (50mg/kg p.o.)	209.78±1.23	186.81±0.98	190.36±0.57	189.56±4.11	196.33±3.33***	198.59±1.82***
SITA (5mg/kg p.o.)	213.67±1.69	190.56±1.65	188.93±3.20	190.28±3.95*	199.71±1.63***	206.12±2.23***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	213.02±2.61	189.08±1.93	191.49±5.30	192.11±4.51**	205.54±2.25***	213.73±2.40***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in diabetic nephropathic rats

5.2.3 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum creatinine in diabetic nephropathic rats

5.2.3.1 Serum creatinine level in non-diabetic rats

The results indicated no fluctuation in serum creatinine level compare to week '0' reading (Figure 5.2.3).

5.2.3.2 Serum creatinine level in diabetic nephropathic rats

Serum creatinine level before induction of diabetes was (0.58±0.14 mg/dl). After induction of diabetes with NICO-STZ the serum creatinine was (1.43±0.13 mg/dl) on 4^{th} week and increase to (1.84±0.19 mg/dl) on 8^{th} week respectively. The increase in serum creatinine indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.2.3).

5.2.3.3 Effect of TRIG (50mg/kg p.o) on serum creatinine level in diabetic nephropathic rats

Before administration of TRIG (50 mg/kg p.o.) the serum creatinine level in diabetic animals was (1.38±0.12mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of TRIG (50 mg/kg p.o.) the serum creatinine level was (1.17±0.17 mg/dl) on 4 week of treatment. The reduction in serum creatinine by 0.21 mg/dl $P< 0.05$ (15.21%) was compared to diabetic animals. The results indicate that TRIG treatment prevented the rise in serum creatinine level and thus at mild nephroprotective effect (Figure 5.2.3).

5.2.3.4 Effect of SITA (5 mg/kg p.o) on serum creatinine level in diabetic nephropathic rats

Before administration of SITA (5 mg/kg p.o.) the serum creatinine level was (1.41±0.16 mg/dl) indicating development of nephropathy in diabetic rats. After treatment of SITA (5 mg/kg p.o.) the serum creatinine level was (1.13±0.19 mg/dl) on the 4 week of treatment. The reduction in serum creatinine by 0.28 mg/dl $P< 0.01$ (19.85%) indicating better prevention of nephropathy compared to TRIG 50 mg/kg p.o. (Figure 5.2.3).

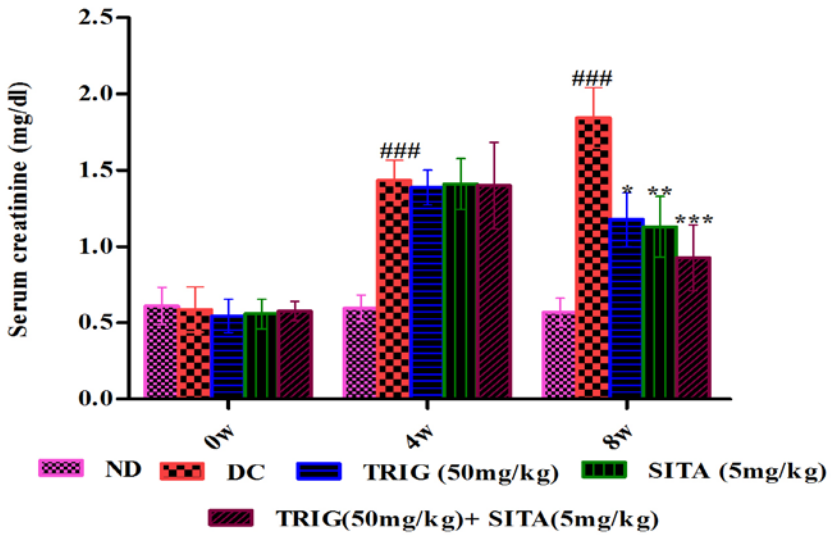
5.2.3.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg, p.o.) on serum creatinine level in diabetic nephropathic rats

The serum creatinine level before concomitant of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was (1.40±0.28 mg/dl). After concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) the serum creatinine was (0.92±0.21 mg/dl). The reduction in serum creatinine by 0.48 mg/dl (34.28%) was significant (*P< 0.001*) compared to that of treatment with individual compound. The results thus indicated that the concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treatment prevented the rise in serum creatinine level and thus at potent nephroprotective effect (Figure 5.2.3).

Table 5.2.3 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum creatinine level in diabetic nephropathic rat

AVG of serum creatinine level in diabetic nephropathic rats		
Parameter / Groups	Weeks	Serum creatinine level (mg/dl)
ND	0 4th 8th	0.61±0.12 0.59±0.08 0.57±0.09
DC	0 4th 8th	0.58±0.14^{ns} 1.43±0.13$^{###}$ 1.84±0.19$^{###}$
TRIG (50mg/kg p.o.)	0 4th 8th	0.54±0.11 1.38±0.12 1.17±0.17*
SITA (5mg/kg p.o.)	0 4th 8th	0.55±0.09 1.41±0.16 1.13±0.19**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	0 4th 8th	0.57±0.06 1.40±0.28 0.92±0.21***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.3 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum creatinine level in diabetic nephropathic rat

5.2.4 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum uric acid level in diabetic nephropathic rats

5.2.4.1 Serum uric acid level in non-diabetic rats

The results indicated no fluctuation in serum uric acid compare to week '0' (Figure 5.2.4).

5.2.4.2 Serum uric acid level in diabetic nephropathic rats

Serum uric acid level before induction of diabetes was (1.90±0.05 mg/dl). After induction of diabetes with NICO-STZ the serum uric acid level was (4.20±0.23 mg/dl) on 4^{th} week and increase to (4.62±0.17mg/dl) on 8^{th} week respectively. The increase in serum uric acid level indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.2.4).

5.2.4.3 Effect of TRIG (50mg/kg p.o) on serum uric acid level in diabetic nephropathic rats

Before administration of TRIG (50 mg/kg p.o.) the serum uric acid level in diabetic animals was (4.15±0.26 mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of TRIG (50 mg/kg p.o.) the serum uric acid level was (3.26±0.14 mg/dl) on 4 week of treatment. The reduction in serum creatinine by 0.89 mg/dl $P< 0.001$ (21.44%) was compared to diabetic animals. The results indicate that TRIG treatment prevented the rise in serum uric acid level and thus at mild nephroprotective effect (Figure 5.2.4).

5.2.4.4 Effect of SITA (5 mg/kg p.o) on serum uric acid level in diabetic nephropathic rats

Before administration of SITA (5 mg/kg p.o.) the serum uric acid level was (4.12±0.10 mg/dl) indicating development of nephropathy in diabetic rats. After treatment of SITA (5 mg/kg p.o.) the serum uric acid level was (2.99±0.10 mg/dl) on the 4 week of treatment. The reduction in serum uric acid level by 1.13 mg/dl $P< 0.001$ (27.42%) indicating better prevention of nephropathy compared to TRIG 50 mg/kg p.o. (Figure 5.2.4).

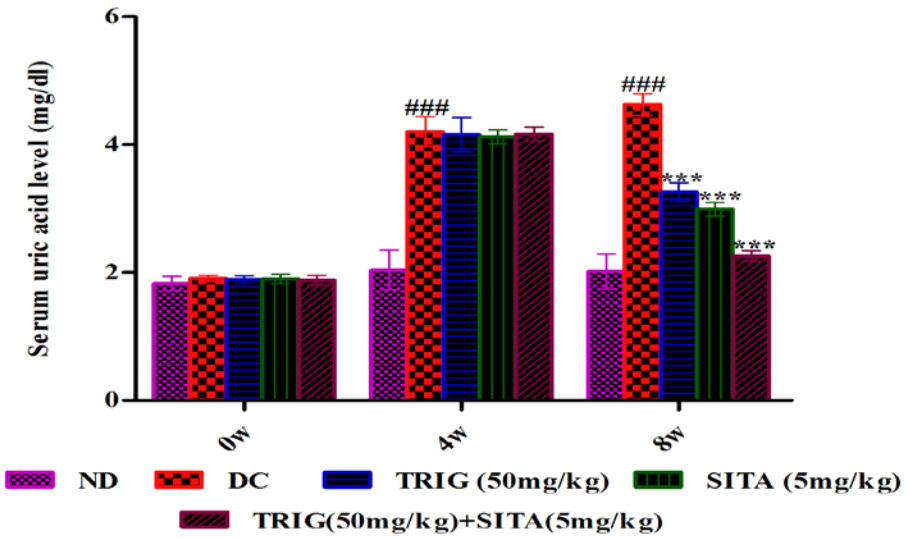
5.2.4.5 Effect of Concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg, p.o) on serum uric acid level in diabetic nephropathic rats

The serum uric acid level before concomitant of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was (4.16±0.10 mg/dl). After concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) the serum uric acid level was (2.26±0.08 mg/dl). The reduction in serum uric acid level by 1.90 mg/dl (45.67 %) was significant ($P < 0.001$) compared to that of treatment with individual compound. The results thus indicated that the concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treatment prevented the rise in serum uric acid level and thus at potent nephroprotective effect (Figure 5.2.4).

Table 5.2.4 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum uric acid level in diabetic nephropathic rats

AVG of serum uric acid level in diabetic nephropathic rats		
Parameter / Groups	Weeks	Serum uric acid (mg/dl)
ND	0 4th 8th	1.82±0.11 2.03±0.31 2.01±0.27
DC	0 4th 8th	1.90±0.05 ns 4.20±0.23 ### 4.62±0.17 ###
TRIG (50mg/kg p.o.)	0 4th 8th	1.88±0.06 4.15±0.26 3.26±0.14 ***
SITA (5mg/kg p.o.)	0 4th 8th	1.90±0.07 4.12±0.10 2.99±0.10 ***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	0 4th 8th	1.87±0.07 4.16±0.10 2.26±0.08 ***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.4 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum uric acid level in diabetic nephropathic rats

5.2.5 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum blood urea nitrogen (BUN) level in diabetic nephropathic rats

5.2.5.1 BUN level in non-diabetic rats

The results indicated no major fluctuation in BUN level compare to week '0' (Figure 5.2.5).

5.2.5.2 BUN level in diabetic nephropathic rats

BUN level before induction of diabetes was (14.70±1.09 mg/dl). After induction of diabetes with NICO-STZ the BUN level was (35.30±3.81 mg/dl) on 4^{th} week and increase to (39.11±3.14 mg/dl) on 8^{th} week respectively. The increase in BUN level indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.2.5).

5.2.5.3 Effect of TRIG (50mg/kg p.o.) on BUN level in diabetic nephropathic rats

Before administration of TRIG (50 mg/kg p.o.) the BUN level in diabetic animals was (33.03±2.52 mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of TRIG (50 mg/kg p.o.) the BUN level was (22.27±2.35 mg/dl) on 4 week of treatment. The reduction in BUN level by 10.76 mg/dl $P< 0.001$ (32.57%) was compared to diabetic animals. The results indicate that TRIG treatment prevented the rise in BUN level and thus at mild nephroprotective effect (Figure 5.2.5).

5.2.5.4 Effect of SITA (5 mg/kg p.o) on BUN level in diabetic nephropathic rats

Before administration of SITA (5 mg/kg p.o.) the BUN level was (34.39±1.92 mg/dl) indicating development of nephropathy in diabetic rats. After treatment of SITA (5 mg/kg p.o.) the BUN level was (21.61±1.98 mg/dl) on the 4 week of treatment. The reduction in BUN level by 12.78 mg/dl $P< 0.001$ (37.16%) indicating better prevention of nephropathy compared to TRIG 50 mg/kg p.o. (Figure 5.2.5).

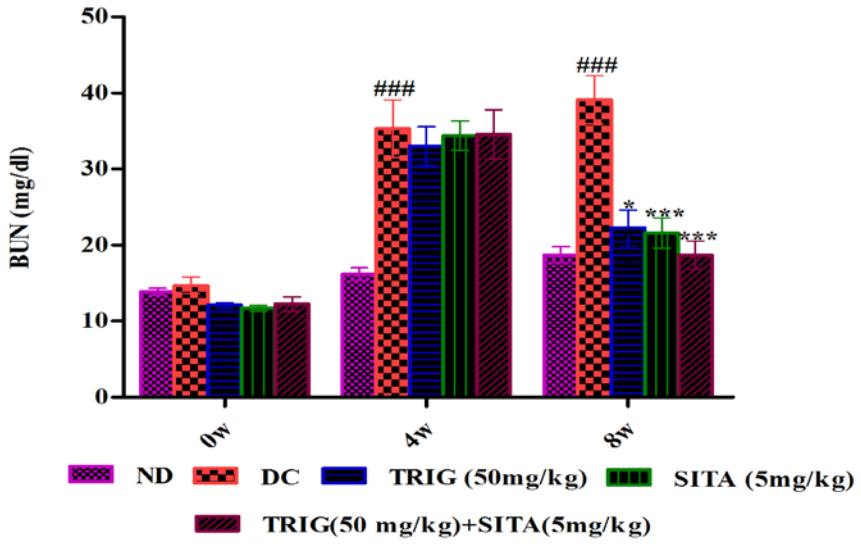
5.2.5.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on BUN level in diabetic nephropathic rats

The BUN level before concomitant of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was (34.54±3.25 mg/dl). After concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) the BUN level was (18.68±1.87 mg/dl). The reduction in BUN level by 15.86 mg/dl (45.91%) was significant ($P< 0.001$) compared to that of treatment with individual compound. The results thus indicated that the concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treatment prevented the rise in BUN level and thus at potent nephroprotective effect (Figure 5.2.5).

Table 5.2.5 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum blood urea nitrogen (BUN) level in diabetic nephropathic rats

AVG of serum BUN level in diabetic nephropathic rats		
Parameter / Groups	Weeks	BUN (mg/dl)
ND	0 4th 8th	13.86±0.47 16.21±0.81 18.71±1.06
DC	0 4th 8th	14.70±1.09 ns 35.30±3.81 ### 39.11±3.14 ###
TRIG (50mg/kg p.o.)	0 4th 8th	12.11±0.37 33.03±2.52 22.27±2.35 ***
SITA (5mg/kg p.o.)	0 4th 8th	11.70±0.40 34.39±1.92 21.61±1.98 ***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	0 4th 8th	12.27±0.93 34.54±3.25 18.68±1.87 ***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.5 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum blood urea nitrogen (BUN) level in diabetic nephropathic rats

5.2.6 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on urine creatinine level in diabetic nephropathic rats

5.2.6.1 Urine creatinine level in non-diabetic rats

The results indicated no major fluctuation in urine creatinine level compare to week '0' (Figure 5.2.6).

5.2.6.2 Urine creatinine level in diabetic nephropathic rats

Urine creatinine level before induction of diabetes was (57.40±1.86 mg/dl). After induction of diabetes with NICO-STZ the urine creatinine level was (24.84±2.08 mg/dl) on 4^{th} week and decrease to (13.81±1.69 mg/dl) on 8^{th} week respectively. The decrease in urine creatinine level indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.2.6).

5.2.6.3 Effect of TRIG (50mg/kg p.o) on urine creatinine level in diabetic nephropathic rats

Before administration of TRIG (50 mg/kg p.o.) the urine creatinine level in diabetic animals was (24.46±2.28 mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of TRIG (50 mg/kg p.o.) the urine creatinine level was (26.08±4.60 $P< 0.05$ mg/dl) on 4 week of treatment. The increase in urine creatinine level by 1.62 mg/dl (6.21%) was compared to diabetic animals. The results indicate that TRIG treatment prevented the decrease in urine creatinine level and thus at mild nephroprotective effect (Figure 5.2.6).

5.2.6.4 Effect of SITA (5 mg/kg, p.o.) on urine creatinine level in diabetic nephropathic rats

Before administration of SITA (5 mg/kg p.o.) the urine creatinine level was (23.77±2.10 mg/dl) indicating development of nephropathy in diabetic rats. After treatment of SITA (5 mg/kg p.o.) the urine creatinine level was (29.48±5.15 mg/dl $P< 0.01$) on the 4 week of treatment. The increase in urine creatinine level by 5.71 mg/dl (19.36%) indicating better prevention of nephropathy compared to TRIG 50 mg/kg p.o. (Figure 5.2.6).

5.2.6.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on urine creatinine level in diabetic nephropathic rats

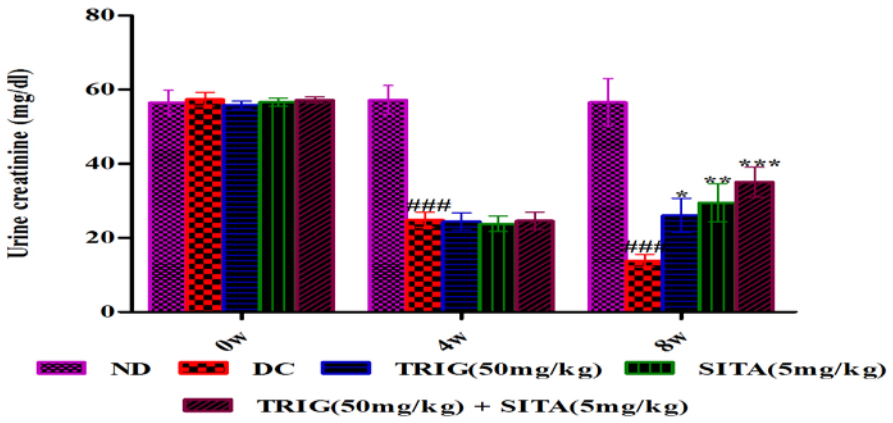
The urine creatinine level before concomitant of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was (24.61±2.29 mg/dl). After concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) the urine creatinine level was (35.03±4.04 mg/dl).

The increase in urine creatinine level by 10.42 mg/dl (29.74%) was significant ($P< 0.001$) compared to that of treatment with individual compound. The results thus indicated that the concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treatment prevented the decrease in urine creatinine level and thus at potent nephroprotective effect (Figure 5.2.6).

Table 5.2.6 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on urine creatinine level in diabetic nephropathic rats

AVG of urine creatinine level in diabetic nephropathic rats		
Parameter / Groups	Weeks	Urine creatinine (mg/dl)
ND	0 4th 8th	56.56±3.29 57.25±3.88 56.63±6.40
DC	0 4th 8th	57.40±1.86 ns 24.84±2.08 ### 13.81±1.69 ###
TRIG (50mg/kg p.o.)	0 4th 8th	56.66±1.11 24.46±2.28 26.08±4.60 *
SITA (5mg/kg p.o.)	0 4th 8th	56.67±1.11 23.77±2.10 29.48±5.15 **
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	0 4th 8th	57.25±0.80 24.61±2.29 35.03±4.04 ***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.6 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on urine creatinine level in NICO-STZ induced diabetic nephropathy in Wistar rats

5.2.7 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on urine volume in diabetic nephropathic rats

5.2.7.1 Urine volume in non-diabetic rats

The results indicated no major fluctuation in urine volume compare to week '0' (Figure 5.2.7).

5.2.7.2 Urine volume in diabetic nephropathic rats

Urine volume before induction of diabetes was (10.66±0.42 ml). After induction of diabetes with NICO-STZ the urine volume was (51.48±2.51 ml) on 4^{th} week and increase to (55.91±2.47ml) on 8^{th} week respectively. The increase in urine volume indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.2.7).

5.2.7.3 Effect of TRIG (50mg/kg p.o) on urine volume in diabetic nephropathic rats

Before administration of TRIG (50 mg/kg p.o.) the urine volume in diabetic animals was (49.50±1.43 ml) which indicated initiation of nephropathy in the diabetic rats. After treatment of TRIG (50 mg/kg p.o.) the urine volume was (35.58±1.25 ml, $P< 0.001$) on 4 week of treatment. The reduction in urine volume by 13.92 ml (28.12%) was compared to diabetic animals. The results indicate that TRIG treatment prevented the rise in urine volume and thus at mild nephroprotective effect (Figure 5.2.7).

5.2.7.4 Effect of SITA (5 mg/kg p.o) on urine volume in diabetic nephropathic rats

Before administration of SITA (5 mg/kg p.o.) the urine volume was (49.75±1.23 ml) indicating development of nephropathy in diabetic rats. After treatment of SITA (5 mg/kg p.o.) the urine volume was (32.41±1.57 ml, $P< 0.001$) on the 4 week of treatment. The reduction in urine volume by 17.34 ml (34.85%) indicating better prevention of nephropathy compared to TRIG 50 mg/kg p.o. (Figure 5.2.7).

5.2.7.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg, p.o) on urine volume in diabetic nephropathic rats

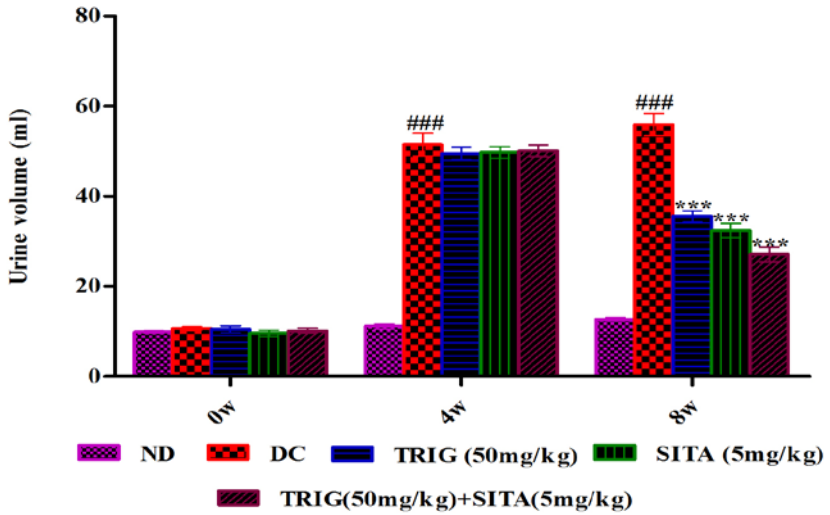
The urine volume before concomitant of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was (50.11±1.27 ml). After concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) the urine volume was (27.08±1.64 ml). The reduction in urine volume by 23.03 (45.95%) was significant ($P< 0.001$) compared to that of treatment with individual compound. The results thus indicated that the concomitant

administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treatment prevented the rise in urine volume and thus at potent nephroprotective effect (Figure 5.2.7).

Table 5.2.7 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on urine volume in diabetic nephropathic rats

AVG of urine volume in diabetic nephropathic rats		
Parameter / Groups	Weeks	Urine volume (ml)
ND	0 4th 8th	9.83±0.30 11.16±0.60 12.75±0.35
DC	0 4th 8th	10.66±0.42^{ns} 51.48±2.51$^{###}$ 55.91±2.47$^{###}$
TRIG (50mg/kg p.o.)	0 4th 8th	10.50±0.76 49.50±1.43 35.58±1.25***
SITA (5mg/kg p.o.)	0 4th 8th	9.58±0.73 49.75±1.23 32.41±1.57***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	0 4th 8th	10.16±0.60 50.11±1.27 27.08±1.64***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.

Figure 5.2.7 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on urine volume in diabetic nephropathic rats

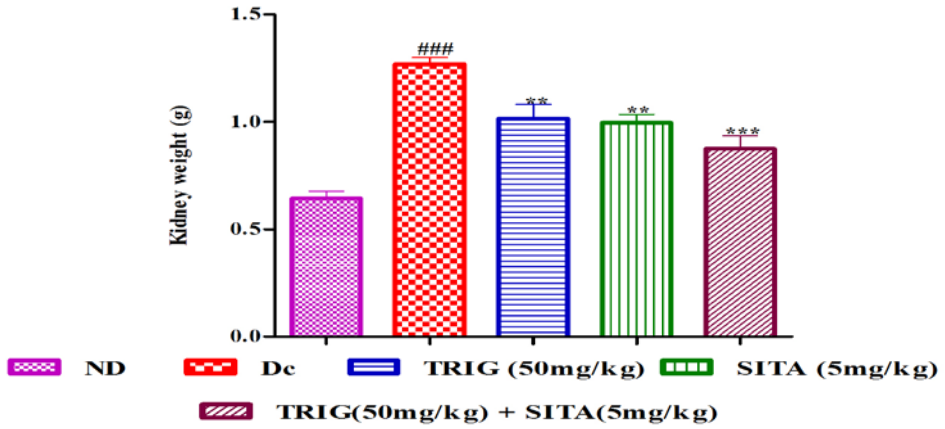
5.2.8 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on kidney weight in diabetic nephropathic rats

At the end day of treatment (week 8) kidney weight in non-diabetic rat was (0.64±0.03 g). On other hand where diabetic rat showed significant (1.26±0.03 g, P< 0.001) increase in kidney weight as compared to non-diabetic kidney. Significant decrease in kidney weight was measured on last day of study period (end of week 8), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 1.01±0.06 g *P< 0.01*, 0.99±0.03 g, *P< 0.01* and 0.87±0.06 g, *P< 0.001* as compared to diabetic rat kidney. Concomitant administration of TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease in kidney weight by (30.95%) compared to individual compound TRIG 19.84 % and SITA 21.42 % (Figure 5.2.8).

Table 5.2.8 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on kidney weight in diabetic nephropathic rats

AVG of kidney weight in diabetic nephropathic rats	
Parameter / Groups	Kidney weight (g)
ND	0.64±0.03
DC	1.26±0.03$^{###}$
TRIG (50mg/kg p.o.)	1.01±0.06**
SITA (5mg/kg p.o.)	0.99±0.03**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	0.87±0.06***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.

Figure 5.2.8 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on kidney weight in diabetic nephropathic rats

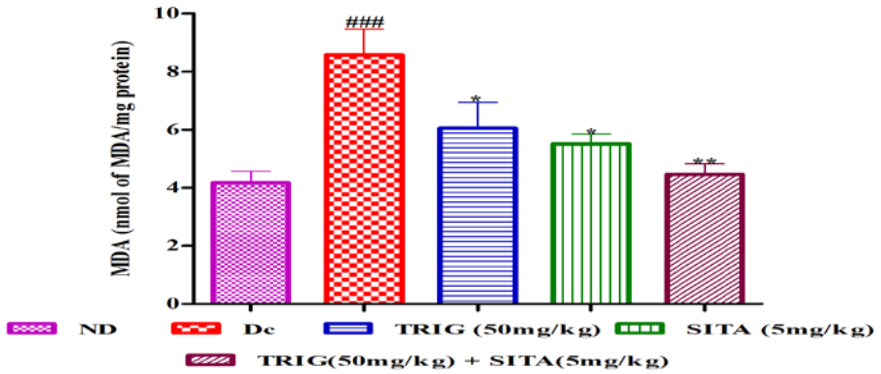
5.2.9 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on malondialdehyde (MDA) level in diabetic nephropathic rat kidney tissue

The level of MDA in non-diabetic rat kidney was (4.17±0.39 nmol of MDA/mg protein). On other hand where diabetic rat kidney showed significant (8.57±0.88 nmol of MDA/mg protein, *P< 0.001*) increase in MDA level as compared to non-diabetic kidney. Significant decrease in MDA level was measured on last day of study period (end of week 8), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 6.05±0.89, *P<0.05*; 5.51±0.33, *P<0.05* and 4.46±0.36, *P<0.01* nmol of MDA/mg protein as compared to MDA level in diabetic rat kidney. Concomitant administration of TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (47.95%) in MDA level compared to individual compound TRIG 29.40% and SITA 35.70% (Figure 5.2.9).

Table 5.2.9 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on malondialdehyde (MDA) level in diabetic nephropathic rat kidney tissue

AVG of malondialdehyde (MDA) level in diabetic nephropathic rats	
Parameter / Groups	**MDA** (nmol of MDA/mg protein)
ND	4.17±0.39
DC	8.57±0.88$^{###}$
TRIG (50mg/kg p.o.)	6.05±0.89*
SITA (5mg/kg p.o.)	5.51±0.33*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	4.46±0.36**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.9 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on malondialdehyde (MDA) level in diabetic nephropathic rat kidney tissue

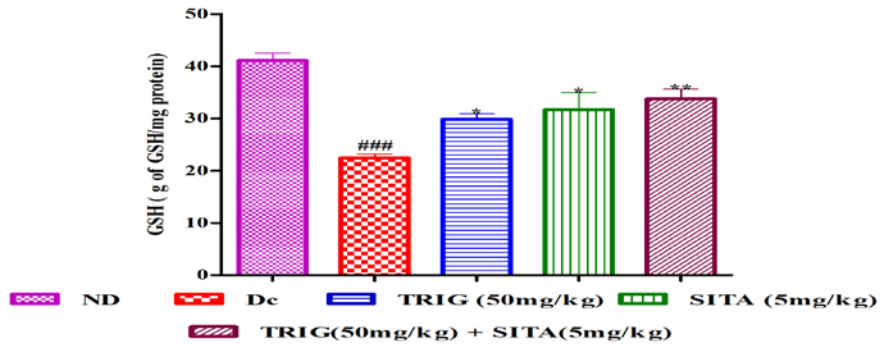
5.2.10 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on reduced glutathione (GSH) level in diabetic nephropathic rat kidney tissue

The level of GSH in non-diabetic rat kidney was (41.09±1.39 g of GSH/mg protein). On other hand where diabetic rat kidney showed significant (22.45±0.76 g of GSH/mg protein, *P< 0.001*) decrease in GSH level as compared to non-diabetic rat kidney. Significant increase in GSH level was measured on last day of study period (end of week 8), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 29.80±1.13, *P<0.05*; 31.69±3.24, *P<0.05* and 33.74±1.88, *P<0.01* g of GSH/mg protein as compared to GSH level in diabetic rat kidney. Concomitant administration of TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (33.46%) in GSH level compared to individual compound TRIG 24.66 % and SITA 29.15% (Figure 5.2.10).

Table 5.2.10 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on reduced glutathione (GSH) level in diabetic nephropathic rat kidney tissue

AVG of reduced glutathione (GSH) level in diabetic nephropathic rats	
Parameter / Groups	**GSH** (g of GSH/mg protein)
ND	41.09±1.39
DC	22.45±0.76$^{###}$
TRIG (50mg/kg p.o.)	29.80±1.13*
SITA (5mg/kg p.o.)	31.69±3.24*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	33.74±1.88**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.10 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on reduced glutathione (GSH) level in diabetic nephropathic rat kidney tissue

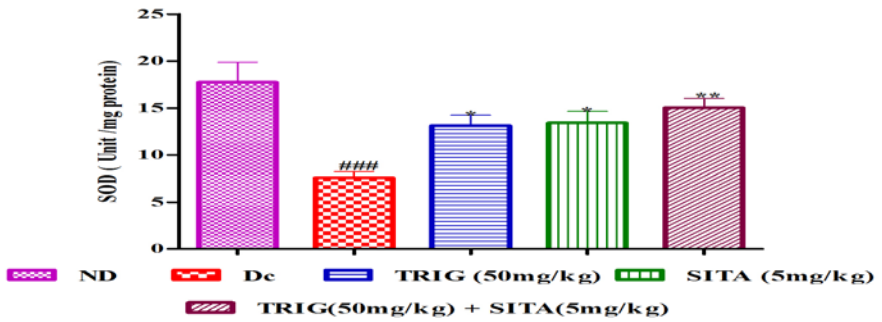
5.2.11 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on superoxide dismutase (SOD) level in diabetic nephropathic rat kidney tissue

The level of SOD in non-diabetic rat kidney was (17.73±2.12 Unit /mg protein). On other hand where diabetic rat kidney showed significant (07.58±0.67Unit /mg protein, *P< 0.001*) decrease in SOD level as compared to non-diabetic rat kidney. Significant increase in SOD level was measured on last day of study period (end of week 8), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 13.14±1.10, *P<0.05*; 13.43±1.22, *P<0.05* and 15.04±0.98, *P<0.01* Unit /mg protein as compared to SOD level in diabetic rat kidney. Concomitant administration of TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (49.60%) in SOD level compared to individual compound TRIG 42.31% and SITA 43.55% (Figure 5.2.11).

Table 5.2.11 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on superoxide dismutase (SOD) level in diabetic nephropathic rat kidney tissue

AVG of superoxide dismutase (SOD) level in diabetic nephropathic rats	
Parameter / Groups	**SOD** (Unit /mg protein)
ND	17.73±2.12
DC	07.58±0.67$^{###}$
TRIG (50mg/kg p.o.)	13.14±1.10*
SITA (5mg/kg p.o.)	13.43±1.22*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	15.04±0.98**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

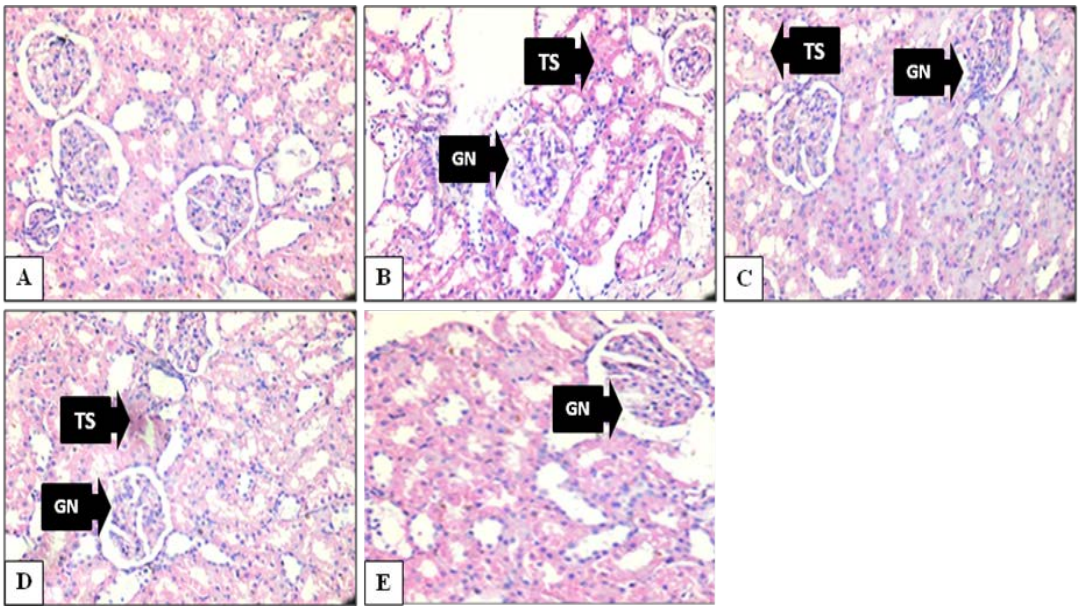


Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.2.11 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on superoxide dismutase (SOD) level in diabetic nephropathic rat kidney tissue

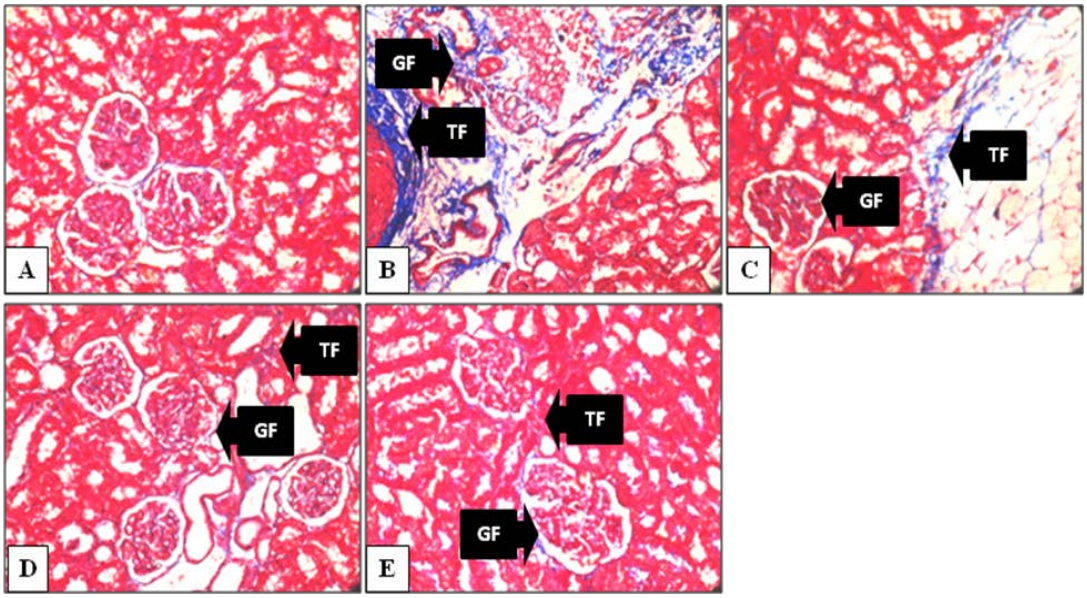
5.2.12 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on histological examination of isolated kidney tissue [H&E and MT stain]

After eight weeks of study examination of renal sections stained with hematoxylin & eosin (H&E) and Masson's trichome (MT) stain. Histopathology of isolated kidney of nondiabetic rat showed (Grade -) normal architecture; Diabetic rat kidney showed (Grade +++) glomerular necrosis, (Grade +++) tubular swelling, (Grade +++) glomerular fibrosis, (Grade +++) peritubular fibrosis; TRIG treated rat kidney showed (Grade ++) glomerular necrosis, (Grade ++) tubular swelling, (Grade +) glomerular fibrosis, (Grade ++) peritubular fibrosis; SITA showed (Grade +) glomerular necrosis, (Grade ++) tubular swelling, (Grade ++) glomerular fibrosis, (Grade +) peritubular fibrosis; TRIG+SITA showed (Grade +) glomerular necrosis, (Grade -) tubular swelling, (Grade +) glomerular fibrosis, (Grade +) peritubular fibrosis. The scoring of grades was as per Dr. Chandrasekhar Mote (MVSC), Innovet Diagnostic Laboratory, Pune. Where (Grade -) normal; (Grade +++) severe; (Grade ++) moderate; (Grade +) mild. Histological study reveals that the TRIG + SITA prevented structural kidney damage (Figure 5.2.12.1, H&E stain and Figure 5.2.12.2, MT stain).



Where, A] Non diabetic group; B] Diabetic group; C] TRIG (50 mg/kg p.o.); D] SITA (5 mg/kg p.o.) and E] TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.). GN- Glomerular necrosis; TS- Tubular swelling. [Magnification 40x].

Figure 5.2.12.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on histological examination of isolated kidney tissue [H&E stain]



Where, A] Non diabetic group; B] Diabetic group; C] TRIG (50 mg/kg p.o.); D] SITA (5 mg/kg p.o.) and E] TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.). GF-glomerular fibrosis, TF- peritubular swelling. [Magnification 40x].

Figure 5.2.12.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on histological examination of isolated kidney tissue [MT stain]

Model 5.3.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) in NICO-STZ induced diabetic neuropathy in Wistar rats

5.3.1.1 Serum glucose level in non-diabetic rats

The results indicated non-significant fluctuation in serum glucose level (Figure 5.3.1).

5.3.1.2 Serum glucose level in diabetic neuropathic rats

Serum glucose level before administration of NICO-STZ in this group was (99.69±1.64 mg/dl). After administration of NICO-STZ the serum glucose level increasing trend and at the end of 4 week after induction of diabetes the serum glucose (429.27±6.95 mg/dl). The vehicle used for dissolving the test compound was double distilled water which could not prevent the rise of serum glucose and at the end 4 weeks of treatment period the serum glucose level was 438.75±8.21 mg/dl (Figure 5.3.1).

5.3.1.3 Effect of TRIG (50mg/kg p.o) on serum glucose level in diabetic neuropathic rats

In the TRIG (50 mg/kg p.o.) treated animals the initial serum glucose level was (422.31±10.21 mg/dl) which was reduced to (261.10±11.34 mg/dl, $P< 0.001$) indicating a decrease of 161.31 mg/dl or 38.17%. The onset of antihyperglycemic effect was evident after 3 week of TRIG (50 mg/kg p.o.) treatment and continuation of treatment showed a trend toward decrease in serum glucose (Figure 5.3.1).

5.3.1.4 Effect of SITA (5 mg/kg p.o) on serum glucose level in diabetic neuropathic rats

In case of SITA (5 mg/kg p.o.) treated animals the serum glucose level before treatment (initial) was (418.27±13.34 mg/dl). After treatment with SITA (5 mg/kg p.o.) the serum glucose level at the 4^{th} week was (242.32±6.27 mg/dl) this reduction of 175.95 mg/dl or 42.06 % indicated significant ($P< 0.001$) antihyperglycemic effect. SITA (5 mg/kg p.o.) appears to be more effective than TRIG (50 mg/kg p.o.) in reducing the serum glucose (Figure 5.3.1).

5.3.1.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on serum glucose level in diabetic neuropathic rats

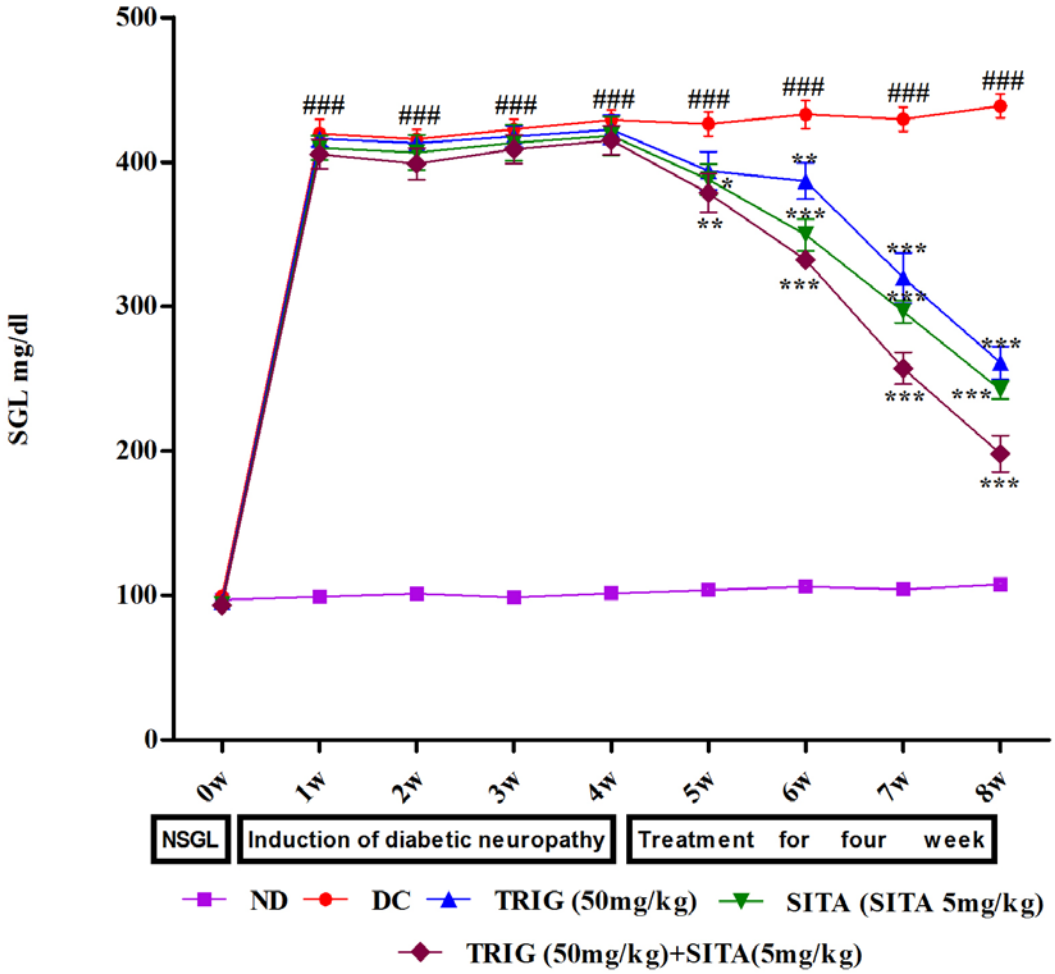
In the concomitant treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treated animals the initial serum glucose level was (414.82±9.82 mg/dl) which was reduced to (198.13±12.70 mg/dl). This reduction of 216.69 mg/dl (52.23 %) was more

than the reduction in serum glucose by the individual drug. Early onset of serum glucose was observed at 2nd week (19.89%) compared of 3rd week of onset in individual treatment group. The results thus indicated synergistic effect of concomitant treatment with TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. (Figure 5.3.1).

Table 5.3.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum glucose level in diabetic neuropathic rats

AVG of serum glucose (mg/dl) levels in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Norm-al SG level	**After induction of diabetes**	**Treatment in weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	96.98±1.89	101.52±2.38	103.71±2.71	106.18±1.89	104.23±1.52	107.49±1.20
DC	99.69±1.64	429.27±6.95$^{###}$	426.44±8.47$^{###}$	432.83±9.79$^{###}$	429.65±8.48$^{###}$	438.75±8.21$^{###}$
TRIG (50mg/kg p.o.)	95.30±2.84	422.31±10.21	393.96±13.17	386.88±12.37**	319.77±17.35***	261.10±11.34***
SITA (5mg/kg p.o.)	93.80±1.97	418.27±13.34	387.80±10.70*	349.52±11.04**	296.47±07.68***	242.32±06.27***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	93.26±2.82	414.82±9.82	378.47±13.18**	332.31±04.58***	257.16±11.02***	198.13±12.70***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.3.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum glucose level in diabetic neuropathic rats

5.3.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in NICO-STZ induced diabetic neuropathy in Wistar rats

5.3.2.1 Body weight in non-diabetic rats

The results indicated increase in body weight compare to week '0' (Figure 5.3.2).

5.3.2.2 Body weight in diabetic neuropathic rats

Before administration of NICO-STZ the body weight of the rat was 206.76±1.40 g. Administration of NICO-STZ resulted in reduction body weight to 164.47±4.97g. This reduction of 42.29 g (20.45 %) was due to the induction of diabetes. The animals appeared weak and polyuria (not recorded) was evident (Figure 5.3.2).

5.3.2.3 Effect of TRIG (50mg/kg p.o) on body weight in diabetic neuropathic rats

Before administration of TRIG (50 mg/kg p.o.) in diabetic rats the body weight was 181.12±3.72 g. After treatment with TRIG (50 mg/kg p.o.) the body weight was 194.05±2.48 g. The results indicated that TRIG treatment arrested the loss of body weight in the diabetic rats. The observed effect appears to be due to control of the hyperglycemia. The rats showed increase in the body weight from the 1st week after TRIG (50 mg/kg p.o.) treatment and after 3rd and 4th week significant ($P< 0.001$) gain in body weight than the initial body weight was observed (Figure 5.3.2).

5.3.2.4 Effect of SITA (5 mg/kg, p.o) on body weight in diabetic neuropathic rats

The initial body weight of diabetic rats was (176.97±2.54 g). After treatment with SITA (5 mg/kg p.o.) body weight increase to (201.43±1.81 g, $P< 0.001$). SITA (5 mg/kg p.o.) appear to be more effective in arresting the loss of body weight compared to that of TRIG 50 mg/kg p.o. (Figure 5.3.2).

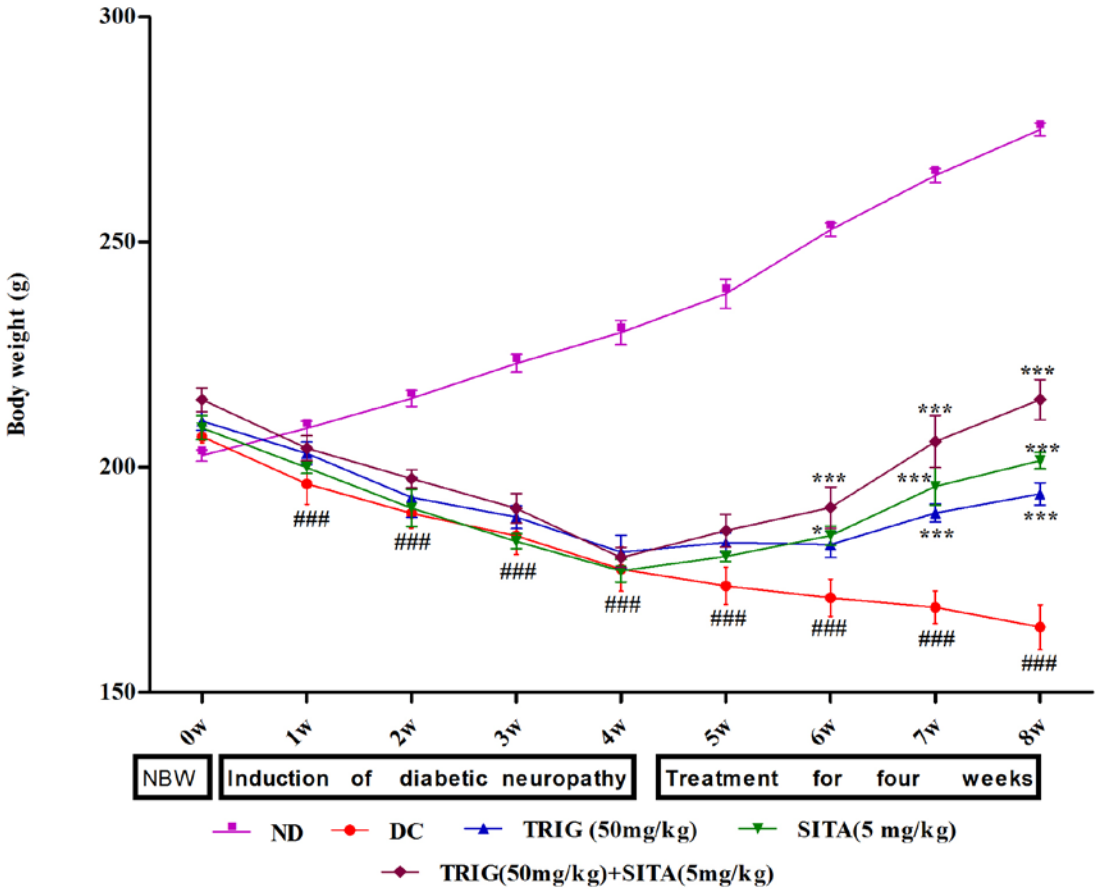
5.3.2.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on body weight in diabetic neuropathic rats

Concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was more effective than the individual compound because the gain in body weight after the treatment was more compared to that of individual drug. The treated diabetic animals showed improvement in health compared to diabetic rats (Figure 5.3.2).

Table 5.3.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight diabetic neuropathic rats

AVG of body weight(g) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Normal SGL	**After induction of diabetes**	**Treatment in week**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	202.58±1.24	229.91±2.70	238.52±3.19	252.73±1.46	264.79±1.51	275.00±1.42
DC	206.76±1.40	177.38±4.92$^{###}$	173.63±4.13$^{###}$	170.99±4.11$^{###}$	168.87±3.64$^{###}$	164.47±4.97$^{###}$
TRIG (50mg/kg p.o.)	210.24±2.07	181.12±3.72	183.24±2.09	182.84±2.95	189.83±2.01***	194.05±2.48***
SITA (5mg/kg p.o.)	208.74±2.66	176.97±2.54	180.19±1.18	184.82±2.12*	195.74±4.17***	201.43±1.81***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	214.97±2.65	179.94±2.23	185.91±3.67	191.00±4.60***	205.69±5.77***	215.01±4.41***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.3.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in diabetic neuropathic rats

5.3.3 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

5.3.3.1 Thermal hyperalgesia in non-diabetic rats

The results indicated increase in paw withdrawal latency compare to week '0' (Figure 5.3.3).

5.3.3.2 Thermal hyperalgesia in diabetic neuropathic rats

Paw withdrawal latency before administration of NICO-STZ in this group was 10.29±0.19 (in sec). After administration of NICO-STZ the paw withdrawal latency decreasing trend and at the end of 4 week after induction of diabetes the paw withdrawal latency was 2.90±0.36 (in sec). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of paw withdrawal latency. And at the end 4 weeks of treatment period the paw withdrawal latency level was 2.37±0.21 (in sec) (Figure 5.3.3).

5.3.3.3 Effect of TRIG (50mg/kg p.o) on thermal hyperalgesia in diabetic neuropathic rats

In the TRIG (50 mg/kg p.o.) treated group the initial paw withdrawal latency was (2.76±0.35 (in sec) which was increase to (4.95±0.10 (in sec), $P< 0.001$) indicating a increase of 2.19 (in sec) or 44.24%. The onset of neuroprotective effect was evident after 2 week of TRIG (50 mg/kg p.o.) treatment and continuation of treatment showed a trend toward increase in paw withdrawal latency (Figure 5.3.3).

