OBSERVATIONS AND RESULTS

Observations and results of the study are presented as follows:

A) Changes in Experimental Dextrose induced Cataract Lenses with respect to-

a) Observation of lens morphology

b) Total Soluble Proteins and Lipid Peroxidation.

c) Antioxidant defense enzymes

d) Aldose reductase (Polyol pathway)

B) Effect of selected medicinal plants on dextrose induced cataractous lenses with reference to-

a) Observation of lens morphology

b) Total Soluble Proteins and Lipid Peroxidation.

c) Antioxidant defense enzymes

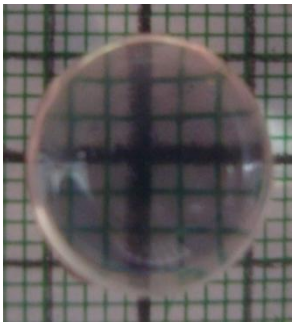
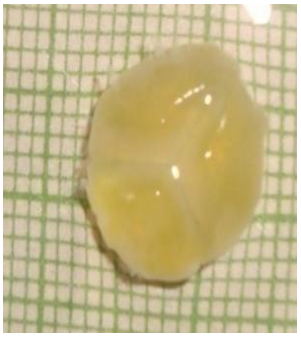
d) Aldose reductase (Polyol pathway)

A) Changes in Experimental Dextrose induced Cataract Lenses:

a) Observation of lens morphology:

At the end of 72 hours, as compared to normal lenses, lenses incubated with 110mM dextrose showed total loss of transparency with development of mature cataract, nearing rupture (grids not visible).

Figure 4:

A.  B. 

A: Normal goat lens incubated in tissue culture medium TC-199. Transparency, clarity of the lens is maintained (grids visible)

B: Lens incubated in 110mM dextrose. Lens shows opacity, swelling and development of mature cataract, nearing rupture (grids not visible)

b) Total Soluble proteins and Lipid Peroxidation:

The results for all the biochemical parameters (Total soluble proteins, MDA, activity of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase) in the normal control lenses and dextrose induced cataract lenses were analysed by student's 't' test and the statistical significance was noted as $p < 0.05$.

Table 1: Lens Total Soluble Proteins and Malondialdehyde (MDA) Levels in Normal Control and Dextrose induced cataract lenses.

Sr. No	Group	N	Total Soluble Proteins (mg / lens) Mean ± SD	MDA (n moles /gm lens) Mean ± SD
1	**Control** (Normal Lenses)	30	344 ± 62.56	9.56 ± 3.43
2	**Dextrose induced Cataract lenses**	30	255 ± 62.46	13.45 ± 3.0
	P - value		< 0.0001	< 0.0001

The total soluble protein content of dextrose induced cataract lenses decreased by 25.8 % as compared to normal control lenses. The decrease was statistically highly significant ($p < 0.0001$).

Lipid peroxidation measured in terms of Malondialdehyde (MDA) levels showed an increase by 28.9 % in the dextrose induced cataract lenses as compared to normal control lenses. The increase in MDA was statistically highly significant ($p < 0.0001$).

c) Activity of Antioxidant defense enzymes and aldose reductase:

Table 2: Specific activity of Superoxide Dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase in Normal Control and Dextrose induced cataract lenses.

Sr. No	Group	N	Superoxide dismutase (units/mg lens) Mean ± SD	Glutathione peroxidase (units/mg lens) Mean ± SD	Glutathione reductase (units/mg lens) Mean ± SD	Aldose reductase (units/mg lens) Mean ± SD
1	**Control** (Normal Lenses)	30	0.41± 0.14	36.16 ± 9.90	13.32 ± 3.55	14.06 ± 7.5
2	**Dextrose** (Experimental Cataract lenses)	30	0.30±0.15	29.71±13.92	10.49 ±2.11	25.01±11.6
	P-value		< 0.01	< 0.05	p< 0.001	< 0.0001

The dextrose induced cataract lenses showed a decrease in the specific activity of enzyme Superoxide dismutase by 26.8% as

compared to control lenses. The decrease was statistically significant ($p < 0.01$).