5.3.3.4 Effect of SITA (5 mg/kg p.o) on thermal hyperalgesia in diabetic neuropathic rats

In case of SITA (5 mg/kg p.o.) treated diabetic animals the paw withdrawal latency before treatment (initial) was (2.81±0.32 (in sec). After treatment with SITA (5 mg/kg p.o.) the paw withdrawal latency at the 4^{th} week was (5.38±0.32 (in sec) this increase of 2.57 (in sec) or 47.76 % indicated significant ($P< 0.001$) neuroprotective effect. SITA (5 mg/kg p.o.) appears to be more effective than TRIG (50 mg/kg p.o.) in increasing the paw withdrawal latency (Figure 5.3.3).

5.3.3.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.)+ SITA (5 mg/kg p.o) on thermal hyperalgesia in diabetic neuropathic rats

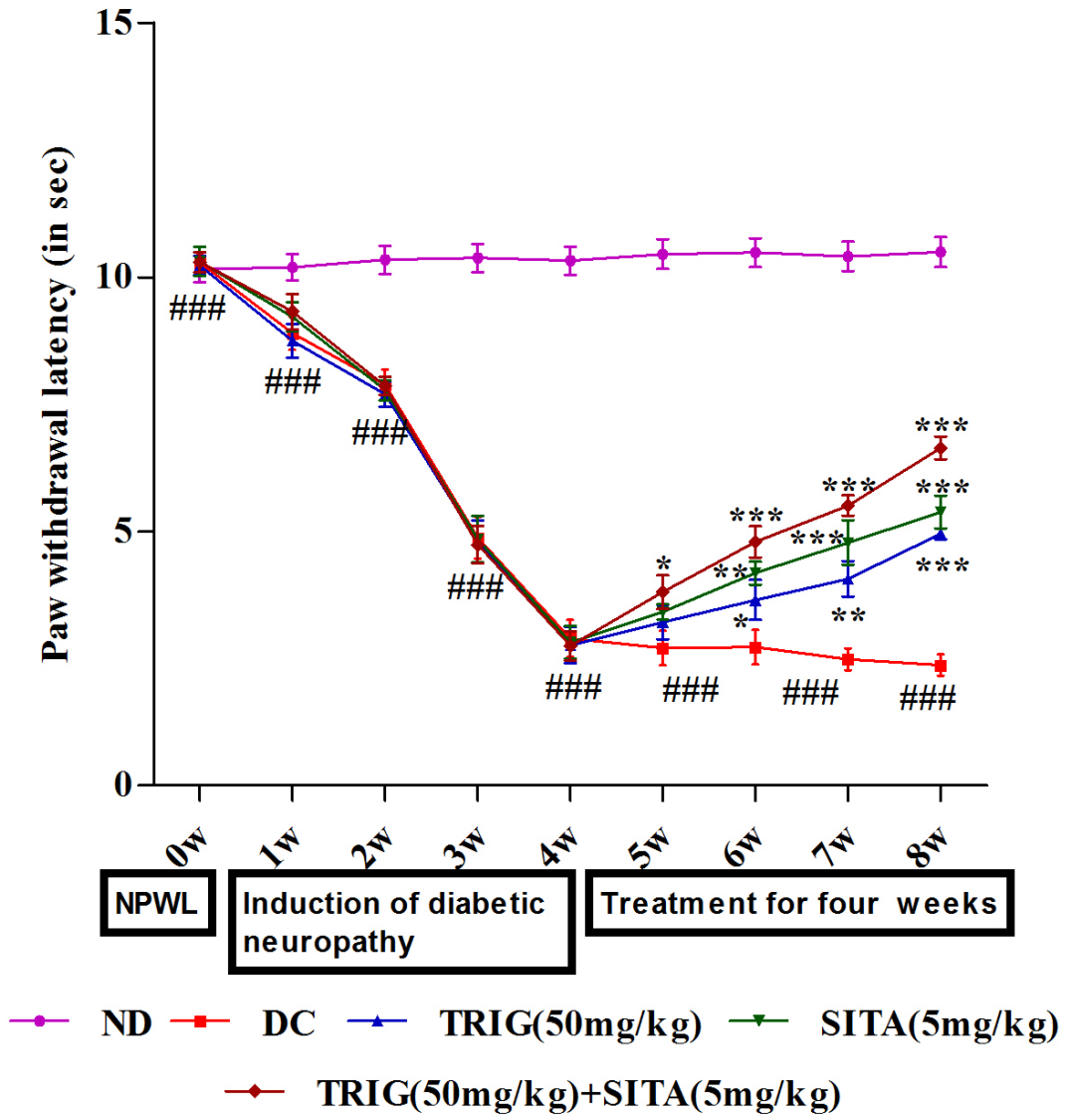
In the concomitant treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treated group the initial paw withdrawal latency was (2.75±0.29 (in sec) which was increased to (6.64±0.22 (in sec). This increase of 3.89 in sec (58.58 %) was more than the increase in paw withdrawal latency by the individual drug. Early onset of paw

withdrawal latency was observed at 1st week (27.63%) compared of 2nd week of onset in individual treatment group. The results thus indicated synergistic effect of concomitant treatment with TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o (Figure 5.3.3).

Table 5.3.3 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

AVG of thermal hyperalgesia (in sec) in diabetic neuropathy (8 weeks model)						
Groups	**Times in week**					
	Normal paw withdrawal latency	**After induction of diabetes**	**Treatment in weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	10.16±0.25	10.33±0.27	10.45±0.28	10.48±0.28	10.41±0.29	10.50±0.26
DC	10.29±0.19	2.90±0.36$^{###}$	2.71±0.34$^{###}$	2.72±0.33$^{###}$	2.48±0.21$^{###}$	2.37±0.21$^{###}$
TRIG (50mg/kg p.o.)	10.23±0.18	2.76±0.35	3.21±0.34	3.65±0.39*	4.07±0.35**	4.95±0.10***
SITA (5mg/kg p.o.)	10.31±0.29	2.81±0.32	3.41±0.15	4.18±0.22**	4.78±0.44***	5.38±0.32***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	10.29±0.20	2.75±0.29	3.80±0.33*	4.79±0.31***	5.51±0.20***	6.64±0.22***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic.

Figure 5.3.3 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

5.3.4 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

5.3.4.1 Mechanical hyperalgesia in non-diabetic rats

The results indicated increase in paw withdrawal threshold compare to week '0' (Figure 5.3.4).

5.3.4.2 Mechanical hyperalgesia in diabetic neuropathic rats

Paw withdrawal threshold before administration of NICO-STZ in this group was 284.50±07.95 (in g). After administration of NICO-STZ the paw withdrawal threshold decreasing trend and at the end of 4 week after induction of diabetes the paw withdrawal threshold 113.52±09.95 (in g). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of paw withdrawal threshold and at the end 4 weeks of treatment period the paw withdrawal threshold was 123.65±12.23 in g (Figure 5.3.4).

5.3.4.3 Effect of TRIG (50mg/kg p.o) on mechanical hyperalgesia in diabetic neuropathic rats

In the TRIG (50 mg/kg p.o.) treated group the initial paw withdrawal threshold was 105.52±08.07 (in g) which was increase to 224.34±06.04 (in g), $P< 0.001$ indicating an increase of 118.82 (in g) or 52.96%. The onset of neuroprotective effect was evident after 1st week of TRIG (50 mg/kg p.o.) treatment and continuation of treatment showed a trend toward increase in paw withdrawal threshold (Figure 5.3.4).

5.3.4.4 Effect of SITA (5 mg/kg p.o) on mechanical hyperalgesia in diabetic neuropathic rats

In case of SITA (5 mg/kg p.o.) treated diabetic animals the paw withdrawal threshold before treatment (initial) was (102.1±05.87 (in g). After treatment with SITA (5 mg/kg p.o.) the paw withdrawal threshold at the 4th week was 240.5±07.03 (in g) this increase of 138.4 (in g) or 57.54 % indicated significant ($P< 0.001$) neuroprotective effect. SITA (5 mg/kg p.o.) appears to be more effective than TRIG (50 mg/kg p.o.) in increasing the paw withdrawal threshold (Figure 5.3.4).

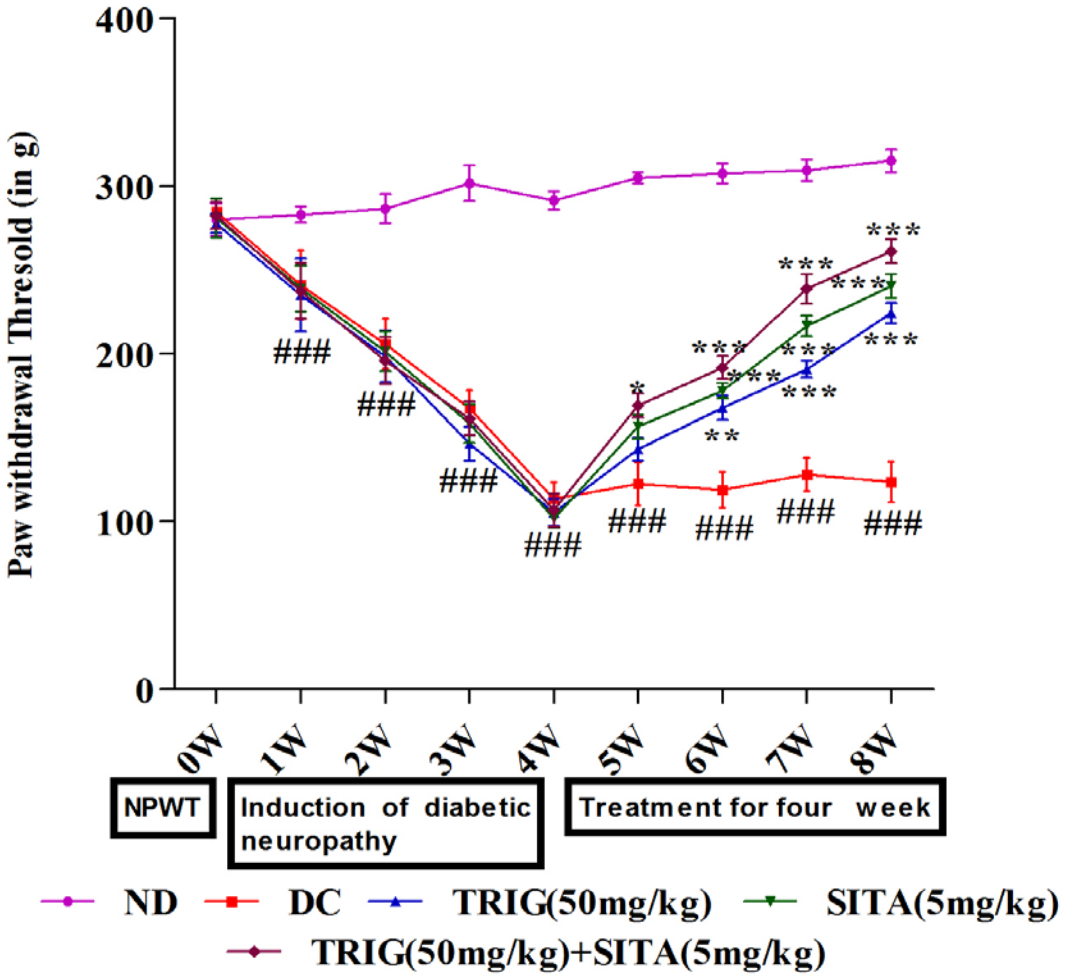
5.3.4.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on mechanical hyperalgesia in diabetic neuropathic rats

In the concomitant treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treated group the initial paw withdrawal threshold was (107.0±09.94 (in g) which was increased to (261.2±07.28 (in g), *P< 0.001.* This increase of 154.2 in g (59.03 %) was more than the increase in paw withdrawal threshold by the individual drug. Early onset of paw withdrawal threshold was observed at 1st week (36.76%) compared of 2nd week of onset in individual treatment group. The results thus indicated synergistic effect of concomitant treatment with TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o (Figure 5.3.4).

Table 5.3.4 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

AVG of mechanical hyperalgesia (in g) in diabetic neuropathy (8 weeks model)						
Groups	**Times in week**					
	Normal paw withdrawal threshold	**After induction of diabetes**	**Treatment in weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	280.0±09.70	291.5±05.45	305.0±03.35	307.5±05.84	309.5±06.41	315.0±06.75
DC	284.50±07.95	113.52±09.95$^{###}$	122.50±12.62$^{###}$	119.10±10.66$^{###}$	128.10±9.78$^{###}$	123.65±12.23$^{###}$
TRIG (50mg/kg p.o.)	277.64±05.70	105.52±08.07	143.56±06.75	167.85±07.19**	190.85±5.04***	224.34±06.04***
SITA (5mg/kg p.o.)	281.0±11.60	102.1±05.87	156.6±07.22	178.0±04.63***	216.5±06.17***	240.5±07.03***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	282.5±08.09	107.0±09.94	169.2±07.10*	191.7±06.92***	238.8±08.80***	261.2±07.28***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic.

Figure 5.3.4 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

5.3.5 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

5.3.5.1 Mechano-tactile allodynia in non-diabetic rats

The results indicated increase in paw withdrawal threshold compare to week '0' (Figure 5.3.5).

5.3.5.2 Mechano-tactile allodynia in diabetic neuropathic rats

Paw withdrawal threshold before administration of NICO-STZ in this group was 69.09±1.96 (in g). After administration of NICO-STZ the paw withdrawal threshold decreasing trend and at the end of 4 week after induction of diabetes the paw withdrawal threshold 25.03±1.25 (in g). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of paw withdrawal threshold and at the end 4 weeks of treatment period the paw withdrawal threshold was 26.73±1.08 in g (Figure 5.3.5).

5.3.5.3 Effect of TRIG (50mg/kg p.o) on mechano-tactile allodynia in diabetic neuropathic rats

In the TRIG (50 mg/kg p.o.) treated group the initial paw withdrawal threshold was 26.52±1.61 (in g) which was increased to 43.64±1.27 (in g), $P< 0.001$ indicating a increase of 17.12 (in g) or 39.23%. The onset of neuroprotective effect was evident after 2nd week of TRIG (50 mg/kg p.o.) treatment and continuation of treatment showed a trend toward increase in paw withdrawal threshold (Figure 5.3.5).

5.3.5.4 Effect of SITA (5 mg/kg p.o) on mechano-tactile allodynia in diabetic neuropathic rats

In case of SITA (5 mg/kg p.o.) treated diabetic animals the paw withdrawal threshold before treatment (initial) was (26.72±1.07 (in g). After treatment with SITA (5 mg/kg p.o.) the paw withdrawal threshold at the 4th week was 46.71±1.00 (in g) this increase of 19.99 (in g) or 42.79 % indicated significant ($P< 0.001$) neuroprotective effect. SITA (5 mg/kg p.o.) appears to be more effective than TRIG (50 mg/kg p.o.) in increasing the paw withdrawal threshold (Figure 5.3.5).

5.3.5.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on mechano-tactile allodynia in diabetic neuropathic rats

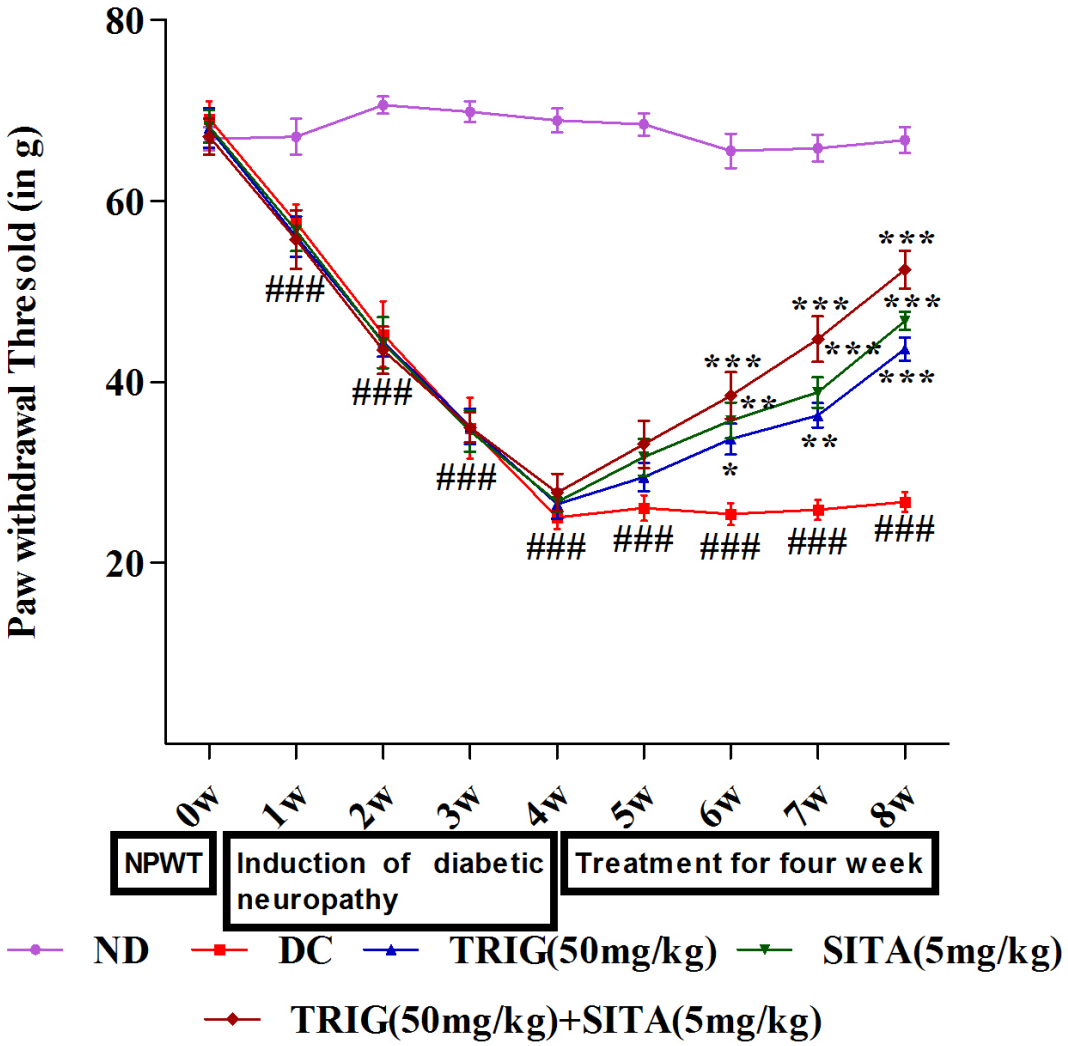
In the concomitant treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treated group the initial paw withdrawal threshold was (27.80±2.05 (in g) which was increased to (52.41±2.07 (in g), P< 0.001. This increase of 24.61 in g (46.95 %) was more than the increase in paw withdrawal threshold by the individual drug. Early

onset of paw withdrawal threshold was observed at 2nd week (27.79 %) compared of 2nd week of onset in individual treatment group. The results thus indicated synergistic effect of concomitant treatment with TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o (Figure 5.3.5).

Table 5.3.5 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

AVG of mechano-tactile allodynia (in g) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Normal paw withdrawal threshold	**After induction of diabetes**	**Treatment in weeks**			
	0	**4**	**5**	**6**	**7**	**8**
ND	66.87±1.29	68.91±1.32	68.47±1.23	65.53±1.88	65.82±1.50	66.74±1.39
DC	69.09±1.96	25.03±1.25$^{###}$	26.06±1.42$^{###}$	25.40±1.20$^{###}$	25.86±1.11$^{###}$	26.73±1.08$^{###}$
TRIG (50mg/kg p.o.)	68.04±2.16	26.52±1.61	29.51±1.60	33.69±1.70*	36.33±1.34**	43.64±1.27***
SITA (5mg/kg p.o.)	68.24±1.81	26.72±1.07	31.69±2.02	35.75±1.96**	38.85±1.68***	46.71±1.00***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	67.14±1.99	27.80±2.05	33.13±2.60	38.50±2.58***	44.74±2.50***	52.41±2.07***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.3.5 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

5.3.6 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on motor nerve conduction velocity (MNCV) in diabetic neuropathic rats

5.3.6.1 Motor nerve conduction velocity in non-diabetic rats

The results indicated increase in motor nerve conduction velocity compare to week '0' (Figure 5.3.6).

5.3.6.2 Motor nerve conduction velocity in diabetic neuropathic rats

Motor nerve conduction velocity before administration of NICO-STZ in this group was 54.35±0.70 (m/s). After administration of NICO-STZ the motor nerve conduction velocity decreasing trend and at the end of 4 week after induction of diabetes the motor nerve conduction velocity 25.04±1.66 (m/s). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of motor nerve conduction velocity and at the end 4 weeks of treatment period the motor nerve conduction velocity was 22.46±2.14 (m/s) (Figure 5.3.6).

5.3.6.3 Effect of TRIG (50mg/kg p.o) on motor nerve conduction velocity in diabetic neuropathic rats

In the TRIG (50 mg/kg p.o.) treated group the initial motor nerve conduction velocity was 25.32±2.29 (m/s) which was increase to 34.74±1.29 (m/s), $P< 0.001$ indicating an increase of 9.42 (in g) or 27.11%. The onset of neuroprotective effect was evident after 3rd week of TRIG (50 mg/kg p.o.) treatment and continuation of treatment showed a trend toward increase in motor nerve conduction velocity (Figure 5.3.6).

5.3.6.4 Effect of SITA (5 mg/kg p.o) on motor nerve conduction velocity in diabetic neuropathic rats

In case of SITA (5 mg/kg p.o.) treated diabetic animals the motor nerve conduction velocity before treatment (initial) was (26.14±1.52 (m/s). After treatment with SITA (5 mg/kg p.o.) the motor nerve conduction velocity at the 4th week was 37.42±1.13 (m/s) this increase of 11.28 (m/s) or 30.14 % indicated significant ($P< 0.001$) neuroprotective effect. SITA (5 mg/kg p.o.) appears to be more effective than TRIG (50 mg/kg p.o.) in increasing the motor nerve conduction velocity (Figure 5.3.6).

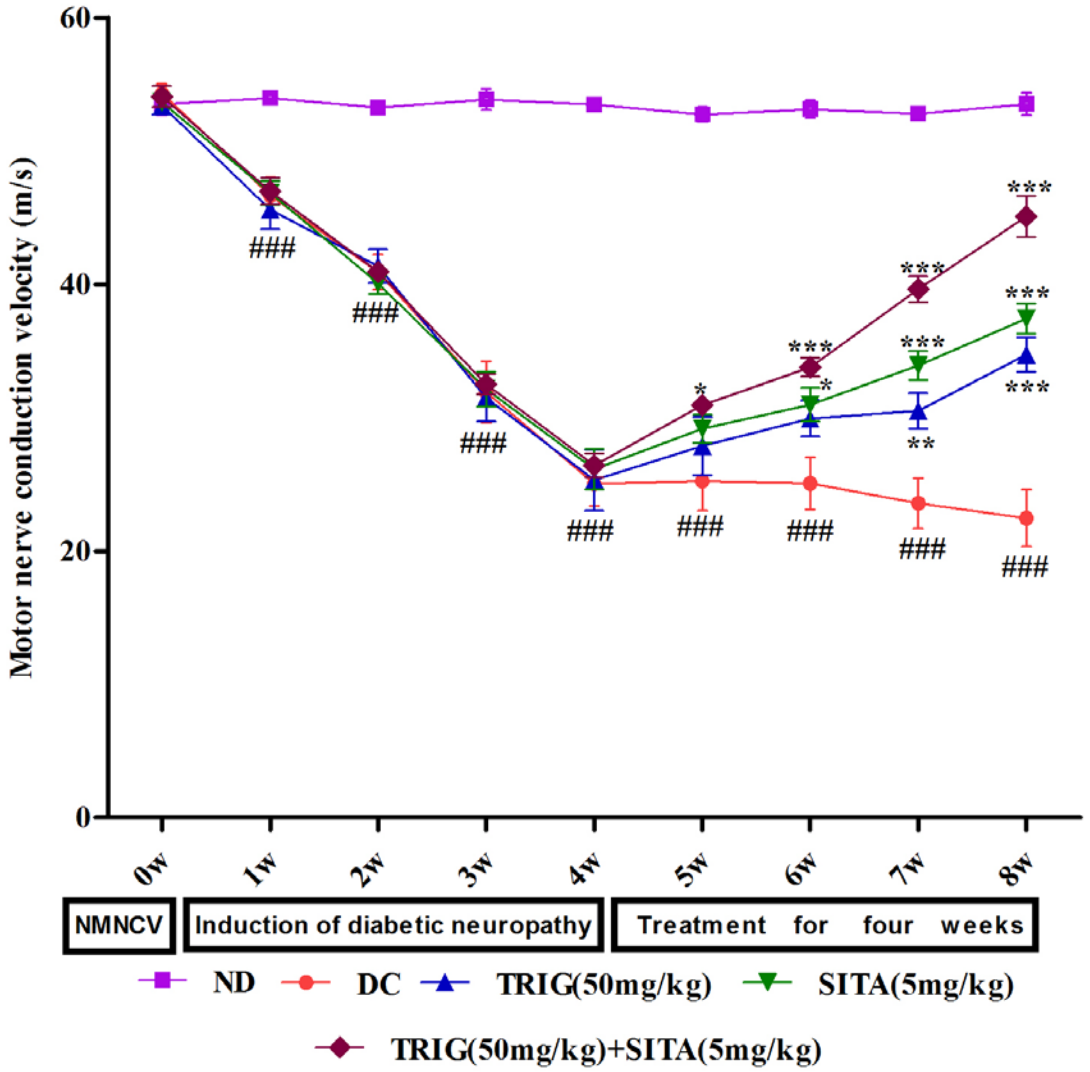
5.3.6.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on motor nerve conduction velocity in diabetic neuropathic rats

In the concomitant treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treated group the initial motor nerve conduction velocity was (26.41±0.90 (m/s) which was increased to 45.10±1.55 (m/s), $P< 0.001$. This increase of 18.69 m/s (41.44 %) was more than the increase in motor nerve conduction velocity by the individual drug. Early onset of motor nerve conduction velocity was observed at 2nd week (21.84%) compared of 3rd week of onset in individual treatment group. The results thus indicated synergistic effect of concomitant treatment with TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o (Figure 5.3.6).

Table 5.3.6 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on motor nerve conduction velocity (MNCV) in diabetic neuropathic rats

AVG of motor MNCV (m/s) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Normal motor nerve conduction velocity	**After induction of diabetes**	**Treatment in week**			
	0	**4**	**5**	**6**	**7**	**8**
ND	53.51±0.82	53.51±0.42	52.75±0.56	53.13±0.67	52.80±0.51	53.52±0.84
DC	54.35±0.70	25.04±1.66$^{###}$	25.25±2.22$^{###}$	25.07±1.95$^{###}$	23.58±1.89$^{###}$	22.46±2.14$^{###}$
TRIG (50mg/kg p.o.)	53.41±0.66	25.32±2.29	27.87±2.17	29.94±1.35	30.51±1.34**	34.74±1.29***
SITA (5mg/kg p.o.)	53.66±0.49	26.14±1.52	29.16±1.06	30.96±1.27*	33.91±1.08***	37.42±1.13***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	54.10±0.79	26.41±0.90	30.94±0.62	33.79±0.71***	39.64±0.98***	45.10±1.55***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.3.6 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on motor nerve conduction velocity (MNCV) in diabetic neuropathic rats

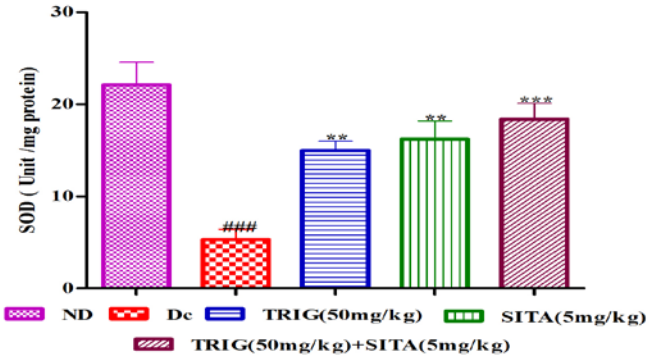
5.3.7 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on superoxide dismutase (SOD) level in diabetic neuropathic rat sciatic nerve

The level of SOD in non-diabetic rat sciatic nerve was (22.12±4.93 Unit /mg protein). On other hand where diabetic rat nerve showed significant (5.30±2.24Unit /mg protein, *P< 0.001*) decrease in SOD level as compared to non-diabetic rat nerve. Significant increase in SOD level was measured on last day of study period (end of week 8), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 14.98±2.02, *P<0.01*; 16.21±3.95, *P<0.01* and 18.40±3.40, *P<0.001* Unit /mg protein as compared to SOD level in diabetic rat nerve. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (71.19%) in SOD level compared to individual compound TRIG 64.61% and SITA 67.30% (Figure 5.3.7).

Table 5.3.7 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on superoxide dismutase (SOD) level in diabetic neuropathic rat sciatic nerve

AVG of superoxide dismutase (SOD) level in diabetic neuropathic rats	
Parameter / Groups	**SOD** (Unit /mg protein)
ND	22.12±4.93
DC	5.30±2.24$^{###}$
TRIG (50mg/kg p.o.)	14.98±2.02**
SITA (5mg/kg p.o.)	16.21±3.95**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	18.40±3.40***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group n=6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.3.7 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on superoxide dismutase (SOD) level in diabetic neuropathic rat sciatic nerve

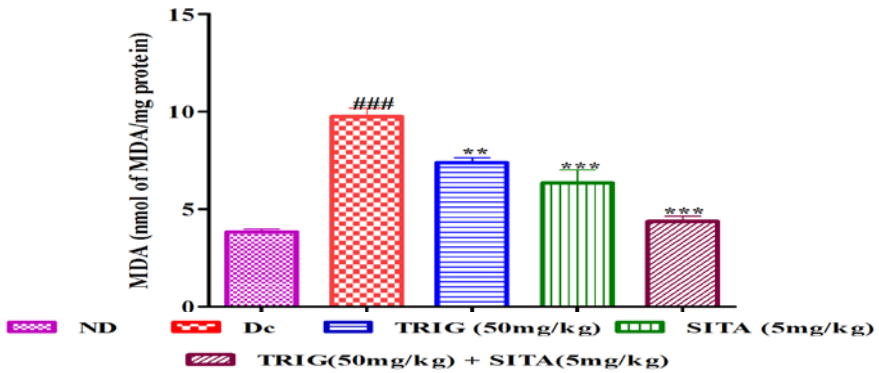
5.3.8 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on malondialdehyde (MDA) level in diabetic neuropathic rat sciatic nerve

The level of MDA in non-diabetic rat sciatic nerve was (3.83±0.31nmol of MDA/mg protein). On other hand where diabetic rat nerve showed significant (9.74±0.87nmol of MDA/mg protein, *P< 0.001*) increase in MDA level as compared to non-diabetic nerve. Significant decrease in MDA level was measured on last day of study period (end of week 8), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 7.39±0.51, *P<0.01*; 6.34±1.34, *P<0.001* and 4.36±0.53, *P<0.001* nmol of MDA/mg protein as compared to MDA level in diabetic rat nerve. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (55.23 %) in MDA level compared to individual compound TRIG 24.12% and SITA 34.90 % (Figure 5.3.8).

Table 5.3.8 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on malondialdehyde (MDA) level in diabetic neuropathic rat sciatic nerve

AVG of malondialdehyde (MDA) level in diabetic neuropathic rats	
Parameter / Groups	**MDA** (nmol of MDA/mg protein)
ND	3.83±0.31
DC	$9.74\pm0.87^{\#\#\#}$
TRIG (50mg/kg p.o.)	$7.39\pm0.51^{**}$
SITA (5mg/kg p.o.)	$6.34\pm1.34^{***}$
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	$4.36\pm0.53^{***}$

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic.

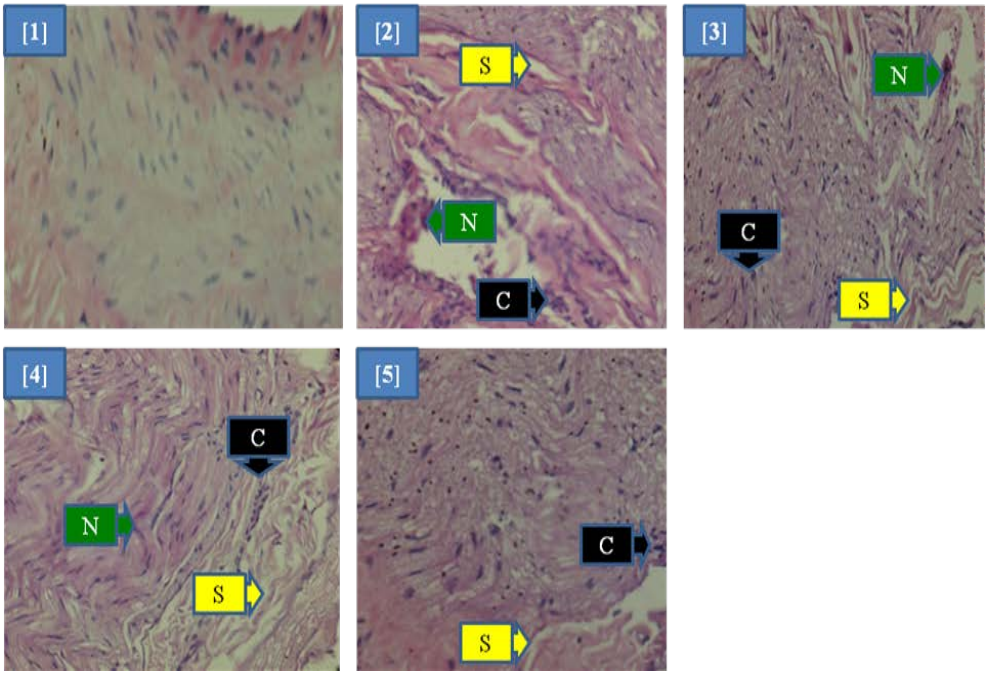


Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic.

Figure 5.3.8 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on malondialdehyde (MDA) level in diabetic neuropathic rat sciatic nerve

5.3.9 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on histological examination of isolated sciatic nerve tissue of diabetic neuropathic rats

Histological examination of rat sciatic nerve sections under light microscopy with Hematoxyline and eosin (H&E) stain were carried out on last day of study period. Isolated rat sciatic nerve of nondiabetic rat showed (Grade -) normal architecture; Diabetic rat isolated sciatic nerve showed (Grade +++) necrosis, (Grade +++) swelling, (Grade +++) congestion, TRIG treated sciatic nerve showed (Grade ++) necrosis, (Grade +) swelling, (Grade +) congestion; SITA showed (Grade ++) necrosis, (Grade +) swelling, (Grade +) congestion and TRIG + SITA treated animals showed (Grade -) necrosis, (Grade +) swelling, (Grade +) congestion. The scoring of grades was as per Dr. Chandrasekhar Mote (MVSC), Innovet Diagnostic Laboratory, Pune. Where (Grade -) normal; (Grade +++) severe; (Grade ++) moderate; (Grade +) mild. Study undertaken demonstrated that concomitant treatment of two drugs (TRIG + SITA) showed promising therapeutic effect against experimental diabetic neuropathy in rats than either drug alone (Figure 5.3.9).



Where, 1] Non diabetic rat; 2] Diabetic rat; 3] TRIG (50 mg/kg p.o.); 4] SITA (5mg/kg p.o.) and 5] TRIG 50 mg/kg p.o. + SITA (5 mg/kg p.o.). N-necrosis, C-congestion, S- swelling. [Magnification 40x].

Figure 5.3.9 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on histological examination of isolated sciatic nerve tissue of diabetic neuropathic rats (H&E stain)

Model 5.4.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on NICO-STZ induced diabetic cardiomyopathy in Wistar rats

5.4.1.1 Serum glucose level in non-diabetic rats

The results indicated non-significant fluctuation in serum glucose level (Figure 5.4.1).

5.4.1.2 Serum glucose level in diabetic cardiomyopathic rats

Serum glucose level before administration of NICO-STZ in this group was (92.05±3.49 mg/dl). After administration of NICO-STZ the serum glucose level increasing trend and at the end of 3 week after induction of diabetes the serum glucose (419.25±9.01 mg/dl). The vehicle used for dissolving the test compound was double distilled water which could not prevent the rise of serum glucose and at the end 8 weeks of treatment period the serum glucose level was 451.17±7.66 mg/dl (Figure 5.4.1).

5.4.1.3 Effect of TRIG (50mg/kg p.o) on serum glucose level in diabetic cardiomyopathic rats

In the TRIG (50 mg/kg p.o.) treated group the initial serum glucose level was (415.98±5.21 mg/dl) which was reduced to (231.23±9.85 mg/dl, $P< 0.001$) indicating a decrease of 184.75 mg/dl or 44.41 %. The onset of antihyperglycemic effect was evident after 3 week of TRIG (50 mg/kg p.o.) treatment and continuation of treatment showed a trend toward decrease in serum glucose (Figure 5.4.1).

5.4.1.4 Effect of SITA (5 mg/kg p.o) on serum glucose level in diabetic cardiomyopathic rats

In case of SITA (5 mg/kg p.o.) treated diabetic animals the serum glucose level before treatment (initial) was (419.96±3.66 mg/dl) on week 3rd. After treatment with SITA (5 mg/kg p.o.) the serum glucose level at the 8th week was (205.24±4.97 mg/dl) this reduction of 214.72 mg/dl or 51.12% indicated significant ($P< 0.001$) antihyperglycemic effect. SITA (5 mg/kg p.o.) appears to be more effective than TRIG (50 mg/kg p.o.) in reducing the serum glucose (Figure 5.4.1).

5.4.1.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg, *p.o*) on serum glucose level in diabetic cardiomyopathic rats

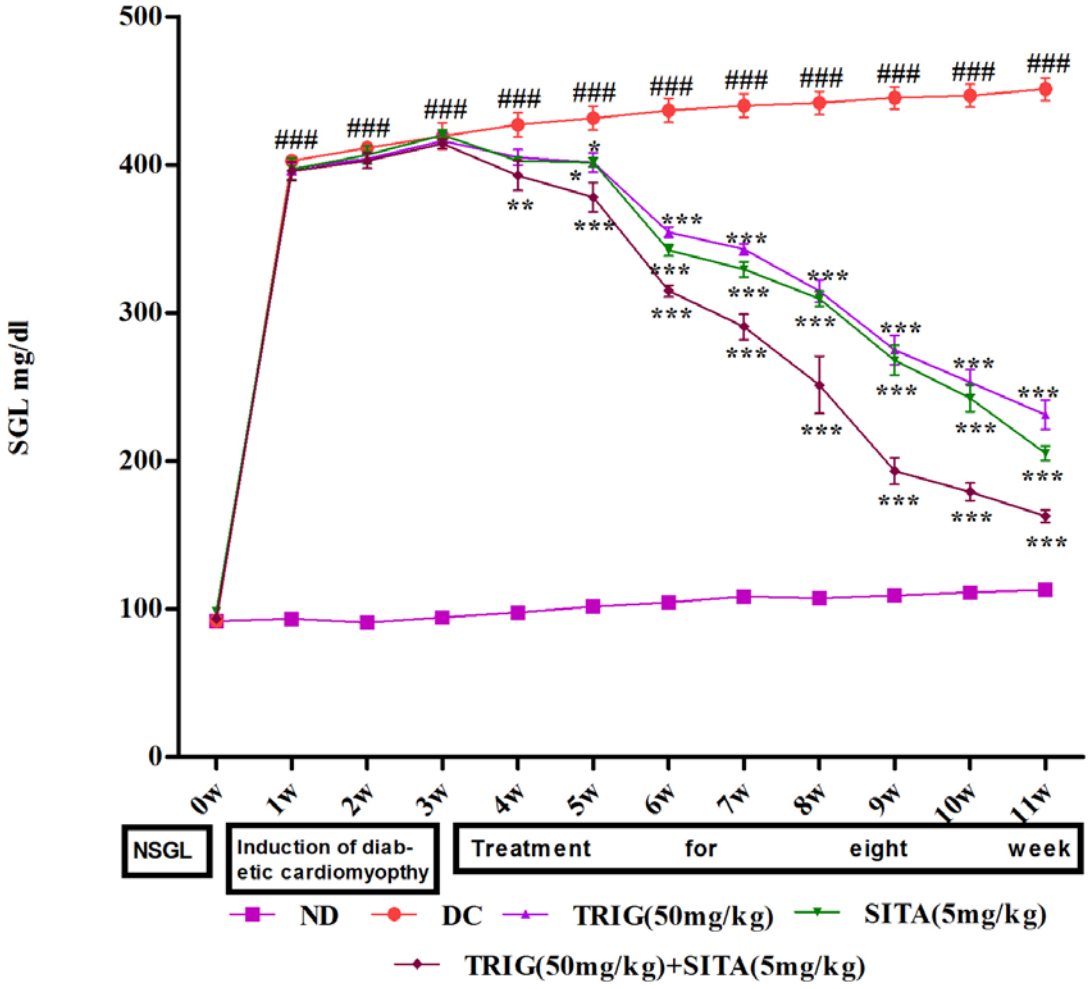
In the concomitant treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) treated group the initial serum glucose level was (414.15±3.43 mg/dl) which was reduced to (162.70±4.26 mg/dl). This reduction of 251.45 mg/dl (60.71 %) was more

than the reduction in serum glucose by the individual drug. Early onset of serum glucose was observed at 3rd week (23.97%) compared of 4th week of onset in individual treatment group. The results thus indicated synergistic effect of concomitant treatment with TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. (Figure 5.4.1).

Table 5.4.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum glucose level in diabetic cardiomyopathic rats

AVG of serum glucose levels (mg/dl) in diabetic cardiomyopathic rats (11 weeks model)										
Groups	**Time in weeks**									
	Nor-mal SGL	**After induct-ion of diabet es**	**Treatment for eight weeks**							
	0W	**3W**	**4W**	**5W**	**6W**	**7W**	**8W**	**9W**	**10W**	**11W**
ND	91.94±2.09	94.48±0.88	97.80±1.24	101.76±2.06	104.62±1.17	108.40±1.32	107.30±2.02	109.30±1.63	111.27±2.30	113.03±1.99
DC	92.05±3.49	419.25±9.01$^{###}$	427.14±8.13$^{###}$	431.48±8.23$^{###}$	436.76±8.14$^{###}$	440.01±7.86$^{###}$	441.74±7.90$^{###}$	445.37±7.55$^{###}$	446.75±7.81$^{###}$	451.17±7.66$^{###}$
TRIG (50mg/kg p.o.)	96.08±2.36	415.98±5.21	405.02±5.22	401.55±6.61*	354.61±3.52***	343.01±3.62***	314.80±7.63***	274.91±9.77***	253.07±8.41***	231.23±9.85***
SITA (5mg/kg p.o.)	98.49±1.83	419.96±3.66	402.67±2.26	401.56±3.00*	342.33±3.66***	329.57±5.13***	309.54±5.15***	267.95±10.1***	242.43±9.27***	205.24±4.97***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	93.45±2.49	414.15±3.43	392.69±10.11**	378.07±10.02***	314.86±3.78***	290.57±8.56***	251.37±19.39**	193.19±9.18***	179.08±5.91***	162.70±4.26***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on serum glucose level in diabetic cardiomyopathic rats

5.4.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in diabetic cardiomyopathic rats

5.4.2.1 Body weight in non-diabetic rats

The results indicated increase in body weight compare to week '0' (Figure 5.4.2).

5.4.2.2 Body weight in diabetic cardiaomyopathic rats

Before administration of NICO-STZ the body weight of the rat was 213.33±3.44 g. Administration of NICO-STZ resulted in reduction body weight to 159.97±4.57g. This reduction of 53.36 g (25.01%) was due to the induction of diabetes. The animals appeared weak and polyuria (not recorded) was evident (Figure 5.4.2).

5.4.2.3 Effect of TRIG (50mg/kg p.o) on body weight in diabetic cardiomyopathic rats

Before administration of TRIG (50 mg/kg p.o.) in diabetic rats the body weight was 195.12±2.91g. After treatment with TRIG (50 mg/kg p.o.) the body weight was 207.35±2.82g. The results indicated that TRIG treatment arrested the loss of body weight in the diabetic rats. The observed effect appears to be due to control of the hyperglycemia. The rats showed increase in the body weight from the 2^{nd} week after TRIG (50 mg/kg p.o.) treatment and after 4^{th} and 5^{th} week significant ($P< 0.001$) gain in body weight than the initial body weight was observed (Figure 5.4.2).

5.4.2.4 Effect of SITA (5 mg/kg p.o) on body weight in diabetic cardiomyopathic rats

The initial body weight of diabetic rats was (200.40±2.45 g). After treatment with SITA (5 mg/kg p.o.) body weight increase to (213.21±3.22 g, $P< 0.001$). SITA (5 mg/kg p.o.) appear to be more effective in arresting the loss of body weight compared to that of TRIG 50 mg/kg p.o. (Figure 5.4.2).

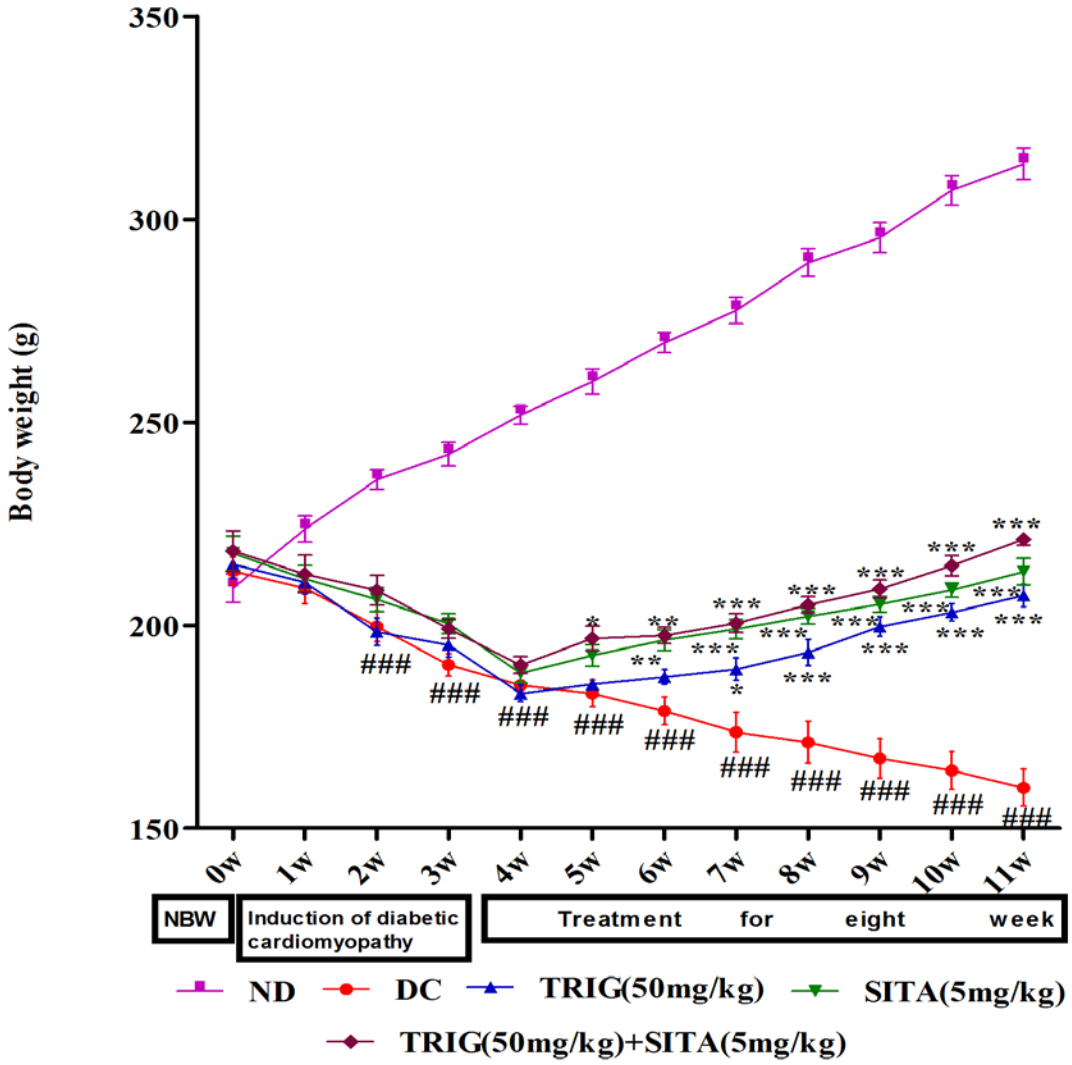
5.4.2.5 Effect of concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o) on body weight in diabetic cardiomyopathic rats

Concomitant administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) was more effective than the individual compound because the gain in body weight after the treatment was more compared to that of individual drug. The treated diabetic animals showed improvement in health compared to diabetic rats (Figure 5.4.2).

Table 5.4.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in diabetic cardiomyopathic rat

AVG of body weight (g) in diabetic cardiomyopathic rats (11 weeks model)										
Groups	**Time in weeks**									
	Nor-mal body weight	**After induct io-n of diabet es**	**Treatment for eight weeks**							
	0W	**3W**	**4W**	**5W**	**6W**	**7W**	**8W**	**9W**	**10W**	**11W**
ND	209.20± 3.51	242.12± 2.91	251.77 ±2.23	260.06±3.11	269.60±2.48	277.55±3.26	289.34±3.38	295.50±3.67	307.14±3.68	313.64±3.88
DC	213.33± 3.44	190.23± 2.77$^{###}$	185.24 ±3.21$^{###}$	183.21±3.26$^{###}$	178.92±3.46$^{###}$	173.65±4.95$^{###}$	171.17±5.16$^{###}$	167.21±4.85$^{###}$	164.22±4.64$^{###}$	159.97±4.57$^{###}$
TRIG (50mg/kg p.o.)	215.04± 3.45	195.12± 2.91	183.23 ±2.06	185.42±1.29	187.26±1.81	189.17±2.77*	193.30±3.21***	199.67±2.46***	203.20±2.12***	207.35±2.82***
SITA (5mg/kg p.o.)	217.75± 4.23	200.40± 2.45	188.21 ±2.48	192.57±2.66	196.34±2.56**	199.08±2.39***	202.20±1.95***	205.16±1.92***	208.66±1.80***	213.21±3.22***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	218.30± 5.01	199.13± 2.38	190.20 ±2.08	196.75±3.04*	197.45±2.01**	200.53±2.32***	205.02±1.97***	208.95±2.21***	214.72±2.47***	221.18±1.39***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.2 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on body weight in diabetic cardiomyopathic rats

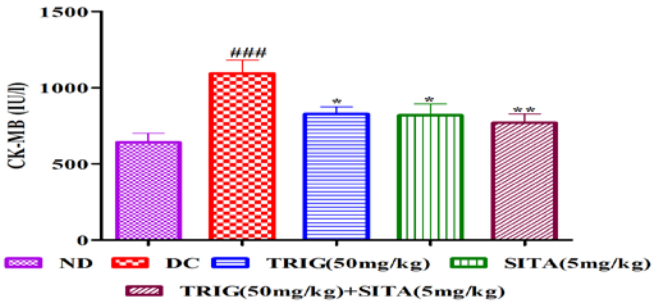
5.4.3.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on creatine kinase (CK-MB) level in diabetic cardiomyopathic rats

The levels of serum CK-MB in non-diabetic rat were (642.1±57.98 IU/l). On other hand where diabetic rat showed significant (1093.0±89.09 IU/l, *P< 0.001*) increase in CK-MB level as compared to non-diabetic rat. Significant decrease in CK-MB level was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 827.9±47.57, *P<0.05*; 818.7±74.91, *P<0.05* and 770.9±56.74 IU/l, *P<0.01* as compared to CK-MB level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (29.46 %) in creatine kinase level compared to individual compound TRIG 24.25% and SITA 25.09 % (Figure 5.4.3.1).

Table 5.4.3.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on creatine kinase (CK-MB) level in diabetic cardiomyopathic rats

AVG of serum creatine kinase (CK-MB) level in diabetic cardiomyopathic rats	
Parameter / Groups	**CK-MB** (IU/l)
ND	642.1±57.98
DC	1093.0±89.09$^{###}$
TRIG (50mg/kg p.o.)	827.9±47.57*
SITA (5mg/kg p.o.)	818.7±74.91*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	770.9±56.74**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.3.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on creatine kinase (CK-MB) level in diabetic cardiomyopathic rats

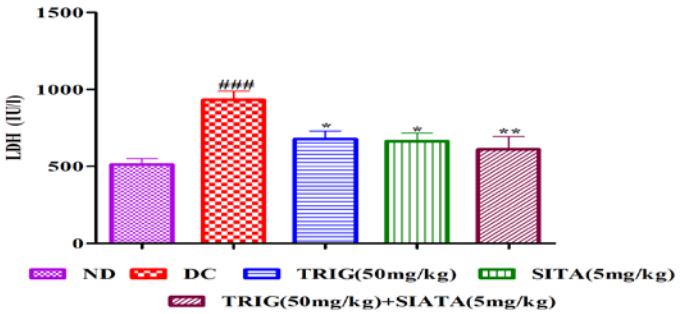
5.4.4.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on lactate dehydrogenase (LDH) level in diabetic cardiomyopathic rats

The levels of serum LDH in non-diabetic rat were (513.1±38.7 IU/l). On other hand where diabetic rat showed significant (932.9±55.4 IU/l, *P< 0.001*) increase in LDH level as compared to non-diabetic rat. Significant decrease in LDH level was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 678.9±51.3, *P<0.05*; 664.8±52.3, *P<0.05* and 609.9±84.91 IU/l, *P<0.01* as compared to LDH level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (34.64%) in lactate dehydrogenase level compared to individual compound TRIG 27.22% and SITA 28.73 % (Figure 5.4.4.1).

Table 5.4.4.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on lactate dehydrogenase (LDH) level in diabetic cardiomyopathic rats

AVG of serum lactate dehydrogenase (LDH) level	
Parameter / Groups	LDH (IU/l)
ND	513.1±38.7
DC	932.9±55.4$^{###}$
TRIG (50mg/kg p.o.)	678.9±51.3*
SITA (5mg/kg p.o.)	664.8±52.3*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)	609.71±84.91**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.4.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on lactate dehydrogenase (LDH) level in diabetic cardiomyopathic rats

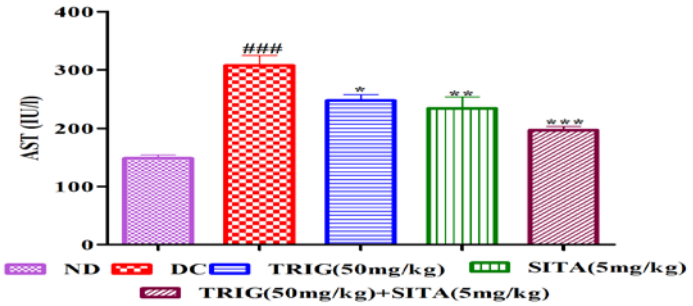
5.4.5.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on aspartate transaminase (AST) level in diabetic cardiomyopathic rats

The levels of serum AST in non-diabetic rat were (148.3±05.85 IU/l). On other hand where diabetic rat showed significant (307.9±17.1 IU/l, *P< 0.001*) increase in AST level as compared to non-diabetic rat. Significant decrease in AST level was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 247.7±10.15, *P<0.05*; 233.8±20.31, *P<0.01* and 196.8±06.30 IU/l, *P<0.001* as compared to LDH level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (36.08 %) in aspartate transaminase level compared to individual compound TRIG 19.55% and SITA 24.06 % (Figure 5.4.5.1).

Table 5.4.5.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on aspartate transaminase (AST) level in diabetic cardiomyopathic rats

AVG of serum aspartate transaminase (AST) level	
Parameter / Groups	AST (IU/l)
ND	148.3±05.85
DC	307.9±17.1$^{###}$
TRIG (50mg/kg p.o.)	247.7±10.15*
SITA (5mg/kg p.o.)	233.8±20.31**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	196.8±06.30***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.5.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on aspartate transaminase (AST) level in diabetic cardiomyopathic rats

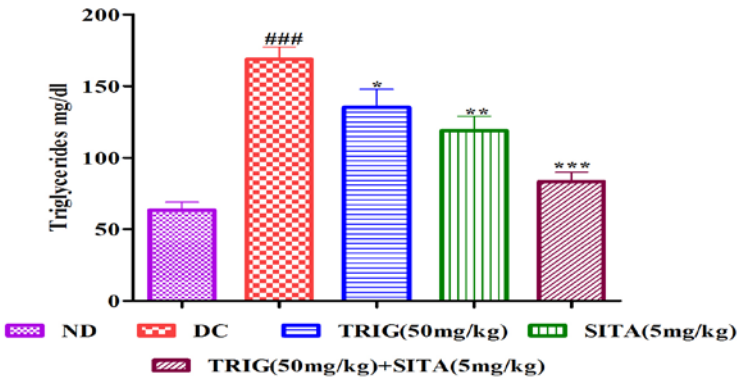
5.4.6.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on triglyceride level in diabetic cardiomyopathic rats

The levels of serum triglyceride in non-diabetic rat were (63.4±5.46 mg/dl). On other hand where diabetic rat showed significant (168.8±8.57 mg/dl, *P< 0.001*) increase in triglyceride level as compared to non-diabetic rat. Significant decrease in triglyceride level was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 135.2±12.55, *P<0.05*; 118.8±10.08, *P<0.01* and 83.28±6.57 mg/dl, *P<0.001* as compared to triglyceride level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (50.66 %) in triglyceride level compared to individual compound TRIG 19.90% and SITA 29.62 % (Figure 5.4.6.1).

Table 5.4.6.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on triglyceride level in diabetic cardiomyopathic rats

AVG of serum triglyceride level in diabetic cardiomyopathic rats	
Parameter / Groups	**Triglyceride (mg/dl)**
ND	63.4±5.465
DC	168.8±8.57$^{###}$
TRIG (50mg/kg p.o.)	135.2±12.55*
SITA (5mg/kg p.o.)	118.8±10.08**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	83.28±6.57***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.6.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on triglyceride level in cardiomyopathic rats

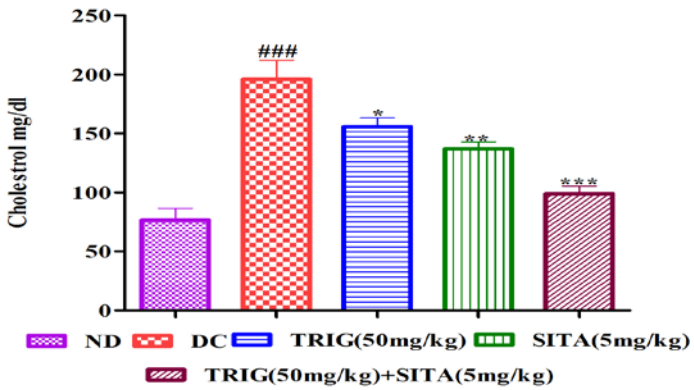
5.4.7.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on cholesterol level in diabetic cardiomyopathic rats

The levels of serum cholesterol in non-diabetic rat were (76.67±10.01 mg/dl). On other hand where diabetic rat showed significant (196.0±16.19 mg/dl, *P< 0.001*) increase in cholesterol level as compared to non-diabetic rat. Significant decrease in cholesterol level was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 155.8±7.39, *P<0.05*; 136.9±5.99, *P<0.01* and 98.81±6.54 mg/dl, *P<0.001* as compared to triglyceride level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (49.58 %) in cholesterol level compared to individual compound TRIG 20.51% and SITA 30.15 % (Figure 5.4.7.1).

Table 5.4.7.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on cholesterol level in diabetic cardiomyopathic rats

AVG of serum cholesterol level in diabetic cardiomyopathic rats	
Parameter / Groups	**Cholesterol (mg/dl)**
ND	76.67±10.01
DC	196.0±16.19$^{###}$
TRIG (50mg/kg p.o.)	155.8±7.39*
SITA (5mg/kg p.o.)	136.9±5.99**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	98.81±6.54***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.7.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on cholesterol level in diabetic cardiomyopathic rats

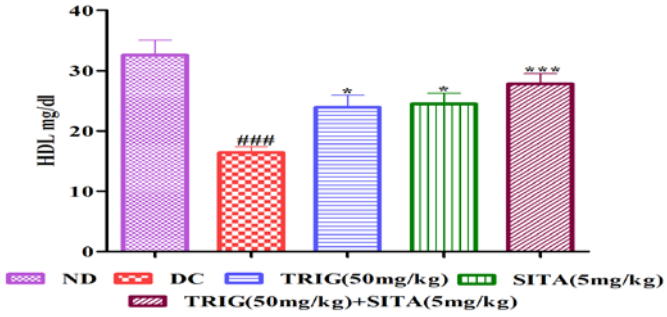
5.4.8.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on high density lipoprotein (HDL) level in diabetic cardiomyopathic rats

The level of high density lipoprotein in non-diabetic rat was (32.58±2.46 mg/dl). On other hand where diabetic rat showed significant (16.4±1.01 mg/dl, *P< 0.001*) decrease in high density lipoprotein level as compared to non-diabetic rat. Significant increase in high density lipoprotein level was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 23.94±2.02, *P<0.05*; 24.46±1.83, *P<0.05* and 27.83±1.69 mg/dl, *P<0.001* as compared to triglyceride level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (41.07%) in high density lipoprotein level compared to individual compound TRIG 31.49 % and SITA 32.95 % (Figure 5.4.8.1).

Table 5.4.8.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on high density lipoprotein (HDL) level in diabetic cardiomyopathic rats

AVG of serum high density lipoprotein in diabetic cardiomyopathic rats	
Parameter / Groups	**HDL (mg/dl)**
ND	32.58±2.46
DC	16.4±1.01$^{###}$
TRIG (50mg/kg p.o.)	23.94±2.02*
SITA (5mg/kg p.o.)	24.46±1.83*
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	27.83±1.69***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.8.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on HDL level in diabetic cardiomyopathic rats

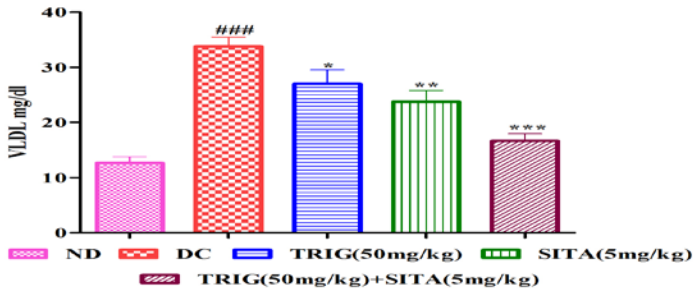
5.4.9.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on very low density lipoprotein (VLDL) level in diabetic cardiomyopathic rats

The level of very low density lipoprotein in non-diabetic rat was (12.68±1.09 mg/dl). On other hand where diabetic rat showed significant (33.78±1.71 mg/dl, *P< 0.001*) increase in very low density lipoprotein level as compared to non-diabetic rat. Significant decrease in high density lipoprotein level was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 27.03±2.51, *P<0.05*; 23.77±2.07, *P<0.01* and 16.66±01.31 mg/dl, *P<0.001* as compared to very low density lipoprotein level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (50.68 %) in very low density lipoprotein level compared to individual compound TRIG 19.98 % and SITA 29.63% (Figure 5.4.9.1).

Table 5.4.9.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on very low density lipoprotein (VLDL) level in diabetic cardiomyopathic rats

AVG of very low density lipoprotein in diabetic cardiomyopathic rats	
Parameter / Groups	VLDL (mg/dl)
ND	12.68±1.09
DC	33.78±1.71$^{###}$
TRIG (50mg/kg p.o.)	27.03±2.51*
SITA (5mg/kg p.o.)	23.77±2.07**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	16.66±01.31***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.9.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on very low density lipoprotein (VLDL) level in diabetic cardiomyopathic rats

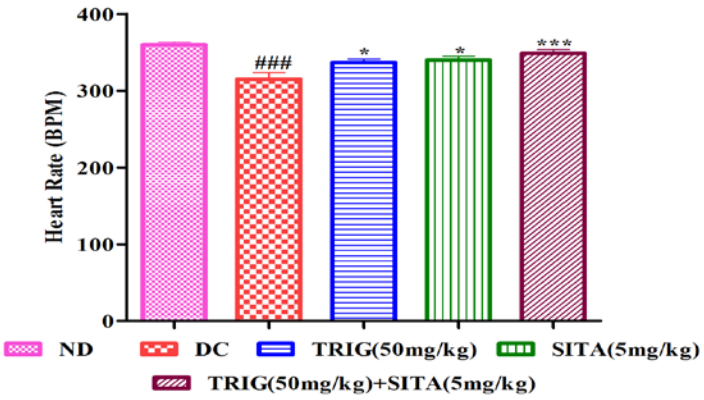
5.4.10.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on heart rate in diabetic cardiomyopathic rats

The heart rate in non-diabetic rat was (359.7±3.99, BPM). On other hand where diabetic rat showed significant (315.2±8.33BPM, *P< 0.001*) decrease in heart rate as compared to non-diabetic rat. Significant increase in heart rate was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 336.8±4.51, *P<0.05*; 339.9±5.14, *P<0.05* and 348.7±4.77 BPM, *P<0.001* as compared to heart rate in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (9.60 %) in heart rate compared to individual compound TRIG 6.41 % and SITA 7.26 % (Figure 5.4.10.1).

Table 5.4.10.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on heart rate in diabetic cardiomyopathic rats

AVG of heart rate in diabetic cardiomyopathic rats	
Parameter / Groups	**Heart rate (BPM)**
ND	359.7±3.99
DC	315.2±8.33$^{###}$
TRIG (50mg/kg p.o.)	336.8±4.51*
SITA (5mg/kg p.o.)	339.9±5.14*
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	348.7±04.77***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, $^{***}P$< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, $^{***}P$< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.

Figure 5.4.10.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on heart rate in diabetic cardiomyopathic rats

5.4.11.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QRS interval in diabetic cardiomyopathic rats

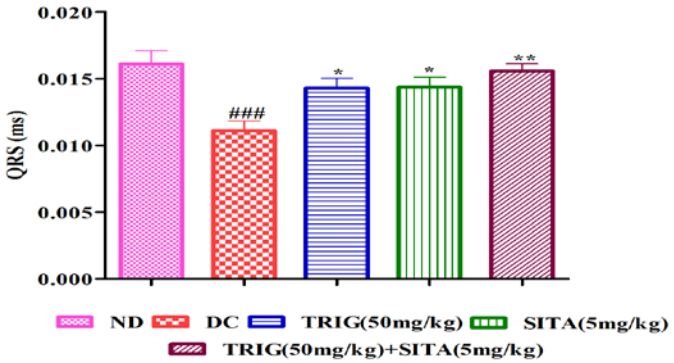
The QRS interval in non-diabetic rat was (0.01610±0.99 ms). On other hand where diabetic rat showed significant (0.01109±0.71ms, *P< 0.001*) decrease in QRS interval as compared to non-diabetic rat. Significant increase in QRS interval was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 0.01431±0.74, *P<0.05*; 0.01437±0.74, *P<0.05* and 0.01555±0.54 ms, *P<0.01* as compared to QRS interval in diabetic rat.

TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (28.68 %) in QRS interval compared to individual compound TRIG 22.50 % and SITA 22.82% (Figure 5.4.11.1).

Table 5.4.11.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QRS interval in diabetic cardiomyopathic rats

AVG of QRS interval in diabetic cardiomyopathic rats	
Parameter / Groups	QRS interval (ms)
ND	0.01610±0.99
DC	0.01109±0.71$^{###}$
TRIG (50mg/kg p.o.)	0.01431±0.74*
SITA (5mg/kg p.o.)	0.01437±0.74*
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	0.01555±0.54**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.11.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QRS interval in diabetic cardiomyopathic rats

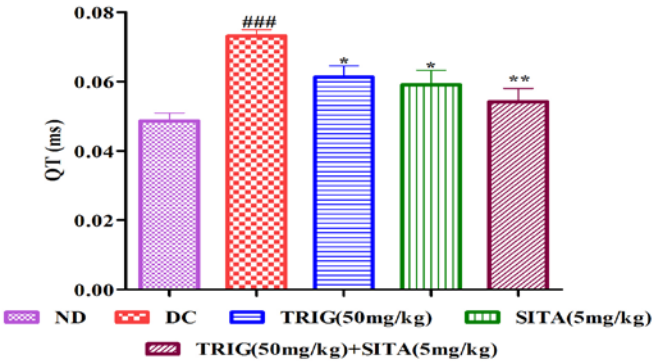
5.4.12.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QT interval in diabetic cardiomyopathic rats

The QT interval in non-diabetic rat was (0.04865±0.22 ms). On other hand where diabetic rat showed significant (0.07314±0.18 ms, *P< 0.001*) increase in QT interval as compared to non-diabetic rat. Significant decrease in QT interval was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 0.06123±0.32, *P<0.05*; 0.05904±0.41, *P<0.05* and 0.05419±0.38 ms, *P<0.01* as compared to QT interval in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (25.90 %) in QT interval compared to individual compound TRIG 16.28% and SITA 19.27% (Figure 5.4.12.1).

Table 5.4.12.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QT interval in diabetic cardiomyopathic rats

AVG of QT interval in diabetic cardiomyopathic rats	
Parameter / Groups	QT interval (ms)
ND	0.04865±0.22
DC	0.07314±0.18$^{###}$
TRIG (50mg/kg p.o.)	0.06123±0.32*
SITA (5mg/kg p.o.)	0.05904±0.41*
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	0.05419±0.38**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared with diabetic control, $^{###}P<0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared with diabetic control, $^{###}P<0.001$ as compared with non diabetic.

Figure 5.4.12.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QT interval in diabetic cardiomyopathic rats

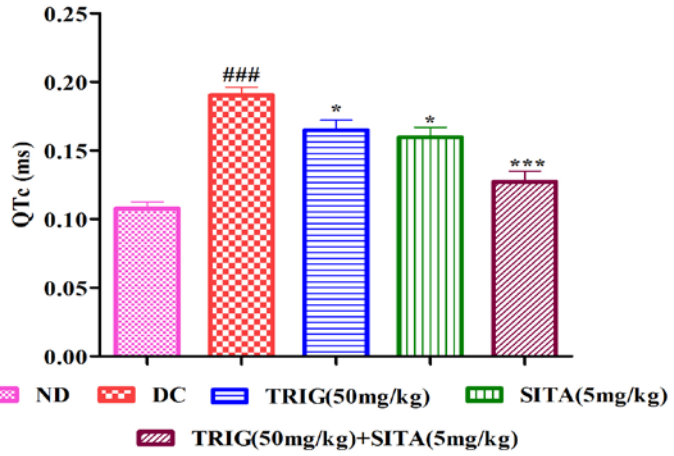
5.4.13.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QTc in diabetic cardiomyopathic rats

The QTc in non-diabetic rat was (0.1077±0.47 ms). On other hand where diabetic rat showed significant (0.1903±0.59 ms, *P< 0.001*) increase in QTc as compared to non-diabetic rat. Significant decrease in QTc was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 0.1649±0.73, *P<0.05*; 0.1598±0.72, *P<0.05* and 0.1272±0.77 ms, *P<0.001* as compared to QTc in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (33.15 %) in QTc compared to individual compound TRIG 13.34% and SITA 16.02 % (Figure 5.4.13.1).

Table 5.4.13.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QTc in diabetic cardiomyopathic rats

AVG of QTc interval in diabetic cardiomyopathic rats	
Parameter / Groups	QTc interval (ms)
ND	0.1077±0.47
DC	0.1903±0.59$^{###}$
TRIG (50mg/kg p.o.)	0.1649±0.73*
SITA (5mg/kg p.o.)	0.1598±0.72*
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	0.1272±0.77***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.13.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on QTc in diabetic cardiomyopathic rats

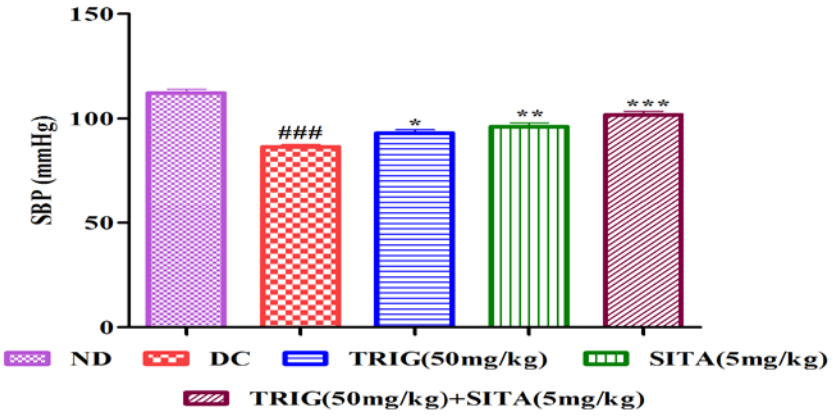
5.4.14.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on systolic blood pressure (SBP) in diabetic cardiomyopathic rats

SBP in non-diabetic rat was (122.2±1.79 mmHg). On other hand where diabetic rat showed significant (86.53±1.03 mmHg, *P< 0.001*) decrease in SBP as compared to non-diabetic rat. Significant decrease in SBP was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 92.98±1.57, *P<0.05*; 96.04±1.84 , *P<0.01* and 101.8±1.67 mmHg, *P<0.001* as compared to SBP in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (15.00 %) in SBP compared to individual compound TRIG 6.93 % and SITA 9.90% (Figure 5.4.14.1).

Table 5.4.14.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on SBP in diabetic cardiomyopathic rats

AVG of systolic blood pressure (SBP) in diabetic cardiomyopathic rats	
Parameter / Groups	**SBP (mmHg)**
ND	122.2±1.79
DC	86.53±1.03$^{###}$
TRIG (50mg/kg p.o.)	92.98±1.57*
SITA (5mg/kg p.o.)	96.04±1.84**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	101.8±1.67***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.

Figure 5.4.14.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on SBP in diabetic cardiomyopathic rats

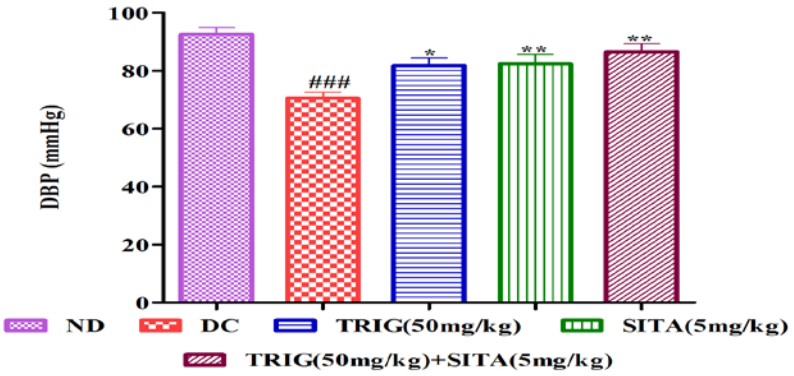
5.4.15.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on diastolic blood pressure (DBP) in diabetic cardiomyopathic rats

DBP in non-diabetic rat was (92.58±2.29 mmHg). On other hand where diabetic rat showed significant (70.54±2.058 mmHg, *P< 0.001*) decrease in DBP as compared to non-diabetic rat. Significant increase in DBP was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 81.76±2.62, *P<0.05*; 82.35±3.31, *P<0.01* and 86.41±2.87 mmHg, *P<0.01* as compared to DBP in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (18.36 %) in DBP compared to individual compound TRIG 13.72 % and SITA 14.34% (Figure 5.4.15.1).

Table 5.4.15.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on DBP in diabetic cardiomyopathic rats

AVG of diastolic blood pressure (DBP) in diabetic cardiomyopathic rats	
Parameter / Groups	DBP (mmHg)
ND	92.58±2.29
DC	70.54±2.058$^{###}$
TRIG (50mg/kg p.o.)	81.76±2.62*
SITA (5mg/kg p.o.)	82.35±3.31**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	86.41±2.87**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.15.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on DBP in diabetic cardiomyopathic rats

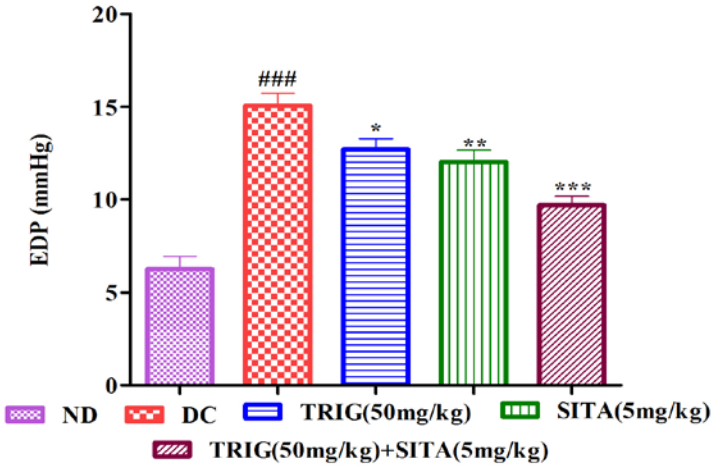
5.4.16.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on end diastolic pressure (EDP) in diabetic cardiomyopathic rats

EDP in non-diabetic rat was (6.25±0.68 mmHg). On other hand where diabetic rat showed significant (15.06±0.67 mmHg, *P< 0.001*) increase in EDP as compared to non-diabetic rat. Significant decrease in EDP was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 12.71±0.57, *P<0.05*; 12.03±0.64, *P<0.01* and 9.69±0.49 mmHg, *P<0.001* as compared to EDP in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant decrease by (35.65 %) in EDP compared to individual compound TRIG 15.60 % and SITA 20.11% (Figure 5.4.16.1).

Table 5.4.16.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on EDP in diabetic cardiomyopathic rats

AVG of end diastolic pressure (EDP) in diabetic cardiomyopathic rats	
Parameter / Groups	EDP (mmHg)
ND	6.25±0.68
DC	15.06±0.67$^{###}$
TRIG (50mg/kg p.o.)	12.71±0.57*
SITA (5mg/kg p.o.)	12.03±0.64**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	9.69±0.49***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.16.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on EDP in diabetic cardiomyopathic rats

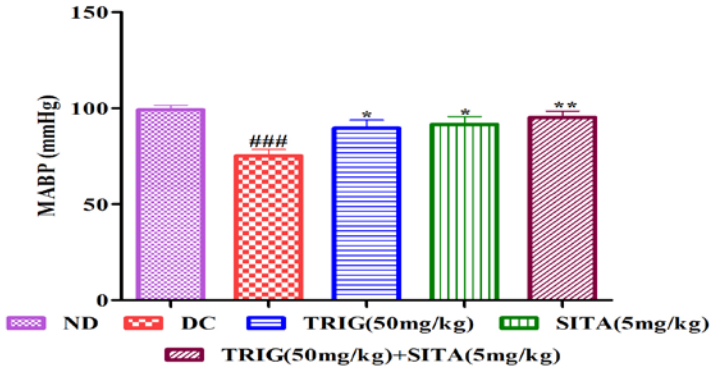
5.4.17.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on mean arterial blood pressure (MABP) in diabetic cardiomyopathic rats

MABP in non-diabetic rat was (99.25±2.28, mmHg). On other hand where diabetic rat showed significant (75.2±3.40 mmHg, *P< 0.001*) decrease in MABP as compared to non-diabetic rat. Significant increase in MABP was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 89.67±4.23, *P<0.05*; 91.54±4.17, *P<0.05* and 95.22±3.31 mmHg, *P<0.01* as compared to MABP in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (21.02 %) in MABP compared to individual compound TRIG 16.13 % and SITA 17.85% (Figure 5.4.17.1).

Table 5.4.17.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on MABP in diabetic cardiomyopathic rats

AVG of mean arterial blood pressure (MABP) in diabetic cardiomyopathic rats	
Parameter / Groups	MABP (mmHg)
ND	99.25±2.28
DC	75.2±3.40$^{###}$
TRIG (50mg/kg p.o.)	89.67±4.23*
SITA (5mg/kg p.o.)	91.54±4.17*
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	95.22±3.31**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.17.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on MABP in diabetic cardiomyopathic rats

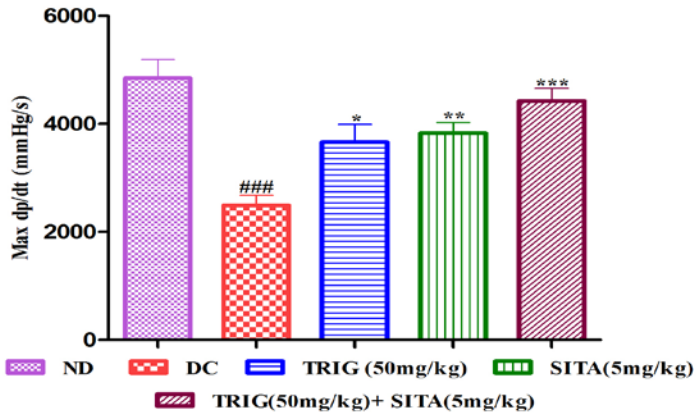
5.4.18.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on max dp/dt in diabetic cardiomyopathic rats

The max dp/dt in non-diabetic rat was (4849.0±345.2, mmHg/s). On other hand where diabetic rat showed significant (2494.0±181.7 mmHg/s, *P< 0.001*) decrease in max dp/dt as compared to non-diabetic rat. Significant increase in max dp/dt was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 3663.0±324.8, *P<0.05*; 3829.0±196.0, *P<0.01* and 4425.0±234.8 mmHg/s, *P<0.001* as compared to max dp/dt in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (43.63 %) in max dp/dt compared to individual compound TRIG 31.91 % and SITA 34.86% (Figure 5.4.18.1).

Table 5.4.18.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on max dp/dt in diabetic cardiomyopathic rats

AVG of max dp/dt in diabetic cardiomyopathic rats	
Parameter / Groups	**max dp/dt (mmHg/s)**
ND	4849±345.2
DC	2494±181.7$^{###}$
TRIG (50mg/kg p.o.)	3663±324.8*
SITA (5mg/kg p.o.)	3829±196.0**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	4425±234.8***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.4.18.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on max dp/dt in diabetic cardiomyopathic rats

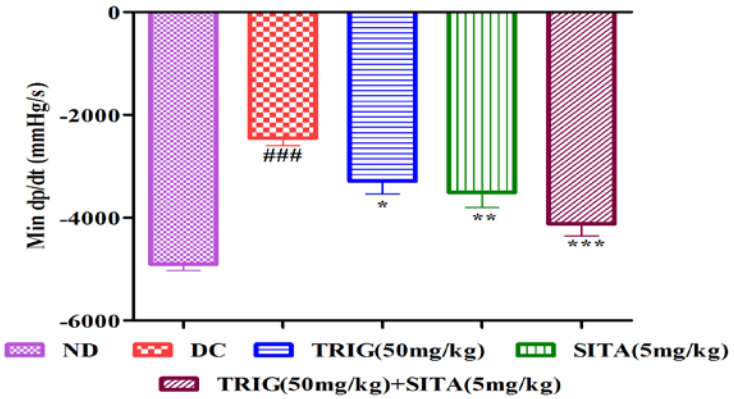
5.4.19.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on min dp/dt in diabetic cardiomyopathic rats

The min dp/dt in non-diabetic rat was (-4905.0±116.3, mmHg/s). On other hand where diabetic rat showed significant (-2454.0±149.9 mmHg/s, *P< 0.001*) decrease in min dp/dt as compared to non-diabetic rat. Significant increase in min dp/dt was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., -3286.0± 249.4, *P<0.05*; -3509.0±294.2, *P<0.01* and 4121.0± 0.77 mmHg/s, *P<0.001* as compared to min dp/dt in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (40.45 %) in min dp/dt compared to individual compound TRIG 25.31% and SITA 30.06% (Figure 5.4.19.1).

Table 5.4.19.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on min dp/dt in diabetic cardiomyopathic rats

AVG of min dp/dt in diabetic cardiomyopathic rats	
Parameter / Groups	**min dp/dt (mmHg/s)**
ND	-4905±116.3
DC	-2454±149.9$^{###}$
TRIG (50mg/kg p.o.)	-3286±249.4*
SITA (5mg/kg p.o.)	-3509±294.2**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	-4121±0.77***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.

Figure 5.4.19.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on min dp/dt in diabetic cardiomyopathic rats

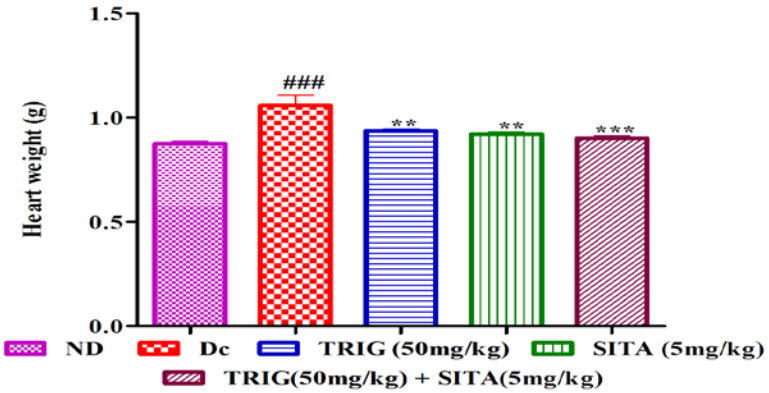
5.4.20.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on heart weight in diabetic cardiomyopathic rats

The heart weight in non-diabetic rat was (0.88±0.012 g). On other hand where diabetic rat showed significant (1.06±0.049 g, *P< 0.001*) increase in heart weight as compared to non-diabetic rat. Significant decrease in heart weight was measured on last day of study period (end of week 11), TRIG 50mg/kg p.o., SITA 5mg/kg p.o. and TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o., 0.94±0.010, *P<0.01*; 0.92 ±0.010, *P<0.01* and 0.90±0.012 g, *P<0.001* as compared to heart weight in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. showed more significant increase by (15.09%) in heart weight compared to individual compound TRIG 11.32% and SITA 13.20% (Figure 5.4.20.1).

Table 5.4.20.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on heart weight in diabetic cardiomyopathic rats

AVG of heart weight in diabetic cardiomyopathic rats	
Parameter / Groups	Heart weight (g)
ND	0.88±0.012
DC	1.06±0.049$^{###}$
TRIG (50mg/kg p.o.)	0.94±0.010**
SITA (5mg/kg p.o.)	0.92 ±0.010**
TRIG (50mg/kg p.o.) +SITA (5mg/kg p.o.)	0.90±0.012***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared with diabetic control, $^{###}P<0.001$ as compared with non diabetic.

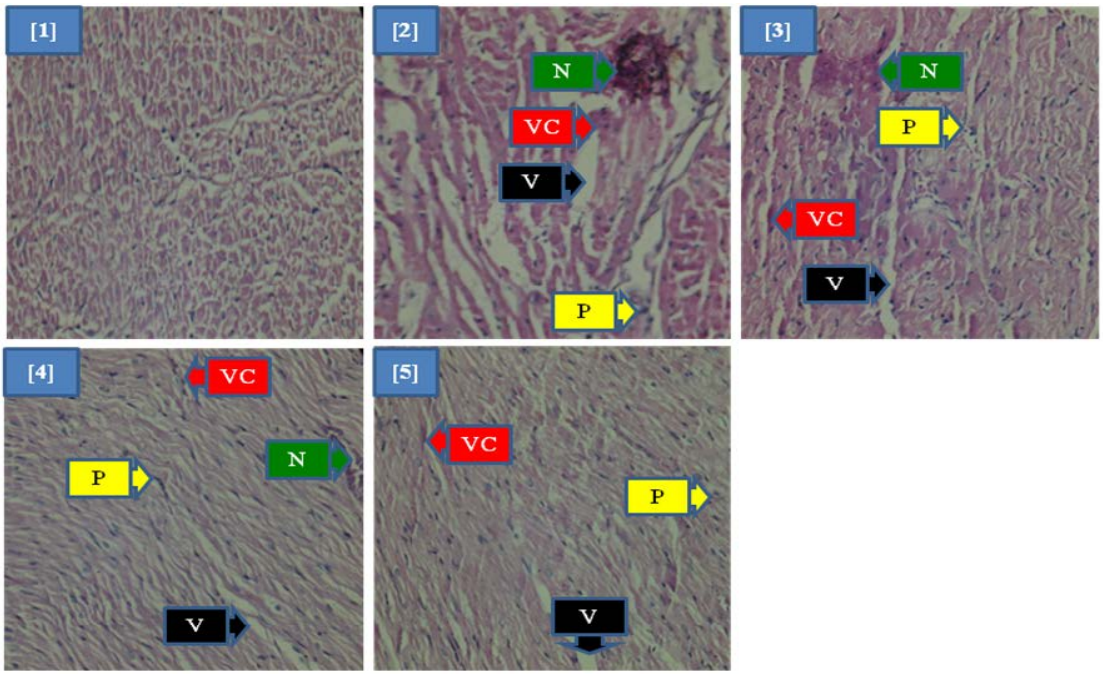


Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared with diabetic control, $^{###}P<0.001$ as compared with non diabetic.

Figure 5.4.20.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on heart weight in diabetic cardiomyopathic rats

5.4.21.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on histological examination of isolated heart in diabetic cardiomyopathic rats (H&E stain)

Histological examination of the rat heart section under light microscopy with Hematoxyline and eosin (H&E) stain were carried out on the last day of study period (last day of week 11). Histopathology of isolated rat heart of nondiabetic group showed (Grade -) normal architecture. Diabetic heart showed (Grade +++) necrosis, (Grade +++) pyknosis, (Grade +++) vascular congestion, (Grade +++) vacuolization; TRIG treated heart showed (Grade++) necrosis, (Grade ++) pyknosis, (Grade +) vascular congestion, (Grade ++) vacuolization; SITA showed (Grade++) necrosis, (Grade +) pyknosis, (Grade +) vascular congestion, (Grade ++) vacuolization; TRIG+SITA treated diabetic heart showed (Grade -) necrosis, (Grade +) pyknosis, (Grade +) vascular congestion, (Grade +) vacuolization. The scoring of grades was as per Dr. Chandrasekhar Mote (MVSC), Innovet Diagnostic Laboratory, Pune. Where (Grade -) normal; (Grade +++) severe; (Grade ++) moderate; (Grade +) mild (figure 5.4.21.1).



Where, 1] Non diabetic group; 2] Diabetic group; 3] TRIG (50 mg/kg p.o.); 4] SITA (5 mg/kg p.o.) and 5] TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.). P-pyknosis, N- necrosis, VC- vascular congestion, V-vacuolization [Magnification 40x].

Figure 5.4.21.1 Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on histological examination of isolated heart in diabetic cardiomyopathic rats (H&E stain)

Model 5.5.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET in NICO-STZ induced diabetic nephropathy in Wistar rats

5.5.1.1 Serum glucose level in non-diabetic rats

The results indicated non-significant fluctuation in serum glucose level compared to week 0 reading (Figure 5.5.1).

5.5.1.2 Serum glucose level in diabetic nephropathic rats

Serum glucose level before administration of NICO-STZ in this group was (99.30±5.29 mg/dl). After administration of NICO-STZ the serum glucose level increasing trend and at the end of 4 week after induction of diabetes the serum glucose (416.71±10.92 mg/dl). The vehicle used for dissolving the test compound was double distilled water which could not prevent the rise of serum glucose and at the end 4 weeks of treatment period the serum glucose level was 428.91±9.77 mg/dl (Figure 5.5.1).

5.5.1.3 Effect of MET (300 mg/kg p.o) on serum glucose level in diabetic nephropathic rats

In the MET (300 mg/kg p.o.) treated group the initial serum glucose level was (414.40±9.11 mg/dl) which was reduced to (207.58±14.62 mg/dl, $P< 0.001$) indicating a decrease of 206.92 mg/dl or 49.90 %. The onset of antihyperglycemic effect was evident after 3rd week of MET (300 mg/kg p.o.) treatment and continuation of treatment showed a trend toward decrease in serum glucose (Figure 5.5.1).

5.5.1.4 Effect of TRIG (50 mg/kg p.o) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o) on serum glucose level in diabetic nephropathic rats

Administration of triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) treated group the initial serum glucose level was (411.40±14.27 mg/dl) which was reduced to (179.39±7.84 mg/dl). This reduction of 232.01 mg/dl (56.39%) was more than the reduction in serum glucose by the individual drug. Early onset of serum glucose was observed at 2nd week (21.93 %) compared of 3rd week of onset in individual treatment group. The results thus indicated synergistic effect of triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) (Figure 5.5.1).

Table 5.5.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum glucose level in diabetic nephropathic rats

AVG of serum glucose levels (mg/dl) in diabetic nephropathy (8 weeks model)						
Groups	**Time in weeks**					
	NSGL	**After induction of diabetes**	**Treatment for four weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	90.95±5.90	95.89±2.28	97.50±1.15	99.83±5.67	101.46±3.84	103.63±3.40
DC	99.30±5.29	416.71±10.92 ###	419.81±8.75 ###	421.35±11.66 ###	425.45±14.94 ###	428.91±09.77 ###
MET (300 mg/kg p.o.)	93.19±7.21	414.40±9.11	375.74±14.74 *	344.19±12.51 ***	274.44±13.15 ***	207.58±14.62 ***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg	92.13±3.32	411.40±14.27	365.50±21.74 **	321.14±24.05 ***	234.58±16.19 ***	179.39±07.84 ***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

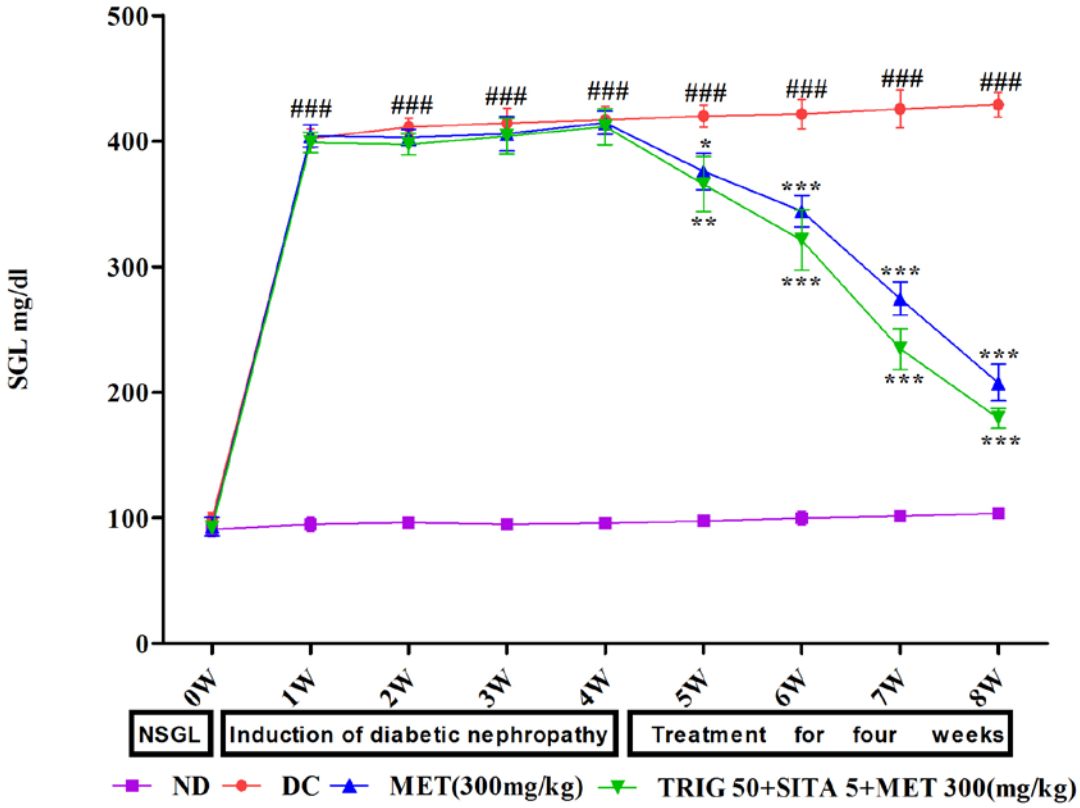


Figure 5.5.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum glucose level in diabetic nephropathic rats

5.5.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic nephropathic rats

5.5.2.1 Body weight in non-diabetic rats

The results indicated increase in body weight compare to week '0' (Figure 5.5.2).

5.5.2.2 Body weight in diabetic nephropathic rats

Before administration of NICO-STZ the body weight of the rat was 210.87±1.42g. Administration of NICO-STZ resulted in reduction body weight to 175.47±2.31g. This reduction of 35.00 g (16.78%) was due to the induction of diabetes. The animals appeared weak and polyuria (not recorded) was evident (Figure 5.5.2).

5.5.2.3 Effect of MET (300 mg/kg p.o) on body weight in diabetic nephropathic rats

The initial body weight of diabetic rats was (187.02±2.13g). After treatment with MET (300 mg/kg p.o.) body weight increase to (207.57±2.20 g). The rats showed increase in the body weight from the 1st week after MET (300 mg/kg p.o.) treatment and after 3rd and 4th week significant ($P< 0.001$) gain in body weight than the initial body weight was observed (Figure 5.5.2).

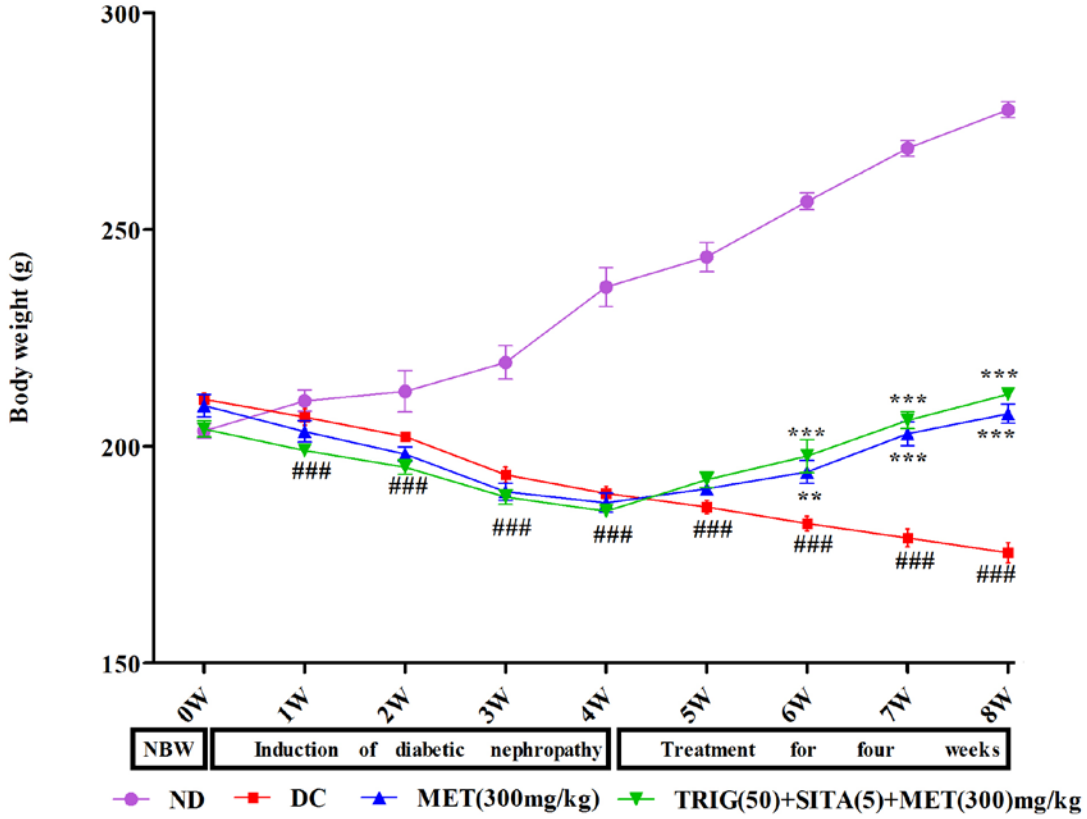
5.5.2.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on body weight in diabetic nephropathic rats

Administration of triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) was more effective than the individual compound because the gain in body weight after the treatment was more compared to that of individual drug. The treated diabetic animals showed improvement in health compared to diabetic rats (Figure 5.5.2).

Table 5.5.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic nephropathic rats

AVG of body weight (g) in diabetic nephropathy (8 weeks model)						
Groups	**Time in weeks**					
	NBW	**After induction of diabetes**	**Treatment for four weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	203.55±1.66	236.80±4.50	243.73±3.38	256.54±1.97	268.78±1.76	277.70±1.83
DC	210.87±1.42	189.19±1.55$^{###}$	186.02±1.52$^{###}$	182.20±1.70$^{###}$	178.90±2.09$^{###}$	175.47±2.31$^{###}$
MET (300 mg/kg p.o.)	209.40±2.56	187.02±2.13	190.26±1.41	194.10±2.64**	202.93±2.74***	207.57±2.20***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	204.02±1.88	185.08±1.53	192.32±1.82	197.78±3.82***	206.04±1.99***	212.07±1.17***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic.

Figure 5.5.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic nephropathic rats

5.5.3 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum creatinine level in diabetic nephropathic rats

5.5.3.1 Serum creatinine in non-diabetic rats

The results indicated no fluctuation in serum creatinine compare to week '0' reading (Figure 5.5.3).

5.5.3.2 Serum creatinine in diabetic nephropathic rats

Serum creatinine level before induction of diabetes was (0.64±0.14 mg/dl). After induction of diabetes with NICO-STZ the serum creatinine was (1.47±0.11mg/dl) on 4^{th} week and increase to (1.88±0.16 mg/dl) on 8^{th} week respectively. The increase in serum creatinine indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.5.3).

5.5.3.3 Effect of MET (300 mg/kg p.o) on serum creatinine level in diabetic nephropathic rats

Before administration of MET (300 mg/kg p.o.) the serum creatinine level in diabetic animals was (1.45±0.22 mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of MET (300 mg/kg p.o.) the serum creatinine level was (1.07±0.10 mg/dl) on 4 week of treatment. The reduction in serum creatinine by 0.38 mg/dl $P< 0.001$ (26.20%) was compared to diabetic animals. The results indicate that MET (300 mg/kg p.o.) treatment prevented the rise in serum creatinine level and thus at strong nephroprotective effect (Figure 5.5.3).

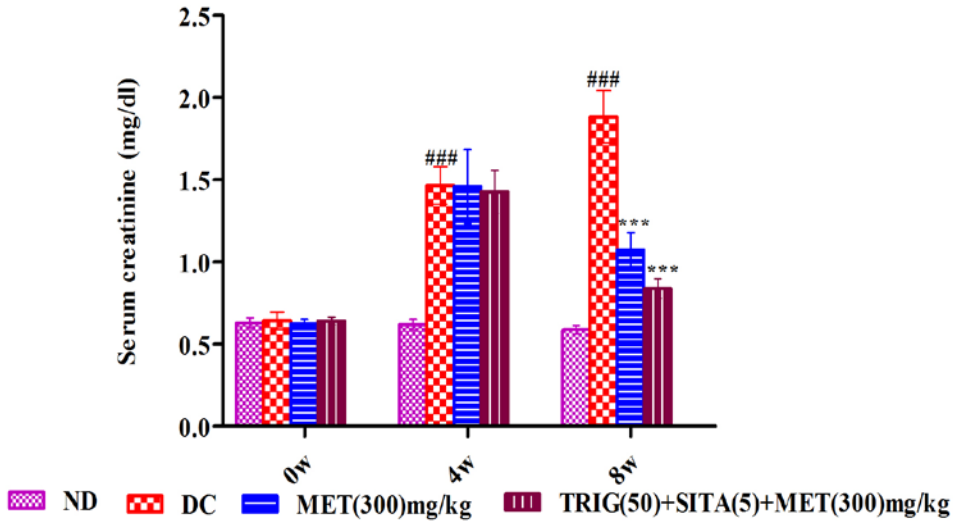
5.5.3.5 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on serum creatinine level in diabetic nephropathic rats

The serum creatinine level before administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) was (1.42±0.13mg/dl). After treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) the serum creatinine was (0.83±0.06 mg/dl). The reduction in serum creatinine by 0.59 mg/dl (41.54 %) was significant ($P< 0.001$) compared to that of treatment with individual compound. The results thus indicated that administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) treatment prevented the rise in serum creatinine level and thus at potent nephroprotective effect (Figure 5.5.3).

Table 5.5.3 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum creatinine level in diabetic nephropathic rats

AVG of serum creatinine level in diabetic nephropathic rats		
Parameter / Groups	Weeks	serum creatinine (mg/dl)
ND	0 4th 8th	0.63±0.03 0.62±0.03 0.59±0.02
DC	0 4th 8th	0.64±0.14^{ns} 1.47±0.11$^{###}$ 1.88±0.16$^{###}$
MET (300 mg/kg p.o.)	0 4th 8th	0.62±0.02 1.45±0.22 1.07±0.10***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	0 4th 8th	0.64±0.02 1.42±0.13 0.83±0.06***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.

Figure5.5.3 Effect of MET alone and triple drug therapy using Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum creatinine level in diabetic nephropathic rats

5.5.4 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum uric acid level in diabetic nephropathic rats

5.5.4.1 Determination of serum uric acid level in non-diabetic rats

The results indicated no major fluctuation in serum uric acid compare to week '0' (Figure 5.5.4).

5.5.4.2 Determination of serum uric acid level in diabetic nephropathic rats

Serum uric acid level before induction of diabetes was (2.03±0.18 mg/dl). After induction of diabetes with NICO-STZ the serum uric acid level was (4.85±0.31mg/dl) on 4^{th} week and increase to (5.15±0.22 mg/dl) on 8^{th} week respectively. The increase in serum uric acid level indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.5.4).

5.5.4.3 Effect of MET (300 mg/kg p.o.) on serum uric acid level in diabetic nephropathic rats

Before administration of MET (300 mg/kg p.o.) the serum uric acid level in diabetic animals was (4.76±0.41 mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of MET (300 mg/kg p.o.) the serum uric acid level was (3.06±0.26 mg/dl) on 4 week of treatment. The reduction in serum creatinine by 1.70 mg/dl, $P< 0.001$ (35.71 %) was compared to diabetic animals. The results indicate that MET (300 mg/kg p.o.) treatment prevented the rise in serum uric acid level and thus at mild nephroprotective effect (Figure 5.5.4).

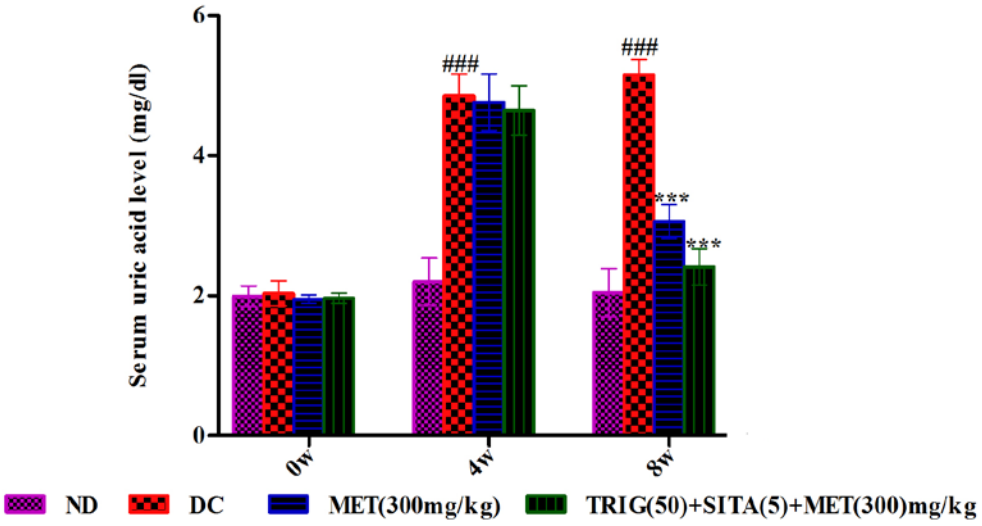
5.5.4.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on serum uric acid level in diabetic nephropathic rats

The serum uric acid level before administration of triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) was (4.65±0.35 mg/dl). After administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) the serum uric acid level was (2.41±0.26 mg/dl). The reduction in serum uric acid level by 2.24 mg/dl (48.17 %) was significant ($P< 0.001$) compared to that of treatment with individual compound. The results thus indicated that the administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treatment prevented the rise in serum uric acid level and thus at potent nephroprotective effect (Figure 5.5.4).

Table 5.5.4 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum uric acid level in diabetic nephropathic rats

AVG of serum uric acid level in diabetic nephropathic rats		
Parameter / Groups	Weeks	uric acid (mg/dl)
ND	0 4th 8th	1.99±0.14 2.20±0.34 2.05±0.35
DC	0 4th 8th	2.03±0.18 ns 4.85±0.31 ### 5.15±0.22 ###
MET (300mg/kg p.o.)	0 4th 8th	1.95±0.06 4.76±0.41 3.06±0.24 ***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	0 4th 8th	1.96±0.07 4.65±0.35 2.41±0.26 ***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.5.4 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum uric acid level in diabetic nephropathic rats

5.5.5 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum blood urea nitrogen (BUN) level in diabetic nephropathic rats

5.5.5.1 BUN level in non-diabetic rats

The results indicated no major fluctuation in BUN level compare to week '0' (Figure 5.5.5).

5.2.5.2 BUN level in diabetic nephropathic rats

BUN level before induction of diabetes was (16.67±0.56 mg/dl). After induction of diabetes with NICO-STZ the BUN level was (37.58±2.77mg/dl) on 4^{th} week and increase to (41.70±2.48 mg/dl) on 8^{th} week respectively. The increase in BUN level indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.5.5).

5.2.5.4 Effect of MET (300 mg/kg p.o.) on BUN level in diabetic nephropathic rats

Before administration of MET (300 mg/kg p.o.) the BUN level in diabetic animals was (36.21±1.94 mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of MET (300 mg/kg p.o.) the BUN level was (21.65±2.36 mg/dl) on 4 week of treatment. The reduction in BUN level by 14.56 mg/dl, $P< 0.001$ (40.20 %) was compared to diabetic animals. The results indicate that MET (300 mg/kg p.o.) treatment prevented the rise in BUN level and thus at strong nephroprotective effect (Figure 5.5.5).

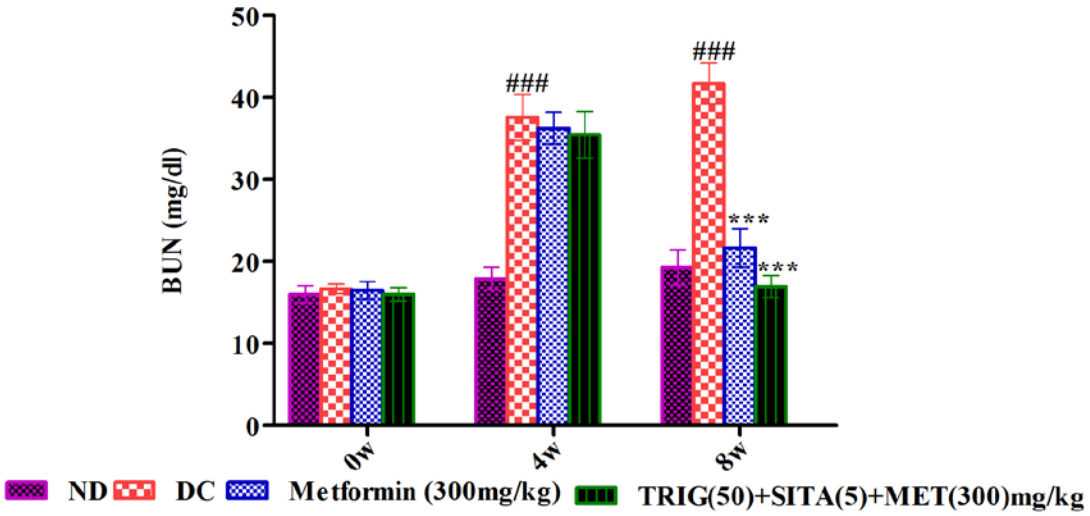
5.2.5.5 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on blood urea nitrogen (BUN) level in diabetic nephropathic rats

The BUN level before administration of triple drug therapy using of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) was (35.45±2.83 mg/dl). After administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) the BUN level was (16.92±1.37 mg/dl). The reduction in BUN level by 18.53 mg/dl (52.27%) was significant ($P< 0.001$) compared to that of treatment with individual compound. The results thus indicated that administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treatment prevented the rise in BUN level and thus at potent nephroprotective effect (Figure 5.5.5).

Table 5.5.5 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum blood urea nitrogen (BUN) level in diabetic nephropathic rats

AVG of blood urea nitrogen (BUN) level in diabetic nephropathic rats		
Parameter / Groups	**Weeks**	**BUN (mg/dl)**
ND	0 4th 8th	15.98±1.05 17.88±1.36 19.24±2.14
DC	0 4th 8th	16.67±0.56 ns 37.58±2.77### 41.70±2.48###
MET (300mg/kg p.o.)	0 4th 8th	16.46±1.09 36.21±1.94 21.65±2.36***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	0 4th 8th	15.98±0.85 35.45±2.83 16.92±1.37***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.5.5 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum blood urea nitrogen (BUN) level in diabetic nephropathic rats

5.5.6 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on urine creatinine level in diabetic nephropathic rats

5.5.6.1 Urine creatinine level in non-diabetic rats

The results indicated no major fluctuation in urine creatinine level compare to week '0' (Figure 5.5.6).

5.5.6.2 Urine creatinine level in diabetic nephropathic rats

Urine creatinine level before induction of diabetes was (59.61±1.92 mg/dl). After induction of diabetes with NICO-STZ the urine creatinine level was (24.46±1.83 mg/dl) on 4^{th} week and decrease to (14.35±0.71 mg/dl) on 8^{th} week respectively. The decrease in urine creatinine level indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.5.6).

5.5.6.3 Effect of MET (300mg/kg p.o) on urine creatinine level in diabetic nephropathic rats

Before administration of MET (300 mg/kg p.o.) the urine creatinine level in diabetic animals was (25.08±2.64 mg/dl) which indicated initiation of nephropathy in the diabetic rats. After treatment of MET (300 mg/kg p.o.) the urine creatinine level was (34.72±3.85 P< 0.001 mg/dl) on 4 week of treatment. The increase in urine creatinine level by 9.64 mg/dl (27.76%) was compared to diabetic animals. The results indicate that MET (300 mg/kg p.o.) treatment prevented the decrease in urine creatinine level and thus at strong nephroprotective effect (Figure 5.5.6).

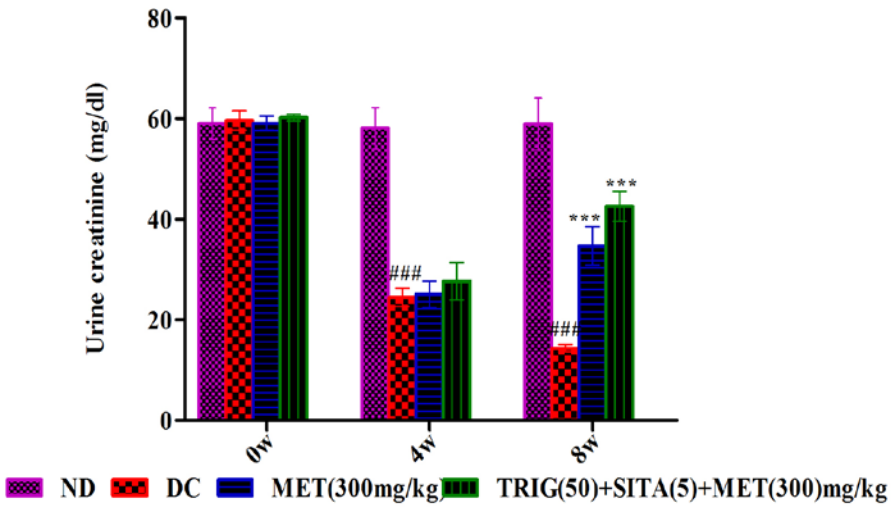
5.5.6.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on urine creatinine level diabetic nephropathic rats

The urine creatinine level before triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) was (27.70±3.76 mg/dl). After administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) the urine creatinine level was (42.59±2.98 mg/dl). The increase in urine creatinine level by 14.89 mg/dl (34.96%) was significant (*P< 0.001*) compared to that of treatment with individual compound. The results thus indicated that administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treatment prevented the decrease in urine creatinine level and thus at potent nephroprotective effect (Figure 5.5.6).

Table 5.5.6 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on urine creatinine level in diabetic nephropathic rats

AVG of urine creatinine level in diabetic nephropathic rats		
Parameter / Groups	Weeks	urine creatinine (mg/dl)
ND	0 4th 8th	59.02±3.10 58.10±4.15 58.95±5.20
DC	0 4th 8th	59.61±1.92^{ns} 24.46±1.83$^{###}$ 14.35±0.71$^{###}$
MET (300mg/kg p.o.)	0 4th 8th	59.12±1.42 25.08±2.64 34.72±3.85***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	0 4th 8th	60.19±0.64 27.70±3.76 42.59±2.98***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.

Figure 5.5.6 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on urine creatinine level in diabetic nephropathic rats

5.5.7 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on urine volume in diabetic nephropathic rats

5.5.7.1 Urine volume in non-diabetic rats

The results indicated no major fluctuation in urine volume compare to week '0' (Figure 5.5.7).

5.5.7.2 Urine volume in diabetic nephropathic rats

Urine volume before induction of diabetes was (11.02±2.17 ml). After induction of diabetes with NICO-STZ the urine volume was (53.05±2.90 ml) on 4^{th} week and increase to (57.57±2.27 ml) on 8^{th} week respectively. The increase in urine volume indicates development of nephropathy in the diabetic rats. The results also indicate that requires 4 weeks of diabetic condition before nephropathy begins in the rats (Figure 5.5.7).

5.5.7.3 Effect of MET (300mg/kg p.o) on urine volume in diabetic nephropathic rats

Before administration of MET (300 mg/kg p.o.) the urine volume in diabetic animals was (52.58±3.23 ml) which indicated initiation of nephropathy in the diabetic rats. After treatment of MET (300 mg/kg p.o.) the urine volume was (31.04±1.05 ml, $P< 0.001$) on 4 week of treatment. The reduction in urine volume by 21.54 ml (40.96 %) was compared to diabetic animals. The results indicate that MET (300 mg/kg p.o.) treatment prevented the rise in urine volume and thus at mild nephroprotective effect (Figure 5.5.7).

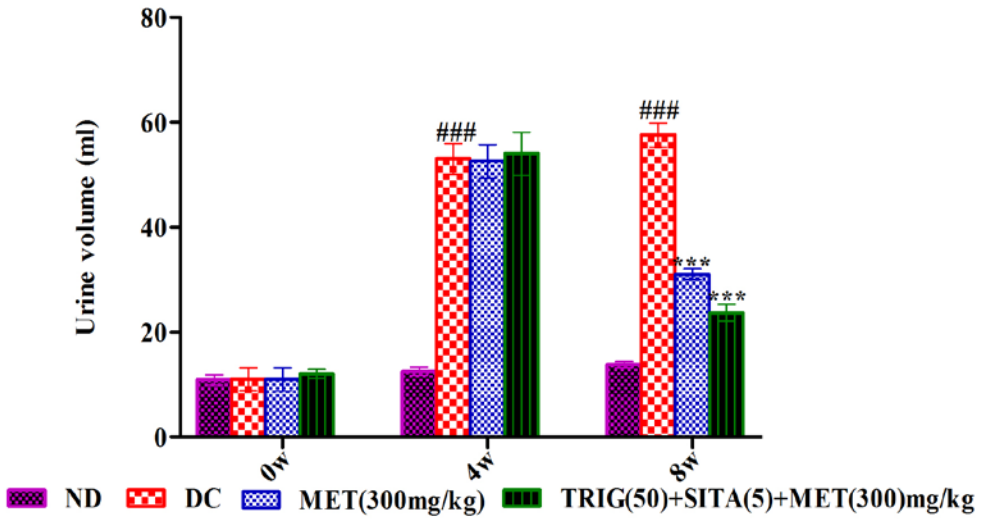
5.5.7.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on urine volume in diabetic nephropathic rats

The urine volume before administration of triple therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg *p.o.*) + MET (300 mg/kg) was (54.04±4.08 ml). After administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg *p.o.*) + MET (300 mg/kg p.o) the urine volume was (23.70±1.65 ml). The reduction in urine volume by 30.34 ml (56.14 %) was significant ($P< 0.001$) compared to that of treatment with individual compound. The results thus indicated that administration of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o) treatment prevented the rise in urine volume and thus at potent nephroprotective effect (Figure 5.5.7).

Table 5.5.7 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on urine volume in diabetic nephropathic rats

AVG of urine volume in diabetic nephropathic rats		
Parameter / Groups	Weeks	urine volume (ml)
ND	0 4th 8th	10.91±0.95 12.51±0.80 13.79±0.61
DC	0 4th 8th	11.02±2.17^{ns} 53.05±2.90$^{###}$ 57.57±2.27$^{###}$
MET (300mg/kg p.o.)	0 4th 8th	11.01±2.20 52.58±3.23 31.04±1.05***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	0 4th 8th	12.04±0.85 54.04±4.08 23.70±1.65***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.5.7 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on urine volume in diabetic nephropathic rats

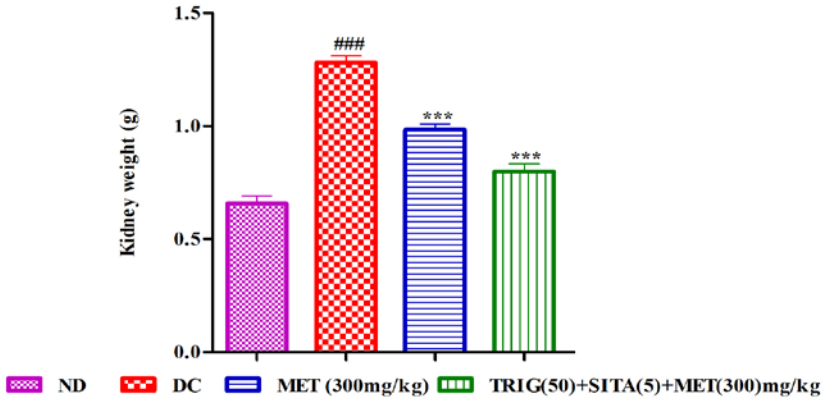
5.5.8 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on kidney weight in diabetic nephropathic rats

At the end day of treatment (week 8) kidney weight in non-diabetic rat was (0.65±0.033g). On other hand where diabetic rat showed significant (1.28±0.030g, *P< 0.001*) increase in kidney weight as compared to non-diabetic kidney. Significant (*P< 0.001*) decrease in kidney weight was measured on last day of study period (end of week 8) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 0.98±0.026g, *P< 0.001* and 0.79±0.034g, *P< 0.001* as compared to diabetic rat kidney. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o.+ MET 300 mg/kg p.o. showed more significant decrease in kidney weight by (38.28%) compared to individual compound MET 23.43 % (Figure 5.5.8).

Table 5.5.8 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on kidney weight in diabetic nephropathic rats

AVG of kidney Weight in diabetic nephropathic rats	
Parameter / Groups	**Kidney Weight (g)**
ND	0.65±0.033
DC	1.28±0.030$^{###}$
MET (300mg/kg p.o.)	0.98±0.026***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	0.79±0.034***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.5.8 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on kidney weight in diabetic nephropathic rats

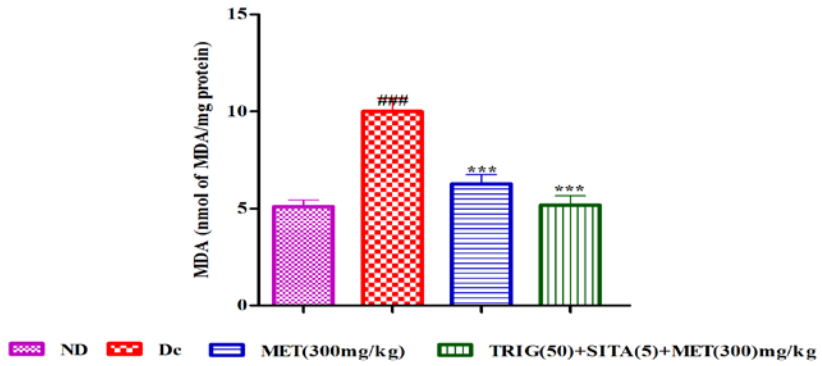
5.5.9 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on malondialdehyde (MDA) level in diabetic nephropathic rat kidney tissue

The level of MDA in non-diabetic rat kidney was (5.094±0.35 nmol of MDA/mg protein). On other hand where diabetic rat kidney showed significant (9.997±0.70 nmol of MDA/mg protein, *P< 0.001*) increase in MDA level as compared to non-diabetic kidney. Significant decrease in MDA level was measured on last day of study period (end of week 8) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.), 6.256±0.48, *P<0.001* and 5.180±0.479, *P<0.001* nmol of MDA/mg protein as compared to MDA level in diabetic rat kidney. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o.+ MET 300 mg/kg p.o. showed more significant decrease by (48.18%) in MDA level compared to individual compound MET 37.42% (Figure 5.5.9).

Table 5.5.9 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on malondialdehyde (MDA) level in diabetic nephropathic rat kidney tissue

AVG of malondialdehyde (MDA) level in diabetic nephropathic rats	
Parameter / **Groups**	**MDA** (nmol of MDA/mg protein)
ND	5.094±0.35
DC	9.997±0.70$^{###}$
MET (300mg/kg p.o.)	6.256±0.48***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	5.180±0.479***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.5.9 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on malondialdehyde (MDA) level in diabetic nephropathic rat kidney tissue

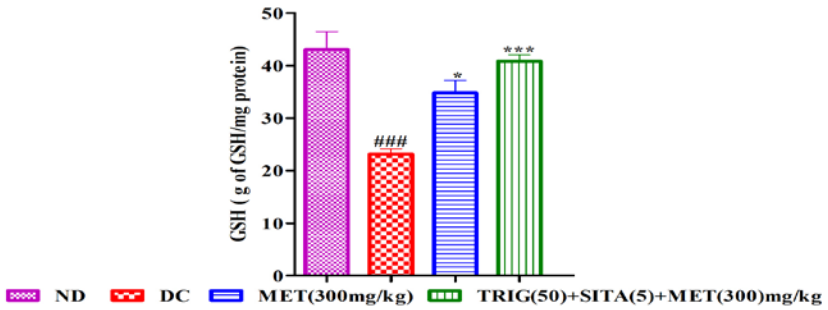
5.5.10 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on reduced glutathione (GSH) level in diabetic nephropathic rat kidney tissue

The level of GSH in non-diabetic rat kidney was (43.04±3.38g of GSH/mg protein). On other hand where diabetic rat kidney showed significant (23.10±1.02g of GSH/mg protein, *P< 0.001*) decrease in GSH level as compared to non-diabetic rat kidney. Significant increase in GSH level was measured on last day of study period (end of week 8) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) 34.77±2.42, *P<0.05* and 40.85±1.18, *P<0.001* g of GSH/mg protein as compared to GSH level in diabetic rat kidney. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300 mg/kg p.o. showed more significant increase by (43.45%) in GSH level compared to individual compound MET 33.56 % (Figure 5.5.10).

Table 5.5.10 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on reduced glutathione (GSH) level in diabetic nephropathic rat kidney tissue

AVG of reduced glutathione (GSH) level in diabetic nephropathic rats	
Parameter / Groups	**GSH** (g of GSH/mg protein)
ND	43.04±3.38
DC	23.10±1.02$^{###}$
MET (300mg/kg p.o.)	34.77±2.42*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	40.85±1.18***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.5.10 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on reduced glutathione (GSH) level in diabetic nephropathic rat kidney tissue

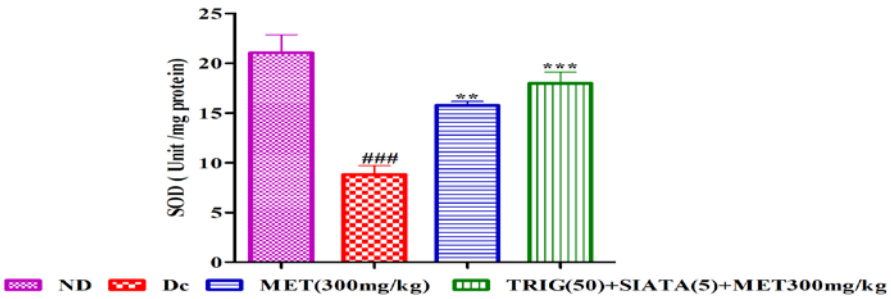
5.5.11 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on superoxide dismutase (SOD) level in diabetic nephropathic rat kidney tissue

The level of SOD in non-diabetic rat kidney was (21.05±1.80 Unit /mg protein). On other hand where diabetic rat kidney showed significant (8.80±0.89 Unit /mg protein, *P< 0.001*) decrease in SOD level as compared to non-diabetic rat kidney. Significant increase in SOD level was measured on last day of study period (end of week 8) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) 15.76±0.38, *P<0.01* and 17.98±1.11, *P<0.001* Unit /mg protein as compared to SOD level in diabetic rat kidney. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300 mg/kg p.o. showed more significant increase by (51.05 %) in GSH level compared to individual compound MET 44.16 % (Figure 5.5.11).

Table 5.5.11 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on superoxide dismutase (SOD) level in diabetic nephropathic rat kidney tissue

AVG of superoxide dismutase (SOD) level in diabetic nephropathic rats	
Parameter / Groups	**SOD** (Unit /mg protein)
ND	21.05±1.80
DC	8.80±0.89$^{###}$
MET (300mg/kg p.o.)	15.76±0.38**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	17.98±1.11***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

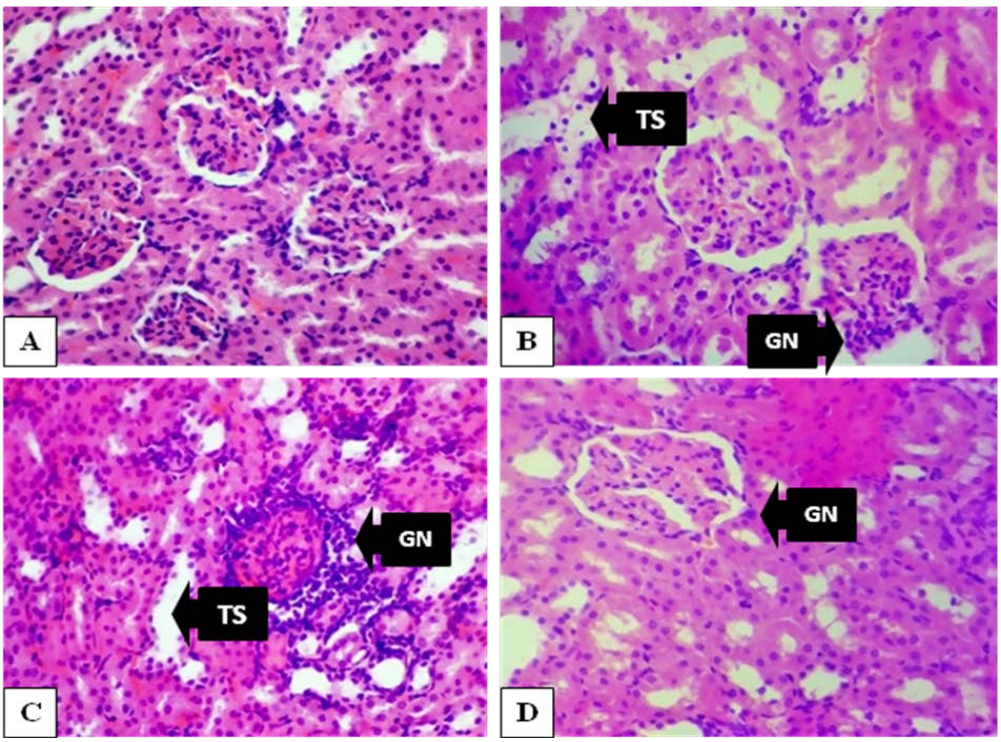


Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.5.11 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on superoxide dismutase (SOD) level in diabetic nephropathic rat kidney tissue

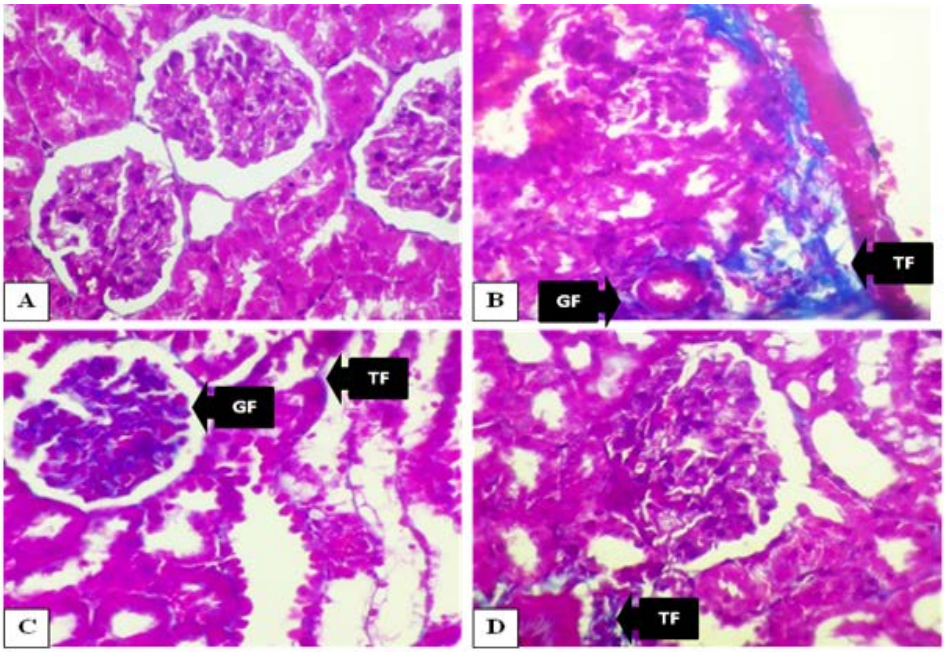
5.5.12 Histopathological examination of isolated renal tissue in diabetic nephropathic rats (H&E stain and MT stain)

After eight weeks of study examination of renal sections stained with hematoxylin & eosin (H&E) and Masson's trichome (MT). Histopathology of isolated kidney of non diabetic rat showed (Grade -) normal architecture; Diabetic rat kidney showed (Grade +++) glomerular necrosis, (Grade +++) tubular swelling, (Grade +++) glomerular fibrosis, (Grade +++) peritubular fibrosis; MET treated rat kidney showed (Grade +) glomerular necrosis, (Grade +) tubular swelling, (Grade +) glomerular fibrosis, (Grade +) peritubular fibrosis; TRIG+SITA+MET showed (Grade +) glomerular necrosis, (Grade -) tubular swelling, (Grade -) glomerular fibrosis, (Grade +) peritubular fibrosis. The scoring of grades was as per Dr. Pralhad Wangikar (MVSC), Prado- Preclinical Research and Development Organization, Pune. Where (Grade -) normal; (Grade +++) severe; (Grade ++) moderate; (Grade +) mild (Figure 5.5.12.1 for H&E stain and 5.5.12.2 for MT stain).



Where, A] Non diabetic; B] Diabetic group; C] MET (300 mg/kg p.o.); D] TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.). GN- Glomerular necrosis; TS- Tubular swelling. [Magnification 40x].

Figure 5.5.12.1 Histopathological examination of isolated renal tissue in diabetic nephropathic rats (H&E stain)



Where, A] Non diabetic; B] Diabetic group; C] MET (300 mg/kg p.o.); D] TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.)+ MET (300 mg/kg p.o.). GF-glomerular fibrosis, TF- peritubular swelling. [Magnification 40x].

Figure 5.5.12.2 Histopathological examination of isolated renal tissue in diabetic nephropathic rats (MT stain)

Model 5.6.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET in NICO-STZ induced diabetic neuropathy in Wistar rats

5.6.1.1 Serum glucose level in non-diabetic rats

The results indicated non-significant fluctuation in serum glucose level (Figure 5.6.1).

5.6.1.2 Serum glucose level in diabetic neuropathic rats

Serum glucose level before administration of NICO-STZ in this group was (97.29±4.81 mg/dl). After administration of NICO-STZ the serum glucose level increasing trend and at the end of 4 week after induction of diabetes the serum glucose (428.36±13.22 mg/dl). The vehicle used for dissolving the test compound was double distilled water which could not prevent the rise of serum glucose and at the end 4 weeks of treatment period the serum glucose level was 434.43±17.74 mg/dl (Figure 5.6.1).

5.6.1.3 Effect of MET (300 mg/kg p.o) on serum glucose level in diabetic neuropathic rats

In the MET (300 mg/kg p.o.) treated group the initial serum glucose level was (425.37±8.59 mg/dl) which was reduced to (212.22±15.13 mg/dl, $P< 0.001$) indicating a decrease of 213.15 mg/dl or 50.10 %. The onset of antihyperglycemic effect was evident after 3 week of MET (300 mg/kg p.o.) treatment and continuation of treatment showed a trend toward decrease in serum glucose (Figure 5.6.1).

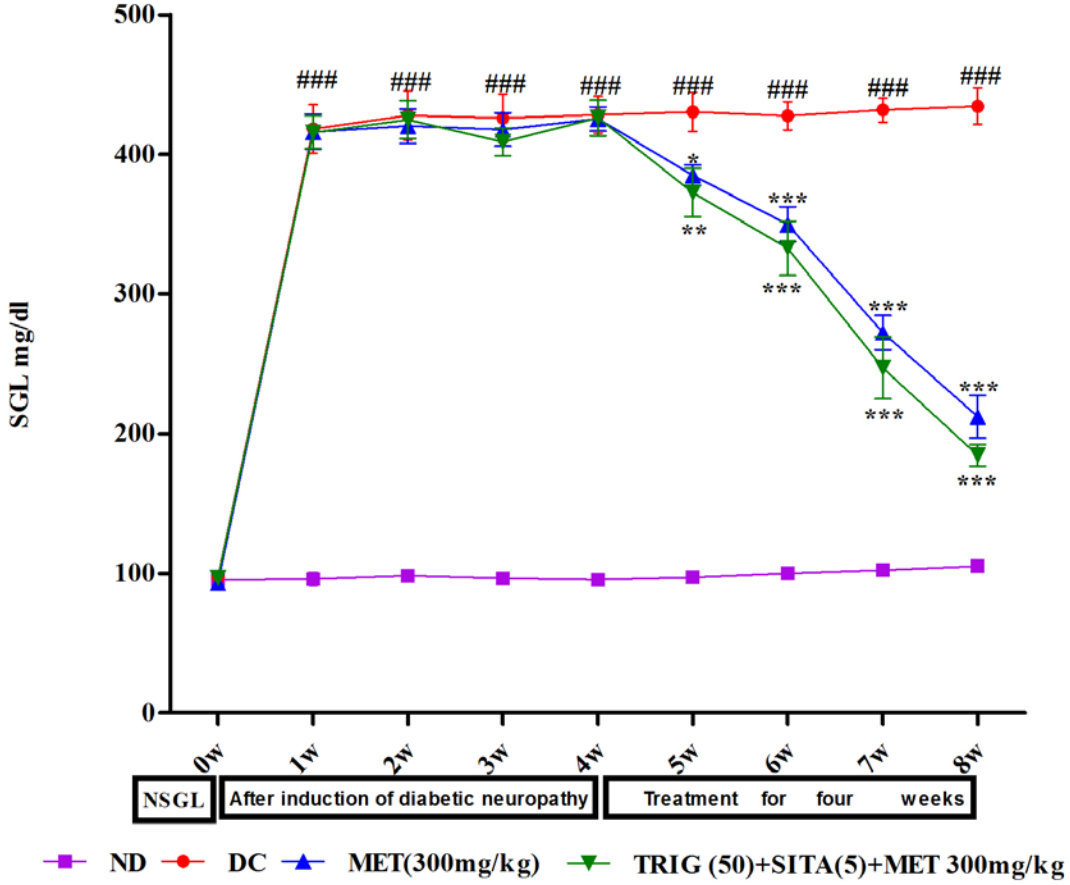
5.6.1.4 Effect of triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5mg/kg p.o.) + MET (300 mg/kg p.o.) on serum glucose level in diabetic neuropathic rats

Triple therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treated group the initial serum glucose level was (426.03±12.79 mg/dl) which was reduced to (184.55±7.69 mg/dl). This reduction of 241.48 mg/dl (56.68 %) was more than the reduction in serum glucose by the individual drug. Early onset of serum glucose was observed at 2^{nd} week (21.90%) compared of 3^{rd} week of onset in individual treatment group. The results thus indicated synergistic effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treatment and continuation of treatment showed a trend toward decrease in serum glucose (Figure 5.6.1).

Table 5.6.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum glucose level in diabetic neuropathic rats

AVG of Serum glucose levels (mg/dl) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	NSGL	**After induction of diabetes**	**Treatment for four weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	95.28±6.50	95.65±2.27	97.31±2.30	100.04±2.12	102.30±2.35	105.22±2.14
DC	97.29±4.81	428.36±13.22###	430.46±14.06###	427.51±10.02###	431.76±08.55###	434.43±17.74###
MET (300mg/kg p.o.)	93.53±5.03	425.37±8.59	384.84±7.26*	349.92±12.52***	272.34±12.41***	212.22±15.13***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	96.72±2.43	426.03±12.79	372.57±17.44**	332.71±19.45***	247.10±22.00***	184.55±7.69***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic.

Figure 5.6.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum glucose level in diabetic neuropathic rats

5.6.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic neuropathic rats

5.6.2.1 Body weight in non-diabetic rats

The results indicated increase in body weight compare to week '0' (Figure 5.6.2).

5.6.2.2 Body weight in diabetic neuropathic rats

Before administration of NICO-STZ the body weight of the rat was 205.43±1.51g. Administration of NICO-STZ resulted in reduction body weight to 162.13±2.46g. This reduction of 43.3 g (21.10%) was due to the induction of diabetes. The animals appeared weak and polyuria (not recorded) was evident (Figure 5.6.2).

5.6.2.3 Effect of MET (300mg/kg p.o) on body weight in diabetic neuropathic rats

Before administration of MET (300 mg/kg p.o.) in diabetic rats the body weight was 174.63±2.27 g. After treatment with MET (300 mg/kg p.o.) the body weight was 204.10±2.20 g. The results indicated that TRIG treatment arrested the loss of body weight in the diabetic rats. The observed effect appears to be due to control of the hyperglycemia. The rats showed increase in the body weight from the 1st week after MET (300 mg/kg p.o.) treatment and after 3rd and 4th week significant (*P< 0.001*) gain in body weight than the initial body weight was observed (Figure 5.6.2).

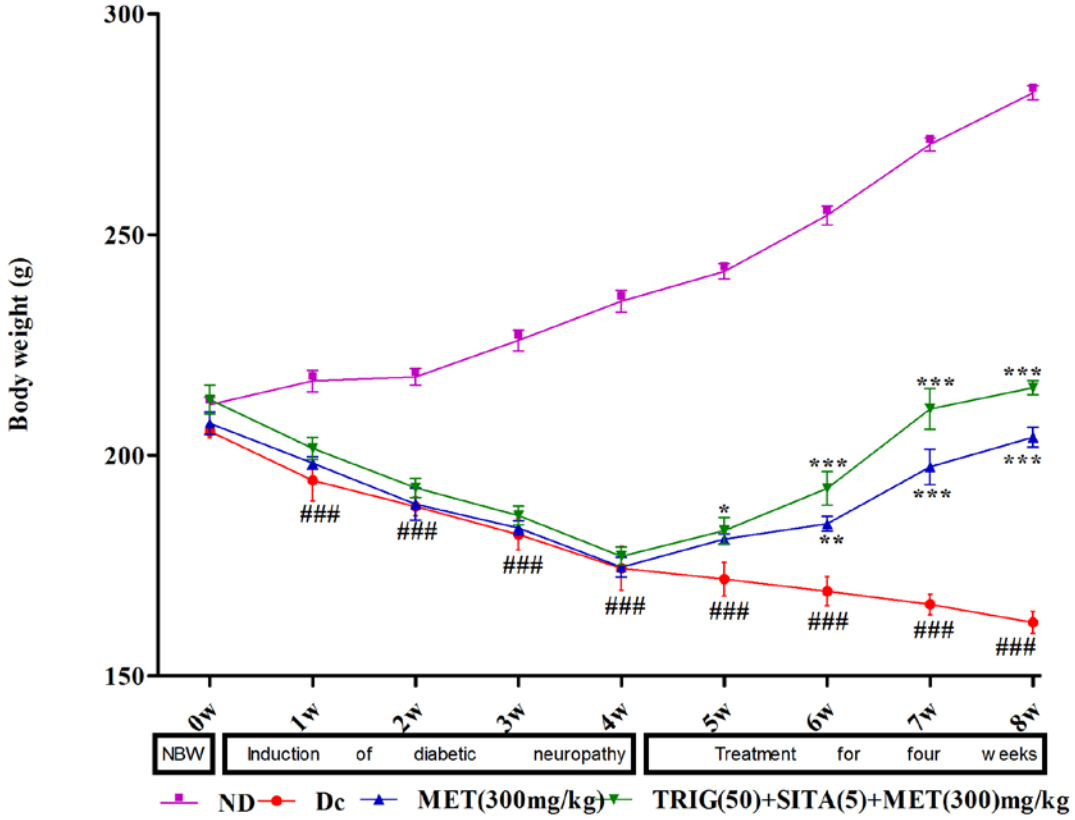
5.6.2.4 Effect TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on body weight in diabetic neuropathic rats

Triple therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.) was more effective than the individual compound because the gain in body weight after the treatment was more compared to that of individual drug. The treated diabetic animals showed improvement in health compared to diabetic rats (Figure 5.6.2).

Table 5.6.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic neuropathic rats

AVG of body weight (g) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Normal Body weight	**After induction of diabetes**	**Treatment for four weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	211.48±1.63	234.91±2.51	241.69±1.75	254.40±2.12	270.46±1.48	282.16±1.60
DC	205.43±1.51	174.38±4.93###	171.97±3.81###	169.16±3.28###	166.20±2.32###	162.13±2.46###
MET (300mg/kg p.o.)	207.24±2.60	174.63±2.27	181.02±1.23	184.48±1.68**	197.40±3.98***	204.10±2.20***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.) + MET (300 mg/kg p.o.)	212.64±3.32	177.11±2.09	182.91±3.00*	192.50±3.81***	210.52±4.64***	215.34±1.57***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.6.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic neuropathic rats

5.6.3 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

5.6.3.1 Thermal hyperalgesia in non-diabetic rats

The results indicated no major change in paw withdrawal latency compare to week '0' (Figure 5.6.3).

5.6.3.2 Thermal hyperalgesia in diabetic neuropathic rats

Paw withdrawal latency before administration of NICO-STZ in this group was 10.43±0.40 (in sec). After administration of NICO-STZ the paw withdrawal latency decreasing trend and at the end of 4 week after induction of diabetes the paw withdrawal latency was 2.91±0.31 (in sec). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of paw withdrawal latency. And at the end 4 weeks of treatment period the paw withdrawal latency level was 2.52±0.20 (in sec) (Figure 5.6.3).

5.6.3.3 Effect of MET (300 mg/kg p.o) on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

In the MET (300 mg/kg p.o.) treated group the initial paw withdrawal latency was (2.89±0.40 (in sec) which was increased to (5.74±0.63 (in sec), $P< 0.001$) indicating a increase of 2.85 (in sec) or 49.65 %. The onset of neuroprotective effect was evident after 2 week of MET (300 mg/kg p.o.) treatment and continuation of treatment showed a trend toward increase in paw withdrawal latency (Figure 5.6.3).

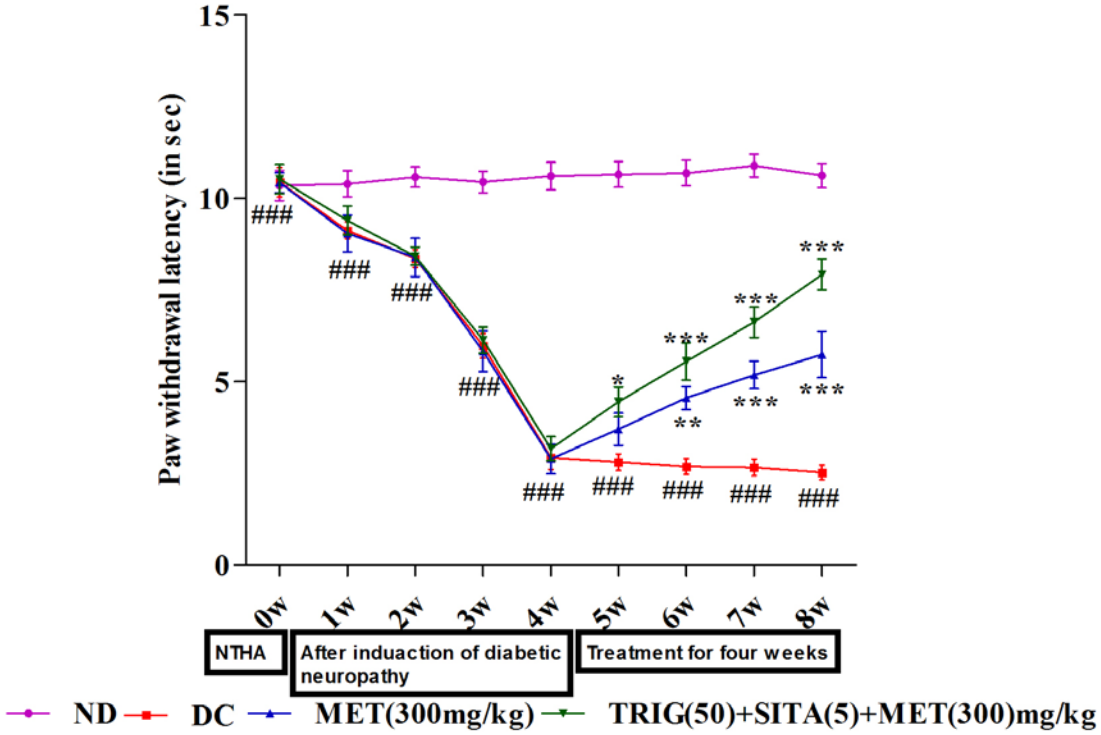
5.6.3.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

Triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treated group the initial paw withdrawal latency was (3.17±0.33 (in sec) which was increased to (7.92±0.42 (in sec). This increase of 4.75 in sec (59.97 %) was more than the increase in paw withdrawal latency by the individual drug. Early onset of paw withdrawal latency was observed at 1st week (28.60%) compared of 2nd week of onset in individual treatment group. The results thus indicated synergistic effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) (Figure 5.6.3).

Table 5.6.3 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

AVG of thermal hyperalgesia (radiant heat test) (in sec) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Normal thermal hyperalgesia	**After Induction of Diabetes**	**Treatment for four weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	10.34±0.41	10.60±0.37	10.65±0.35	10.68±0.35	10.89±0.31	10.62±0.33
DC	10.43±0.40	2.91±0.31$^{###}$	2.80±0.22$^{###}$	2.68±0.22$^{###}$	2.66±0.23$^{###}$	2.52±0.20$^{###}$
MET (300mg/kg p.o.)	10.42±0.27	2.89±0.40	3.70±0.44	4.55±0.32**	5.18±0.38***	5.74±0.63***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.	10.52±0.40	3.17±0.33	4.44±0.41*	5.55±0.51***	6.62±0.41***	7.92±0.42***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.6.3 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on thermal hyperalgesia (radiant heat test) in diabetic neuropathic rats

5.6.4 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

5.6.4.1 Mechanical hyperalgesia (Randall Selitto paw pressure test) in non-diabetic rats

The results indicated no major change in paw withdrawal threshold compare to week '0' (Figure 5.6.4).

5.6.4.2 Mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

Paw withdrawal threshold before administration of NICO-STZ in this group was 286.05±948 (in g). After administration of NICO-STZ the paw withdrawal threshold decreasing trend and at the end of 4 week after induction of diabetes the paw withdrawal threshold 115.87±10.25 (in g). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of paw withdrawal threshold and at the end 4 weeks of treatment period the paw withdrawal threshold was 127.05±12.54 in g (Figure 5.6.4).

5.6.4.3 Effect of MET (300 mg/kg p.o) on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

In the MET (300 mg/kg p.o.) treated group the initial paw withdrawal threshold was 105.72±5.64 (in g) which was increased to 253.20±8.26 (in g), $P< 0.001$ indicating a increase of 147.48 (in g) or 58.24 %. The onset of neuroprotective effect was evident after 1st week of MET (300 mg/kg p.o.) treatment and continuation of treatment showed a trend toward increase in paw withdrawal threshold (Figure 5.6.4).

5.6.4.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

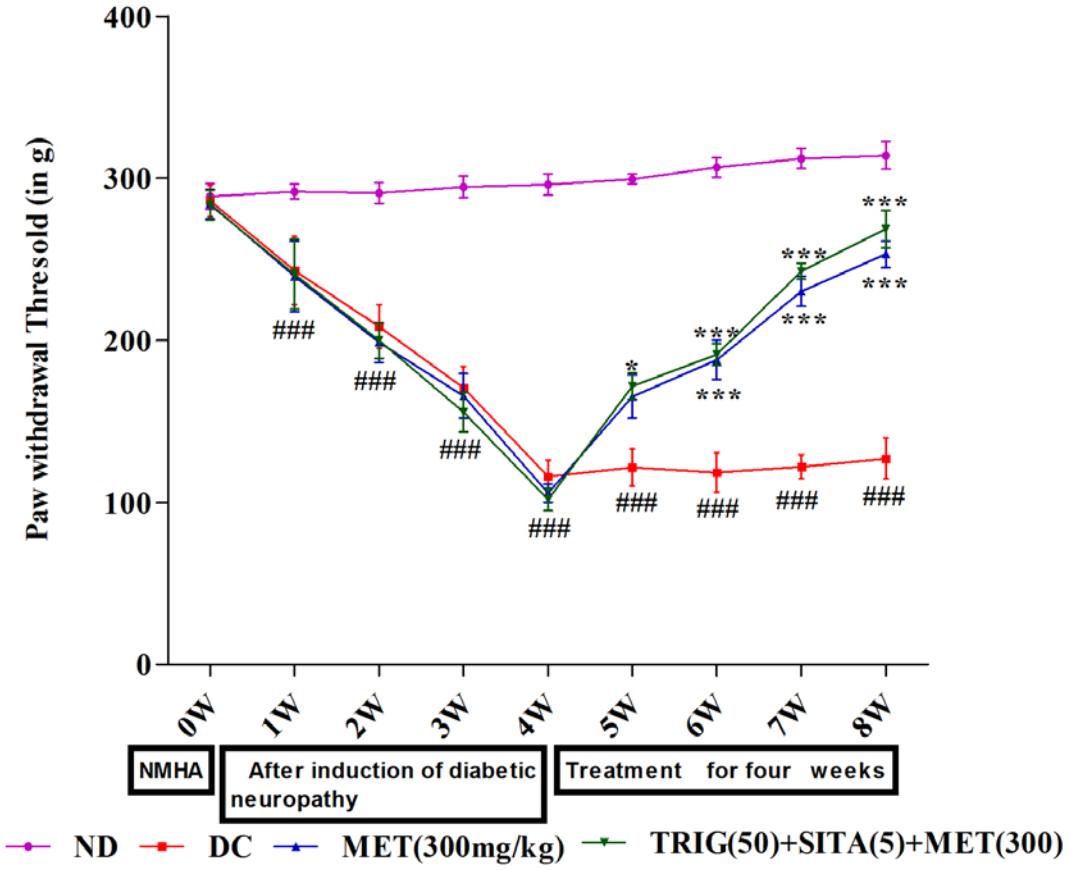
Triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg) treated group the initial paw withdrawal threshold was (101.82±6.94 (in g) which was increased to (268.55±11.53 (in g), $P< 0.001$. This increase of 166.73 in g (62.08 %) was more than the increase in paw withdrawal threshold by the individual drug. Early onset of paw withdrawal threshold was observed at 1st week (40.69%) compared of 1st week of onset in individual treatment group. The results thus

indicated synergistic effect of treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) (Figure 5.6.4).

Table 5.6.4 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

AVG of mechanical hyperalgesia (Randall Selitto paw pressure test) (in g) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Normal Mechanic-al Hyperalg sia	**After Induaction of diabetes**	**Treatment for four weeks**			
	0	**4**	**5**	**6**	**7**	**8**
ND	288.85±7.99	296.15±6.52	299.50±2.92	306.70±6.11	312.10±6.24	314.05±8.67
DC	286.05±948	115.87±10.25$^{###}$	121.65±11.42$^{###}$	118.55±12.18$^{###}$	122.10±7.42$^{###}$	127.05±12.54$^{###}$
MET (300mg/kg p.o.)	284.15±8.98	105.72±5.64	165.25±13.29	187.95±12.13***	230.25±9.29***	253.20±8.26***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET 300 mg/kg p.o.	283.55±9.19	101.82±6.94	171.70±8.13*	191.35±6.74***	242.65±4.92***	268.55±11.53***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.6.4 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mechanical hyperalgesia (Randall Selitto paw pressure test) in diabetic neuropathic rats

5.6.5 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

5.6.5.1 Mechano-tactile allodynia (Von-Frey hair test) in non-diabetic rats

The results indicated no major change in paw withdrawal threshold compare to week '0' (Figure 5.6.5).

5.6.5.2 Mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

Paw withdrawal threshold before administration of NICO-STZ in this group was 73.01±2.68 (in g). After administration of NICO-STZ the paw withdrawal threshold decreasing trend and at the end of 4 week after induction of diabetes the paw withdrawal threshold 27.84±1.80 (in g). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of paw withdrawal threshold and at the end 4 weeks of treatment period the paw withdrawal threshold was 24.40±1.17 in g (Figure 5.6.5).

5.6.5.3 Effect of MET (300 mg/kg p.o) on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

In the MET (300 mg/kg p.o.) treated group the initial paw withdrawal threshold was 24.85±1.67 (in g) which was increase to 45.98±1.71 (in g), $P< 0.001$ indicating an increase of 21.13 (in g) or 45.95%. The onset of neuroprotective effect was evident after 2nd week of MET (300 mg/kg p.o.) treatment and continuation of treatment showed a trend toward increase in paw withdrawal threshold (Figure 5.6.5).

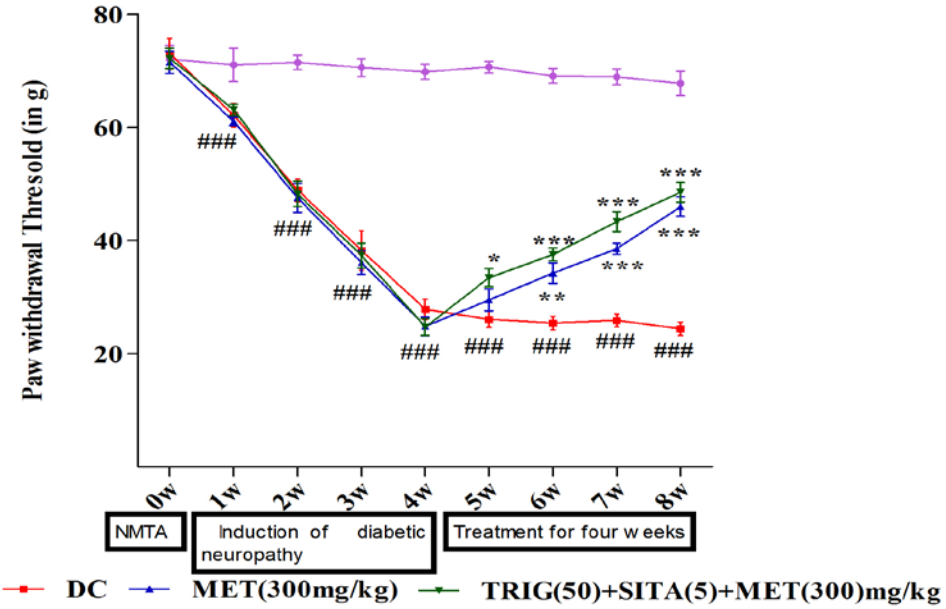
5.6.5.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

In the triple therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treated group the initial paw withdrawal threshold was (24.72±1.47 (in g) which was increased to (48.51±1.75 (in g), $P< 0.001$. This increase of 24.80 in g (49.04%) was more than the increase in paw withdrawal threshold by the individual drug. Early onset of paw withdrawal threshold was observed at 1st week (26.03%) compared of 2nd week of onset in individual treatment group. The results thus indicated synergistic effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) (Figure 5.6.5).

Table 5.6.5 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

AVG of mechano-tactile allodynia (Von-Frey hair test) (in g) in diabetic neuropathy (8 weeks model)						
Groups	**Time in weeks**					
	Normal Mechano-tactile allodynia	**After Induction of diabetes**	**Treatment for four weeks**			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	72.00±2.49	69.79±1.34	70.62±1.04	69.08±1.34	68.91±1.42	67.77±2.18
DC	73.01±2.68	27.84±1.80$^{###}$	26.06±1.42$^{###}$	25.40±1.20$^{###}$	25.86±1.11$^{###}$	24.40±1.17$^{###}$
MET (300mg/kg p.o.)	71.48±1.97	24.85±1.67	29.50±1.94	34.22±1.80**	38.53±0.98***	45.98±1.71***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.	72.18±1.82	24.72±1.47	33.42±1.61*	37.47±1.14***	43.31±1.76***	48.51±1.75***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}$ $P< 0.001$ as compared with non diabetic.

Figure 5.6.5 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mechano-tactile allodynia (Von-Frey hair test) in diabetic neuropathic rats

5.6.6 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on motor nerve conduction velocity (MNCV) in diabetic neuropathic rats

5.6.6.1 Motor nerve conduction velocity in non-diabetic rats

The results indicated no major change in motor nerve conduction velocity compare to week '0' (Figure 5.6.6).

5.6.6.2 Motor nerve conduction velocity in diabetic neuropathic rats

Motor nerve conduction velocity before administration of NICO-STZ in this group was 59.35±2.08 (m/s). After administration of NICO-STZ the motor nerve conduction velocity decreasing trend and at the end of 4 week after induction of diabetes the motor nerve conduction velocity 25.83±1.84 (m/s). The vehicle used for dissolving the test compound was double distilled water which could not prevent the decrease of motor nerve conduction velocity and at the end 4 weeks of treatment period the motor nerve conduction velocity was 21.88±1.87 (m/s) (Figure 5.6.6).

5.6.6.3 Effect of MET (300 mg/kg p.o) on motor nerve conduction velocity in diabetic neuropathic rats

In the MET (300 mg/kg p.o) treated group the initial motor nerve conduction velocity was 25.48±1.85 (m/s) which was increase to 40.25±2.60 (m/s), $P< 0.001$ indicating a increase of 14.77 (in g) or 36.69%. The onset of neuroprotective effect was evident after 2nd week of MET (300 mg/kg p.o) treatment and continuation of treatment showed a trend toward increase in motor nerve conduction velocity (Figure 5.6.6).

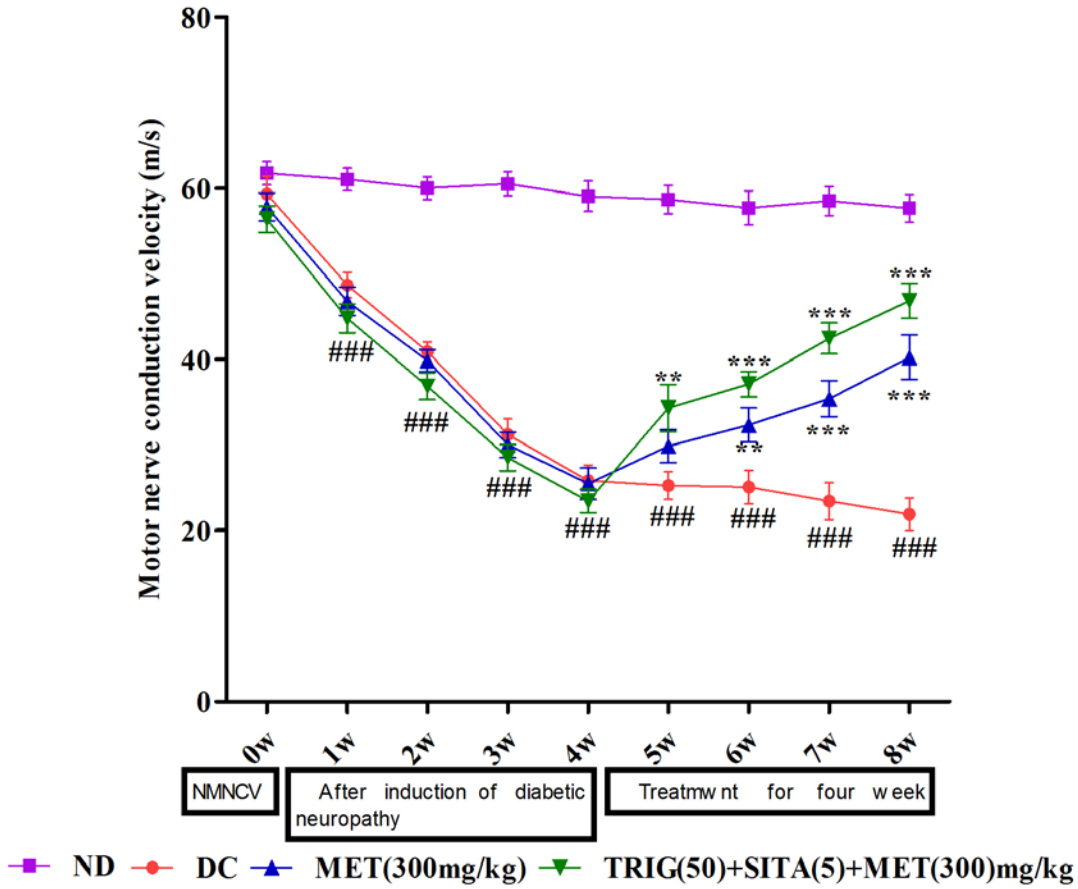
5.6.6.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on motor nerve conduction velocity (MNCV) in diabetic neuropathic rats

In case of triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) treated group the initial motor nerve conduction velocity was (23.44±1.36 (m/s) which was increased to 46.87±2.07 (m/s), $P< 0.001$. This increase of 23.43 m/s (49.98%) was more than the increase in motor nerve conduction velocity by the individual drug. Early onset of motor nerve conduction velocity was observed at 1st week (31.72 %) compared of 2nd week of onset in individual treatment group. The results thus indicated synergistic effect of treatment with TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) (Figure 5.6.6).

Table 5.6.6 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on motor nerve conduction velocity (MNCV) in diabetic neuropathic rats

AVG of motor nerve conduction velocity (m/s) in diabetic neuropathy (8 weeks model)						
Groups	**Normal MNCV**	**After induction of diabetes**	**Time in weeks**			
			Treatment for four weeks			
	0W	**4W**	**5W**	**6W**	**7W**	**8W**
ND	61.77±1.34	59.10±1.78	58.69±1.65	57.74±1.97	58.54±1.69	57.70±1.65
DC	59.35±2.08	25.83±1.84 ###	25.26±1.65 ###	25.07±1.95 ###	23.41±2.17 ###	21.88±1.87 ###
MET (300mg/kg p.o.)	57.86±1.61	25.48±1.85	29.86±1.96	32.33±1.99 **	35.42±2.11 ***	40.25±2.60 ***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	56.41±1.54	23.44±1.36	34.33±2.77 **	37.12±1.49 ***	42.47±1.83 ***	46.87±2.07 ***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic.

Figure 5.6.6 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on motor nerve conduction velocity (MNCV) in diabetic neuropathic rats

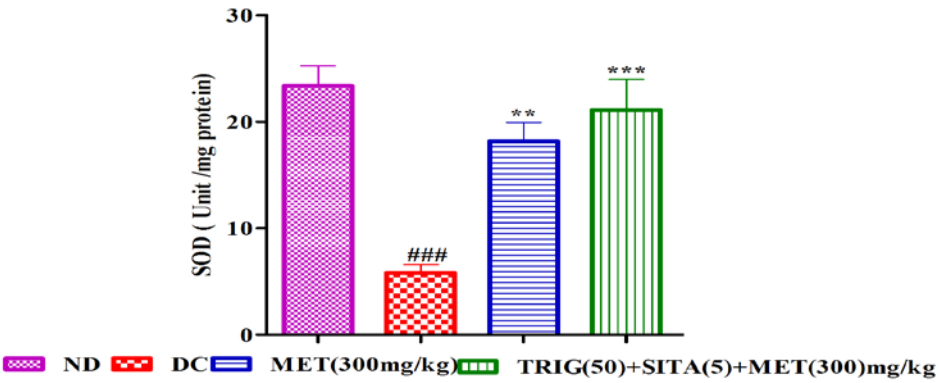
5.6.7 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on superoxide dismutase (SOD) level in diabetic neuropathic rats

The level of SOD in non-diabetic rat sciatic nerve was (23.39±1.86 Unit /mg protein). On other hand where diabetic rat nerve showed significant (5.82±0.75Unit /mg protein, *P< 0.001*) decrease in SOD level as compared to non-diabetic rat nerve. Significant increase in SOD level was measured on last day of study period (end of week 8) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg), 18.17±1.79, *P<0.01* and 21.14±2.84, *P<0.001* Unit /mg protein as compared to SOD level in diabetic rat nerve. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o.+ MET 300 mg/kg p.o. showed more significant increase by (72.46 %) in SOD level compared to individual compound MET 67.96% (Figure 5.6.7).

Table 5.6.7 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on superoxide dismutase (SOD) level in diabetic neuropathic rats

AVG of superoxide dismutase (SOD) in diabetic neuropathic rats	
Parameter / Groups	**SOD** (Unit /mg protein)
ND	23.39±1.86
DC	5.82±0.75$^{###}$
MET (300mg/kg p.o.)	18.17±1.79**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	21.14±2.84***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group n=6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.6.7 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on superoxide dismutase (SOD) level in diabetic neuropathic rats

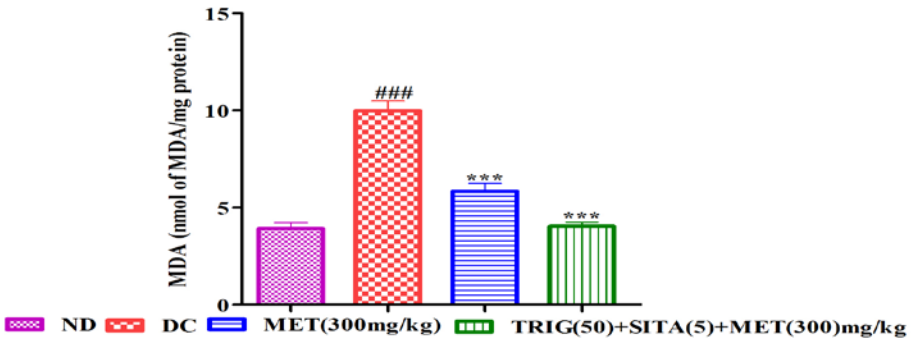
5.6.8 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on malondialdehyde (MDA) level in diabetic neuropathic rat sciatic nerve

The level of MDA in non-diabetic rat sciatic nerve was (3.91±0.31nmol of MDA/mg protein). On other hand where diabetic rat nerve showed significant (9.98±0.50 nmol of MDA/mg protein, *P< 0.001*) increase in MDA level as compared to non-diabetic nerve. Significant decrease in MDA level was measured on last day of study period (end of week 8) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg), 5.84±0.41, *P<0.001* and 4.03±0.22, *P<0.001* nmol of MDA/mg protein as compared to MDA level in diabetic rat nerve. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300 mg/kg p.o. showed more significant decrease by (59.61 %) in MDA level compared to individual compound MET 41.48 % (Figure 5.6.8).

Table 5.6.8 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on malondialdehyde (MDA) level in diabetic neuropathic rat sciatic nerve

AVG of malondialdehyde (MDA) level in diabetic neuropathic rats	
Parameter / Groups	**MDA** (nmol of MDA/mg protein)
ND	3.91±0.31
DC	9.98±0.50$^{###}$
MET (300mg/kg p.o.)	5.84±0.41***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o.)	4.03±0.22***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

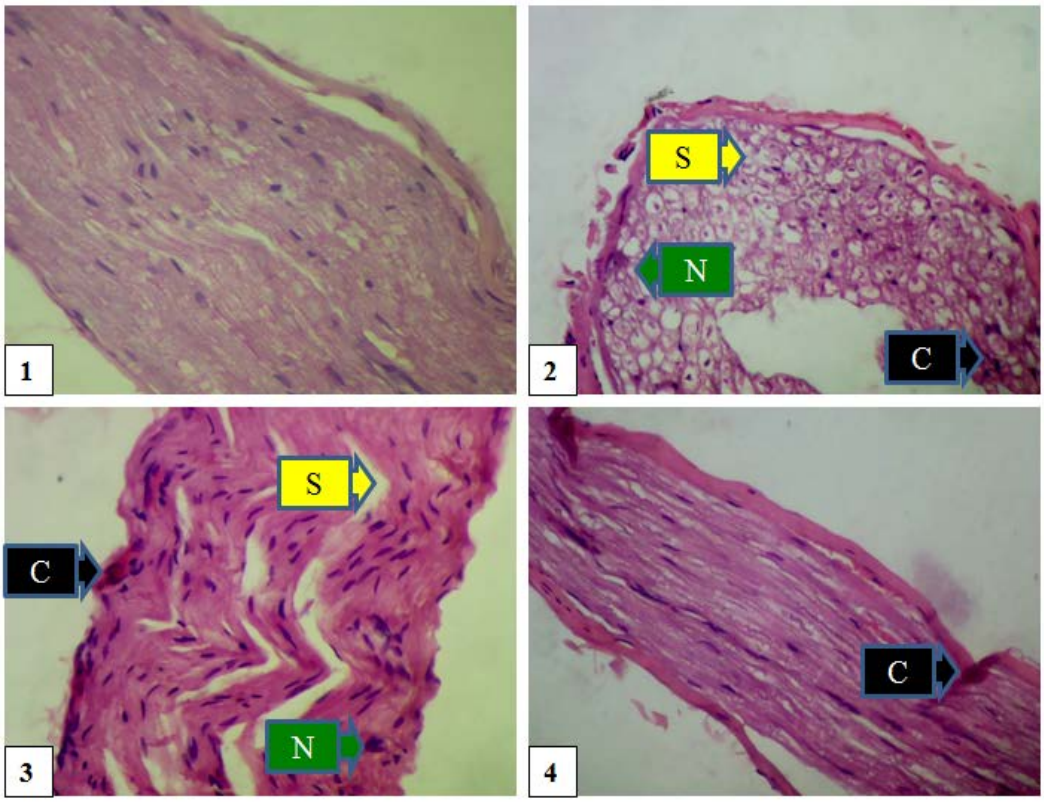


Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.6.8 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on malondialdehyde (MDA) level in diabetic neuropathic rat sciatic nerve

5.6.9 Histopathological examination of isolated sciatic nerve tissue of diabetic neuropathic rats (H&E stain)

After eight weeks of study examination of sciatic nerve sections stained with hematoxylin & eosin (H&E). Histology of isolated sciatic nerve of non-diabetic rat showed (Grade -) normal architecture; Diabetic rat showed (Grade +++) necrosis; (Grade +++) swelling; (Grade +++) congestion; MET showed (Grade +) necrosis; (Grade +) swelling; (Grade +) congestion; TRIG+SITA+MET treated animals showed (Grade -) necrosis; (Grade -) swelling; (Grade +) congestion. The scoring of grades was as per Dr. Pralhad Wangikar (MVSC), Prado- Preclinical Research and Development Organization, Pune. Where (Grade -) normal; (Grade +++) severe; (Grade ++) moderate; (Grade +) mild (Figure 5.6.9.1).



Where, 1] Non diabetic rat; 2] Diabetic rat; 3] MET (300 mg/kg p.o.); 4] TRIG 50 mg/kg p.o. + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.). N-necrosis, C-congestion, S- swelling. ([Magnification 40x].

Figure 5.6.9.1 Histopathological examination of isolated sciatic nerve tissue of diabetic neuropathic rats (H&E stain)

Model 5.7.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET in NICO-STZ induced diabetic cardiomyopathy in Wistar rats

5.7.1.1 Serum glucose level in non-diabetic rats

The results indicated no major fluctuation in serum glucose level (Figure 5.7.1).

5.7.1.2 Serum glucose level in diabetic cardiomyopathic rats

Serum glucose level before administration of NICO-STZ in this group was (92.04±7.18 mg/dl). After administration of NICO-STZ the serum glucose level increasing trend and at the end of 3 week after induction of diabetes the serum glucose (439.08±21.13 mg/dl). The vehicle used for dissolving the test compound was double distilled water which could not prevent the rise of serum glucose and at the end 8 weeks of treatment period the serum glucose level was 457.36±16.78 mg/dl (Figure 5.7.1).

5.7.1.3 Effect of MET (300 mg/kg p.o) on serum glucose level in diabetic cardiomyopathic rats

In the MET (300 mg/kg, p.o.) treated group the initial serum glucose level was (438.23±20.84 mg/dl) which was reduced to (182.93±9.63 mg/dl, $P< 0.001$) indicating a decrease of 255.30 mg/dl or 58.25%. The onset of antihyperglycemic effect was evident after 4th week of MET (300 mg/kg, p.o.) treatment and continuation of treatment showed a trend toward decrease in serum glucose (Figure 5.7.1).

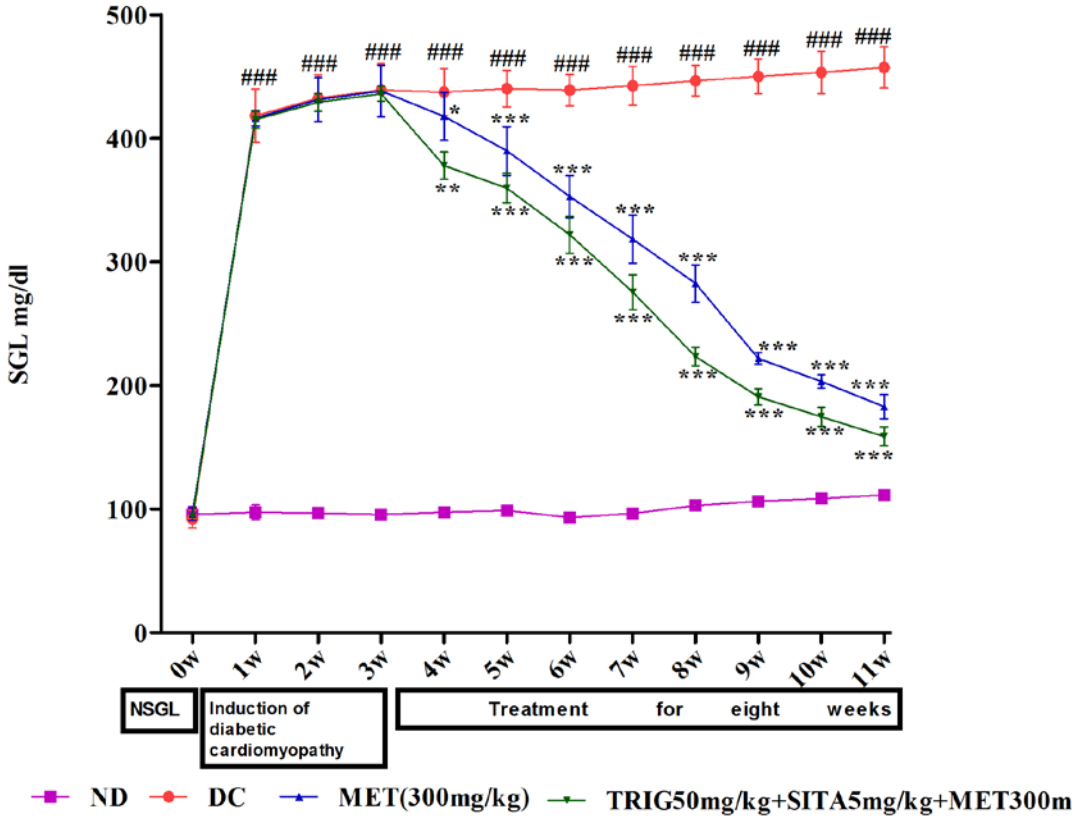
5.7.1.4 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on serum glucose level in diabetic cardiomyopathic rats

Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300 mg/kg p.o. treated group the initial serum glucose level was (436.00±6.02 mg/dl) which was reduced to (158.89±7.57 mg/dl). This reduction of 277.11 mg/dl (63.55 %) was more than the reduction in serum glucose by the individual drug. Early onset of serum glucose was observed at 2nd week (17.49%) compared of 3rd week of onset in individual treatment group. The results thus indicated synergistic effect of TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300 mg/kg p.o compared to metformin alone (Figure 5.7.1).

Table 5.7.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum glucose level in diabetic cardiomyopathic rats

AVG of serum glucose levels (mg/dl) in diabetic cardiomyopathic rats (11 weeks model)										
Groups	**Time in weeks**									
	Normal SGL	**After induction of diabetes**	**Treatment for eight weeks**							
	0W	**3W**	**4W**	**5W**	**6W**	**7W**	**8W**	**9W**	**10W**	**11W**
ND	95.60± 6.32	95.55 ±4.28	97.49± 1.63	98.82± 4.11	93.22± 2.88	96.57± 3.54	102.91 ±1.97	106.36±2.27	108.50 ±2.10	111.48 ±2.25
DC	92.04± 7.18	439.08±21.13###	437.43 ±18.96 ###	440.13 ±14.74 ###	439.10 ±12.68 ###	442.45 ±15.73 ###	446.53 ±12.29 ###	450.12±14.05###	453.27 ±17.20 ###	457.36 ±16.78 ###
MET (300 mg/kg p.o.)	96.05± 5.17	438.23±20.84	417.60 ±19.39 *	389.78 ±19.71 ***	352.94 ±17.09 ***	318.46 ±19.43 ***	282.54 ±15.18 ***	221.77±4.64***	203.35 ±5.43***	182.93 ±9.63***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.) + MET (300 mg/kg p.o.)	94.29± 2.17	436.00±6.02	377.95 ±10.96 **	359.74 ±11.87 ***	321.96 ±14.95 ***	275.62 ±14.13 ***	223.37 ±7.51***	190.83±6.62***	174.68 ±7.78***	158.89 ±7.57***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on serum glucose level in diabetic cardiomyopathic rats

5.7.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic cardiomyopathic rats

5.7.2.1 Body weight in non-diabetic rats

The results indicated increase in body weight compare to week '0' (Figure 5.7.2).

5.7.2.2 Body weight in diabetic cardiaomyopathic rats

Before administration of NICO-STZ the body weight of the rat was 215.66±3.34 g. Administration of NICO-STZ resulted in reduction body weight to 154.63±4.13 g. This reduction of 61.03 g (28.29 %) was due to the induction of diabetes. The animals appeared weak and polyuria (not recorded) was evident (Figure 5.7.2).

5.7.2.3 Effect of MET (300 mg/kg p.o) on body weight in diabetic cardiomyopathic rats

Before administration of MET (300 mg/kg p.o.) in diabetic rats the body weight was 192.24±2.21g. After treatment with MET (300 mg/kg p.o.) the body weight was 207.54±1.81 g. The results indicated that MET treatment arrested the loss of body weight in the diabetic rats. The observed effect appears to be due to control of the hyperglycemia. The rats showed increase in the body weight from the 2^{nd} week after MET (300 mg/kg p.o.) treatment and after 3^{rd} and 4^{th} week significant ($P< 0.001$) gain in body weight than the initial body weight was observed (Figure 5.7.2).

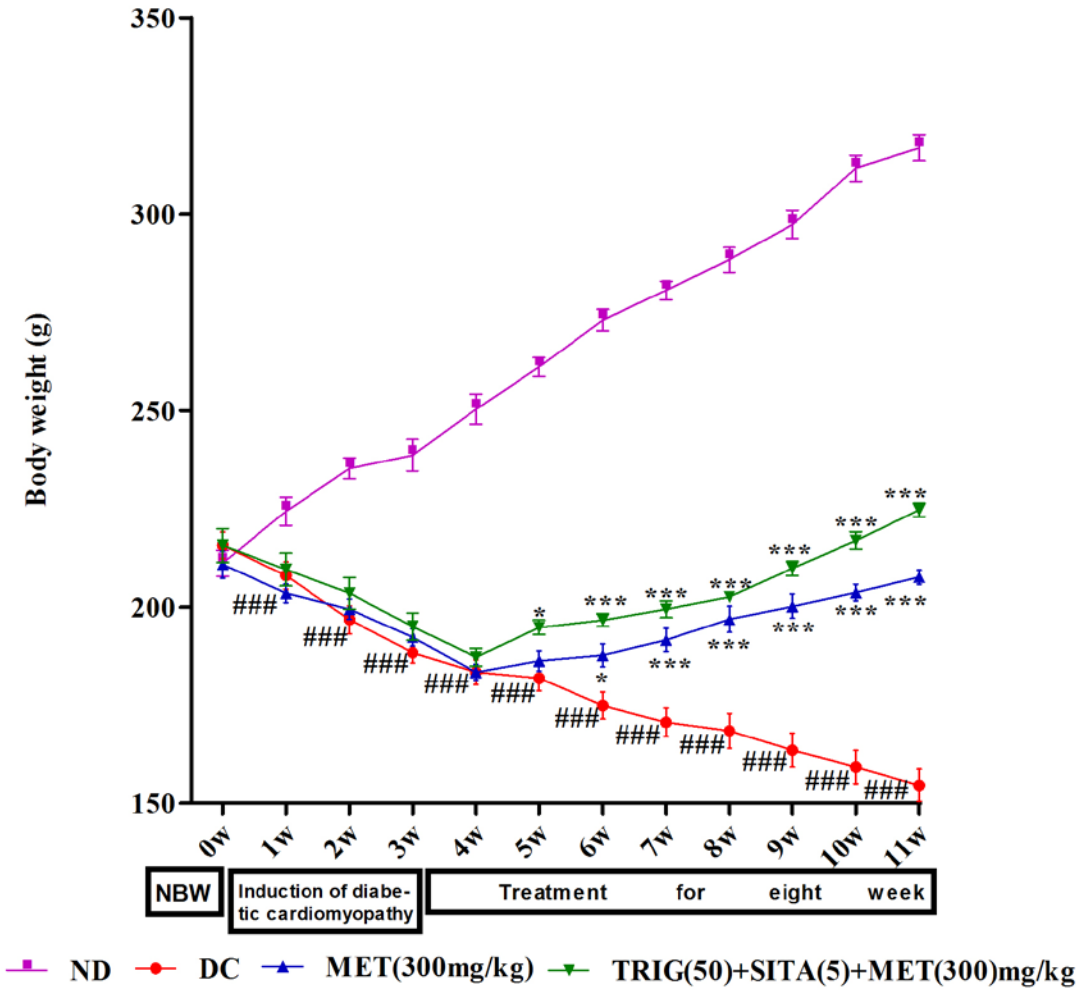
5.4.2.5 Effect of TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.) on body weight in diabetic cardiomyopathic rats

Triple drug therapy using TRIG (50 mg/kg p.o.) + SITA (5 mg/kg *p.o.*) + MET (300 mg/kg p.o.) was more effective than the individual compound because the gain in body weight after the treatment was more compared to that of individual drug. The treated diabetic animals showed improvement in health compared to diabetic rats (Figure 5.7.2).

Table 5.7.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic cardiomyopathic rats

AVG of body weight (g) in diabetic cardiomyopathic rats (11 weeks model)										
Groups	**Time in weeks**									
	Norm-al body weight	**After induction of diabetes**	**Treatment for eight weeks**							
	0W	**3W**	**4W**	**5W**	**6W**	**7W**	**8W**	**9W**	**10W**	**11W**
ND	211.16±3.34	238.62±4.05	250.27±3.87	261.06±2.39	272.94±2.74	280.55±2.36	288.34±3.16	297.33±3.56	311.64±3.31	316.80±3.30
DC	215.66±3.34	188.36±2.63###	183.41±3.14###	181.87±3.19###	174.92±3.39###	170.65±3.66###	168.44±4.44###	163.54±4.30###	159.22±4.21###	154.63±4.13###
MET (300 mg/kg p.o.)	210.75±3.42	192.24±2.21	183.38±2.03	186.24±2.58	187.67±2.94*	191.58±2.98***	196.87±3.32***	200.16±3.07***	203.66±2.11***	207.54±1.81***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.) + MET (300 mg/kg p.o.)	215.63±4.32	194.96±3.51	187.20±2.27	194.75±1.86*	196.61±1.66***	199.37±2.13***	202.52±1.16***	209.78±1.82***	216.88±2.20***	224.68±1.75***

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.2 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on body weight in diabetic cardiomyopathic rats

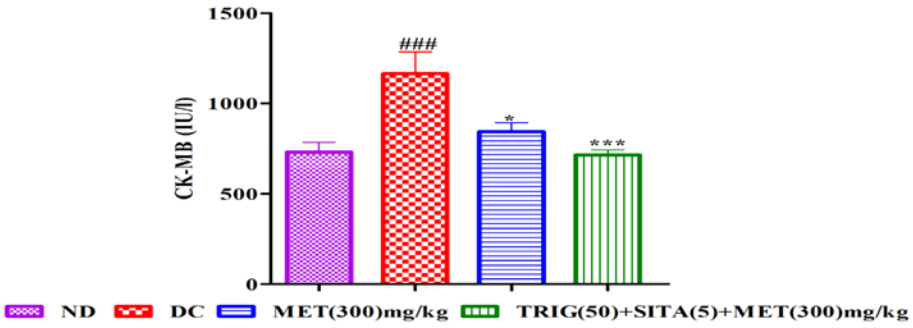
5.7.3.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on creatine kinase (CK-MB) level in diabetic cardiomyopathic rats

The level of serum CK-MB in non-diabetic rats was (731.5±57.79 IU/l). On other hand where diabetic rat showed significant (1165±120.48 IU/l, *P< 0.001*) increase in CK-MB level as compared to non-diabetic rat. Significant decrease in CK-MB level was measured on last day of study period (end of week 11) with MET 300 mg/kg p.o. and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 844.9±49.63, *P<0.05* and 715.7±26.19 IU/l, *P<0.001* as compared to CK-MB level in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o.+ MET (300mg/kg p.o) showed more significant decrease by (38.56 %) in creatine kinase level compared to individual compound MET 300 mg/kg 27.47% (Figure 5.7.3.1.).

Table 5.7.3.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on creatine kinase (CK-MB) level in diabetic cardiomyopathic rats

AVG of serum creatine kinase-MB level in diabetic cardiomyopathic rats	
Parameter / Groups	**CK-MB (IU/l)**
ND	731.5±57.79
DC	1165.0±120.48 ###
MET (300mg/kg p.o.)	844.9±49.63*
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	715.7±26.19***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; *P< 0.05, **P< 0.01, *** P< 0.001 as compared with diabetic control, ###P< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; *P< 0.05, **P< 0.01, *** P< 0.001 as compared with diabetic control, ###P< 0.001 as compared with non diabetic.

Figure 5.7.3.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on creatine kinase (CK-MB) level in diabetic cardiomyopathic rats

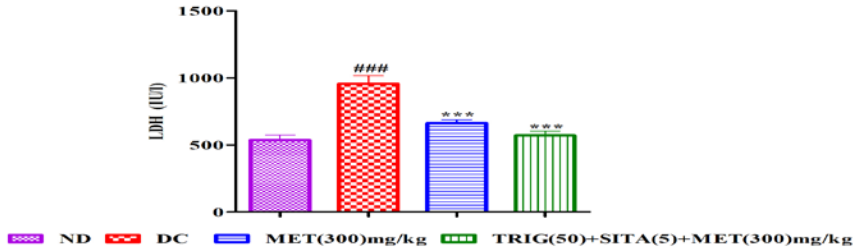
5.7.4.1 Effect of MET alone and triple drug therapy using TRIG + SITA + metformin MET on lactate dehydrogenase (LDH) level in diabetic cardiomyopathic rats

The level of serum LDH in non-diabetic rat was (537.1±37.76 IU/l). On other hand where diabetic rat showed significant (956.9±61.36 IU/l, *P< 0.001*) increase in LDH level as compared to non-diabetic rat. Significant decrease in LDH level was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 663.5±47.17, *P<0.001* and 572.4±31.23 IU/l, *P<0.001* as compared to LDH level in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. +MET 300mg/kg p.o. showed more significant decrease by (40.18 %) in lactate dehydrogenase level compared to individual compound MET 300 mg/kg p.o. 30.66% (Figure 5.7.4.1.).

Table 5.7.4.1 Effect of MET alone and triple drug therapy using TRIG + SITA + metformin MET on lactate dehydrogenase (LDH) level in diabetic cardiomyopathic rats

AVG of serum lactate dehydrogenase (LDH) level in diabetic cardiomyopathic rats	
Parameter / Groups	**LDH (IU/l)**
ND	537.1±37.76
DC	956.9±61.36 $^{###}$
MET (300mg/kg p.o.)	663.5±47.17***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	572.4±31.23***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.4.1 Effect of MET alone and triple drug therapy using TRIG + SITA + metformin MET on lactate dehydrogenase (LDH) level in diabetic cardiomyopathic rats

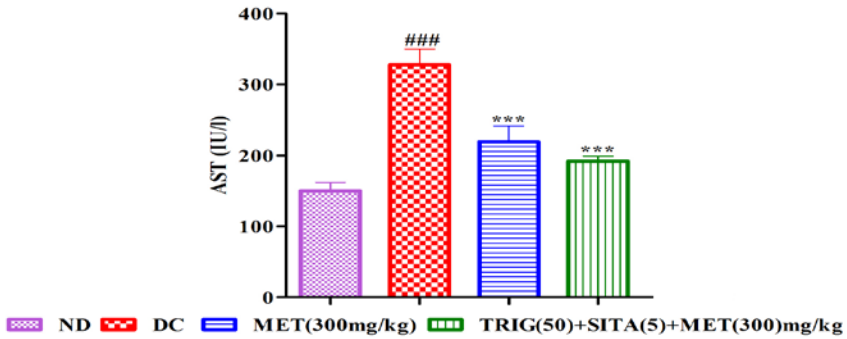
5.7.5.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on aspartate transaminase (AST) level in diabetic cardiomyopathic rats

The level of serum AST in non-diabetic rats was (150.1±11.85 IU/l). On other hand where diabetic rat showed significant (327.9±22.37 IU/l, *P< 0.001*) increase in AST level as compared to non-diabetic rat. Significant decrease in AST level was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 219.4±22.04, *P<0.001*; 192.2±6.78, IU/l *P<0.001* as compared to LDH level in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o.+ MET 300mg/kg p.o. showed more significant decrease by (41.38 %) in aspartate transaminase level compared to individual compound MET 300 mg/kg p.o. 33.08% (Figure 5.7.5.1).

Table 5.7.5.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on aspartate transaminase (AST) level in diabetic cardiomyopathic rats

AVG of serum aspartate transaminase (AST) level in diabetic cardiomyopathic rats	
Parameter / Groups	**AST (IU/l)**
ND	150.1±11.85
DC	327.9±22.37$^{###}$
MET (300mg/kg p.o.)	219.4±22.04***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	192.2±6.78***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.5.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on aspartate transaminase (AST) level in diabetic cardiomyopathic rats

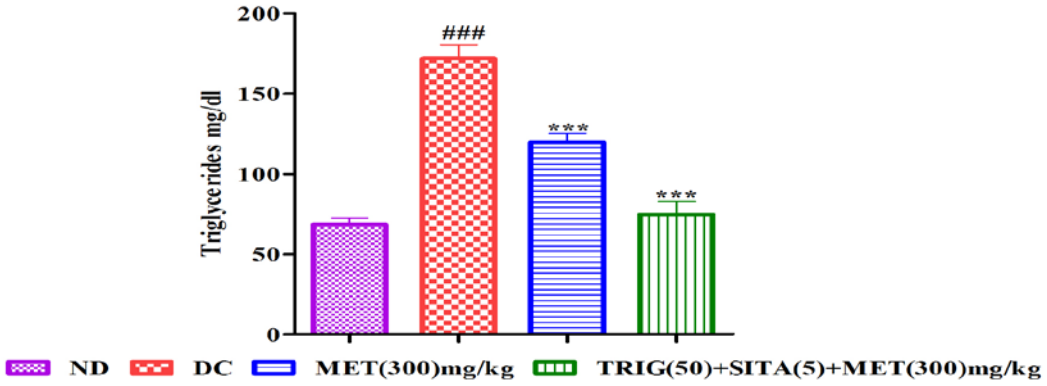
5.7.6.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on triglyceride level in diabetic cardiomyopathic rats

The level of serum triglyceride in non-diabetic rats was (68.49±4.04 mg/dl). On other hand where diabetic rat showed significant (171.9±8.36 mg/dl, *P< 0.001*) increase in triglyceride level as compared to non-diabetic rat. Significant decrease in triglyceride level was measured on last day of study period (end of week 11), MET 300 mg/kg p.o. and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300mg/kg p.o.), 119.8±5.39, *P<0.001* and 74.71±8.09 mg/dl, *P<0.001* as compared to triglyceride level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o.+ MET 300mg/kg p.o. showed more significant decrease by (56.53%) in triglyceride level compared to individual compound MET 300mg/kg p.o. 30.30% (Figure 5.7.6.1).

Table 5.7.6.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on triglyceride level in diabetic cardiomyopathic rats

AVG of serum triglyceride in diabetic cardiomyopathic rats	
Parameter / Groups	**Triglyceride** (mg/dl)
ND	68.49±4.04
DC	171.9±8.36$^{###}$
MET (300mg/kg p.o.)	119.8±5.39***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	74.71±8.09***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.6.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on triglyceride level in diabetic cardiomyopathic rats

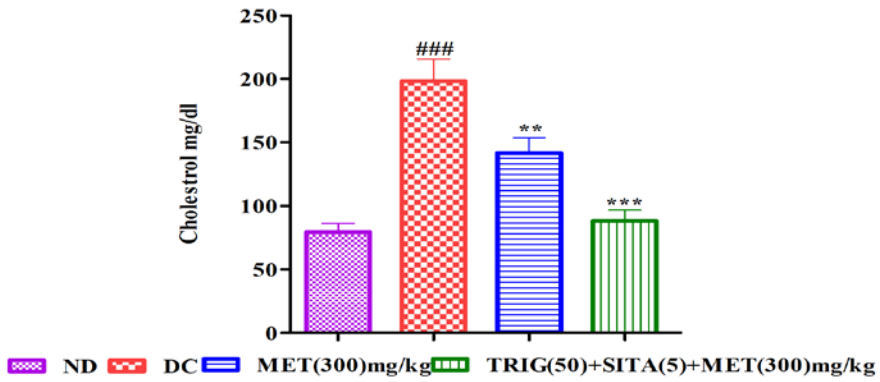
5.7.7.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on cholesterol level in diabetic cardiomyopathic rats

The level of serum cholesterol in non-diabetic rats was (79.47±6.90 mg/dl). On other hand where diabetic rat showed significant (198.4±17.13 mg/dl, *P< 0.001*) increase in cholesterol level as compared to non-diabetic rat. Significant decrease in cholesterol level was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 132.2±11.04, *P<0.01* and 88.39±8.28 mg/dl, *P<0.001* as compared to triglyceride level in diabetic rat. TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. +MET 300 mg/kg p.o. showed more significant decrease by (55.44 %) in cholesterol level compared to individual compound MET 300mg/kg p.o 33.36 % (Figure 5.7.7.1).

Table 5.7.7.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on cholesterol level in diabetic cardiomyopathic rats

AVG of serum cholesterol in diabetic cardiomyopathic rats	
Parameter / Groups	**Cholesterol (mg/dl)**
ND	79.47±6.90
DC	198.4±17.13$^{###}$
MET (300mg/kg p.o.)	132.2±11.04**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	88.39±8.28***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.7.1 Effect of MET alone and triple drug therapy using trigonelline (TRIG) + sitagliptin (SITA) +metformin (MET) on cholesterol level in diabetic cardiomyopathic rats

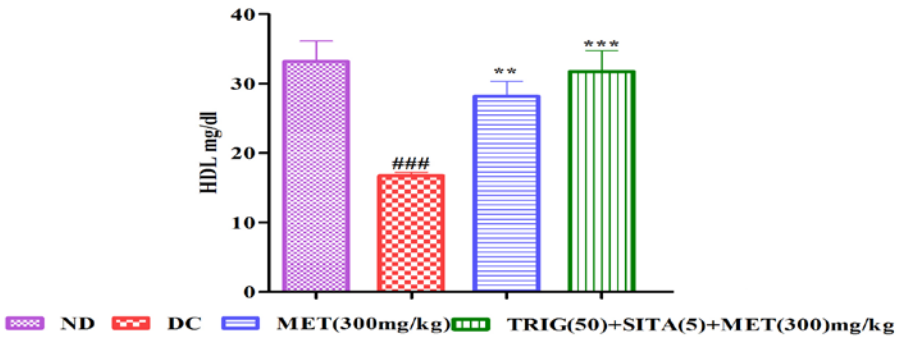
5.7.8.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on high density lipoprotein (HDL) level in diabetic cardiomyopathic rats

The level of high density lipoprotein in non-diabetic rats was (33.18±2.93 mg/dl). On other hand where diabetic rat showed significant (16.73±0.49 mg/dl, *P< 0.001*) decrease in high density lipoprotein level as compared to non-diabetic rat. Significant increase in high density lipoprotein level was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 28.17±2.13, *P<0.01* and 31.74±3.00 mg/dl, *P<0.001* as compared to high density lipoprotein level in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o showed more significant increase by (47.29%) in high density lipoprotein level compared to individual compound MET 300mg/kg p.o. 40.61% (Figure 5.7.8.1).

Table 5.7.8.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on high density lipoprotein (HDL) level in diabetic cardiomyopathic rats

AVG of serum high density lipoprotein in diabetic cardiomyopathic rats	
Parameter / Groups	**HDL (mg/dl)**
ND	33.18±2.93
DC	16.73±0.49$^{###}$
MET (300mg/kg p.o.)	28.17±2.13**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	31.74±3.00***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.8.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on high density lipoprotein (HDL) level in diabetic cardiomyopathic rats

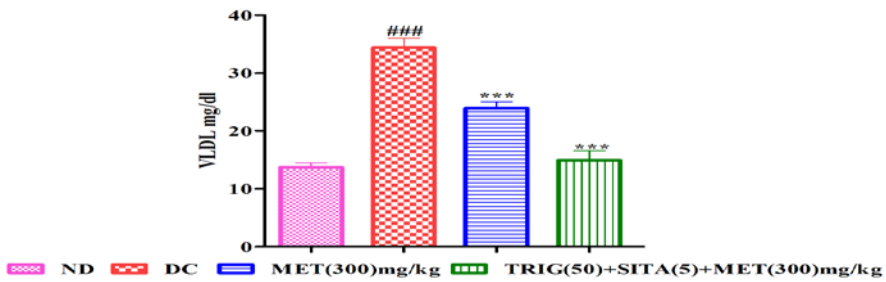
5.7.9.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on very low density lipoprotein (VLDL) level in diabetic cardiomyopathic rats

The level of very low density lipoprotein in non-diabetic rats was (13.70±0.80 mg/dl). On other hand where diabetic rat showed significant (34.38±1.67 mg/dl, *P< 0.001*) increase in very low density lipoprotein level as compared to non-diabetic rat. Significant decrease in very low density lipoprotein level was measured on last day of study period (end of week 11), (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.)+ SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 23.97±1.07, *P<0.001* and 14.94±1.61mg/dl, *P<0.001* as compared to very low density lipoprotein level in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. +MET 300mg/kg p.o. showed more significant decrease by (56.54%) in very low density lipoprotein level compared to individual compound MET 300mg/kg p.o. 30.27% (Figure 5.7.9.1).

Table 5.7.9.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on very low density lipoprotein (VLDL) level in diabetic cardiomyopathic rats

AVG of very low density lipoprotein in diabetic cardiomyopathic rats	
Groups \ **Parameter**	**VLDL** (mg/dl)
ND	13.70±0.80
DC	34.38±1.67$^{###}$
MET (300mg/kg p.o.)	23.97±1.07***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	14.94±1.61***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.

Figure 5.7.9.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on very low density lipoprotein (VLDL) level in diabetic cardiomyopathic rats

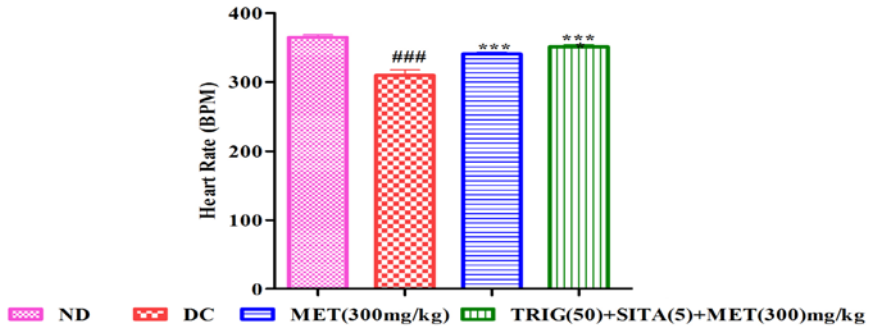
5.7.10.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on heart rate in diabetic cardiomyopathic rats

The heart rate in non-diabetic rat was (365.1±3.31, BPM). On other hand where diabetic rat showed significant (309.6±8.12, *P< 0.001* BPM) decrease in heart rate as compared to non-diabetic rat. Significant increase in heart rate was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 340.5±3.07, *P<0.001* and 351.5±2.66 *P<0.001*, BPM as compared to heart rate in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o. showed more significant increase by (11.92%) in heart rate compared to individual compound MET 300mg/kg p.o. 9.07% (Figure 5.7.10.1).

Table 5.7.10.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on heart rate in diabetic cardiomyopathic rats

AVG of heart rate in diabetic cardiomyopathic rats	
Parameter / Groups	**Heart rate** (BPM)
ND	365.1±3.31
DC	309.6±8.12$^{###}$
MET (300mg/kg p.o.)	340.5±3.07***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	351.5±2.66***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.10.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on heart rate in diabetic cardiomyopathic rats

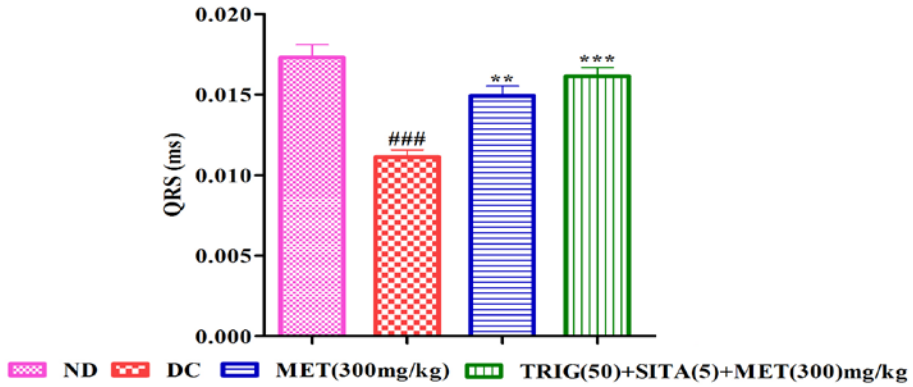
5.7.11.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QRS interval in diabetic cardiomyopathic rats

The QRS interval in non-diabetic rat was (0.01730±0.79 ms). On other hand where diabetic rat showed significant (0.01112±0.45ms, *P< 0.001*) decrease in QRS interval as compared to non-diabetic rat. Significant increase in QRS interval was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and (TRIG 50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 0.01492±0.61, *P<0.01* and 0.01614±0.53 ms, *P<0.001* as compared to QRS interval in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o. showed more significant increase by (31.10%) in QRS interval compared to individual compound MET 300mg/kg p.o 25.46% (Figure 5.7.11.1).

Table 5.7.11.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QRS interval in diabetic cardiomyopathic rats

AVG of QRS interval in diabetic cardiomyopathic rats	
Parameter / Groups	**QRS (ms)**
ND	0.01730±0.79
DC	0.01112±0.45$^{###}$
MET (300mg/kg p.o.)	0.01492±0.61**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	0.01614±0.53***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.

Figure 5.7.11.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QRS interval in diabetic cardiomyopathic rats

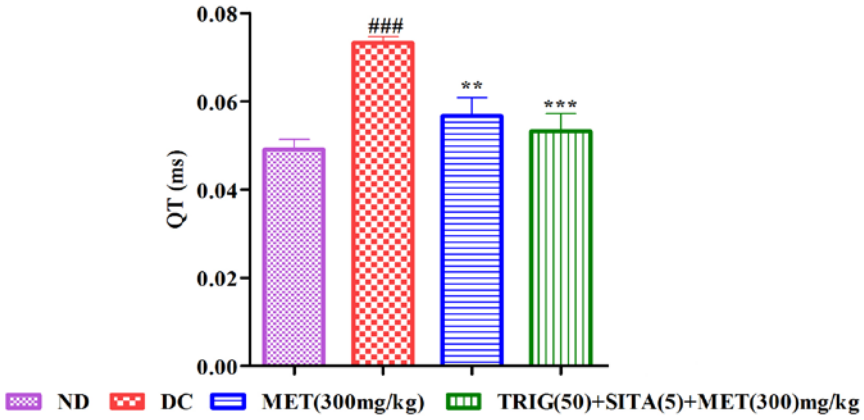
5.7.12.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QT interval in diabetic cardiomyopathic rats

The QT interval in non-diabetic rat was (0.04904±0.22 ms). On other hand where diabetic rat showed significant (0.07323±0.01 ms, *P< 0.001*) increase in QT interval as compared to non-diabetic rat. Significant decrease in QT interval was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 0.05666±0.42, *P<0.01* and 0.05324±0.39 ms, *P<0.001* as compared to QT interval in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o. showed more significant decrease by (27.29%) in QT interval compared to individual compound MET 300mg/kg p.o 22.62 % (Figure 5.7.12.1).

Table 5.7.12.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QT interval in diabetic cardiomyopathic rats

AVG of QT interval in diabetic cardiomyopathic rats	
Parameter / Groups	QT (ms)
ND	0.04904±0.22
DC	0.07323±0.01$^{###}$
MET (300mg/kg p.o.)	0.05666±0.42**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	0.05324±0.39***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.12.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QT interval in diabetic cardiomyopathic rats

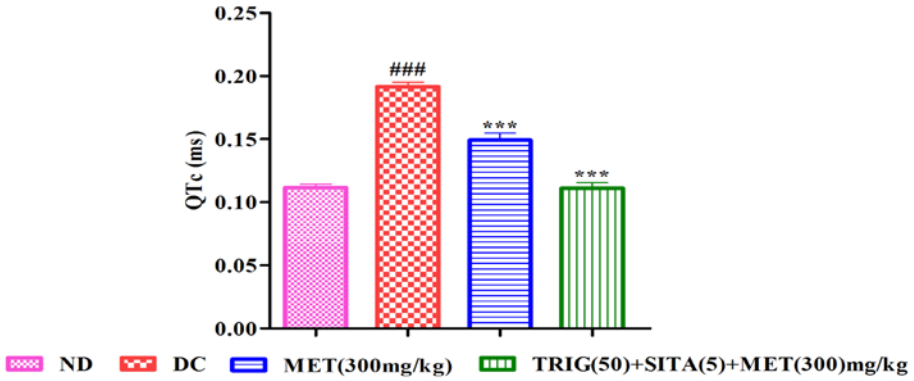
5.7.13.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QTc interval in diabetic cardiomyopathic rats

The QTc in non-diabetic rat was (0.1117±0.24 ms). On other hand where diabetic rat showed significant (0.1915±0.37 ms, *P< 0.001*) increase in QTc as compared to non-diabetic rat. Significant decrease in QTc was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 0.1493±0.54, *P<0.001* and 0.1113±0.43 ms, *P<0.001* as compared to QTc in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o. showed more significant decrease by (41.87%) in QT interval compared to individual compound MET 300mg/kg p.o 22.03 % (Figure 5.7.13.1).

Table 5.7.13.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QTc interval in diabetic cardiomyopathic rats

AVG of QTc interval in diabetic cardiomyopathic rats	
Parameter / Groups	**QTc (ms)**
ND	0.1117±0.24
DC	0.1915±0.37$^{###}$
MET (300mg/kg p.o.)	0.1493±0.54***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	0.1113±0.43***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.13.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on QTc interval in diabetic cardiomyopathic rats

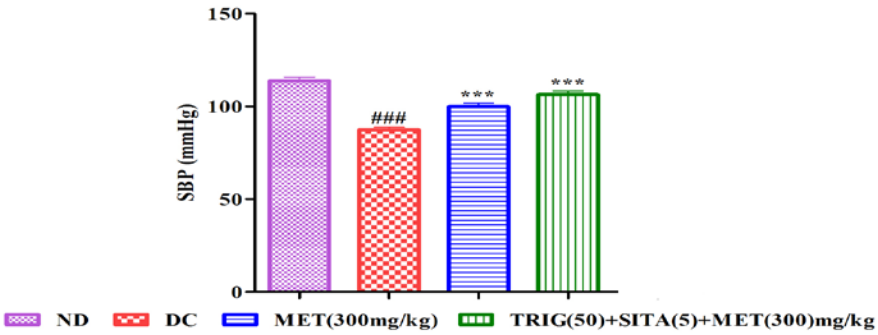
5.7.14.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on SBP in diabetic cardiomyopathic rats

SBP in non-diabetic rat was (113.8±1.87 mmHg). On other hand where diabetic rat showed significant (87.38±1.32 mmHg, *P< 0.001*) decrease in SBP as compared to non-diabetic rat. Significant decrease in SBP was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 99.88±1.85, *P<0.001* and 106.3±1.98 mmHg, *P<0.001* as compared to SBP in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. +MET 300mg/kg p.o. showed more significant increase by (17.79%) in SBP compared to individual compound MET 300mg/kg p.o. 12.51% (Figure 5.7.14.1).

Table 5.7.14.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on SBP in diabetic cardiomyopathic rats

AVG of SBP in diabetic cardiomyopathic rats	
Parameter / Groups	**SBP (mmHg/s)**
ND	113.8±1.87
DC	87.38±1.32$^{###}$
MET (300mg/kg p.o.)	99.88±1.85***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	106.3±1.98***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.14.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on SBP in diabetic cardiomyopathic rats

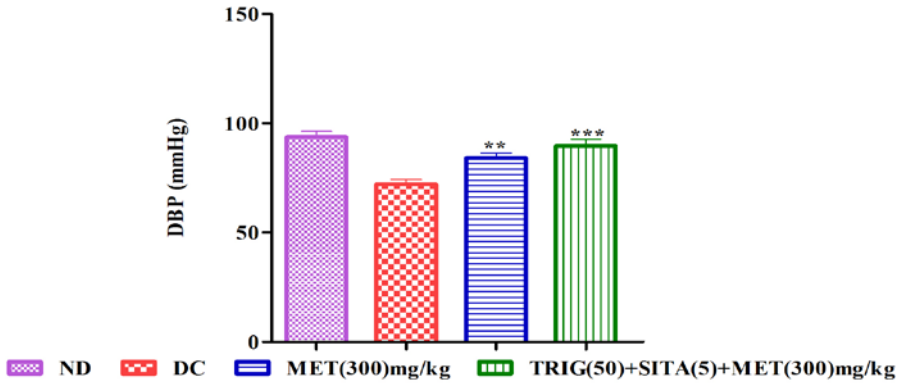
5.7.15.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on DBP in diabetic cardiomyopathic rats

DBP in non-diabetic rat was (93.67±2.29 mmHg). On other hand where diabetic rat showed significant (72.04±2.16 mmHg, *P< 0.001*) decrease in DBP as compared to non-diabetic rat. Significant increase in DBP was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 84.13±2.22, *P<0.01* and 89.67±2.98 mmHg, *P<0.01* as compared to DBP in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o. showed more significant increase by (19.66 %) in DBP compared to individual compound MET 300 mg/kg p.o. 14.37% (Figure 5.7.15.1).

Table 5.7.15.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on DBP in diabetic cardiomyopathic rats

AVG of DBP in diabetic cardiomyopathic rats	
Parameter / Groups	**DBP (mmHg/s)**
ND	93.67±2.29
DC	72.04±2.16$^{###}$
MET (300mg/kg p.o.)	84.13±2.22**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	89.67±2.98***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.15.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on DBP in diabetic cardiomyopathic rats

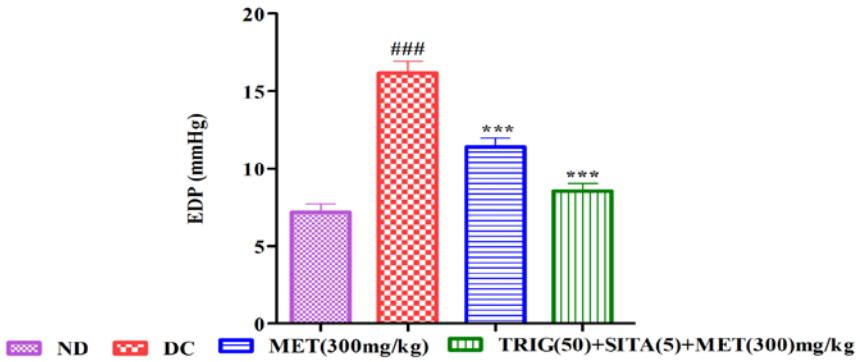
5.7.16.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on EDP in diabetic cardiomyopathic rats

EDP in non-diabetic rat was (7.18±0.52 mmHg). On other hand where diabetic rat showed significant (16.17±0.76 mmHg, *P< 0.001*) increase in EDP as compared to non-diabetic rat. Significant decrease in EDP was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 11.41±0.55, *P<0.001* and 8.55±0.49 mmHg, *P<0.001* as compared to EDP in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o. showed more significant decrease by (47.12 %) in EDP compared to individual compound MET 300mg/kg p.o. 29.43% (Figure 5.7.16.1).

Table 5.7.16.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on EDP in diabetic cardiomyopathic rats

AVG of EDP in diabetic cardiomyopathic rats	
Parameter / Groups	**EDP (mmHg)**
ND	7.18±0.52
DC	16.17±0.76$^{###}$
MET (300mg/kg p.o.)	11.41±0.55***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	8.55±0.49***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.16.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on EDP in diabetic cardiomyopathic rats

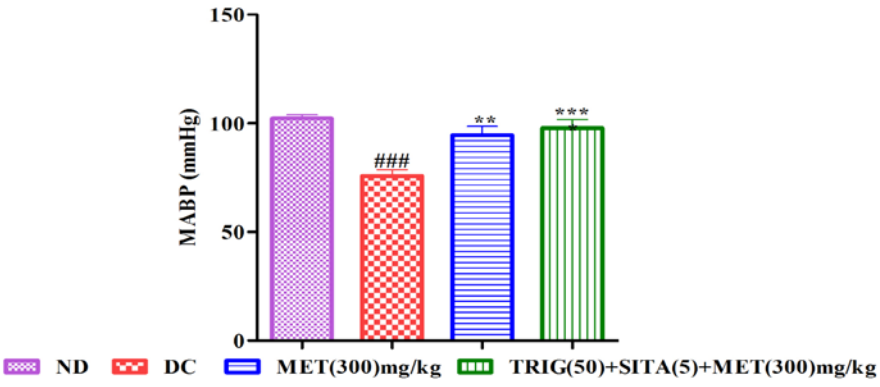
5.7.17.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mean arterial blood pressure (MABP) in diabetic cardiomyopathic rats

MABP in non-diabetic rat was (102.2±1.60, mmHg). On other hand where diabetic rat showed significant (75.79±2.85 mmHg, *P< 0.001*) decrease in MABP as compared to non-diabetic rat. Significant increase in MABP was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 94.57±3.95, *P<0.01* and 97.85±3.82 mmHg, *P<0.001* as compared to MABP in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. + MET 300mg/kg p.o. showed more significant increase by (22.54 %) in MABP compared to individual compound MET 300mg/kg p.o. 19.85% (Figure 5.7.17.1).

Table 5.7.17.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mean arterial blood pressure (MABP) in diabetic cardiomyopathic rats

AVG of mean arterial blood pressure (MABP) in diabetic cardiomyopathic rats	
Parameter / Groups	**MABP (mmHg/s)**
ND	102.2±1.60
DC	75.79±2.85$^{###}$
MET (300mg/kg p.o.)	94.57±3.95**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	97.85±3.82***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.17.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on mean arterial blood pressure (MABP) in diabetic cardiomyopathic rats

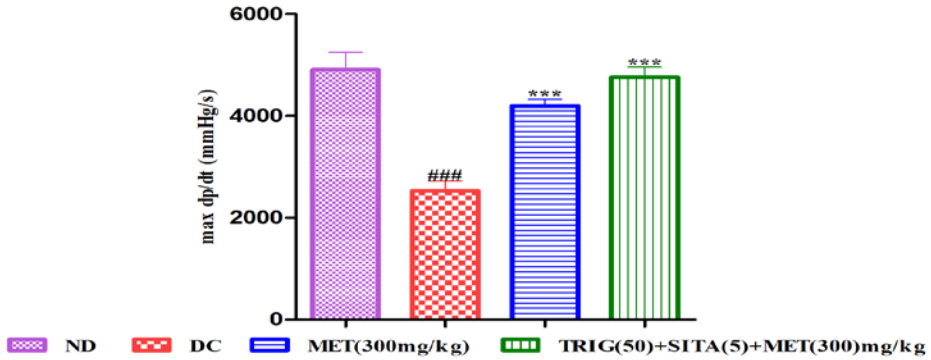
5.7.18.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on max dp/dt in diabetic cardiomyopathic rats

The max dp/dt in non-diabetic rat was (4909±341.8, mmHg/s). On other hand where diabetic rat showed significant (2530.0±181.7 mmHg/s, *P< 0.001*) decrease in max dp/dt as compared to non-diabetic rat. Significant increase in max dp/dt was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 4202±124.8, *P<0.001* and 4759±199.5 mmHg/s, *P<0.001* as compared to max dp/dt in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. +MET 300mg/kg p.o. showed more significant increase by (46.83 %) in max dp/dt compared to individual compound MET 300mg/kg p.o. 39.79% (Figure 5.7.18.1).

Table 5.7.18.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on max dp/dt in diabetic cardiomyopathic rats

AVG of max dp/dt in diabetic cardiomyopathic rats	
Parameter / Groups	max dp/dt (mmHg/s)
ND	4909±341.8
DC	2530±192.5$^{###}$
MET (300mg/kg p.o.)	4202±124.8***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	4759±199.5***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P$< 0.05, $^{**}P$< 0.01, *** P< 0.001 as compared with diabetic control, $^{###}P$< 0.001 as compared with non diabetic.

Figure 5.7.18.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on max dp/dt in diabetic cardiomyopathic rats

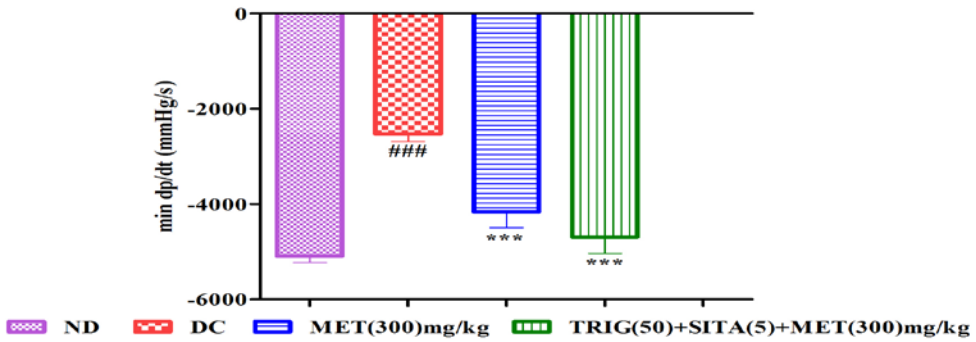
5.7.19.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on min dp/dt in diabetic cardiomyopathic rats

The min dp/dt in non-diabetic rat was (-5092±134.9, mmHg/s). On other hand where diabetic rat showed significant (-2525±161.1 mmHg/s, *P< 0.001*) decrease in min dp/dt as compared to non-diabetic rat. Significant increase in min dp/dt was measured on last day of study period (end of week 11) with MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), -4159±336.6, *P<0.001* and - 4693±337.5 mmHg/s, *P<0.001* as compared to min dp/dt in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. +MET 300mg/kg p.o. showed more significant increase by (46.19%) in min dp/dt compared to individual compound MET 300mg/kg p.o. 39.28% (Figure 5.7.19.1).

Table 5.7.19.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on min dp/dt in diabetic cardiomyopathic rats

AVG of min dp/dt in diabetic cardiomyopathic rats	
Parameter / Groups	min dp/dt (mmHg/s)
ND	-5092±134.9
DC	-2525±161.1$^{###}$
MET (300mg/kg p.o.)	-4159±336.6***
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	-4693±337.5***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.



Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.19.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on min dp/dt in diabetic cardiomyopathic rats

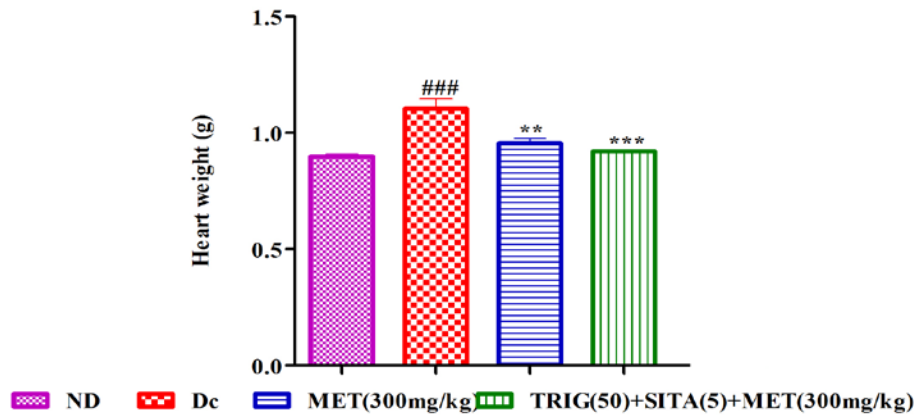
5.7.20.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on heart weight in diabetic cardiomyopathic rats

The heart weight in non-diabetic rat was (0.90±0.009g). On other hand where diabetic rat showed significant (1.10±0.043g, *P< 0.001*) increase in heart weight as compared to non-diabetic rat. Significant decrease in heart weight was measured on last day of study period (end of week 11), MET (300 mg/kg p.o.) and TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.), 0.96±0.021, *P<0.01*; 0.92±0.004g, *P<0.001* as compared to heart weight in diabetic rat. Triple drug therapy using TRIG 50 mg/kg p.o. + SITA 5 mg/kg p.o. +MET 300mg/kg p.o. showed more significant decrease by (16.36%) in heart weight compared to individual compound MET 300mg/kg p.o. 12.72% (Figure 5.7.20.1).

Table 5.7.20.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on heart weight in diabetic cardiomyopathic rats

AVG of heart weight (g) in diabetic cardiomyopathic rats	
Parameter / Groups	**Heart weight (g)**
ND	0.90±0.009
DC	1.10±0.043$^{###}$
MET (300mg/kg p.o.)	0.96±0.021**
TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.)+ MET (300 mg/kg p.o.)	0.92±0.004***

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

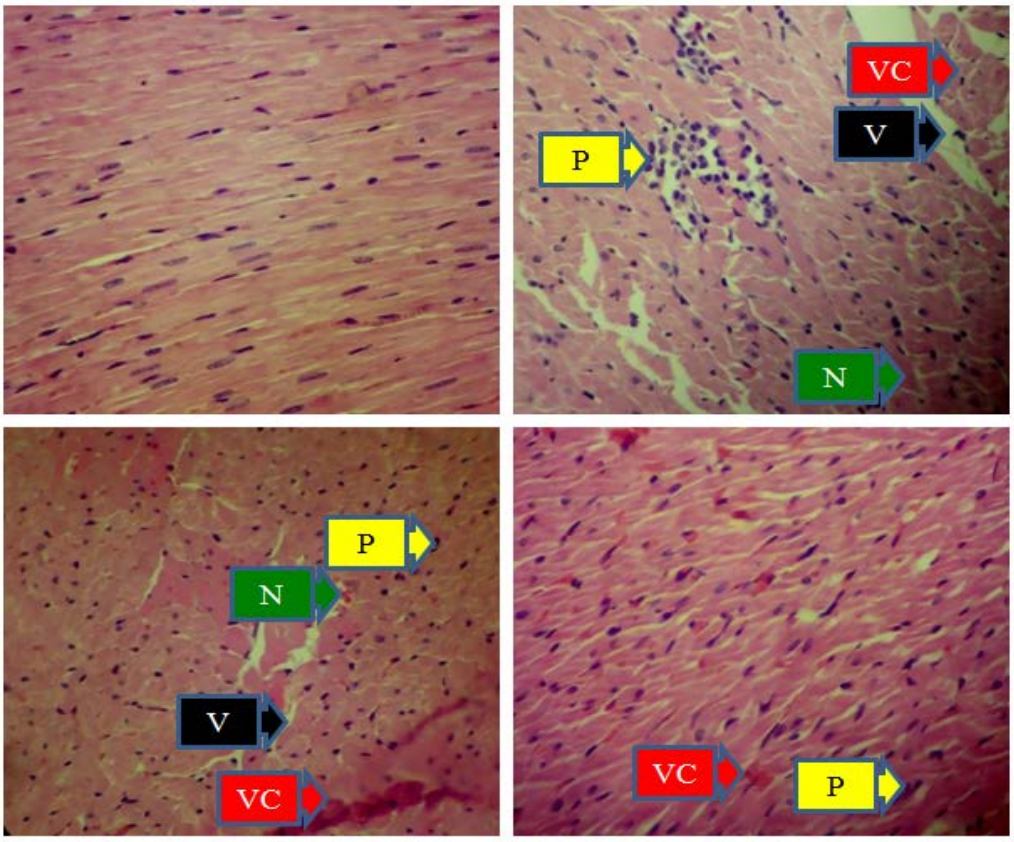


Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{###}P< 0.001$ as compared with non diabetic.

Figure 5.7.20.1 Effect of MET alone and triple drug therapy using TRIG + SITA + MET on heart weight in diabetic cardiomyopathic rats

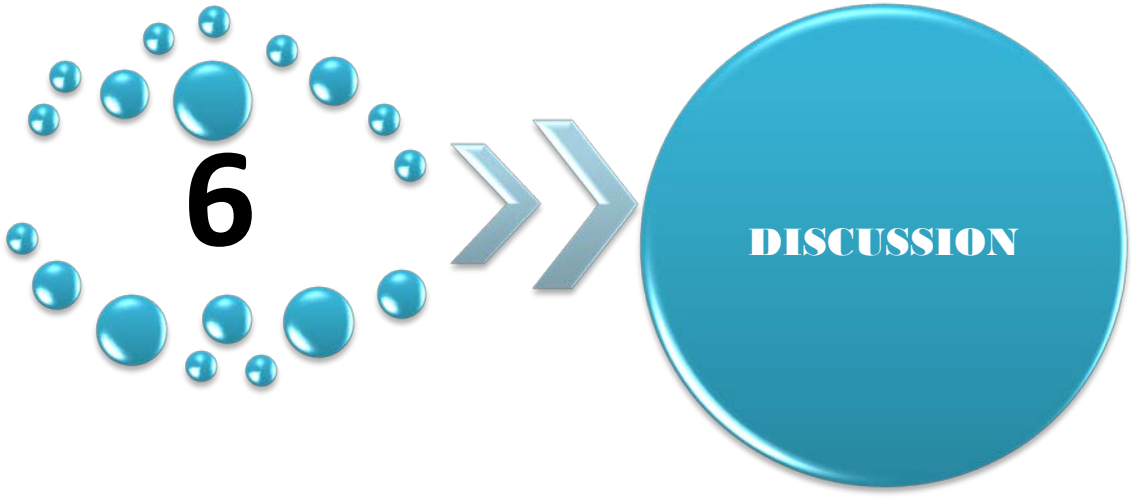
5.7.21.1 Histopathological examination of isolated cardiac tissue of diabetic cardiomyopathic rats [H&E stain]

After eleven weeks of study examination of isolated heart tissue sections stained with hematoxylin & eosin (H&E). Histopathology of isolated rat heart of nondiabetic rat showed (Grade -) normal architecture. Diabetic rat heart showed (Grade+++) necrosis, (Grade +++) pyknosis, (Grade +++) vascular congestion, (Grade +++) vacuolization; MET showed (Grade++) necrosis, (Grade +) pyknosis, (Grade +) vascular congestion, (Grade ++) vacuolization; TRIG+SITA+MET treated diabetic heart showed (Grade-) necrosis, (Grade +) pyknosis, (Grade +) vascular congestion, (Grade -) vacuolization. The scoring of grades was as per Dr. Pralhad Wangikar (MVSC), Prado- Preclinical Research and Development Organization, Pune. Where (Grade -) normal; (Grade +++) severe; (Grade ++) moderate; (Grade +) mild Figure 5.7.21.1).



Where, 1] Non diabetic group; 2] Diabetic group; 3] MET (300 mg/kg p.o.); 4] TRIG (50 mg/kg p.o.) + SITA (5 mg/kg p.o.) + MET (300 mg/kg p.o.). P-pyknosis, N- necrosis, VC- vascular congestion, V-vacuolization. [Magnification 40x].

Figure 5.7.21.1 Histopathological examination of isolated cardiac tissue of diabetic cardiomyopathic rats (H&E stain)


6
DISCUSSION

6. DISCUSSION

Streptozotocin (STZ) (2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose) is a naturally occurring compound, produced by the soil bacterium *Streptomyces achromogenes* that exhibits broad spectrum of antibacterial properties. It is a mixture of α-and β- stereoisomer's that appears pale yellow or off- white crystalline powder. In terms of solubility, it is very soluble in water, ketones and lower alcohols, but slightly soluble in polar organic solvents. Streptozotocin has a molecular formula of $C_8H_{15}N_3O_7$, molecular weight of 265 g/mol and the structure is composed of nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other end [Eleazu *et al*., 2013]. STZ is a cytotoxic glucose analogue. After its discovery, it was being used as a chemotherapeutic alkylating agent in the treatment of metastasizing pancreatic islet cell tumors and other malignancies [Lenzen, 2007]. In the year 1963, Rakieten and colleagues reported that STZ is diabetogenic [Rakieten *et al*., 1993]. From that time of discovery till date, STZ has been one of the chemical agent for the induction of diabetes in experimental animals [Eleazu *et al*., 2013].

STZ exerts cytotoxic effect on pancreatic β-cells and its effects can be seen within seventy two hours after administration depending on the dose administered [Hassan *et al*., 2010]. Hyperglycemia causes oxidative damage by the generation of reactive oxygen species plays a pivotal role in the development of diabetes complications, both microvascular and macrovascular [Morgan *et al*., 1994; Giacco and Brownlee, 2010].

Administration of both streptozotocin (STZ) and nicotinamide (NICO) has been proposed to induce experimental type 2 diabetes in the rat. STZ is well known to cause pancreatic β-cell damage, whereas NICO is administered to rats to partially protect insulin-secreting cells against STZ. STZ is transported into β-cells via the glucose transporter GLUT 2 and causes DNA damage. However, exaggerated activity of this enzyme results in depletion of intracellular nicotinamide adenine dinucleotide NAD (+) and ATP and the insulin-secreting cells undergo necrosis. The protective action of NICO is due to the inhibition of poly (ADP-ribose) polymerase (PARP-1) activity (PARP-1). NICO inhibits this enzyme, preventing depletion of NAD (+) and ATP in cells exposed to STZ. Moreover, NICO serves as a precursor of NAD (+) and thereby additionally increases intracellular NAD (+) levels [Szkudelski, 2012].

Trigonelline (TRIG), the major isolated component of seeds of *Trigonella foenum graceum* L., is commonly used to treat diabetes in China [Zhou *et al*., 2013]. TRIG is found in various plants and in some animal species including sea urchins and jellyfish [Hiroshi, 2006]. Trigonelline is a plant alkaloid which is claimed to have antihyperglycemic, antiseptic, hypocholesterolemic, anticarcinogenic, antimigraine activities [Zhou *et al*., 2012]. TRIG is reported to have antihyperglycemic effect in alloxan induced diabetes in mice [Shah *et al*., 2006].

Food and Drug Administration (U.S.) in October 2006 has approved sitagliptin (SITA) as first dipeptidyl peptidase-IV (DPP-IV) inhibitor for the treatment of type 2 diabetes [White, 2008]. After ingestion of meal it enhances levels of incretin hormones like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) [Sanusi, 2009]. Both GLP-1 and GIP hormones enhance glucose-dependent insulin biosynthesis and release of glucagon-like peptide-1, additionally inhibit glucagon secretion and delays gastric emptying [Kolika, 2012]. Moreover in animal models DDP-IV inhibitors are shown to enhance glycemic control, insulin secretion and proliferation and differentiation of pancreatic beta cells [Moritoh *et al*., 2009]. These effects may be beneficial in preserving the pancreatic beta cell mass and function [Matveyenko, 2009].

Although both trigonelline (TRIG) and sitagliptin (SITA) are effective in reducing the hyperglycemia. The extent to which this reduction of blood glucose is achieved is not adequate. The serum glucose level still remains high. Than the threshold levels of fasting (70-100 mg/dl) and postprandial (<140mg/dl). Although reduction in serum glucose by 20% or more is considered as threshold for deciding antihyperglycemic effect in the animals. In clinical conditions necessitate tight control of sugars in human diabetics. Therefore in clinical practice more than one oral hypoglycemic drug without insulin or with insulin are used. No reports are available on the concomitant administration of two drugs i.e. (TRIG + SITA) and triple drug therapy using (TRIG + SITA + MET) on microvascular and macrovascular complications in preclinical studies. It is necessary to study the effect of one bioactive compound + two synthetic drugs in the prevention of progression of diabetic complications.

Combination therapy refers to administration of two or more drugs to treat disease. Combination therapy consists of different pros likewise, slow development of resistance, cost effectiveness, less treatment failure rate, low case fatality ratios [Chou *et al*., 2010]. However, mechanistic combination may link with better effects.

Moreover, diseases can be cured possibly by acting through different mechanistic pathways [Kalra *et al*., 2010]. Therefore, in present investigation concomitant effect two (TRIG + SITA) and three drug therapy using (TRIG + SITA + MET) was justified for the evalution of diabetic nephropathy, neuropathy and cardiomyopathic complications in Wistar rats.

In the present study, streptozotocin injection (65mg/kg i.p.) produced injury to the pancreatic β- cells which resulted hyperglycemia. Nicotinamide (110mg/kg p.o) was administered prior to streptozotocin to avoid severe damage to the pancreatic β- cells which demonstrated stable, moderate hyperglycemia and glucose intolerance.

In the present study, acute administration of TRIG (100 mg/kg, p.o.), SITA (10 mg/kg, p.o.) alone showed maximum reduction in serum glucose level at 6h post administration (27.48% and 29.65%) respectively. Apparently although the reduction was more than 20% and satisfied the criteria of assessing antihyperglycemic effect but both the drugs failed to restore the concomitant administration of this two drugs in three doses design was envisaged. The extent of reduction of serum glucose level in diabetic animal revealed that TRIG (50 mg/kg p.o.) +SITA (5 mg/kg p.o.) i.e. in the ratio of 50:50% was more effective. Hence further experiments were carried out in using this preparation. Acute study has some limitations in case of onset and duration of action for knowing we go for chronic study.

In the present study, subacute administration of TRIG + SITA (50% + 50%) resulted in antihyperglycemic effect for 28 days. The result indicated that for long-term treatment, TRIG + SITA (50% + 50%) was more effective than either drug alone, since it is possible that beneficial effects of the TRIG + SITA (50% + 50%) on β-cells can result in improvement of glycemia control. However, it is highly plausible that the beneficial effects of this combination on glycemic regulation and β-cell mass are at least partially mediated via increased regeneration of pancreas and GLP-I, GIP signaling. This hypothesis would be consistent with previous reports that trigonelline enhanced pancreatic regeneration in alloxan induced diabetes in mice [Shah *et al*., 2006]. Previous study of sitagliptin in rodent model has shown reduced glycemia in insulinopenic model such as streptozotocin treated rats [Green *et al*., 2006]. In 2011, Yoem et al. reported that sitagliptin preserves the β-cell population in the islets in non-obese and obese diabetic mice [Yeom *et al*., 2011]. In addition, others have shown that mice deficient in DPP-4 and elevated levels of circulating GLP-I and GIP are resistant to STZ-induced beta cell destruction [Mu *et al*., 2006].

Diabetic rats showed significant and progressive reduction in the body weight due to negative nitrogen balance and muscle waste, which are symptom of diabetes [Kulkarni *et al*., 2012]. In present study administration of TRIG +SITA (50%+50%) for period of 28 days in diabetic rats showed significant increase in body weight compared to either drug alone. Results indicated that long term treatment administration of TRIG +SITA (50%+50%) was more effective in the prevention of weight loss than either drug alone.

Kulkarni et al., (2012) reported ethanolic extract of fenugreek seeds showed improved serum insulin level. Sitagliptin improves β-cell function and increases insulin synthesis and its release [Gallwitz, 2007]. In present study, subacute treatment of administration of TRIG + SITA (50%+50%) for 28 days resulted in increased level of serum insulin than alone, which may due to stimulation of release of insulin from the pancreas to improve peripheral glucose utilization.

Glycosylated hemoglobin (HbA_{1c}) has served as an indicator of glycemic control, HbA_{1c} level has been reported to be increased in patients with diabetes mellitus [Edwards *et al*., 1998]. The excess glucose present in the blood reacts with hemoglobin to form HbA_{1c}. Hence, estimation of glycosylated hemoglobin is a well accepted biochemical parameter useful for the diagnosis and management of the disease [Adaramoye, 2012]. Present study demonstrated that concomitant administration of two drugs TRIG + SITA (50%+50%) for 28 days resulted in decreased level of HbA1c than either drug alone. DPP-4 inhibitors used as monotherapy in type 2 diabetic patients, reduced HbA1c levels by an average of -0.8%. These compounds are also effective for chronic glucose control when added to the treatment of diabetic patients receiving metformin, thiazolidinediones, sulphonylureas and insulin. The effects of DPP-4 inhibitors appear to be additive [Powers and Dalessio, 2011]**.**

Histological examination of isolated diabetic rat pancreata showed (Grade +++) destruction while TRIG+SITA (50%+50%) treated pancreata showed (Grade ++) destruction in isolated pancreas compared to either drug alone. Thus this study indicated that TRIG +SITA (50%+50%) preserve the β -cell proportion.

Diabetic nephropathy in type 2 diabetes has become the single most important cause of end stage of renal disease worldwide [Ntemka *et al*., 2011]. Preventing the progression of diabetic nephropathy has been a tough goal in biomedical research [Cristina *et al*., 2011]. Increased levels of serum glucose, creatinine, BUN, uric acid

are the markers for diabetic nephropathy that can be commonly seen in this complication [Idonije *et al*., 2011]. Intraperitonelly administration of NICO-STZ in Wistar rats produced marked and sustained increase in glucose level [Li *et al*., 2011]. STZ caused diabetes by the rapid depletion of β-cells of pancreas [Oguanobi *et al*., 2012; Saini and Sharma, 2013]. Moreover, when administered along with nicotinamide, it causes minor damage to pancreatic β-cells [Masiello *et al*., 1998].

High glucose level is the main factor responsible for structural alteration at the renal level in diabetic nephropathic rats [Somani *et al*., 2012]. Mesangial cells produce endogenous TGF-β1 under high glucose conditions which increase glucose uptake and glucose transport by inducing over expression of mRNA and protein GLUT-1. Ultimately, it initiates glucose induced metabolic abnormalities in mesangial cells [Francesco and Loreto, 2005]. Diabetic Control and Complication Trial Research Group in 1993 elucidated that hyperglycemia is directly linked to diabetic microvascular complications, particularly in kidney [Yamabe *et al*., 2006].

In the present study, triple drug therapy using TRIG (50mg/kg p.o.) + SITA (5mg/kg p.o.) +MET (300mg/kg p.o.) showed significant reduction in serum glucose level than treatment with concomitant therapy of two drugs (TRIG+SITA) and either drug alone after four weeks of treatment. Previous report showed that trigonelline improved pancreatic regeneration in alloxan induced diabetes in mice [Shah *et al*., 2006]. Earlier findings suggest that hypoglycemic effect of trigonelline may be mediated by stimulating insulin synthesis and/or secretion from the beta pancreatic cells of Langerhans [Dijk *et al*., 2009]. Sitagliptin enhance release of incretin hormone GLP-1 from small intestine that stimulates insulin secretion from the pancreas [Hocher *et al*., 2012]. Literature has also revealed that ratio of pancreatic β-cells is maintained by sitagliptin in diabetic mice [Yeom *et al*., 2011]. Metformin is a biguanides that lowers serum glucose level primarily by decreasing hepatic glucose output and reducing insulin resistance [Rossetti *et al*., 1990]. NICO-STZ induced type 2diabetes caused a severe weight loss due to degradation or loss of proteins which contribute to the body weight [Barik *et al*., 2008; Pierre *et al*., 2012; Ruiz-Ortega *et al*., 2003]. In the present study kidney weight in diabetic rat was significantly increased as compared to normal rat. Administration of triple drug therapy using TRIG+SITA+MET significantly increased body and decreased kidney weight. These findings suggest that triple drug therapy may prevent kidney hypertrophy.

The abnormalities in kidney functions progress by alteration in renal haemodynamics which leads to proteinuria, glomerulosclerosis and renal dysfunction [Jefferson *et al*., 2008]. Management of renal hemodynamic abnormality and reduction of proteinuria are important to prevent the decline of kidney function. Degradation of protein and nucleic acid results in the formation of non-protein nitrogenous compounds such as urea and creatinine [Firdous *et al*., 2013]. A significant elevation in serum creatinine, uric acid and BUN levels is indicative of impaired renal function in diabetic animals. These are independent predictor for diabetic nephropathy [Mustafa *et al*., 2012]. The present study demonstrated that chronic administration of TRIG+SITA+MET improved renal function, which was evident from the lowered serum uric acid, creatinine and blood urea nitrogen levels compared to concomitant therapy of two drugs (TRIG+SITA) and either drug alone treated rats.

Glomerular filtration rate is calculated by creatinine levels in blood and urine which is indication of renal function. High creatinine level in urine is ideal while high blood creatinine level indicate alteration in the kidney function. GFR is ultimate indication for assessment of excretory functions of kidney [Woodhouse *et al*., 2005; Li *et al*., 2009; Owolabi and Omogbai, 2011]. Significant decrease in urine volume observed in the study; probably appear as a result of the normalization of serum glucose level. The present study demonstrated that administration of triple drug therapy TRIG+SITA+MET significantly increased urine creatinine level and decreased urine volume than either drug alone treated groups.

In present study kidney weight in diabetic rats significantly increased as compared to normal rats. Chronic administration of triple drug therapy using TRIG+SITA+MET significantly increased body weight and decreased kidney weight compared to concomitant administration of two drugs (TRIG + SITA) and either drug alone. These findings suggest that triple drug therapy TRIG+SITA+MET may prevent kidney hypertrophy.

Previous study suggests that oxidative stress is provoked in diabetic nephropathy [Matough *et al*., 2012]. Metabolic activity within the nephron generates a large amount of reactive oxygen species (ROS) that are balanced by antioxidant enzymes and free radical scavenging systems [Sayed, 2012]. Various biological effects such as peroxidation of cell membrane lipids, oxidation of proteins, renal vasoconstriction and damage to DNA are aggravated by ROS [Noeman *et al*., 2011]. High glucose level is associated with oxidative stress through generation of ROS from the mitochondrial

electron transport chain [Yu *et al*., 2011]. Alterations in glucose metabolism disturb several pathways such as polyol pathway, advanced glycation and uncoupling of nicotinamide adenine dinucleotide phosphate oxidase (NADPH) [Krishan and Chakkarwar, 2011; Elmarakby and Sullivan, 2012].

Oxygen free radicals exert their cytotoxic effects on membrane phospholipids resulting in the formation of lipid peroxidation product malondialdehyde (MDA) [Cheng *et al*., 2011]. MDA is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation [Allegra *et al*., 2002; Somani *et al*., 2012; Ghule *et al*., 2012]. Present study showed that the renal concentration of MDA was elevated and that of GSH, SOD decreased in diabetic animals. Treatment with triple drug therapy using TRIG+SITA+MET significantly decreased MDA content and increased SOD, GSH concentration than concomitant therapy of two drugs (TRIG+SITA) and either drug alone. Elevated concentration of MDA is due to reactive oxygen species (ROS). Moreover decreased SOD activity and GSH concentration may be due to defense mechanism against oxygen free radicals.

Trigonelline ameliorated diabetic hypertensive nephropathy by suppression of oxidative stress in kidney and reduction in renal cell apoptosis and fibrosis in streptozotocin induced neonatal diabetic (nSTZ) rats [Ghule *et al*., 2012]. Several mechanisms might be responsible for the protective effects of sitagliptin against renal damage in diabetic nephropathy; sitagliptin treatment attenuated renal dysfunction and structural damage by suppression of renal oxidative stress by activating AMP-activated protein kinase (AMPK) together with activation of FoxO3a signaling in rats [Joo *et al*., 2013; Vaghasiya *et al*., 2011; Cristina *et al*., 2013; Li *et al*., 2009]. Metformin reduced renal oxidative stress by activation AMPK [Viollet *et al*., 2012].

In previous study, non diabetic rat kidneys showed normal morphology of glomerular and tubular architecture [Soria *et al*., 2011]. Kidneys of diabetic rat showed glomerular damage and severe destruction of tubules occurs due to factors such as glomerular hyperplasia, tubular hypertrophy and interstitial expansion [Kiran *et al*., 2012]. In the present study, histological examination of isolated diabetic rat kidney showed glomerular necrosis (Grade +++), tubular swellings (Grade +++), glomerular fibrosis ((Grade +++) and peritubular tubular fibrosis (Grade +++). Administration of TRIG+SITA+MET showed renal prevention the damage was mild (+) in nature compared to concomitant therapy of two drugs (TRIG+SITA) and alone drug treated kidneys. The result of present study showed that triple therapy drug administration of

TRIG+SITA+MET compared to concomitant administration of two drugs and either drug alone had better renoprotective effect in diabetic nephropathic rats.

Diabetic neuropathy threatens quality of life affecting function, mood and sleep pattern of the majority of affected patients [Griebeler *et al*., 2012]. About 33.5% of patients with diabetic neuropathy are strongly related to serious mortality and mobility rate [Brannagan, 2012]. Diabetic neuropathy is characterized by clinical features like allodynia, hyperalgesia due to elevated nociceptive response, reduced motor nerve conduction velocity and reduced threshold to painful stimuli [Fuchs *et al*., 2010]. The underlying mechanisms of persistent pain in diabetic patients remain poorly understood [Mariee *et al*., 2013; Galeotti *et al*., 2014]. Treatment of painful diabetic neuropathy represents a major unmet medical need [Mariee *et al*., 2013].

STZ induced diabetic neuropathy is well known model for especially in rats [Are *et al*., 2011]. In the present study, administration of triple drug therapy using TRIG (50mg/kg p.o) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o) for four weeks, it showed significant reduction in serum glucose level and increases in body weight compared to concomitant administrion of two drug and either drug alone. Trigonelline reduced serum glucose level it might be due to regeneration in pancreatic β-cells [Kulkarni *et al*., 2012]. It has been reported that sitagliptin enhance release of GLP-1 that stimulates insulin secretion from pancreatic beta cells [Gallwitz, 2007]. Metformin inhibit hepatic glucose production and enhance peripheral glucose utilization [Cheng *et al*., 2006].

In the study undertaken, behavioral techniques to distinguish nociceptor functions in diabetic rats was used. For behavioral studies Von Frey hairs, Randall Selitto and tail flick are reported methods to measure mechanical hyperalgesia, thermal hyperalgesia in preclinical studies [Sandkuhler, 2009]. STZ causes damage in sensory and motor fibers resulting in reduction in pain threshold [Kappelle *et al*., 1993]. In the present study significant increase in pain threshold was observed in the TRIG (50mg/kg p.o) + SITA (5mg/kg p.o.)+ MET (300mg/kg p.o) treated rats compared to concomitant therapy of two drugs (TRIG+SITA) and either drug alone. It is apparent that triple drug therapy protect pain threshold might be due to reduction in motor and sensory damage. Accordance with previous study of enzyme dipeptidyl peptidase IV (DPP- 4) inhibitors showed amelioration in pain threshold due to reverse alteration in damaged motor and sensory fibers in rats [Davidson *et al*., 2008; Zhou *et al*., 2012]. Metformin

increase in pain threshold might be due to reduced oxidative stress in diabetic neuropathic rat model [Sharma *et al*., 2012].

Reduced MNCV in diabetic rats is due to high glucose level which leads to neuronal dysfunction and nerve reperfusion to cause the endoneurial hypoxia [Stevens *et al*., 2000]. Other finding suggest that stz induced neuropathy resulted in structural changes leads to endoneurial edema, increased intraneural pressure, decreased blood flow to nerves; ischemias leads to axonal degeneration and finally decrease in MNCV [Morani *et al*., 2012]. Present study demonstrated that MNCV was decreased in diabetic rats. Administration of TRIG+SITA+MET showed marked increase in MNCV compared to concomitant therapy of two drugs and either drug alone. It is apparent that triple drug therapy TRIG+SITA+MET protects neuronal dysfunction (sciatic nerve) by alteration in morphological and neuronal architecture. Trigonelline restored the MNCV might be due to reduced oxidative stress. Metformin inhibits tissue advanced glycation end product formation, which in turn may contribute to influence the nerve function by modifying cell signaling and nerve blood flow [Nagilla *et al*., 2014]. In 2011, Davidson reported that improvement in MNCV by DPP-IV inhibitor alogliptin to improved vascular relaxation in response to calcitonin gene related peptide in mice.

High glucose level is responsible for generation of reactive oxygenase species (ROS) that leads to imbalance between radical production and radical scavenging system resulted in generation of oxidative stress [Abraham *et al*., 2008; Esfandiari *et al*., 2007]. The elevated levels of oxidative stress in diabetic rats responsible for the vascular impairment that resulted in endoneurial hypoxia thus causes impaired neuronal function which are the primary features of diabetic neuropathy. Accumulation of ROS increases lipid, DNA, and protein peroxidation, induces cellular apoptosis and reduces nerve blood flow [Han *et al*., 2013]. MDA is endogenous biomarker mainly responsible for oxidative damage causing vascular endothelial dysfunction [Bandeira *et al*., 2013]. SOD provides protection to all aerobic cells by scavenging superoxide radicals and hydrogen peroxide (H_20_2). Present study showed increased level of MDA and reductions in the activity of SOD in sciatic nerve of diabetic rats as compared to nondiabetic rats. Increased MDA level in stress condition, is responsible for lipid membrane destruction resulted tissue injury, which resembles with the reported literature [Pandey and Rizvi, 2010; Andrea *et al*., 2003]. In the present study administration of triple drug therapy using

TRIG+SITA+MET showed decreased level of MDA and amelioration in the level of SOD as compared to concomitant drug therapy of two drugs and either drug alone. Previous findings suggest that trigonelline partially normalizing glucose homeostasis and restored altered enzyme activities [Genet *et al*., 1999; Bakuradze *et al*., 2010]. Trigonelline reduced oxidative stress by restored altered enzymes activity in rat [Genet *et al*., 1999; Bakuradze *et al*., 2010]. Sitagliptin showed antioxidant property which might be due to redued overproduction of reactive oxygenase species. Antioxidant effect of metformin due to maintain hyperglycemic homeostasis, improved neuronal signaling by decline ROS levels [Nagilla *et al*., 2014].

TRIG effect in diabetic neuropathy may be partly attributed to it regulation of GLP-1R/p 38 MAPK signaling pathway. GLP- 1R has regenerative effect on peripheral nervous system via reduced ROS production [Temraz *et al*., 2006; Tohda *et al*., 1999]. DPP-4 inhibitors might be act through inhibition of polyol pathway which leads to improved nerve blood flow and nerve fiber damage in patient with diabetic neuropathy [Sakamoto *et al*., 2013].

In the present study histological examination of isolated sciatic nerve of diabetic rat showed severe necrosis (Grade +++), swelling (Grade +++) and congestion (Grade +++). Administration of TRIG+SITA+MET treated rats showed (+) damage compared to concomitant and either drug alone. Results of present study demonstrated that treatment with TRIG+SITA+MET showed significant attenuation in progression diabetic neuropathy in rats. Most of parameters tested showed correlation to glycemic control. Hence appropriate glycemic control will result in lessening of diabetic neuropathy.

Chronic hyperglycemia is an independent risk factor for development of cardiomyopathy [Leung *et al*., 2013; Pappachan *et al*., 2013; Chow *et al*., 2014]. Diabetic cardiomyopathy is characterized by cardiac fibrosis, cellular necrosis, cardiac hypertrophy, decreased ventricular compliance, systolic and diastolic dysfunction [Soetikno *et al*., 2012]. Cardiovascular disease is a common complication of diabetes responsible for 80% of the mortality in the diabetic population [Fowler *et al*., 2008].

Previously it was reported that echocardiogram of diabetic rats showed several alterations in cardiac function as compared to non diabetic rats most of them related to QT and QTc interval prolongation and decreased QRS complex [Clemente *et al*., 2012]. QT interval represent the time period between ignition of depolarization and

end of repolarization of the ventricular myocardium [Clemente *et al*., 2012]. Moreover, heart rate corrected interval (QTc) prolongation is associated with morbidity and mortality in diabetes [Timar *et al*., 2013].We found similar results in current investigation; diabetic rats showed significant prolongation in QT, QTc interval whereas QRS complex was decreased. Concomitant administration of TRIG+SITA+MET showed significant reduction in prolongation of QT and QTc interval and considerable improvement in QRS complex as compared to concomitant therapy of two drugs (TRIG+SITA) and either drug alone. Inversion of P wave was observed in present study which is an indication of abnormality of SA node in diabetic rats whereas concomitant treated group did not show inversion of P wave. Chronic hyperglycemia may produce ventricular instability, as manifested by QT and QTc prolongation and reduction in QRS complex [Howarth *et al*., 2009]. In the present study treatment with triple drug therapy using TRIG+SITA+MET prevented pathologic alterations in ECG indicating its protective effect on cell membrane function.

Cardioprotective effect of TRIG+SITA+MET against NICO-STZ induced cardiomyopathy in Wistar rats were observed in present study. There were significant decrease in systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure in diabetic rats. The decrease might be due to myocardial necrosis or hypertrophy. TRIG+SITA+MET treated animals showed reduction of myocardial necrosis. Moreover triple drug therapy showed significant increase in SBP, DBP and MABP as compared to concomitant therapy of two drugs (TRIG+SITA) and monotherapy.

The technique of left ventricular (LV) analysis in rats has been introduced recently [Radovits *et al*., 2009]. The max dP/dt and min dP/dt pressure developed during contraction and relaxation. In the present investigation, significant decrease in both pressure max dp/dt and min dp/dt in diabetic rats. Administration of triple drug therapy showed significant improvement in LV max dp/dt and min dp/dt. The observed findings thus suggest that triple drug therapy using TRIG+SITA+MET provided sufficient contractile reserve.

Chronic sustained hyperglycemia leads to increase in the levels of myocardial enzymes like CKMB (creatine kinase isoenezyme), LDH (lactate dehydrogenase), and AST (Aspartate amino transferase). Creatine kinase has three isoforms; MM (CK-MM), MB (CK-MB) and BB (CK-BB) [Kemp *et al*., 2004]. Myocardium contain CK-

MB is a more sensitive indicator for necrosis or ischemia [Edet *et al*., 2009]. Increased level of LDH indicates cardiac muscle damage [Patel and Goyal, 2011]. It has been previously reported that elevated level of AST enzyme is an indication of myocardial injury [Aslani *et al*., 2013]**.** In the present investigation, levels of all three myocardial enzymes were significantly elevated in diabetic rats as compared with non diabetic rats. Triple drug therapy using TRIG+SITA+MET showed significant decrease in the levels of CK-MB, LDH and AST as compared to concomitant therapy of two drugs (TRIG+SITA) and monotherapy. Triple drug therapy might have showed additive effect in protection of myocardial necrosis/ ischemia, damage and injury by decreasing the levels of myocardial enzymes.

Increased levels of serum triglyceride, cholesterol, very low density lipoprotein and down regulation of high density lipoprotein were observed in diabetic rats in present study which indicated that hyperglycemia interfere with metabolism or biosynthesis of lipid. These findings were consistent with previous reports [Kain *et al*., 2010; Kim *et al*., 2012]. Administration triple drug therapy of TRIG+SITA+MET normalized the serum lipid profile. Lipid lowering action of trigonelline can be attributed to inhibition of key enzymes like hexokinase, glucokinase, pyruvate kinase, malic enzyme, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, sorbitol dehydrogenase in the liver which led to a significant decrease in triglyceride, cholesterol and an increase in the HDL level [Hamden *et al*., 2013; Zhou *et al*., 2013].

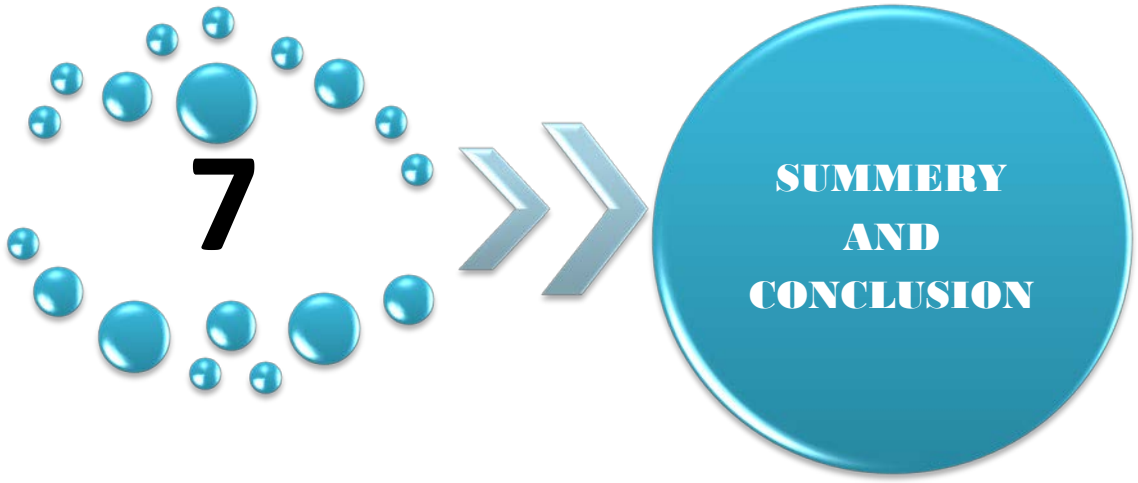
Lipase is an essential enzyme that is important for the conversion of triglycerides from the lipoproteins of blood to fatty tissues, heart and muscles. Previously it was reported that lipase activity was increased in the intestine of diabetic rat that increased lipid absorption from intestine these consequences cause hypercholesterolemia and hyperlipidemia in the serum [Howarth *et al*., 2009]. Trigonelline decreased lipase level in the intestine by inhibiting hydrolysis of dietary triglyceride into monoglycerides and free fatty acids as it lowered the cholesterol, low density lipoprotein, triglyceride and increased levels of high density lipoprotein in rat serum [Howarth *et al*., 2009].Previous findings demonstrated that levels of cholesterol, low density lipoprotein, triglycerides were decreased with sitagliptin as GLP-1 influences intestinal triglyceride absorption, potentially by inhibiting gastric lipase [Shehata *et al*., 2013; Sakamoto *et al*., 2013].Metformin reversed the altered lipid levels due to its antiatheroslerotic effect in rat [Zhongju *et al*., 2014].

In the present study histological examination of isolated diabetic rat heart showed (Grade +++), necrosis, (Grade +++) pyknosis, (Grade +++) congestion, (Grade +++) vacuolization while treatment with triple drug therapy showed (Grade +) damage compared to concomitant therapy of two drugs and monotherapy. Thus this study indicated that TRIG+SITA+MET offered protection of the heart of diabetic animals.

Cardioprotective effect of fenugreek seed is preliminary due to presence of saponins, 4 hydroxyisoleucine, trigonelline and high fiber contents [Petit *et al*., 1995; Sauvaire *et al*., 1998; Raghuram *et al*., 1994; Ali *et al*., 1995]. Fenugreek seed maintains level of lipid profile and counteracts the oxidative stress of myocardial infract in rats [Murugesan *et al*., 2011]. Additionally fenugreek seed powder has been shown to normalize the activity of creatinine kinase in liver, skeletal muscle and heart of diabetic rats [Genet *et al*., 1999]. Hamden et al, (2013) reported that trigonelline protect liver and kidney function in diabetic rat. Trigonelline ameliorates diabetic hypertensive nephropathy by suppression of oxidative stress and maintain level of TNF alpha in neonatal diabetes [Ghule *et al*., 2012].

It has been reported that sitagliptin a DPP-4 inhibitor exhibited protective effect on tissue injury of heart, lung and kidney in rat [Joo *et al*., 2013]. Chronic treatment with sitagliptin showed reduction in myocardial infracts size with GLP-1 receptor-PKA pathway through glucose dependant manner [Hausenloy *et al*., 2013]. DPP-4 inhibitors facilitate potential of GLP-1 to provide cardiac protection against ischemia [Read *et al*., 2010].Trigonelline showed antihyperglycemic activity by elevating the levels of insulin via regenerating pancreatic beta cells [Arif *et al*., 2014]. Sitagliptin utilizes an increased level of insulin for the facilitation of action potential of GLP-1 expression in heart tissue by activation of PKA pathway. Cardioprotective effect metformin have been reported to be mediated by its activation of AMP-activated protein kinase (AMPK) in mice [Calvert *et al*., 2008].

Thus it is concluded that triple drug therapy using trigonelline + sitagliptin + metformin was more effective than two drug therapy (trigonelline + sitagliptin) in the prevention of progression of diabetic microvascular (neuropathy, nephropathy) and macrovascular (cardiomyopathy) complications in laboratory animals compared to monotherapy. Present investigation thus provided the basic preclinical data supporting the effectiveness of triple drug therapy in the diabetic animals.


7
SUMMERY
AND
CONCLUSION

7. SUMMARY AND CONCLUSION

In the present investigation, trigonelline (TRIG) and sitagliptin (SITA) were selected for evalution for antihyperglycemic activity.

- Antihyperglycemic activity of TRIG, SITA alone and their concomitant administration of TRIG+SITA in NICO-STZ induced diabetes in Wistar rats.
- In present acute study, diabetic rats treated with TRIG (25, 50 and 100 mg/kg p.o) and SITA (2.5, 5 and 10 mg/kg p.o.) showed dose dependent reduction in serum glucose level. The doses of TRIG (100 mg/kg) and SITA (5mg/kg) were found to be effective. For further study we kept TRIG (100 mg/kg) and SITA (10mg/kg) as 100% dose.
- For further acute study we have concomitantly administered TRIG (100mg/kg p.o.) and SITA (10mg/kg p.o.) in the combination of doses as 70%+30%, 50%+50%, 30%+70%. Administration of TRIG 50% + SITA 50% viz; TRIG (50 mg/kg p.o) + SITA (5 mg/kg p.o.) were found to be more effective than other concomitants.
- The onset of antihyperglycemic effect of TRIG+SITA was at 2h; peak effect at 6h but the antihyperglycemic effect waned at 24h. Concomitant administration of TRIG+SITA (50%+50%) showed maximum peak reduction at 6h compared to other concomitant treated diabetic rats.
- In subacute study diabetic rats treated with TRIG+SITA (50%+50%) for 28 days showed significant maximum reduction in serum glucose level, while as preventing loss of body compared to other concomitant doses.
- TRIG+SITA (50%+50%) significantly lowered HbA1c compared to other concomitants in diabetic rats on last day of study period.
- The serum insulin was significantly increased in the concomitant TRIG+SITA (50%+50%) treated diabetic rats on last day of study period compared to others concomitants.

Thus we concluded that concomitant administration of TRIG (50 mg/kg p.o) + SITA (5 mg/kg p.o.) for the period of four weeks in nicotinamide-streptozotocin induced diabetes in Wistar rats showed significant decrease the level of serum glucose, HbA1c and increased serum insulin, prevent loss of body weight with improvement in damaged pancreatic islets. Thus, TRIG+SITA have additive antihyperglycemic activity in NICO-STZ induced diabetes in Wistar rats.

In the present investigation, TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and their concomitant therapy of two drugs (TRIG 50mg/kg p.o. + SITA 5mg/kg p.o.) were selected for evalution of diabetic nephropathy in Wistar rats.

- Diabetic rats treated with (TRIG 50mg/kg + SITA 5mg/kg) showed maximum significant reduction in serum glucose level compared to TRIG and SITA alone.
- Treatment with (TRIG+SITA) significantly inhibits the decreased body weight and increased kidney weight compared to TRIG and SITA alone.
- In present study, diabetic rats showed alteration in the levels of serum creatinine, uric acid, blood urea nitrogen, urine creatinine and urine volume. Four week treatment with (TRIG+SITA) showed significant reverse alteration in the levels of serum creatinine, uric acid, blood urea nitrogen, urine creatinine and urine volume compared to TRIG and SITA alone.
- Concomitant treatment with (TRIG+SITA) significantly inhibited this alteration in antioxidant enzymes compared to either drug alone.

Findings of present investigation showed that concomitant therapy of two drugs TRIG 50mg/kg p.o. + SITA 5mg/kg p.o. compared to either drug alone have better renoprotective effects in diabetic rats. However, the concomitant administration of trigonelline along with sitagliptin not only attenuated the glucose homeostasis but also showed significant decrease in kidney weight, serum creatinine, BUN, serum uric acid, urine volume, the level of lipid peroxidation product, MDA and significant increase in the urine creatinine, activity of endogenous antioxidants such as SOD and GSH. Results obtained from histopathological study confirmed that concomitant administration prevented kidney damage, which provided structural support for the renal shielding effect.

In the present investigation TRIG (50 mg/kg p.o), SITA (5 mg/kg p.o.) alone and their concomitant therapy of two drugs (TRIG 50mg/kg + SITA 5mg/kg) were selected for evalution of diabetic neuropathy in Wistar rats.

- Diabetic neuropathic rats treated with TRIG+SITA showed maximum significant reduction in serum glucose level compared to TRIG and SITA alone.
- Treatment with TRIG+SITA significantly prevented loss of body weight.
- Intraperitoneal administration of NICO-STZ resulted significant decrease in pain threshold, whereas treatment with TRIG+SITA significantly inhibited this decreased level of pain threshold elevated by Randall Selitto paw pressure test, Von-Frey hair test and radiant heat test.
- Decrease in the motor nerve conduction velocity in diabetic neuropathic rat. This decreased level was significantly restored by TRIG+SITA compared to TRIG and SITA alone.
- Alteration in the level of antioxidant enzymes of isolated sciatic nerve tissue in diabetic neuropathic rats. Treatment with TRIG+SITA significantly inhibited this alteration in antioxidant enzymes.

It is concluded that concomitant therapy of two drugs TRIG+SITA not only decreased serum glucose level but also showed significant decrease MDA level and significant increase in pain threshold, MNCV and level of SOD. Histopathological analysis of rat sciatic nerve showed minimum pathological changes. Thus, additive effect of concomitant administration of TRIG+SITA might be able to improve multiple mechanisms involved in the pathophysiologic events of diabetic neuropathy.

In the present investigation TRIG (50 mg/kg p.o.), SITA (5 mg/kg p.o.) alone and concomitant therapy of two drugs (TRIG 50mg + SITA 5mg/kg p.o) were selected for evalution of diabetic cardiomyopathy in Wistar rats.

- TRIG+SITA showed maximum reduction in serum glucose level compared to TRIG and SITA alone.
- TRIG+SITA significantly lowered the levels of cardiac markers viz; CK-MB, AST and LDH compared to TRIG and SITA alone.
- Diabetic cardiomyopathic rats showed significant increase in triglyceride, cholesterol, very low density lipoprotein, where as significant decrease in high density lipoprotein. Concomitant therapy of two drugs TRIG+SITA showed significant decrease in triglyceride, cholesterol, very low density lipoprotein, where as significant increase high density lipoprotein compared to TRIG and SITA alone.
- Concomitant treatment with TRIG+SITA in diabetic cardiomyopathic rats showed significant increase heart rate and QRS interval, where as significantly decreased QTc interval compared to TRIG and SITA alone.
- Concomitant administration of TRIG+SITA on haemodynamic parameters showed significant increase in SBP, DBP, MABP, max dp/dt, min dp/dt where as significantly decreased in EDP compared to TRIG and SITA alone.
- TRIG+SITA showed significant inhibition in decrease body weight where as significant inhibition in increase in the heart weight.

Present investigation suggest that concomitant therapy of two drugs TRIG+SITA in diabetic cardiomyopathic rats showed significant antihyperglycemic and antihyperlipidemic activity which remarkably restored the alteration in ECG, heamodynamic and cardiac biomarkers enzymes with heart histopathology resulted improvement in myocardial damage. Thus, TRIG+SITA treated rat showed additive effect in diabetic cardiomyopathic rats.

In present investigation MET 300 mg/kg p.o alone and triple drug therapy using TRIG 50mg/kg + SITA 5mg/kg + MET 300mg/kg was selected for the evaluation of activity in diabetic nephropathy.

- Activity of MET 300 mg/kg and triple drug therapy using TRIG 50mg/kg+ SITA 5mg/kg + MET 300mg/kg on diabetic nephropathy was investigated in NICO-STZ induced diabetic Wistar rats.
- Diabetic rats treated triple drug therapy using TRIG+SITA+MET showed significant decrease in serum glucose level compared to MET alone.
- Diabetic rats treated with treated with triple drug therapy using TRIG+SITA+MET showed significant prevention in loss of body weight where as significant decrease in kidney weight compared to MET alone.
- In present study, diabetic rats showed alteration in the levels of serum creatinine, uric acid, blood urea nitrogen, urine creatinine and urine volume. Four week treatment with TRIG+SITA+MET showed significant reverse alteration in the levels of serum creatinine, uric acid, blood urea nitrogen, urine creatinine and urine volume compared to MET alone.
- Diabetic rats treated with TRIG+SITA+MET showed significantly increase in SOD, GSH where as decrease in MDA antioxidant activity compared to MET alone.

Hence to conclude, the present investigation showed renoprotective activity via modulation of various endogenous antioxidant defense systems along with biomarkers by controlling hyperglycaemia.

In present investigation MET 300 mg/kg alone and triple drug therapy using TRIG 50mg/kg+ SITA 5mg/kg+ MET 300 mg/kg was selected for the evaluation of activity in diabetic neuropathy.

- Activity of MET 300 mg/kg alone and triple drug therapy using TRIG 50mg/kg+ SITA 5mg/kg + MET 300 mg/kg in diabetic neuropathy was investigated in NICO-STZ induced diabetic Wistar rats.
- Diabetic rats treated with triple drug therapy using TRIG+SITA+MET showed significantly reduced serum glucose level compared to MET alone.
- Treatment with triple drug therapy using TRIG+SITA+MET significantly prevent loss of body weight.
- Intraperitoneal administration of NICO-STZ resulted in significant decrease in pain threshold, whereas treatment with TRIG+SITA+MET significantly increase compared to MET alone.
- Diabetic rat treated with TRIG+SITA+MET showed significant increase in motor nerve conduction velocity compared to MET alone.
- Diabetic rats treated with TRIG+SITA+MET showed significant increase SOD while as decrease in MDA.

Findings of present investigation indicate neuroprotective role of triple drug treatment in diabetic neuropathic rats. Modulations of antioxidant enzymes and tight control of hyperglycemia.

In present investigation MET 300 mg/kg alone and triple dose using TRIG 50mg/kg+ SITA 5mg/kg+ MET 300 mg/kg was selected for the evaluation of activity on diabetic cardiomyopathy.

- Activity of MET 300 mg/kg alone and triple drug therapy using TRIG 50mg/kg+ SITA 5mg/kg+ MET 300 mg/kg in diabetic cardiomyopathy was investigated in NICO-STZ induced diabetic Wistar rats.
- Diabetic rats treated with triple drug therapy using TRIG+SITA+MET showed significant decrease in serum glucose level compared to MET alone.
- Diabetic rats treated with TRIG+SITA+MET showed significant prevention in loss of body weight where as significant decrease in heart weight.
- Level of cardiac markers CK-MB, AST and LDH was significantly decreased in diabetic cardiomyopathic rats. Diabetic rats treated with TRIG+SITA+MET showed significant reverse alteration in the levels of cardiac markers compared to MET alone.
- Hyperlipidemia was observed in diabetic rats. This diabetes induced hyperlipidemia was more significantly decreased by TRIG+SITA+MET compared to MET alone.
- Diabetic rats treated with TRIG+ SITA+ MET changes in haemodynamic parameters showed significant increase in SBP, DBP, MABP, max dp/dt, min dp/dt where as significant decrease in EDP compared to MET alone.

We conclude that treatment with TRIG+ SITA+ MET effective in prevention of progression of diabetic cardiomyopathy via attenuation in hyperglycemia, hyperlipidemia and cardiac markers.

Thus it is concluded that triple drug therapy using TRIG+SITA+MET (one bioactive compound trigonelline+ two synthetic drugs sitagliptin and metformin) was effective in the prevention of progression of diabetic microvascular (neuropathy, nephropathy) and macrovascular (cardiomyopathy) complications in laboratory animals compared to concomitant/ monotherapy. Present investigation is just the beginning of advance research techniques in the development of new multiple therapies for prevention of diabetes and its complications.


8
HIGHLIGHTS
OF
WORK

8. HIGHLIGHTS OF WORK

Increase/decrease when compared to non diabetic group

Increase/decrease when compared to diabetic group

****P*<0.001, ***P*<0.01, **P*<0.05 when compared to diabetic group. ###*P*<0.001 when compared to normal group.

1. Effect of trigonelline (TRIG) and sitagliptin (SITA) in nicotinamide (NICO) –streptozotocin (STZ) induced diabetes in Wistar rats					
Sr. No	**Parameters/ Groups**	**Diabetic group**	**TRIG (50mg/kg p.o.)**	**SITA (5mg.kg p.o.)**	**TRIG (50mg/kg p.o.)+ SITA (5mg.kg p.o.)**
1	**Serum glucose level at 6h (mg/dl)(Acute study 24h)**	### ▲	*** ▼	*** ▼	*** ▼
2	**Serum glucose level (mg/dl) (Subacute study 28 days)**	### ▲	*** ▼	*** ▼	*** ▼
3	**Body weight (g)**	### ▼	*** ▲	*** ▲	*** ▲
4	**Serum insulin level (g)**	### ▼	*** ▲	** ▲	*** ▲
5	**HbA1c level (%)**	### ▲	** ▼	* ▼	*** ▼

2. Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) in NICO-STZ induced diabetic nephropathy in Wistar rats [8 week study model]					
Sr. No	**Parameters/ Groups**	**Diabetic group**	**TRIG (50mg/kg p.o.)**	**SITA (5mg.kg p.o.)**	**TRIG (50mg/kg p.o.)+ SITA (5mg.kg p.o.)**
1	**Serum glucose level (mg/dl) (Acute study 24h)**	### 	*** 	*** 	*** 
2	**Body weight (g)**	### 	*** 	*** 	*** 
3	**Serum creatinine (mg/dl)**	### 	* 	** 	*** 
4	**Serum uric acid (mg/dl)**	### 	*** 	*** 	*** 
5	**Blood urea nitrogen (mg/dl)**	### 	*** 	*** 	*** 
6	**Urine creatine level (mg/dl)**	### 	* 	** 	*** 
7	**Urine volume (ml)**	### 	*** 	*** 	*** 
8	**Kidney weight (g)**	### 	** 	** 	*** 
9	**MDA (nmol of MDA/mg protein)**	### 	* 	* 	** 
10	**GSH (g of GSH/ mg protein)**	### 	* 	* 	** 
11	**SOD (Unit/ mg protein)**	###	*	*	**

3. Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) in NICO-STZ induced diabetic neuropathy in Wistar rats [8 week study model]					
Sr. No	**Parameters/ Groups**	**Diabetic group**	**TRIG (50mg/kg p.o.)**	**SITA (5mg.kg p.o.)**	**TRIG (50mg/kg p.o.)+ SITA (5mg.kg p.o.)**
1	**Serum glucose level (mg/dl)**	### 	*** 	*** 	*** 
2	**Body weight (g)**	### 	*** 	*** 	*** 
3	**Thermal hyperalgesia (in sec) [Radiant heat test]**	### 	*** 	*** 	*** 
4	**Mechanical hyperalgesia (in g) [Randall Sellito paw pressure test]**	### 	*** 	*** 	*** 
5	**Mechano-tactile allodynia (in g) [Von-Frey hair test]**	### 	*** 	*** 	*** 
6	**Motor nerve conduction velocity (m/s)**	### 	*** 	*** 	*** 
7	**SOD (Unit/ mg protein)**	### 	** 	** 	** 
8	**MDA (nmol of MDA/mg protein)**	### 	** 	*** 	*** 

4. Effect of TRIG, SITA and concomitant therapy of two drugs (TRIG+SITA) on NICO-STZ induced diabetic cardiomyopathy in Wistar rats [11 Week study Model]					
Sr. No	**Parameters/ Groups**	**Diabetic group**	**TRIG (50mg/kg p.o.)**	**SITA (5mg.kg p.o.)**	**TRIG (50mg/kg p.o.)+ SITA (5mg.kg p.o.)**
1	**Serum glucose level (mg/dl)**	### 	*** 	*** 	*** 
2	**Body weight (g)**	### 	*** 	*** 	*** 
3	**CK-MB (IU/l)**	### 	* 	* 	** 
4	**LDH (IU/l)**	### 	* 	* 	** 
5	**AST (IU/l)**	### 	* 	** 	*** 
6	**Triglyceride (mg/dl)**	### 	* 	** 	*** 
7	**Cholesterol (mg/dl)**	### 	* 	** 	*** 
8	**HDL (mg/dl)**	### 	* 	* 	*** 
9	**VLDL (mg/dl)**	### 	* 	** 	*** 
10	**Heart rate**	### 	* 	* 	*** 
11	**QRS interval (ms)**	###	*	*	**

12	**QT interval (ms)**	### 	* 	* 	** 
13	**QTc interval (ms)**	### 	* 	* 	*** 
14	**SBP (mmHg)**	### 	* 	** 	*** 
15	**DBP (mmHg)**	### 	* 	** 	** 
16	**EDP (mmHg)**	### 	* 	** 	*** 
17	**MABP (mmHg)**	### 	* 	* 	** 
18	**max dP/dt (mmHg/s)**	### 	* 	** 	*** 
19	**min dP/dt (mmHg/s)**	### 	* 	** 	*** 
20	**Heart weight (g)**	### 	** 	** 	*** 

5. Effect of MET alone and triple drug therapy using TRIG + SITA + MET in NICO-STZ induced diabetic nephropathy in Wistar rats

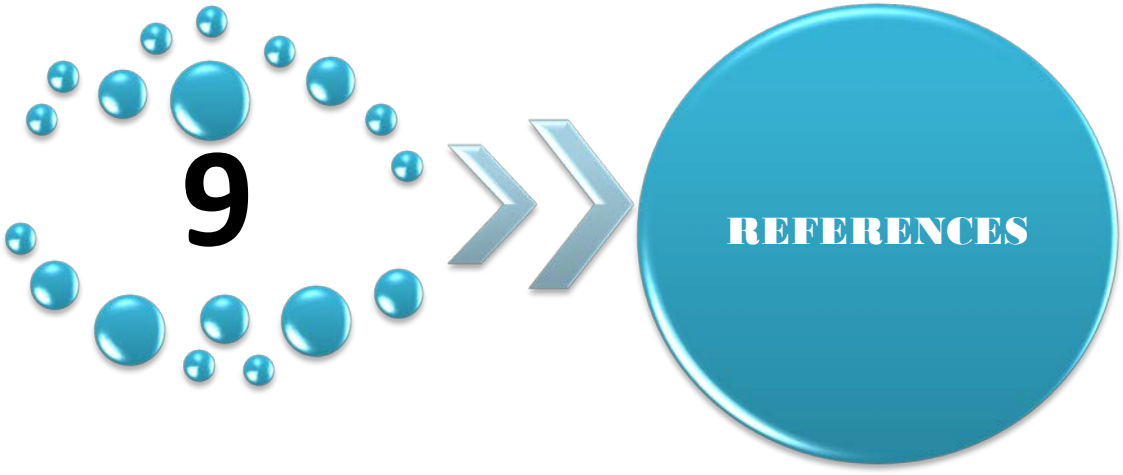
[8 week study model]

Sr. No	Parameters/ Groups	Diabetic group	MET (300mg/kg p.o.)	TRIG (50mg/kg p.o.)+ SITA (5mg.kg p.o.)+ MET (300mg/kg p.o.)
1	Serum glucose level (mg/dl)	### 	*** 	*** 
2	Body weight (g)	### 	*** 	*** 
3	Serum creatinine (mg/dl)	### 	*** 	*** 
4	Serum uric acid (mg/dl)	### 	*** 	*** 
5	Blood urea nitrogen (mg/dl)	### 	*** 	*** 
6	Urine creatine level (mg/dl)	### 	*** 	*** 
7	Urine volume (ml)	### 	*** 	*** 
8	Kidney weight (g)	### 	*** 	*** 
9	MDA (nmol of MDA/mg protein)	### 	*** 	*** 
10	GSH (g of GSH/ mg protein)	### 	* 	*** 
11	SOD (Unit/ mg protein)	### 	** 	*** 

6. Effect of MET alone and triple drug therapy using TRIG + SITA + MET in NICO-STZ induced diabetic neuropathy in Wistar rats [8 week study model]				
Sr. No	**Parameters/ Groups**	**Diabetic group**	**MET (300mg/kg p.o.)**	**TRIG (50mg/kg p.o.)+ SITA (5mg.kg p.o.)+ MET (300mg/kg p.o.)**
1	**Serum glucose level (mg/dl)**	### 	*** 	*** 
2	**Body weight (g)**	### 	*** 	*** 
3	**Thermal hyperalgesia (in sec) [Radiant heat test]**	### 	*** 	*** 
4	**Mechanical hyperalgesia (in g) [Randall Sellito paw pressure test]**	### 	*** 	*** 
5	**Mechano-tactile allodynia (in g) [Von-Frey hair test]**	### 	*** 	*** 
6	**Motor nerve conduction velocity (m/s)**	### 	*** 	*** 
7	**SOD (Unit/ mg protein)**	### 	** 	*** 
8	**MDA (nmol of MDA/mg protein)**	### 	*** 	*** 

7. Effect of MET alone and triple drug therapy using TRIG + SITA + MET in NICO-STZ induced diabetic cardiomyopathy in Wistar rats [11week study model]				
Sr. No	**Parameters/ Groups**	**Diabetic group**	**MET (300mg/kg p.o.)**	**TRIG (50mg/kg p.o.)+ SITA (5mg.kg p.o.)+ MET (300mg/kg p.o.)**
1	**Serum glucose level (mg/dl)**	### 	*** 	*** 
2	**Body weight (g)**	### 	*** 	*** 
3	**CK-MB (IU/l)**	### 	* 	*** 
4	**LDH (IU/l)**	### 	*** 	*** 
5	**AST (IU/l)**	### 	*** 	*** 
6	**Triglyceride (mg/dl)**	### 	*** 	*** 
7	**Cholesterol (mg/dl)**	### 	** 	*** 
8	**HDL (mg/dl)**	### 	** 	*** 
9	**VLDL (mg/dl)**	### 	*** 	*** 
10	**Heart rate**	### 	*** 	*** 
11	**QRS interval (ms)**	### 	** 	*** 

12	**QT interval (ms)**	### 	** 	*** 
13	**QTc interval (ms)**	### 	*** 	*** 
14	**SBP (mmHg)**	### 	*** 	*** 
15	**DBP (mmHg)**	### 	** 	*** 
16	**EDP (mmHg)**	### 	*** 	*** 
17	**MABP (mmHg)**	### 	** 	*** 
18	**max dP/dt (mmHg/s)**	### 	*** 	*** 
19	**min dP/dt (mmHg/s)**	### 	*** 	*** 
20	**Heart weight (g)**	### 	** 	*** 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REFERENCES

9. REFERENCES

- Abbasi F, Carantoni M, Chen YD. Further evidence for central role of adipose tissue in the antihyperglycemic effect of metformin. Diabetes Care 1998; 21: 1301-1305.

- Abbott CA, Malik RA, Ross E, Kulkarni J, Boulton A. Prevalence and characteristics of painful diabetic neuropathy in a large community- based diabetic population in the U.K. Diabetes Care 2011; 34: 2220-2224.

- Abor MM, Rahman AE. Hypoglycemic and hypolipidemic effect of fenugreek in Different Forms on Experimental Rats. World Applied Sciences Journal 2014; 29 (7): 835-841.

- Abraham NG, Tsenovoy PL, McClung J, Drummond GS. Heme oxygenase: a target gene for anti-diabetic and obesity. Current Journal of Pharmaceutical Design 2008; 14: 412-421.

- Achenbach P, Bonifacio E, Koczwara K, Ziegler A. Natural history of type 1 diabetes. Diabetes 2005; 54(2): S25-S31.

- Adaramoye OA. Antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds, in Wistar rats. African Health Sciences 2012; (4): 498-506.

- Ahmed AM. History of diabetes mellitus. Saudi Medical Journal 2002; 23(4): 373-378.

- Akinboboye O, Idris O, Akinkugbe O. Trends in coronary artery disease and associated risk factors in Sub-Saharan Africans. Journal of Human Hypertension 2003; 17: 381-387.

- Akram M. Diabetes mellitus type II: treatment strategies and options: a review. Journal of Diabetes and Metabolism 2013; 4(9): 2-9.

- Al-Habori M, Raman A. Antidiabetic and hypocholesterolemic effects of fenugreek. Phytotherapeutic Research 1998; 12(4): 233-242.

- Alhaider AA, Korashy HM, Sayed-Ahmed MM, Mobark M, Kfoury H, Mansur MA. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through modulation of oxidative stress genes expression. Chemico-Biological Interactions 2011; 192(2): 233-242.

- Ali A, Iqbal F, Taj A, Iqbal Z, Amin MJ, Iqbal QA. Prevalence of microvascular complications in newly diagnosed patients with Type 2 diabetes. Pakistan Journal of Medical Science 2013; 29(4): 899-902.

- Ali A, Khan AK, Hassan Z. Characterization of the hypoglycemic effects of *Trigonella foenum gracecum* seed. Planta Medica 1995; 61(4): 358-360.

- Allegra M, Gentile C, Tesoriere L, Livrea MA. Protective effect of melatonin against cytotoxic actions of malondialdehyde: an in vitro study on human erythrocytes. Journal of Pineal Research 2002; 32(3): 187-193.

- Allred KF, Yackley KM, Vanamala JV, Allerd CD. Trigonelline is a novel phytoestrogen in coffee beans. The Journal of Nutrition 2009: 1833-1838.

- Almeida AA, Farah A, Silva DA, Nuran EA, Gloria MB. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. Journal of Agriculture and Food Chemistry 2006; 15(54):8738-8743.

- Alter M, Kretschmer A, Websky KV, Tsuprykov O, Reichetzeder C, Simon A, Stach JP, Hocher B. Early urinary and plasma biomarkers for experimental diabetic nephropathy. Clinical Laboratory 2012; 58: 659-671.

- Andrea M, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocrine Reviews 2003; 25(4): 612-628.

- Apaijai N, Pintana H, Chattipakorn SC, Chattipakorn N. Effects of vildagliptin versus sitagliptin, on cardiac function, heart rate variability and mitochondrial function in obese insulin-resistant rats. British Journal of Pharmacology 2013; 169(5): 1048-1057.

- Appel A. Detecting and controlling diabetic nephropathy: what do we know? .Cleveland Clinic Journal of Medicine 2013; 80(4): 209-217.

- Araki A, Ito HI, Hattori A, Inoue J, Sato T, Shiraki M, Orimo HE. Risk factors for development of retinopathy in elderly Japanese patients with diabetes mellitus. Diabetes Care 1993; 16(8): 1184-1186.

- Are PC, Reddy RR, Puchchakayala G. Hypoglycemic and antidiabetic activity of *Glochidion velutinum* on streptozotocin-nicotinamide induced type 2 diabetic rats. European Journal of Biological Sciences 2011; 3 (4): 126-130.

- Arif T, Sharma B, Gahlaut A, Kumar V, Dabur R. Anti-diabetic agents from medicinal plants: a review. Chemical Biology Letters 2014; 1(1): 1-13.

- Aronson D. Revascularization for coronary artery disease in diabetes mellitus: Angioplasty, stents and coronary artery bypass grafting. Revision of Endocrinal Metabolic Disordorders 2010; 11(1): 1-17.

- Arora S, Bodhankar SL, Mohan V, Thakurdesai PA. Effects of reconstructed composition of *Trigonella foenum graceum* L. seeds in animal model of diabetic nephropathy with and without renal ischemia reperfusion in rats. Indian Journal of Pharmacology 2012; 8: 321-332.

- Arshag DM, Bernbaum M, Albert SG. Narrative Review: a rational approach to starting insulin therapy. Annals of Internal Medicine 2006; 145: 125-134.

- Aryangat AV, Gerich JE. Type 2 diabetes: postprandial hyperglycemia and increased cardiovascular risk. Vascular Health Risk Management 2010; 6: 145-145.

- Aslani MR, Mohri M, Movassaghi AR. Serum troponin I as an indicator of myocarditis in lambs affected with foot and mouth disease. Veterinary Research 2013; 4(1): 59-62.

- Aswar U, Mohan V, Bodhankar S. Effect of trigonelline on fertility in female rats. International journal of Green Pharmacy 2009; 3 (3): 220-223.

- Avila C, Taboada S, Pons L. Antarctic marine chemical ecology: what is next? Marine Ecology 2008; 29:1-7.

- Ayoola O. Recent advances in childhood diabetes mellitus. Annals of Ibadan Postgraduate Medicine 2008; 6(2): 9-20.

- Baily CJ, Turner RC. Metformin. The New England Journal of Medicine 1996; 334 (9): 574-579.

- Bailey CJ. Biguanides and NIDDM. Diabetes Care 1992; 15(6): 755-772.

- Bakris GL, Fonseca V, Katholi RE, McGill JB, Messerli FH, Phillips RA, Raskin P, et al. Metabolic effects of carvedilol vs metoprolol in patients with type 2 diabetes mellitus and hypertension: A randomized controlled trial. Journal of American Medical Association 2004; 292: 2227-2236.

- Bakuradze T, Lang R, Hormann T, Stiebitz H, Butof G, Lantz I et al. Antioxidant effectiveness of coffee extracts and selected constituents in cell-free systems and human colon cell lines. Molecular Nutrition and Food Research 2010; 54 (12): 1734-1743.

- Bandeira SM, Fonseca LJ, Guedes GS, Rabelo LA, Goulart MO, Vasconcelos SL. Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus. International Journal of Molecular Sciences 2013; 14: 3265-3284.

- Barik R, Jain S, Qwatra D, Joshi A, Tripathi GS, Goyal R. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced in type-II diabetes in rats. Indian Journal Pharmacology 2008; 40: 19-22.

- Barreras AM, Turner CG. Angiotensin II receptor blockers. Baylor University Medical Center Proceedings 2003; 16: 123-126.

- Basch E. Therapeutic applications of fenugreek. Alternative Medicine Review 2003; 8(1): 20-27.

- Bastaki S. Diabetes mellitus and its treatment. International Journal of Diabetes & Metabolism 2005; 13: 111-134.

- Battershill AJ, Scott LJ. Telmisartan: a review of its use in the management of hypertension. Drugs 2006; 66(1): 51-83.

- Battiprolu PK, Gillette TG, Wang ZV, Lavandero S, Hill JA. Diabetic cardiomyopathy: mechanisms and therapeutic targets. Drug Discovery Today Disease Mechanism 2010; 7(2): 137-143.

- Beconi MG, Reed JR, Teffera Y, Xia YQ, Kochansky CJ, Liu DQ et al. Disposition of the dipeptidyl peptidase 4 inhibitor sitagliptin in rats and dogs. Drug Metabolism and Disposition 2006; 35: 525-532.

- Bell PM, Hadden DR. Metformin. Endocrinology and Metabolism clinics of North America 1997; 26(3): 523-537.

- Bell SH. Diabetic cardiomyopathy. Diabetes care 2003; 26(10): 2949-2951.

- Belle V, Ken TC, Matthias G. Herrath V. Type 1 Diabetes: etiology, immunology and therapeutic strategies TOM L. Physiology Revision 2011; 91: 79-118.

- Belvis A, Pelone F, Biasco A, Ricciardi W, Volpe M. Can primary care professional's adherence to evidence based medicine tools improve quality of care in type 2 diabetes mellitus? A systematic review. Journal of Diabetes Research Clinical Practice 2009; 85: 119-131.

- Best RM, Chakravarthy U. Diabetic retinopathy in pregnancy. British Journal of Ophthalmology 1997; 81: 249-251.

- Bhatia K, Kaur F, Atif F, Ali M, Rehman H, Rahman S et al. Aqueous extract of *Trigonella foenum-graecum* L. ameliorates additive urotoxicity of buthionine sulfoximine and cyclophosphamide in mice. Food and Chemical Toxicology 2006; 44(10): 1744-1750.

- Bhattacharya S, Dey D, Roy SS. Molecular mechanism of insulin resistance. Journal Biosciences 2007; 32: 405-413.

- Bhinge JR, Patil SL, Bhalgat CM. Development AND validation of a stability indicating RP-HPLC method for simultaneous determination of sitagliptin and metformin in tablet dosage form. International Journal of Research in Pharmaceutical and Biomedical Sciences 2013; 4(2): 590-596.

- Blankenship GW, Skyler J. Diabetic retinopathy: a general survey. Diabetes Care 1978; 1(2): 1278-137.

- Boaz M, Leibovitz E, Dayan YB, Wainstein J. Functional foods in the treatment of type 2 diabetes: olive leaf extract, turmeric and fenugreek, a

qualitative review. Functional Foods in Health and Disease 2011; 1(11): 472-481.

- Boulton AJ, Vinik AI, Arezzo A, Brill V, Feldman EL, Freeman R et al. Diabetic neuropathies. Diabetes Care 2005; 28(4): 956-962.

- Boyle PJ, Freeman JS. Application of incretin mimetic and dipeptidyl peptidase IV inhibitors in managing type 2 diabetes mellitus. The Journal of the American Osteopathic Association 2007; 107(3): S10-S16.

- Brajendra K, Arvind K. Diabetes mellitus: Complications and therapeutics. Medical Science Monitoring Journal 2006; 12(7): 130-147.

- Brannagan TH. Current issues in peripheral neuropathy. Journal of Peripheral Nervous System 2012; 17: 1-3.

- Bugger H, Abel ED. Rodent models of diabetic cardiomyopathy. Disease Models & Mechanisms 2009; 2; 454-466.

- Burnier M. Angiotensin II type 1 receptor blockers. Circulation 2001; 103: 904-912.

- Cade WD. Diabetes related microvascular and macrovascular diseases in the physical therapy setting. Physical Therapy 2008; 88(11): 1322-1335.

- Calvert JW, Gundewar S, Jha S, Greer JM, Bestermann WH, Tian R et al. Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK- eNOS-mediated signaling. Diabetes 2008; 57: 696-705.

- Campbell IW, Day C. Sitagliptin enhancing incretin action: sitagliptin. British Journal of Diabetes and Vascular Disease 2007; 7: 134-39.

- Cao X, Li H, Tao H, Wu N, Yu L, Zhang D et al. Metformin inhibits vascular calcification in female rat aortic smooth muscle cells via the AMPK-eNOS-NO pathway. Endocrinology 2013; 154(10): 3680-3689.

- Cavlan D, Vijayaraghavan S, Gelding S, Drake W. GH replacement causing acute hyperglycaemia and ketonuria in a type 1 diabetic patient. Endocrinology, Diabetes and Metabolism 2013; 1-4: DOI: 10.1530/EDM-13-0047.

- Ceriello A, Kilpatrick ES. Glycemic variability: both sides of the story. Diabetes care 2013; 36 (2): 272-275.

- Chaplan SR, Bach FW, Pogrel JW, Chung JM; Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. Journal of Neuroscience Methods 1994; 53: 55-63.

- Chatterjee S, Kumar M, Kumar A. Chemomodulatory effect of *Trigonella foenum graecum* (L.) seed extract on two stage mouse skin carcinogenesis. Toxicology International. 2012; 19(3): 287-294.

- Cheng AY, Cheng I, Fantus G. Oral antihyperglycemic therapy for type 2 diabetes mellitus. Canadian Medical Association or its licensors 2006; 172(2): 213-226.

- Cheng J, Wang F, Yu DF, Wu PF, Chen JG. The cytotoxic mechanism of malondialdehyde and protective effect of carnosine via protein cross-linking/mitochondrial dysfunction/reactive oxygen species/MAPK pathway in neurons. European Journal of Pharmacology 2011; 650: 184-194.

- Cheng JT, Huang CC, Liu M, Tzeng TF, Chang CJ. Novel mechanism for plasma glucose–lowering action of metformin in streptozotocin-induced diabetic rats. Diabetes 2006; 55: 819-825.

- Cheung KW, Wong SF. Gestational diabetes mellitus update and review of literature. Reproductive System Sexual Disorder 2011; Doi 10.4172/2161-038X.S2-002.

- Cheung NW. The management of gestational diabetes. Vascular Health Risk Management 2009; 5(1): 153-164.

- Chiasson JL, Aris-Jilwan N, Belanger R, Bertrand S, Beauregard H, Ekoe JM et al, Fournier H, Diagnosis and treatment of diabetic ketoacidosis and the hyperglycemic hyperosmolar state. Canadian Medical Association Journal 2003; 168(7): 859-866.

- Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. Journal of Diabetes Science and Technology 2008; 2(6): 1130-1138.

- Chou TM. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Research 2010; 70(2); 440-446.

- Chow EJ, Baker KS, Lee SJ, Flowers ME, Cushing-Haugen KL, Inamoto Y et al. Influence of conventional cardiovascular risk factors and lifestyle characteristics on cardiovascular disease after hematopoietic cell transplantation. Journal of Clinical Oncology 2014; 32(3): 191-198.

- Cittadini A, Napoli R, Monti MC, Rea D, Longobardi S, Netti PA et al. Metformin prevents the development of chronic heart failure in the SHHF rat model. Diabetes 2012; 61: 944-953.

- Claydon A, Campbell-Richards D, Hill M. Living with diabetes: A qualitative review of minority ethnic groups in a deprived London borough. Journal of Diabetes Nursing 2013; 17 (3): 95-100.

- Clemente D, Pereira T, Ribeiro S. Ventricular repolarization in diabetic patients: characterization and clinical implications. Arquivos Brasileiros De Cardiologia 2012; 99(5): 1015-1022.

- Cockram CS. The epidemiology of diabetes mellitus in the Asia-Pacific region. Hong Kong Medical Journal 2000; 6: 43-52.

- Cooper ME. Interaction of metabolic and hemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia 2001; 44(11): 1957-1972.

- Cristina M, Lemos ET, Vala H, Fernandes R, Oliveria J, Teixeira F, Teixeira F, Reis F. Diabetic nephropathy amelioration by a low dose sitagliptin in an animal model of type 2 Diabetes (Zucker diabetic fatty Rat). Experimental Diabetes Research 2011; 10: 1-12.

- Daniel D, Chris E, Peter K. Fresh from the Pipeline: Sitagliptin. Nature Reviews Drug Discovery 2007; 6: 109-110.

- Davidson EP, Coppey LJ, Dake B, Yorek MA. Treatment of streptozotocin-induced diabetic rats with alogliptin: effect on vascular and neural complications. Experimental Diabetes Research 2007; 1-7: doi:10.1155/2011/810469.

- Debono M, Cachia E. The impact of Cardiovascular Autonomic Neuropathy in diabetes: Is it associated with left ventricular dysfunction. Autonomic Neuroscience: Basic and Clinical 2007; 132:1-7.

- Dedov V, Maslova O, Suntsov Y, Bolotskaia L, Milenkaia T, Besmertnaia L. Prevalence of diabetic retinopathy and cataract in adult patients with type 1 and type 2 diabetes in Russia. Review of Diabetic Studies 2009; 6(2): 124-129.

- DeFronzo RA, Goodman AM. The multicenter metformin study group: efficacy of metformin in patients with non-insulin dependent diabetes mellitus. The New England Journal of Medicine 1995; 333: 541-549.

- Dijk AV, Lthof MR, Meeuse J, Elin SE, Heine RH, Vandam R. Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. Diabetes Care 2009; 32(6): 1023-1025.

- Dimitropoulos G, Tahrani AA, Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. World Journal of Diabetes 2014; 15: 5(1): 17-39.

- Dokken BB. The Pathophysiology of cardiovascular disease and diabetes: beyond blood pressure and lipids. Diabetes Spectrum 2008; 21(3): 160-165.

- Drucker D, Easley C, Kirkpatrick P. Sitagliptin. Nature Reviews 2007; 7: 109-110.

- Duttaroy A, Kanakaraj P, Osborn BL, Schneider H, Pickeral OK, Chen C, Zhang G, Kaithamana S, Singh M, Schulingkamp R, Crossan D. Development of a long-acting insulin analog using albumin fusion technology. Diabetes 2005; 54: 251-258.

- Earle E, Viberti GC. Familial, hemodynamic and metabolic factors in the predisposition to diabetic kidney disease. Kidney International 1994; 44: 434-437.

- Edet EE, Akpanabiatu MI, Eno AE, Umoh IB, Itam EH. Effect of *Gongronema Latifolium* crude leaf extract on some cardiac enzymes of alloxan-induced diabetic rats. African Journal of Biochemistry Research 2009; 3(11): 366-369.

- Edwards, CR. Baird, JD (1998). Frier, BM. Shephard, J. Toft, AD. Devidson's principles and practice of medicine. Endocrine and metabolic diseases, including diabetes mellitus. Churchill Livingstone, Edinburgh, 17th edition; 669-774.

- Efimov A, Sokolova L, Sokolova M. Diabetes mellitus and coronary artery diseases. Diabetologia Croatica 2001; 30(4): 115-120.

- Eknoyan G. A history of diabetes mellitus - a disease of the kidneys that became a kidney disease. Journal of Nephrology 2006; 19 (10): S71-S74.

- Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. Journal of Diabetes and Metabolic Disorders 2013; 2-7:http://www.jdmdonline.com/content/12/1/60.

- Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovascular Therapeutics 2012; 30: 49-59.

- Erejuwa OO, Sulaiman SA, Wahab MH, Sirajudeen KN, Salleh SD, Gurtu S. Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. International Journal of Bioligiacal Sciences 2011; 7(2): 244-252.

- Errera MH, Kohly RP, Cruz L. Pregnancy-associated retinal diseases and their management. Survey of Ophthalmology 2012; 58(2): 127-142.

- Esfandiari E, Shekari F, Shekari F, Esfandiari M. The effect of salt stress on antioxidant enzymes' activity and lipid peroxidation on the wheat seedling. Notulae Botanicae Horti Agrobotanici Clug- Napoca 2007; 35 (1): 48-56.

- Faeste CK, Christians U, Egaas E, Jonsche KR. Characterization of potential allergens in fenugreek (*Trigonella foenum-graecum*) using patient sera and MS-based proteomic analysis. Journal of Proteomics 2010; 73(7): 1321-1333.

- Farhangkhoee H, Khan ZA, Kaura H, Xin X, Chena S, Chakrabarti S. Vascular endothelial dysfunction in diabetic cardiomyopathy: Pathogenesis and potential treatment targets. Pharmacology and Therapeutics 2006; 111: 384-399.

- Ferries FL, Chew EY, Hoogwear BJ. Serum lipid s and diabetic retinopathy. Diabetes Care 1996; 19(11): 1291-1293.

- Firdous MS, Paul S, Bag AK. Effect of *Sechium edule* on chemical induced kidney damage in experimental animals. Bangladesh Journal of Pharmacology 2013; 8: 28-35.

- Fleischer J, Yderstraede K, Gulichsen E, Jakobsen PK, Lervang HH, Eldrup E, Hans. Cardiovascular autonomic neuropathy is associated with macrovascular risk factors in type 2 diabetes: new technology used for routine large-scale screening ads new insight. Journal of Diabetes Science and Technology 2014; 1-7: Doi: 10.1177/1932296814528616.

- Fong D, Cavallerano JD, Aiello L, Ferris FL, Gardner TW, Klein R et al. Retinopathy in diabetes. Diabetes Care 2004; 27(1): S84-S87.

- Fotopoulos C. Marketing, In: G. A. Petropoulos (Ed.). Fenugreek - The genus Trigonella, Taylor and Francis, London and New York. 2002: 183-195.

- Fowler MJ. Microvascular and macrovascular complications of diabetes. Clinical Diabetes 2008; 26(2): 77-82.

- Francesco PS, Loreto G. Pathogenetic mechanisms of diabetic nephropathy. American Society of Nephrology 2005; 16: S30- S33.

- Fraser GL, Gaudreau GA, Clarke PB, Menard DP, Perkins MN. Antihyperalgesic effects of ð-opoids agonists in a rat model of chronic inflammation. British Journal of Pharmacology 2000; 129: 1688-1672.

- Freedman BI, Parekh RS, Kao L. Genetic basis of non-diabetic end-stage renal disease. Seminer Nephrology 2010; 30(2): 101-110.

- Fuch D, Birklein F Reeh PW, Sauer SK. Sensitized peripheral nociception in experimental diabetes of the rat. Pain 2010; 151: 496-505.

- Fulgencio JP, Kohl C, Girard J, Pegorier JP. Effect of metformin on fatty acid and glucose metabolism in freshly isolated hepatocytes and on specific gene expression in cultured hepatocytes. Biochemical Pharmacology 2001; 62: 439-446.

- Gale EA. The discovery of type 1 diabetes. Diabetes 2001; 50: 217-226.

- Galeotti N, Maidecchi A, Mattoli L, Burico M, Ghelardini C. St. John's Wort seed and feverfew flower extracts relieve painful diabetic neuropathy in a rat model of diabetes. Fitotherapia 2014; 92: 23-33.

- Gallwitz B. Review of sitagliptin phosphate: a novel treatment for type 2 diabetes. Vascular Health and Risk Management 2007; 3(2): 203-210.

- Garg K, Tripathi CD, Kumar S. Clinical review of sitagliptin: a DPP-4 Inhibitor. Journal of the Association of Physicians of India 2013; 645-649.

- Genet S, Kale RK, Baquer NZ. Effects of vanadate, insulin and fenugreek (*Trigonella foenum graecum*) on creatine kinase levels in tissues of diabetic rat. Indian Journal of Experimental Biology 1999; 37(2): 200-202.

- Ghule A, Jadhav S, Bodhankar S. Trigonelline ameliorates diabetic hypertensive nephropathy by suppression of oxidative stress in kidney and reduction in renal cell apoptosis and fibrosis in streptozotocin induced neonatal diabetic (nSTZ) rats. International Immunopharmacology 2012; 14(4): 740-748.

- Giacco F. Brownlee M. Oxidative stress and diabetic complications. Circulation Research 2010; 107: 1058-1070.

- Gillow JT, Gibson JM, Dodson PM. Hypertension and diabetic retinopathy-what's the story? British Journal of Ophthalmology 1999; 83: 1083-1087.

- Green BD, Flatt PR, Bailey CJ. Dipeptidyl peptidase IV (DPP IV) inhibitors: a newly emerging drug class for the treatment of type 2 diabetes. Diabetes and Vascular Disease Research 2006; 3(3): 159-165.

- Griebeler ML, Tsapas A, Brito JP, Wang Z, Phung OJ, Montori VM et al. Pharmacologic interventions for painful diabetic neuropathy: an umbrella systematic review and comparative effectiveness network meta-analysis (Protocol). Systematic Reviews 2012; 1(61): 2-7.

- Gulichsen E, Fleischer J, Ejskjaer N, Eldrup E, Tarnow L. Screening for diabetic cardiac autonomic neuropathy using a new handheld device. Journal of Diabetes Science and Technology 2012; 6(4): 966-972.

- Hamadi SA. Effect of trigonelline and ethanol extract of Iraqi Fenugreek seeds on oxidative stress in alloxan diabetic rabbits. Journal of the Association of Arab Universities for Basic and Applied Sciences 2012; 12: 23-26.

- Hamden KH, Mnafgui K, Amri Z, Aloulou A, Elfeki A. Inhibition of key digestive enzymes related to diabetes and hyperlipidemia and protection of liver-kidney functions by trigonelline in diabetic rats. Scientia Pharmaceutica 2013; 81: 233-246.

- Hammer KM, Pedersen SA. Elevated seawater levels of CO_2 change the metabolic fingerprint of tissues and hemolymph from the green shore crab

Carcinus maenas. Comparative Biochemistry and Physiology 2012; 7: 292-302.

- Han JW, Sin MY, Yoon YS. Cell therapy for diabetic neuropathy using adult stem or progenitor cells. Diabetes Metabolism Journal 2013; 37:91-105.

- Hardern RD, Quinn ND. Emergency management of diabetic ketoacidosis in adults. Emergency Medicine Journal 2003; 20: 210-213.

- Hassan Z, Yam MF, Ahmad M, Yusof M. Antidiabetic properties and mechanism of action of *Gynura procumbens* water extract in streptozotocin-induced diabetic rats. Molecules 2010; 15: 9008-9023.

- Hausenloy DJ, Whittington HJ, Wynne AM, Begum SS, Theodorou L, Riksen N. Dipeptidyl peptidase-4 inhibitors and GLP-1 reduce myocardial infarct size in a glucose-dependent manner. Cardiovascular Diabetology 2013; 12(154): 2-9.

- Hayat SA, Patel B, Khattar RS, Malik RA. Diabetic cardiomyopathy: mechanisms, diagnosis and treatment. Clinical Science 2004; 107: 539-557.

- Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, Smet M et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clinical Pharmacology and Therapeutics 2005; 78(6): 675- 688.

- Hirakawa N, Okauchi R, Miura Y, Karumi Y. Considering potential bioactivity, trigonelline has inhibited the invasiveness of cancer cells in vitro. Journal of Bioscience, Biotechnology and Biochemistry 2005; 63(3): 653-658.

- Hiroshi A. Metabolism of alkaloids in coffee plants. Brazilian Journal of Plant Physiology 2006; 18(1): 1-8.

- Hocher B, Reichetzeder C, Alter ML. Renal and cardiac effects of DPP-4 inhibitors- from preclinical development to clinical research. Kidney and Blood Pressure Research 2012; 36: 65-84.

- Hostetter TH. Prevention of the development and progression of renal disease. Journal of the American Society of Nephrology 2003; 14: S144-S147.

- Houghton PJ, Howes MJ. Natural products and derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson's disease. Neurosignals 2005; 14: 6-22.

- Howard BV, Lee ET, Best LG, Ratner RE, Galloway JM, Resnik HE et al. Coronary heart disease risk equivalence in diabetes depends on concomitant risk factors. Diabetes Care 2006; 29: 391-397.

- Howarth FC, Adeghate E, M Jacobson. Heart rate and QT interval in streptozotocin-induced diabetic rat. Journal of Medical Sciences 2009; 2(3): 108-118.

- Huizinga MM, Peltier A. Painful diabetic neuropathy: a management-centered review. Clinical Diabetes 2007; 25(1): 6-15.

- Hung JW, Liou CW, Wang PW, Yeh SH, Lin LW, Lo SK et al. Effect of 12-week Tai Chi Chuan exercise on peripheral nerve modulation in patients with type 2 diabetes mellitus. Journal of Rehabilitation Medicine 2009; 41(11): 924-929.

- Idiculla J, Nithyanandam S, Joseph M, Mohan A, Vasu U, Sadiq M. Serum lipids and diabetic retinopathy: A cross-sectional study. Indian Journal of Endocrinology and Metabolism 2012; 16: S492-S494.

- Idkaidek N, Arafat T, Melhim M, Alawneh J, Hakooz N. Metformin IR versus XR pharmacokinetics in humans. Bioequivalence & Bioavailability 2011; 3(10): 233-235.

- Idonije BO, Festus O, Oluba OM. Plasma glucose, creatinine and urea levels in Type 2 diabetic patient attending a Nigerian Teaching Hospital. Research Journal of Medical Sciences 2011; 5(1): 1-3.

- Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes. Journal of the American Medical Association 2002; 287: 360-372.

- Iribarren C, Karter AJ, Go AS, Ferrara A, Liu J Y, Sidney S, Selby J V. Glycemic control and heart failure among adult patients with diabetes. Circulation 2001; 103: 2668-2673.

- Ismail NA, Kasem OM, Abou-El-Asrar M, Samahy MH. Epidemiology and management of type 1 diabetes mellitus at the Ain shams university pediatric hospital. Journal of Egypt Public Health Association 2008; 83(1): 107-132.

- Jakubowicz DJ, Seppala M, Jakubowicz S, Rodriguez-Armas O, Rivas-Santiago A, Koistinen H, Koistinen R et al. Insulin reduction with metformin increases luteal phase serum glycodelin and insulin-like growth factor-binding protein 1 concentrations and enhances uterine vascularity and blood flow in the polycystic ovary syndrome. The Journal of Clinical and Endocrinology Metabolism 2001; 86: 1126-1133.

- Janghorbani M, Rezvanian H, Kachooei A, Ghorbani A, Chitsaz A, Izadi F et al. Peripheral neuropathy in type 2 diabetes mellitus in Isfahan, Iran: prevalence and risk factors. International Journal of Diabetes and Metabolism 2006; 14: 126-133.

- Jayadev R, Patlolla JM, Swamy MV, Rao CV. Diosgenin, a steroid saponin of *Trigonella foenum graecum* (Fenugreek), inhibits Azoxymethane-induced aberrant crypt foci formation in F344 Rats and induces apoptosis in HT-29

human colon cancer cells. Cancer Epidemiology, Biomarkers & Prevention 2004; 4(13): 1392-1396.

- Jefferson JA, Shankland SJ, Pichler RH. Proteinuria in diabetic kidney disease: a mechanistic viewpoint. Kidney International 2008; 74: 22-36.

- Jensen TS. Anticonvulsants in neuropathic pain: rationale and clinical evidence. European Journal of Pain 2002; 6: 61-68.

- Jerums G, Maclsaac RJ. Treatment of microalbuminuria in patients with type 2 diabetes mellitus. Treatment Endocrinology 2002; 1(3): 163-173.

- John SG, Gallagher RT, Denn M, Peter RM. Simultaneous quantitation of metformin and sitagliptin from mouse and human dried blood spots using laser diode thermal desorption tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2011; 55: 544-551.

- Johnson K, Schurr K. Sitagliptin: a DPP-4 inhibitor for the treatment of type 2 diabetes mellitus. Clinical Medicine Insights: Therapeutics 2011; 3: 81-92.

- Joo KW, Kim S, Ahn S, Chin HJ, Chae D, Lee J et al. Dipeptidyl peptidase IV inhibitor attenuates kidney injury in rat remnant kidney. BMC Nephrology 2013; 14(98): 2-11.

- Joseph MP, Varughese GI, Sriraman R, Arunagirinathan G. Diabetic cardiomyopathy: Pathphysiology, diagnostic evaluation and management. World Journal of Diabetes 2013; 4(5): 177-179.

- Joshi JG, Handler P. Biosynthesis of trigonelline. The Journal of Biological Chemistry 1960; 235 (10): 2981-2983.

- Joshi MC, Dorababu M, Prabha T, Kumar M, Goel RK. Effects of pterocarpus marsupium on NIDDM-induced rat gastric ulceration and mucosal offensive and defensive factors. Indian Journal Pharmacology 2004; 36: 296-302.

- Joshi S. A review of insulin and insulin regimens in type 2 diabetes. South Africa Family Practice 2009; 51(2): 97-102.

- Kafle D, Singh N, Singh S, Singh N , Bhargav V, Singh AK. Persistent hyperglycemia generating reactive oxygen species in renal cells, a probable cause of inflammation in type 2 diabetic nephropathy subjects. Biomedical Research 2012; 23(4): 501-504.

- Kain V, Kumar S, Puranik AS, Sitasawad SL. Azelnidipine protects myocardium in hyperglycemia-induced cardiac damage. Cardiovascular Diabetology 2010; 9(82): 2-9.

- Kakadiya J, Patel D, Shah N. Effect of hesperidin on renal complication in experimentally induced renal damage in diabetic Sprague dawley rats. Journal of Eco Biotechnology 2010; 2: 45-50.

- Kakkar R, Mantha S, Radhi J, Prasad K, Kalra J. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clinical Sciences 1998; 94: 623-632.

- Kalantzis G, Angelou M, Poulakou-Rebelakou E. Diabetic retinopathy: an historical assessment. Hormones (Athens) 2006; 5(1): 72-75.

- Kalra S, Kalra B, Agrawal N. Review. Combination therapy in hypertension: An update. Diabetic Metabolic Syndrome 2010; 2(44): 2-11.

- Kandhare AD, Raygude KS, Ggosh P, Ghule AE, Bodhankar SL. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitotherapia 2012; 83: 650-659.

- Kappelle AC, Bravenboer B, Buren TV, Traber TJ, Erkelens DW, Gispen WH. Amelioration by the Ca^{2+} antagonist, nimodipine of an existing neuropathy in the streptozotocin-induced, diabetic rat. British Journal of Pharmacology 1993; 108: 780-785.

- Kastenbauer T, Irsigler P, Sauseng S, Grimm A, Prager R. The prevalence of symptoms of sensorimotor and autonomic neuropathy in type 1 and type 2 diabetic subjects. Journal of Diabetes Complications 2004; 18(1): 27-31.

- Kaur J. An overview of diabetic neuropathy. Annual Review & Research in Biology 2013; 3(4): 994-1012.

- Kemp M, Donovan J, Higham H, Hooper J. Biochemical markers of myocardial injury. British Journal of Anaesthesia 2004; 93(1): 63-73.

- Kempler P. Review: Autonomic neuropathy: a marker of cardiovascular risk. British Journal of Diabetes & Vascular Disease 2003; 3(84): 84-90.

- Khalil E. Biochemical and Histopathological studies on the influence of aqueous extract of fenugreek seed (*Trigonella foenum graceum*) on alloxan diabetic male rats. The Egyption Journal of Hospital Medicine 2004; 15: 83-94.

- Khandoker AH, Jelinek HF, Palaniswami M. Heart rate variability and complexity in people with diabetes associated cardiac autonomic neuropathy. Engineering in Medicine and Biological Society 2008; 4696 - 4699: Doi 10.1109 /IEMBS. 2008.4650261.

- Kiencke S, Handschin R, Dahlen R, Muser J, Brunner-Larocca HP, Schumann J et al. Pre-clinical diabetic cardiomyopathy: prevalence, screening, and outcome. European Journal of Heart Failure 2010; 12(9): 951-957.

- Kim KS, Oh DH, Kim JY, Lee BG, You JS, Chang KJ. Taurine ameliorates hyperglycemia and dyslipidemia by reducing insulin resistance and leptin

level in Otsuka Long-Evans Tokushima fatty (OLETF) rats with long-term diabetes. Experimental Journal of Molecular Medicine 2012; 44(11): 665-673.

- King P, Ian Peacock I, Donnelly R. The UK Prospective Diabetes Study (UKPDS): clinical and therapeutic implications for type 2 diabetes. British Journal of Clinical Pharmacology 1999; 48: 643-648.

- Kiran G, Nandini CD, Ramesh HP, Salimath PV. Progression of early phase diabetic nephropathy in streptozotocin induced diabetic rats: Evalution of various kidney related parameters. Indian Journal of Experimental Biology 2012; 50: 133-140.

- Klatt S, Fromm MF, Konig J. Transporter-mediated drug- drug interactions with oral antidiabetic drugs. Pharmaceutics 2011; 3: 680-705.

- Klein BE, Moss SE, Klein R, Surawicz TS. The Wisconsin epidemiologic study of diabetic retinopathy. XIII. Relationship of serum cholesterol to retinopathy and hard exudate.Optholmology1991; 98(8): 1261-1265.

- Klip A, Leiter L. Cellular mechanism of action of metformin. Diabetes Care 1990; 13: 696-704.

- Kolika C, Doupis J. Incretin-Based Therapy: a powerful and promising weapon in the treatment of type 2 diabetes mellitus. Diabetes Therapeutics 2012; 2(2): 101-121.

- Korish A, Sallam R. Change in renal angiotensin II in the response to caffeic acid phenethyle ester in diabetic rats and its implications on diabetic nephropathy. Bulletin Alexandria Faculty of Medicine 2007; 43(2): 429-436.

- Kouroumichakis I, Papanas N, Zarogoulidis P, Liakopoulos V, Maltezos E, Mikhailidis DP. Fibrates: Therapeutic potential for diabetic nephropathy. European Journal of Internal Medicine 2012; 23: 309-316.

- Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs 2005; 65(3): 385-411.

- Krishan P, Chakkarwar VA. Diabetic nephropathy: aggressive involvement of oxidative stress. Journal Pharmaceutical Education Research 2011; 2(1): 35-41.

- Kulkarni CP, Bodhankar SL, Ghule AE, Movan V, Thakurdesai PA. Antidiabetic activity of *Trigonella Foenum graecum* L. seeds extract (IND01) in neonatal streptozotocin- induced (n-STZ) rats. Diabetologia Croatica 2012; 41(1): 29-40.

- Kumar H, More SV, Han SD, Choi JY, Choi DK. Promising therapeutics with natural bioactive compounds for improving learning and memory- a review of randomized trials. Molecules 2012; 17: 10503-10539.

- Landin K, Tengborn L, Smith U. Metformin and metoprolol CR treatment in non-obese men. Journal of Internal Medicine 1994; 235: 335-341.

- Lenzen S. Alloxan and streptozotocin diabetes. Diabetologia 2007; 51:21 6-226.

- Leung GM, Lam KS. Diabetic complications and their implications on health care in Asia. Hong Kong Medical Journal 2000; 6(1): 61-68.

- Li Q, Xiao Y, Gong H, Shen D, Zhu F, Wu Q, Chen H, Zhong H. Effect of puerarin on the expression of extracellular matrix in rats with streptozotocin-induced diabetic nephropathy. The National Medical Journal of India 2009; 22 (1): 9-12.

- Li TH, Wu ZD, Davey AK, Wang J. Antihyperglycemic effects of baicalin on streptozotocin-nicotinamide induced diabetic rats. Phytotherapy Research 2011; 25(2): 189-194.

- Lim LS, Wong TY. Lipids and diabetic retinopathy. Expert Opinion Biological Therapy 2012; 12(1): 93-105.

- Lin MV, Bishop G, Benito-Herrero M. Diabetic ketoacidosis in type 2 diabetics: a novel presentation of pancreatic adenocarcinoma. Journal of General Internal Medicine 2009; 25(4): 369-373.

- Lin S, Yang J, Wu G, Liu M, Luan X, Lv Q, Zhao H, Hu J. Preventive effect of taurine on experimental type II diabetic nephropathy. Journal of Biomedical Science 2010; 17(1): 3-8.

- Lutz H, Muchmore DB, Douglas B. Ultrafast-acting insulins: state of the art. Journal of Diabetes Science and Technology 2012; 6(4): 728-742.

- Maldonado MR, Otiniano ME, Lee R, Rodriguez L, Balasubramanyam A. Characteristics of ketosis-prone diabetes in a multiethnic indigent community. Ethnicity & Disease 2004; 14: 243-249.

- Manoria PC, Manoria P, Manoria P, Paraskar SK. A cardiologist's view point on gliptin: the new star on the horizon of diabetes. Cardiology 2013; 17(6): 264-268.

- Maric A. Metformin-more than 'Gold standard' in the treatment of type 2 diabetes mellitus. Diabetologia Croatica 2010; 39(3): 95-104.

- Mariee AD, Abd-Allah GM, El-Yamany MF. Renal oxidative stress and nitric oxide production in streptozotocin-induced diabetic nephropathy in rats: the possible modulatory effects of garlic (*Allium sativum* L.). Biotechology and Application of Biochemistry 2009; 52(3): 227- 232.

- Marzilli M, Merz NB, Boden WE, Bonow RO. Obstructive coronary atherosclerosis and ischemic heart disease: an elusive link! Journal of the American College of Cardiology 2012; 60(11): 951-956.

- Masiello P, Broca C, Gross R, Roye M, Manteghetti M; Hillaire-Buys D. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 1998; 47: 224-229.

- Mathur P, Choudhary M, Consumption pattern of fenugreek seeds in Rajasthani families. Journal of Human Ecology 2009; 25(1): 9-12.

- Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos University Medical Sciences Journal 2012; 12(1): 5-18.

- Matthaei S, Stumvoli M, Kellerer M, Haring HU. Pathophysiology and pharmacological treatment of insulin resistance. Endocrine Review 2000; 21: 585-618.

- Matveyenko AV, Dry S, Cox HI, Moshtaghian A, Gurlo T, Galasso R, Butler AE, Butler PC. Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes. Diabetes 2009; 58: 1604-1615.

- Mbanya JC, Motala AA, Sobngwi E, Assah FK, Enoru ST. Diabetes in sub-Saharan Africa. Lancet 2010; 375: 2254-266.

- McCormack J, Johns K, Tildesley H. Metformin contraindications should be contraindicated. Commentary 2005; 173(5): 502-504.

- McCormick MC, Kydd AC, Read PA, Ring LS, Bond SJ, Hoole SP et al. Chronic dipeptidyl peptidase-4 inhibition with sitagliptin is associated with sustained protection against ischemic left ventricular dysfunction in a pilot study of patients with type 2 diabetes mellitus and coronary artery disease. Circulation: Cardiovascular Imaging 2014; 7: 274-281.

- McGuire DK, Inzucchi SE. New drugs for the treatment of diabetes mellitus: part I: thiazolidinediones and their evolving cardiovascular implications. Circulation 2008; 117: 440-449.

- Meghwal M, Goswami TK. A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. Journal of Food Process Technology 2012; 3(9): 3-10.

- Mehrafarin A, Rezazadeh SH, Naghdi BH, Noormohammadi GH, Zand E, Qaderi A. A review on biology, cultivation and biotechnology of fenugreek (*Trigonella foenum-graecum* L.) as a valuable medicinal plant and multipurpose. Journal of Medicinal Plants 2011; 10(37): 6-24.

- Menato G, Signorile A. Current management of gestational diabetes mellitus. Expert Review of Obstetrics and Gynecology 2008; 3(1): 73-91.

- Mianzhi Z, Min X, Junbo G, Xiaoyue T. Schisandra chinensis fruit extract attenuates albuminuria and protects podocyte integrity in a mouse model of streptozotocin-induced diabetic nephropathy. Journal of Ethnopharmacology 2012; 141: 111-118.

- Miki T, Yuda S, Kouzu H, Miura T. Diabetic cardiomyopathy: pathophysiology and clinical features. Heart Failure Review 2013; 18:149-166.

- Miljanovic B, Glynn RJ, Nathan DM, Manson JE, Schaumberg DA. A prospective study of serum lipids and risk of diabetic macular edema in type 1 diabetes. Diabetes 2004; 53: 2883-2892.

- Misera HP, Fridocich.The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. Journal of Biological Chemistry 1972; 3170-3175.

- Mogasen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease with emphasis on the stage of incipient diabetic nephropathy. Diabetes 1983; 2: 64-78.

- Moosa AS, Rashid UR, Asadi AZ, Ara N, Uddin MM, Ferdaus A. Hypolipidemic effects of fenugreek seed powder. Bangladesh Journal of Pharmacology 2006; 1: 64-67.

- Morani AS, Bodhankar SL, Mohan V, Thakurdesai P. Ameliorative effects of standardized extract from *Trigonella foenum-graecum* L. seeds on painful peripheral neuropathy in rats. Asian Pacific Journal of Tropical Medicine 2012; 385- 390.

- Morgan NG, Cable HC, Newcombe NR, Williams GT. Treatment of cultured pancreatic β-cells with streptozotocin induces cell death by apoptosis. Bioscience Reports 1994; 14(5): 243-250.

- Morgon MS, Depirre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochemical and Biophysics Acta 1979; 582: 67-78.

- Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H. Combining a dipeptidyl peptidase-4 inhibitor, alogliptin, with pioglitazone improves glycaemic control, lipid profiles and β-cell function in *db/db* mice. British Journal of Pharmacology 2009; 157: 415-426.

- Mu J, Woods J, Zhou Y, Roy RS, Li Z, Zycband E et al . Chronic Inhibition of Dipeptidyl Peptidase-4 with a Sitagliptin Analog Preserves Pancreatic-Cell Mass and Function in a Rodent Model of Type 2 Diabetes. Diabetes 2006; 55: 1695-704.

- Mullaicharam AR, Deori G, Maheswari UR. Medicinal values of fenugreek -a review. Research Journal of Pharmaceutical, Biological and Chemical 2013; 4(1): 1304-1313.

- Muraka S, Mohaved MR. Diabetic cardiomyopathy. Journal of Cardiac Failure, 2010; 16: 971-979.

- Murugesan M, Revathi R, Manju M. Research article cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats. Indian Journal of Pharmacology 2011; 43(5): 516-551.

- Mustafa HZ, Javad H, Mohmoodi M,Gholamhossein H, Hashemi B, Mohammad HZ. The effects of *Persian shallot* extract on the levels of some blood biochemical parameters in streptozotocin-induced diabetic rats. African Journal of Agriculture Research 2012; 7(22): 3308-3313.

- Nagilla B, Reddy PK. Neuroprotective and antinociceptive effect of curcumin in diabetic neuropathy in rats. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(5): 131-138.

- Narender T, Khaliq T, Madhur G. Naturally occurring antihyperglycemic and antidyslipidemic agents. Research Signpost 2011; 37(2): 155-185.

- Neumiller JJ, Campbell RK. Saxagliptin: a dipeptidyl peptidase 4 inhibitor for the treatment of type 2 diabetes mellitus. American Journal of Health System Pharmacy 2010; 67: 1515-1525.

- Neumiller JJ. Differential chemistry (structure), Mechanism of action, and pharmacology of GLP-1 receptor agonist and DPP-4 inhibitors. Journal of American Pharmacists Association 2009; 49: 16-29.

- Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D et al. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. Circulation 2004; 109: 962-965.

- Noeman SA, Hamooda HE, Baalash AA. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetology and Metabolic Syndrome 2011; 3 (17): 2-8.

- Ntemka A, Iliadis F, Papanikolaou NA, Grekas D. Network-centric analysis of genetic predisposition in diabetic nephropathy. Hippokratia 2011; 15 (3): 232-237.

- Nyenwe EA, Jerkins TW, Umpierrez GE, Kitabchi AE. Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes. Metabolism Clinical and Experimental 2011; 60: 1-23.

- Oguanobi NI, Chijioke CP, Ghasi S. Anti-diabetic effect of crude leaf extracts of *Ocimum gratissimum* in neonatal streptozotocin-induced Type-2 model diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(5): 77- 83.

- Olaiya CO, Soetan KO. A review of the health benefits of fenugreek (*Trigonella foenum-graecum* L.): nutritional, biochemical and pharmaceutical perspectives. American Journal of Social Issues and Humanities 2014: 3-12.

- Olokoba AB, Olusegun A. Obateru, Lateefat B. Type 2 diabetes mellitus: a review of current trends. Oman Medical Journal 2012; 27 (4): 269-273.

- Olugbenga EA, Salako BL. Diabetic nephropathy a review of the natural history, burden, risk factors and treatment. Journal of National Medical Association 2004; 96 (11): 1445-1454.

- Olusola AM, Adekoya AI, Olanrewaju OJ. Comparative evaluation of physicochemical properties of some commercially available brands of metformin Hcl tablets in Lagos, Nigeria. Journal of Applied Pharmaceutical Science 2012; 2 (02): 41-44.

- Owolabi OJ, Omogbai EK. Co-administration of glibenclamide and amlodipine induces resistance to hyperglycemic treatment in streptozotocin induced adapted/non adapted diabetic Rats. Clinical Experimental Pharmacology 2011; 1(1): 2-7.

- Pacher P, Liaudet L, Francisco GS, Mabley JG. The role of poly (Adp-Ribose) polymerase activation in the development of myocardial and endothelial dysfunction in diabetes. Diabetes 2002; 51: 514-521.

- Padwal RS, Gabr RQ, Sharma AM, Langkaas AG, Birch DW, Karmali S. Effect of gastric bypass surgery on the absorption and bioavailability of metformin. Diabetes Care 2011; 34(1): 1295-1300.

- Pandey A, Tripathi P, Pandey R, Srivastava R, Goswami S. Alternative therapies useful in the management of diabetes: a systematic review. Journal of Bioallied Sciences 2011; 3(4): 504-5012.

- Pappachan JM, Varughese GI, Sriraman R, Arunagirinathan G. Diabetic cardiomyopathy: pathophysiology, diagnostic evaluation and management. World Journal of Diabetes 2013; 4(5): 177-189.

- Parmindar K, Kushwah AS, Ravinder P. Current therapeutic strategy in diabetic neuropathy. International Research Journal of Pharmacy 2012; 3(3): 22-29.

- Pastromas S. Diabetes Mellitus: Dipeptidyl Peptidase 4 Inhibitors and Cardiovascular Outcomes. Hospital Chronicles 2014; 9(2): 1-5.

- Patel SS, Goyal RK. Prevention of diabetes-induced myocardial dysfunction in rats using the juice of *Emblica Officinalis* fruits. Experimental Journal of Clinical Cardiology 2011; 16(3):87-91.

- Pathak AK, Sinha PK, Sharma J. Diabetes- a historical review. Journal of Drug Delivery & Therapeutics 2013; 3(1): 83-84.

- Pathak R, Bridgeman MB. Dipeptidyl peptidase-4(DPP-4) inhibitors in the management of diabetes. Drug class Review 2010; 35(9): 509-513.

- Pedersen ML, Jacobsen JL, Lynge AR. Micro- and macrovascular complications among Greenlanders and Danes with type 2 diabetes mellitus in Nuuk, Greenland. International Journal of Circumpolar Health 2010; 69(2): 195-207.

- Perilli G, Saraceni C. Diabetic ketoacidosis: a review and update. Current Emergency and Hospital Medicine Reports 2013; 1:10-17.
  Doi 10.1007/s40138-012-0001-3.

- Petit PR, Sauvaire YD, Hillaire-Buys DM. Steroid saponins from fenugreek seeds: extraction, purification and pharmacological investigation on feeding behavior and plasma cholesterol. Steroids 1995; 60(10): 674-680.

- Petropoulos GA. Fenugreek -The genus Trigonella. London and New York 2000: 255-285.

- Pierre W, Gildas AJ, Ulrich MC, Modeste WN, Benoit NT, Albert K. Hypoglycemic and hypolipidemic effects of *Bersama engleriana* leaves in nicotinamide/streptozotocin induced type 2 diabetic rats. BMC Complementary and Alternative Medicine 2012; 12: 2-6.

- Poole C, Bushey B, Foster C, Campbell B, Willougbhy D, Kreider et al. The effects of a commercially available botanical supplement on strength, body composition, power output and hormonal profiles in resistance-trained males. International Journal Sport Nutritation 2010; 7 (34): 2-9.

- Pop-Busui R. Cardiac autonomic neuropathy in diabetes. Diabetes Care 2010; 33(2): 434-441.

- Powers, AC. Dalession, D. (2011). Goodman and Gillman's, Endocrine pancreas and pharmacotherapy of diabetes mellitus and hypoglycemia. The pharmacological basis of therapeutics. Mc Grive-Hill, USA, 12th edition; 1237-1273.

- Prentki M, Nolan C. Islet β-cell failure in type 2 diabetes. The Journal of Clinical Investigation 2006; 116(7): 1802-1812.

- Qi MY, Chen K, Liu HR, Su YH, Yu SQ. Protective effect of icariin on the early stage of experimental diabetic nephropathy induced by streptozotocin via modulating transforming growth factor β-1 and type IV

collagen expression in rats. Journal of Ethnopharmacology 2011; 138: 731-736.

- Radovits T, Korkmaz S, Loganathan S, Barnucz E, Bomicke T, Arif R et al. Comparative investigation of the left ventricular pressure-volume relationship in rat models of type 1 and type 2 diabetes mellitus. American Journal of Physiology 2009; 297: 125-133.

- Raghuram TC, Sharma RD, Sivakumar B, Sahay BK. Effect of fenugreek seeds on intravenous glucose disposi-tion in non-insulin dependent diabetic patients. Phytotherapy Research 1994; 8(2): 83-86.

- Rakieten L, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of Streptozotocin (NSC-37917). Cancer Chemotherapy Reports 1993; 29: 91-98.

- Ramana KV. Aldose reductase: new insights for an old enzyme. Bimolecular Concepts 2011; 2(1-2): 103-114.

- Raskin J1, Pritchett YL, Wang F, D'Souza DN, Waninger AL, Iyengar S et al. A double-blind, randomized multicenter trial comparing duloxetine with placebo in the management of diabetic peripheral neuropathic pain. Pain Medicine 2005; 6(5): 346-356.

- Ravid M, Neumann L, Lishner M. Plasma lipids and the progression of nephropathy in diabetes mellitus type II: effect of ACE inhibitors. Kidney International 1995; 47: 907-910.

- Read PA, Khan FZ, Heak PM, Hoole SP, Dutka DP. Dpp-4 inhibition by sitagliptin improves the myocardial response to dobutamine stress and mitigates stunning in a pilot study of patients with coronary artery disease. Circulation Cardiovascular Imaging 2010; 3: 195-201.

- Reema M, Pradeep R. Diabetic retinopathy: an Indian perspective. Indian Journal of Medical Research 2007; 125: 297-310.

- Richard AH, Nathan MS, Beattie P. Oral agents for the treatment of type 2 diabetes mellitus: pharmacology, toxicity and treatment. Annals of Emergency Medicine 2001; 38:1: 68-78.

- Robert F, Fendri S, Hary L, Lacroix C, Andrejak M, Lalau JD. Kinetics of Plasma and erythrocyte metformin after acute administration in healthy subjects. Diabetes metabolism 2003; 29(3): 279-283.

- Rodica PB. Cardiac autonomic neuropathy in diabetes. Diabetes care 2010; 33(2): 434-441.

- Rohilla A, Tiwari SK, Rohilla S, Kushnoor A. Diabetic nephropathy: pathogenesis, prevention and treatment. European Journal of Experimental Biology 2011; 1(4): 72-80.

- Rojas LB, Gomes MB. Metformin: an old but still the best treatment for type 2 diabetes. Diabetology & Metabolic Syndrome2013; 5(6): 2-15.

- Rolim LC, Jose, Desouza ST2, Dib SA. Tests for early diagnosis of cardiovascular autonomic neuropathy: critical analysis and relevance. Frontiers in Endocrinology 2013. Doi: 10.3389/fendo.2013.00173.

- Rosenstock J, Sankoh S. Glucose-lowering activity of the dipeptidyl peptidase-4 inhibitor saxagliptin in drug-naive patients with type 2 diabetes. Journal of Diabetes Obesity & Metabolism 2008; 10: 376-386.

- Rossetti L, Fronzo RA, Gherzi R, Stein P, Andraghetti G, Falzetti G et al. Effect of metformin treatment on insulin action in diabetic rats: in vivo and in vitro correlations. Metabolism 1990; 39(4): 425-35.

- Ruiz-Ortega M, Ruperez M, Esteban V, Lorenzo S, Mezzano S, Plara JJ, et al. Angiotensin II increases connective tissue growth factor in the kidney. American Journal Pathology 2003; 163(5): 1937-1947.

- Ryan EA. Diagnosing gestational diabetes. Diabetologia 2011; 54: 480-486.

- Saini S, Sharma S. Antidiabetic effect of *Helianthus annuus* L., seeds in ethanolic extract in streptozotocin- nicotinamide induced type 2 diabetes mellitus. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(2): 382- 387.

- Sakamoto Y, Oyama JI, Ikeda H, Kuroki S, Gondo G, Iwamoto T et al. Effects of sitagliptin beyond glycemic control: focus on quality of life. Cardiovascular Diabetology 2013; 12(35): 2-9.

- Sandhu JS, Frasher DR. The metabolic origin of trigonelline in the rat. Journal of Biochemical Society 1981; 200: 495-500.

- Sandkuhler J. Models and mechanisms of hyperalgesia and allodynia. Physiological Reviews 2009; 89: 707-758.

- Sanusi H. Role of incretin on diabetes mellitus. Acta Medica Indonesia 2009; 41(4): 205-212.

- Sauvaire Y, Petit C, Broca. 4-hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. Diabetes 1998; 47(2): 206-210.

- Sawicki PT, Didjurgeit U, Muhlhauser ID, Bender R, Heinemann L, Berger M. Smoking is associated with progression o f diabetic nephropathy. Diabetes Care 1994; 17(2): 126-131.

- Sayed AA. Thymoquinone and proanthocyanidin attenuation of diabetic nephropathy in rats. European Review of Medical and Pharmacological Sciences 2012; 16: 808-815.

- Scheen AJ. Cardiovascular effects of dipeptidyl peptidase-4 inhibitors: from risk factors to clinical outcomes. Postgraduate Medicine 2013; 125(3): 7-20.

- Scheen AJ. Clinical pharmacokinetics of metformin.Clinical Pharmacokinetics 1996; 30(5): 359-371.

- Schmid H. New options in insulin therapy. Journal of Pediatrics 2007; 83(5):S146-S154.

- Shah S, Bodhankar S, Badole S, Mohan V. Effect of trigonelline: an active compound from *Trigonella foenum graecum* L. in alloxan induced diabetes in mice. Journal of Cell and Tissue Research 2006; 6(1): 585-590.

- Shah SN, Bodhankar SL, Bhonde R, Mohan V. Hypoglycemic Activity of the combination of active ingredients isolated from *Trigonella foenum graceum* in alloxan induced diabetic mice. Pharmacologyonline 2006; 1: 65-82.

- Shapiro K, Gong W. Natural products used for diabetes. Journal of American Pharmacists Association 2002; 42(2): 217-226.

- Sharma AK, Sharma A, Kumari R, Kishore K, Sharma D, Srinivasan B et al. Sitagliptin, sitagliptin and metformin, or sitagliptin and amitriptyline attenuate streptozotocin -nicotinamide induced diabetic neuropathy in rats. Journal of Biomedical Research 2012; 26: 1-10.

- Sharma V, McNeill JH. Diabetic cardiomyopathy: where are we 40 years later? Canadian Journal of Cardiology 2006; 22(4): 305-308.

- Shehata MM, Abd El-Kader ZY, Abd El Al Ahmed, Abbas NA. Comparison of effect of stiagliptin and glimepiride on glycemic control and potential cardiovascular complications in diabetic albino rats. Zagazig University of Medical Journal 2013; 19(13): 625-636.

- Shekelle PG, Rich MW, Morton SC, Atkinson CS, Tu W, Maglione M et al. Efficacy of angiotensin converting enzyme inhibitors and β-blockers in the management of left ventricular systolic dysfunction according to race, gender, and diabetic status: A meta analysis of major clinical trials. Journal of the American College of Cardiology 2003; 41: 1529-1538.

- Shinde P, Patil P, Bairagi V. Herbs in pregnancy and lactation: a review appraisal. International Journal of Pharmaceutical Sciences and Research 2012; 3(9): 3001-3006.

- Shotton HR, Clarke S, Lincoln J. The effectiveness of treatments of diabetic autonomic neuropathy is not the same in autonomic nerves supplying different organs. Diabetes 2003; 52:157-164.

- Silva SB, Costa JP, Pintado ME, Ferreira DC, Sarmento B. Antioxidants in the prevention and treatment of diabetic retinopathy- a review. Journal of Diabetes and Metabolism 2010; 1(3):2-10; Doi 10.4172/2155-6156.1000111.

- Singh S, Patel D, Patel NR, Kumar K, Kumar M. Insulin oral delivery may be possible. International Journal of Pharma Professional's Research 2010; 1(1): 46-51.

- Sivaprasad S, Gupta B, Evans J. Prevalence of diabetic retinopathy in various ethnic groups: a worldwide perspective. Survey of Ophthalmology 2012; 57 (4): 338-370.

- Slater TF, Sawyer BC.The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in rat liver fraction in vitro. Biochemical Journal 1971; 123: 805-814.

- Smit R, Neeraj K, Kothiyal P. Traditional medicinal plants used for the treatment of diabetes. International journal of Pharmaceutical and Phytopharmacological Research 2013; 3 (3): 171-175.

- Smith E. Therapeutic applications of fenugreek. Alternative Medicine Review 2003; 8(1): 20-27.

- Soetikno V, Sari FR, Sukumaran V, Lakshmanan AP, Mito S, Harima M, Thandavarayan RA et al. Curcumin prevents diabetic cardiomyopathy in streptozotocin-induced diabetic rats: Possible involvement of PKC–MAPK signaling pathway. European Journal of Pharmaceutical Sciences 2012; 47: 604-614.

- Somani R, Singhai A, Shivgunde P, Jain D. *Asparagus racemosus wild* (Liliaceae) ameliorates early diabetic nephropathy in STZ induced diabetic rats. Indian Journal of Experimental Biology 2012; 50: 469-475.

- Soria CL, Gomez MR, Olvera LG, Gonazaler RG, Pacheco IR, Corona MA, et al.Transcriptomic analysis in diabetic nephropathy of streptozotocin-induced diabetic rats. International Journal of Molecular Sciences 2011; 12: 8431-8448.

- Sridevi V, Giridhar P. Influence of altitude variation on trigonelline content during ontogeny of *Coffea Canephora* fruit. The Journal of Food Studies 2013; 2(1): 62-74.

- Srivastava S, Saxena GN, Keshwani P, Gupta R. Comparing the efficacy and safety profile of sitagliptin versus glimepiride in patients of type 2 diabetes mellitus inadequately controlled with metformin alone. Journal of Association of Physician of India 2012; 60: 27-30.

- Steele KP, Ickert-Bond SM, Zarre S, Wojciechowski MF. Phylogeny and character evolution in medicago (leguminosae): evidence from analyses of plastid TRNk/MATk and nuclear ga3 ox1 sequences. American Journal Botany 2010; 97(7): 1142-1155.

- Sterne J. Treatment of diabetes mellitus with N, N-dimethylguanylguanidine (LA. 6023, glucophage).Therapie 1959; 14: 625-630.

- Stevens MJ, Obrosova I, Cao X, Huysen CV, Greene DA. Effects of $_{DL}$-α-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. Diabetes 2000; 49: 1006-1015.

- Stumvoll M, Nurjhan N, Perriello G. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. The New England Journal of Medicine 1995; 333 (9): 550-554.

- Symeonides P, Koulouris S, Ekaterini Vratsista E, Triantafyllou K, Loannidis G, Thalassinos N. Both ramipril and telmisartan reverse indices of early diabetic cardiomyopathy: a comparative study. European Journal of Echocardiography 2007; 8: 480-486.

- Szkudelski T. Streptozotocin-nicotinamide induced diabetes in the rat. Journal of Experimental Biology and Medicine 2012; 237(5): 481-490.

- Temraz TA, Houssen WE, Jaspars M. A pyridinium derivative from Red Sea Soft corals inhibited voltage-activated potassium conductances and increased excitability of rat cultured sensory neurones. BMC Pharmacology 2006; 6(10): 1-19.

- Tesfaye S. Epidemiology and etiology of diabetic peripheral neuropathies. Advanced studies in Medicine 2004; 1(10): S1014-S1021.

- Thaisetthawatkul P, Dyck PJ. Treatment of diabetic and nondiabetic lumbosacral radiculoplexus neuropathy. Current Treatment Options in Neurology 2010; 12(2): 95-99.

- Therasa S, Sivaraj C, Sivakumar E, Thirumalai T, David E. *Trigonella foenum graceum* seed extract Inhibit uptake of glucose across rat everted gut sacs in vitro. International Journal of Pharmacy and Technology Research 2010; 2(1): 359-362.

- Thoda C, Kuboyama T, Komatsu K. Search for natural products related to regeneration of the neuronal network. Neurosignals 2005; 14(2): 34-45.

- Timar R, Popescu S, Simu M, Diaconu L, Timar B. QTc interval and insulin resistance in type 2 diabetes mellitus. European Journal of Pharmaceutical Sciences 2013; 9(12): 70-77.

- Tohda C, Nakamura N, Komatsu K, Hattori M. Trigonelline-induced neurite outgrowth in human neuroblastoma SK-N-SH cells. Biology and Pharmaceutical Bulletin 1999; 22(7): 679-682.

- Tremblay AJ, Lamarche B, Deacon CF, Weisnagel SJ, Couture P. Effect of sitagliptin therapy on postprandial lipoprotein levels in patients with type 2 diabetes. Diabetes Obesity and Metabolism 2011; 13(4): 366-373.

- Trevisan R, Dodesini AR, Lepore G. Lipids and renal disease. Journal of The American Society of Nephrology 2006; 17: S145–S147.

- Trevisan R, Vedovato M, Mazzon C, Coracina A, Iori E, Tiengo A, Del PS. Concomitance of diabetic retinopathy and proteinuria accelerates the rate of decline of kidney function in type 2 diabetic patients. Diabetes Care 2002; 25(11): 2026-2031.

- Tripathi UN, Chandra D. The plant extracts of *Momordica charantia* and *Trigonella foenum-graecum* have antioxidant and antihyperglycemic properties for cardiac tissue during diabetes mellitus. Oxidative Medicine and Cellular Longevity 2009; 2(5): 290-296.

- Unger J. Incretins: clinical perspectives, relevance and applications for the primary care physician in the treatment of patients with type 2 diabetes mellitus. Mayo Clinic Proceedings 2010; 85(12): S38-S49.

- Vaghasiya J, Sheth N, Bhalodia Y, Manek R. Sitagliptin protects renal ischemia reperfusion induced renal damage in diabetes. Regulatory Peptides 2011; 166: 48-54.

- Venkatraman R. Sunit C. Singhi. Hyperglycemic hyperosmolar non-ketotic syndrome. Indian Journal of Pediatrics 2006; 73: 55-60.

- Vinik A, Erbas VT. Recognizing and treating diabetic autonomic neuropathy. Cleveland Clinic Journal of Medicine 2001; 68(11): 929-944.

- Vinje NE, Namork E, Lovik M. Cross-allergic reactions to legumes in Lupin and fenugreek-sensitized mice Scandinavian Journal of Immunology 2012; 76: 387-397.

- Viollet BG, Garcia NS, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview Benoit. Clinical Science 2012; 122(6): 253-270.

- Visnagri A, Kandhare AD, Ghosh P; Ghule AE, Bodhankar SL et al. Elucidation of ameliorative effect of Co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomedicine & Aging Pathology 2012; 2: 309-316.

- Wada J, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. Clinical Science 2013; 124(3): 139-152.

- Waget A, Cabou C, Masseboeuf M, Cattan P, Armanet M, Karaca M et al. Physiological and pharmacological mechanisms through which the DPP-IV inhibitor sitagliptin regulates glycemia in mice. Endocrinology 2011; 152(8): 1-11.

- Wanner C. Importance of hyperlipidemia and therapy in renal patients. Nephrol Dialysis Transplantation 2000; 15 (5): 92-96.

- Westergaard T, Wohlfahrt J, Aaby P, Melbye M. Population based study of rates of multiple pregnancies in Denmark. British Medical Journal 1997; 314(15): 775-795.

- White JR. Dipeptidyl peptidase-IV inhibitors: pharmacological profile and clinical Use. Clinical Diabetes 2008; 26(2): 53-57.

- White MF, Kahn CR. The insulin receptor and the molecular mechanism of insulin action. Journal of Clinical Investigation 1988; 82: 1151-1156.

- Wolfensberger TJ, Hamilton AM. Diabetic retinopathy an historical review. Seminars in Ophthamology 2001; 16(1): 2-7.

- Woodhouse S, Batten W, Hendrick H, Malek PA. The glomerular filtration rate: an important test for diagnosis, staging and treatment of chronic kidney disease. Laboratory Medicine 2005; 37(4): 244-246.

- Wright J, Hutchison A. Cardiovascular disease in patients with chronic kidney disease. Vascular Health and Risk Management 2009:5:713-722.

- Xue W, Lei J, Li X, Zhang R. *Trigonella foenum graecum* seed extract protects kidney function and morphology in diabetic rats via its antioxidant activity. Nutrition Research 2011; 31: 555-562.

- Yamabe N, Yokozawa T, Oya T, Mujo K. Therapeutic potential of (-) Epigallocatechin 3- O-Gallate on renal damage in diabetic nephropathy model rats. Journal of Pharmacology and Experimental Therapeutics 2006; 319 (1): 228- 236.

- Yamada S. Insulin glulisine in the management of diabetes. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2009; 2: 111-115.

- Yanai H, Adachi H, Hamasaki H, Masui Y, Yoshikawa R, Moriyama S, Mishima S, Sako A. Effects of 6-month sitagliptin treatment on glucose and lipid metabolism, blood pressure, body weight and renal function in type 2 diabetic patients: a chart-based analysis. Journal of Clinical and Medical Research 2012; 4(4): 251-258.

- Yau JW, Orchard, Rogers S, Porta M, Kawasaki R, Rema M et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 2012, 35: 556-564.

- Yehia BR, Epps KC, Golden SH. Diagnosis and management of diabetic ketoacidosis in adults. Hospital Physician 2008; 21-26.

- Yeom JA, Kim ES, Park HS, Ham DS, Sun C, Kim JW et al. Both sitagliptin analogue & pioglitazone preserve the β-cell proportion in the islets with

different mechanism in non-obese and obese diabetic mice. BMB Reports 2011; 44(11): 713-718.

- Yu T, Jhun BS, Yoon Y. High-glucose stimulation increases reactive oxygen species production through the calcium and mitogen-activated protein kinase-mediated activation of mitochondrial fission. Antioxid Redox Signal 2011; 14 (3): 425-437.

- Yuyun MF, Khaw KT, Luben R, Welch A, Bingham S et al. A prospective study of microalbuminuria and incident coronary heart disease and its prognostic significance in a British population. American Journal of Epidemiology 2003; 59(3): 284-293.

- Zbigniew Z. Entrapment neuropathies. Advances in Palliative Medicine 2010; 9(3): 103-108.

- Zellweger MJ, Hachamovitch RH, Kang X, Hayes SW, Friedman JD, Guido G. Prognostic relevance of symptoms versus objective evidence of coronary artery disease in diabetic patients. European Heart Journal 2004; 25: 543-550.

- Zhang L, Cannell MB, Phillips AR, Cooper GJ, Ward ML. Altered calcium homeostasis does not explain the contractile deficit of diabetic cardiomyopathy. Diabetes 2008; 57: 2158- 2166.

- Zheng ZQ, Hayashibe E, Ashihara H. Changes in trigonelline (*N - methylnicotinic acid*) content and nicotinic acid metabolism during germination of mungbean (*Phaseolus aureus*) seeds. Journal of Experimental Botany 2005; 56(416): 1615-1623.

- Zhongju T, Zherong XU, Qifeng G, Weizhen W, Yunmei Y. Gliquidone versus metformin: differential effects on aorta in streptozotocin induced diabetic rats. Cinical Medicine Journal 2014; 127(7): 1298-1303.

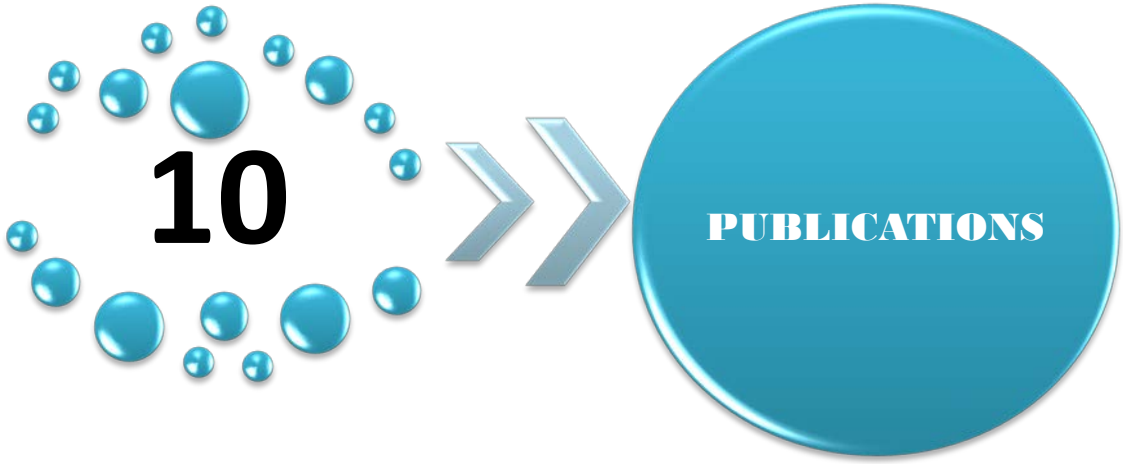
- Zhou J, Chan L, Zhou S. Trigonelline: a plant alkaloid with therapeutic potential for diabetes and central nervous system disease. Current Medicinal Chemistry 2012; 19(21): 3523-3531.

- Zhou J, Zhou S, Zeng S. Experimental diabetes treated with trigonelline: effect on β cell and pancreatic oxidative parameters. Fundamentals Clinical Pharmacology 2013; 27 (3): 279-287.

- Zhou SF. Drugs behave as substrates, inhibitors and inducers of human Cytochrome P4503A4. Current Drug Metabolism 2008; 9: 310-22.

- Ziegler D, Reljanovic M, Mehnert H, Gries FA. Alpha-lipoic acid in the treatment of diabetic polyneuropathy in Germany: current evidence from clinical trials. Experimental and Clinical Endocrinology and Diabetes 1999; 107(7): 421-430.

- Zulfiqar HK, Liaqat IA, Syed A, Gulzar A. Nutritional value and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) from two regions of Pakistan. Pakistan Journal of Food Science 2013; 23(3): 144-147.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PUBLICATIONS

10. PUBLICATIONS

List of International of Publications:

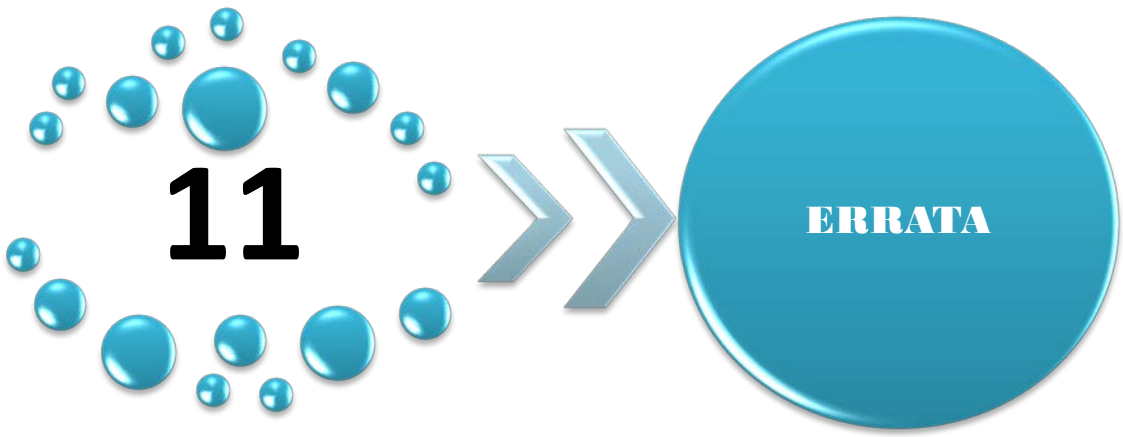
1. Kamble H.V., Bodhankar S.L. Antihyperglycemic activity of trigonelline and sitagliptin in nicotinamide-streptozotocin induced diabetes in Wistar rats. Biomedicine and Aging Pathology 2013; 3:125-130.

2. Kamble H.V., Bodhankar S.L. Trigonelline and sitagliptin attenuates nicotinamide-streptozotocin induced diabetic nephropathy in Wistar rats. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(4): 583-589.

3. Kamble H.V., Bodhankar S.L. Concomitant administration of trigonelline and sitagliptin attenuates nicotinamide-streptozotocin induced diabetic neuropathy in Wistar rats. Journal of Chemical and Pharmaceutical Research 2014; 6(2): 616-624.

Accepted International Publication:

1. Kamble H.V., Bodhankar S.L. Cardioprotective effect of concomitant administration of trigonelline and sitagliptin on cardiac biomarkers, lipid levels, electrocardiographic and heamodynamic modulation on cardiomyopathy in diabetic Wistar rats. Biomedicine and Aging Pathology 2014.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