Glutathione peroxidase specific activity was reduced by 17.7 % in dextrose induced cataract lenses as compared to control lenses. The decrease was statistically significant ($p < 0.05$).

A decrease of 21.2% in the specific activity of Glutathione reductase was noted in dextrose induced cataract lenses as compared to control lenses with a significance of $p < 0.001$.

Specific activity of Aldose reductase was increased by 43.6 % in dextrose induced cataract lenses as compared to normal control lenses. The rise was statistically highly significant ($p < 0.0001$).

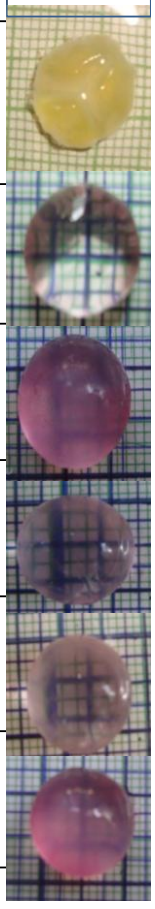
B) Effect of selected medicinal plants on dextrose induced cataractous lenses –

a) Observation of lens morphology:

At the end of 72 hours, as compared to dextrose induced cataract lenses, lenses incubated with 110mM dextrose in the presence of each medicinal plant extract showed significant maintenance of lens transparency and integrity.

Figure 5: **Observation of lens morphology when incubated with medicinal plant extracts and vitamin c respectively**

GROUP	TRANSPARENCY & CLARITY
B) Dextrose	Opaque, mature cataract, nearing rupture (grids not visible)
c) Dextrose + S.cumini	Transparency, lens integrity maintained (grids visible)
D) Dextrose + A. marmelos	Opacity, but lens integrity maintained (grids faintly visible)
E)Dextrose + A.Sativum	Transparency, lens integrity maintained (grid visible)
F)Dextrose +E.officinalis	Transparency, clarity maintained (grids visible)
G)Dextrose + Vit. C	Lens integrity maintained , opacity (grids faintly visible)



Analysis of variance (ANOVA) was performed for each biochemical parameter (Total soluble proteins, MDA, activity of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase) in the **dextrose and medicinal plant extract groups.**

Post hoc analysis was performed using **Tukey's test**

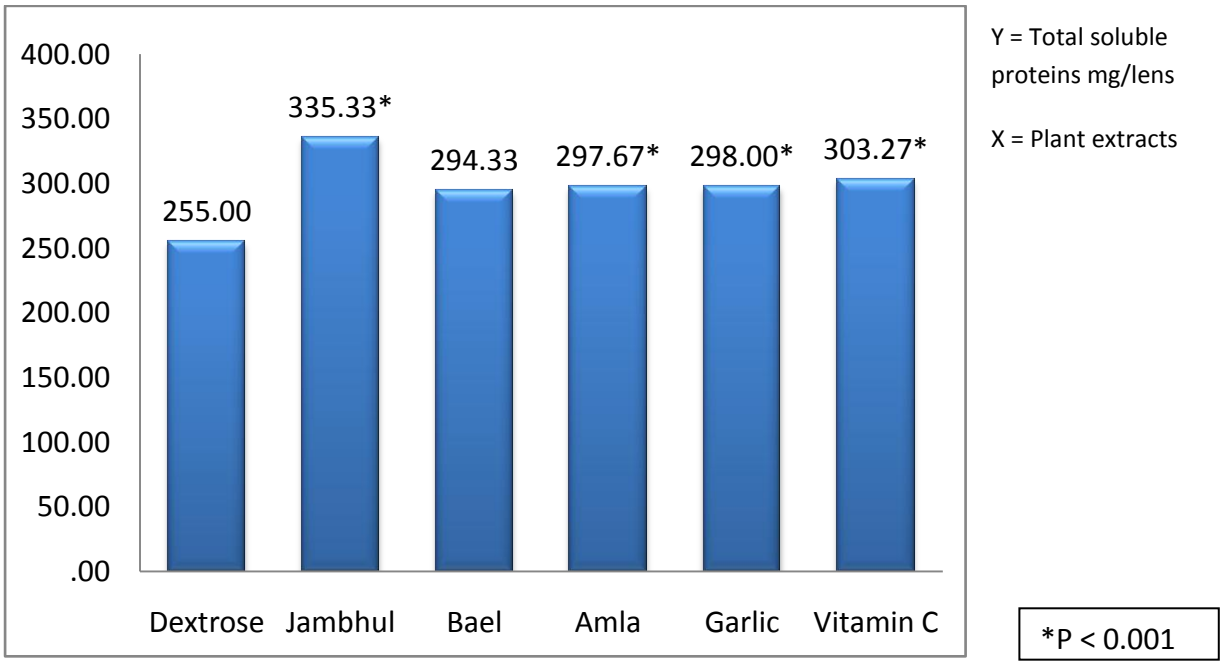
bi) Total Soluble Proteins:

Table 3: Total Soluble lens proteins (mg/lens) in experimental diabetic cataract lenses incubated with S.cumini (jambhul), A.marmelos (bael), E.officinalis (amla), A.sativum (garlic) water extracts and Vitamin C respectively, compared with Dextrose group.

Group	N	Mean (mg/lens)	SD ±
Dextrose	30	255.00	62.46
Jambhul	30	335.33	74.03
Bael	30	294.33	34.90
Amla	30	297.67	70.13
Garlic	30	298.00	34.58
Vitamin C	30	303.27	40.20
ANOVA: $F(5, 174) = 6.44$, $p<0.001$			

A one-way ANOVA was conducted to compare the effect of the jambhul, bael, garlic, amla aqueous extracts and vitamin C on Lens Total soluble proteins in the presence of dextrose. There was a significant effect of these medicinal plant extracts and vitamin C. [F (5, 174) = 6.44, p < 0.001].

Figure 6: Total Soluble Proteins (mg/lens) in all groups



Post hoc comparisons using the Tukey HSD test indicated that the mean score for the total soluble proteins in groups with jambhul extract (M = 335.33, SD = 74.0), amla (M= 297.67, SD = 70.14), garlic extract (M= 298, SD = 34.58) and Vitamin C (M= 303.2, SD = 40.2) was significantly different than the dextrose induced cataract group (M = 255,

SD = 62.4). However, the difference in the bael extract group (M = 294, SD = 34.9) was not statistically significant.

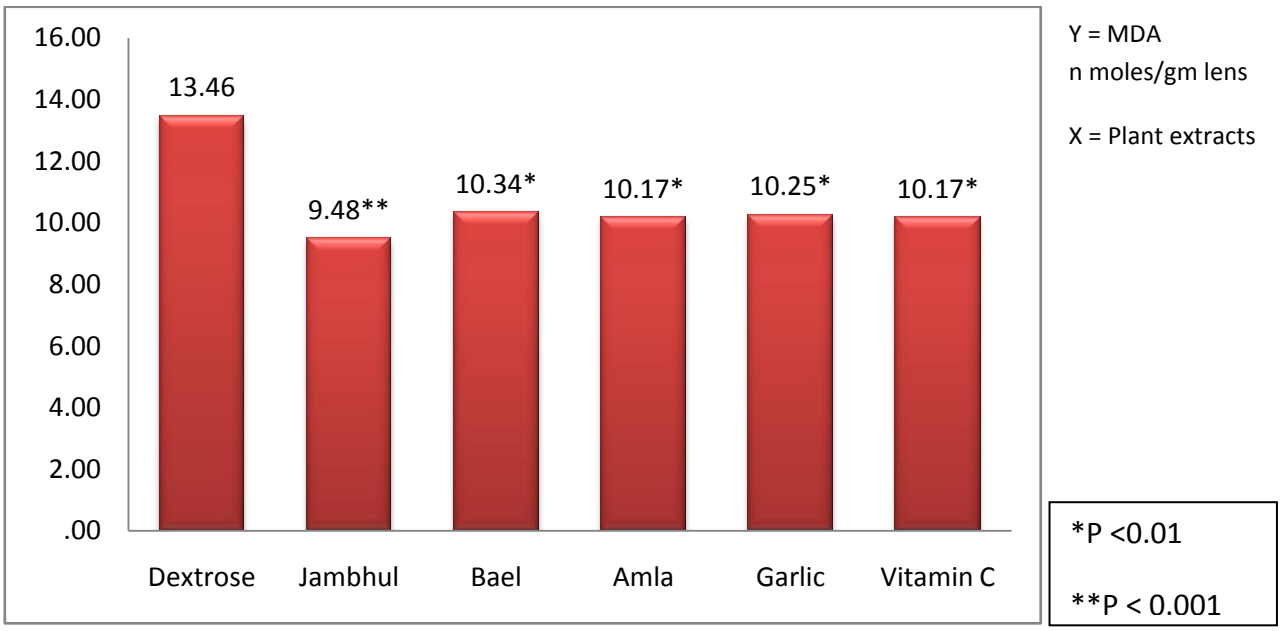
b- ii) Malondialdehyde (MDA) levels in lens:

Table 4: Malondialdehyde (MDA) levels (n moles/gm lens) as an index of lipid peroxidation in experimental diabetic cataract lenses incubated with S.cumini (jambhul), A.marmelos (bael), E.officinalis (amla), A.sativum (garlic) water extracts and Vitamin C respectively, compared with Dextrose group.

Group	N	Mean (n moles/gm lens)	SD ±
Dextrose	30	13.46	3.06
Jambhul	30	9.48	2.44
Bael	30	10.34	3.26
Amla	30	10.17	3.12
Garlic	30	10.25	3.56
Vitamin C	30	10.17	3.33
ANOVA: $F(5, 174) = 6.02$, $p < 0.001$			

A one-way ANOVA conducted to compare the effect of the jambhul, bael, garlic, amla aqueous extracts and vitamin C on MDA levels in the presence of dextrose showed a significant effect of these medicinal plant extracts and vitamin C.[F (5, 174) = 6.44, p < 0.001].

Figure 7: **Malondialdehyde (MDA) levels (n moles/gm lens) in all groups**



Post hoc comparisons using the Tukey HSD test indicated that the mean score for the Malondialdehyde levels in groups with Bael extract (M = 10.34, SD = 3.26), amla (M= 10.17, SD = 3.12), garlic extract (M= 10.25, SD = 3.56) and Vitamin C (M= 10.17, SD = 3.33) was significantly different (p < 0.01) than the dextrose induced cataract group (M = 13.46, SD = 3.06). The difference in the jambhul extract group

(M = 9.48, SD = 2.44) was statistically highly significant ($p < 0.001$) when compared with dextrose and bael extract groups.

c) Activity of Antioxidant defense enzymes and aldose reductase:

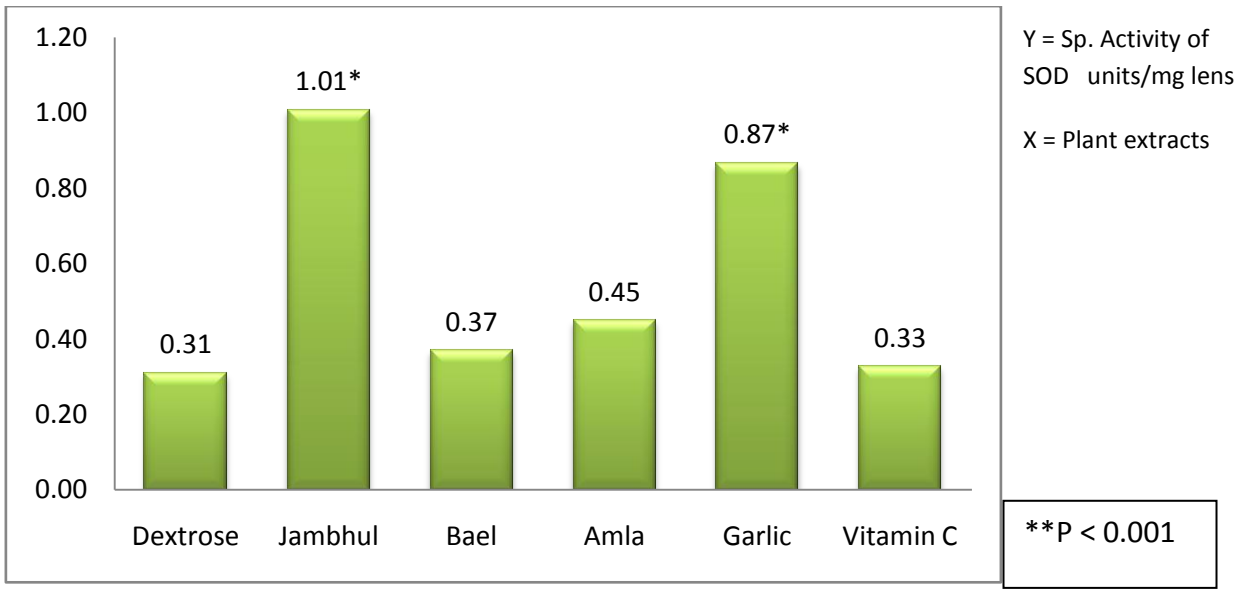
c i) Specific activity of Superoxide dismutase:

Table 5: Specific activity of Superoxide dismutase (units/mg lens)in experimental diabetic cataract lenses incubated with S.cumini (jambhul), A.marmelos (bael), E.officinalis (amla), A.sativum (garlic) water extracts and Vitamin C respectively, compared with Dextrose group.

Group	N	Mean (units/mg lens)	SD ±
Dextrose	30	0.31	0.15
Jambhul	30	1.01	0.28
Bael	30	0.37	0.12
Amla	30	0.45	0.16
Garlic	30	0.87	0.37
Vitamin C	30	0.33	0.19
ANOVA: $F(5, 174) = 51.41$, $p < 0.001$			

A one-way ANOVA conducted to compare the effect of the jambhul, bael, garlic, amla aqueous extracts and vitamin C on specific activity of Superoxide dismutase in the presence of dextrose showed a significant effect of these medicinal plant extracts and vitamin C. [F (5, 174) = 51.41, p < 0.001].

Figure 8: **Specific activity of Superoxide dismutase (units/mg lens) in all groups**



Post hoc comparisons using the Tukey HSD test indicated that the increase in the mean score for the Specific activity of Superoxide dismutase in groups with jambhul extract (M = 1.01, SD = 0.28) and garlic extract (M= 0.87, SD = 0.37) was significantly increased (p < 0.001) than the dextrose induced cataract

group (M = 0.31, SD = 0.15). The increase in the mean scores of bael, amla and Vitamin C groups was not significant when compared to dextrose group.

The increase in Specific activity of Superoxide dismutase in jambhul extract group was also highly significant ($p < 0.001$) over bael (M = 0.37, SD = 0.12), amla (M = 0.45, SD = 0.16) and Vitamin C (M = 0.33, SD = 0.19) groups but not significant over garlic group.

The difference in the mean score of garlic group was highly significant ($p < 0.001$) over bael, amla and Vitamin C groups.

The amla extract group had a highly statistical significance ($p < 0.001$) over the vitamin C group when compared for its Superoxide dismutase specific activity.

c ii) Specific activity of Glutathione Peroxidase

Table 6: Glutathione Peroxidase specific activity (units/mg lens) in experimental diabetic cataract lenses incubated with S.cumini (jambhul), A.marmelos (bael), E.officinalis (amla),A.sativum (garlic) water extracts and Vitamin C respectively, compared with Dextrose group.

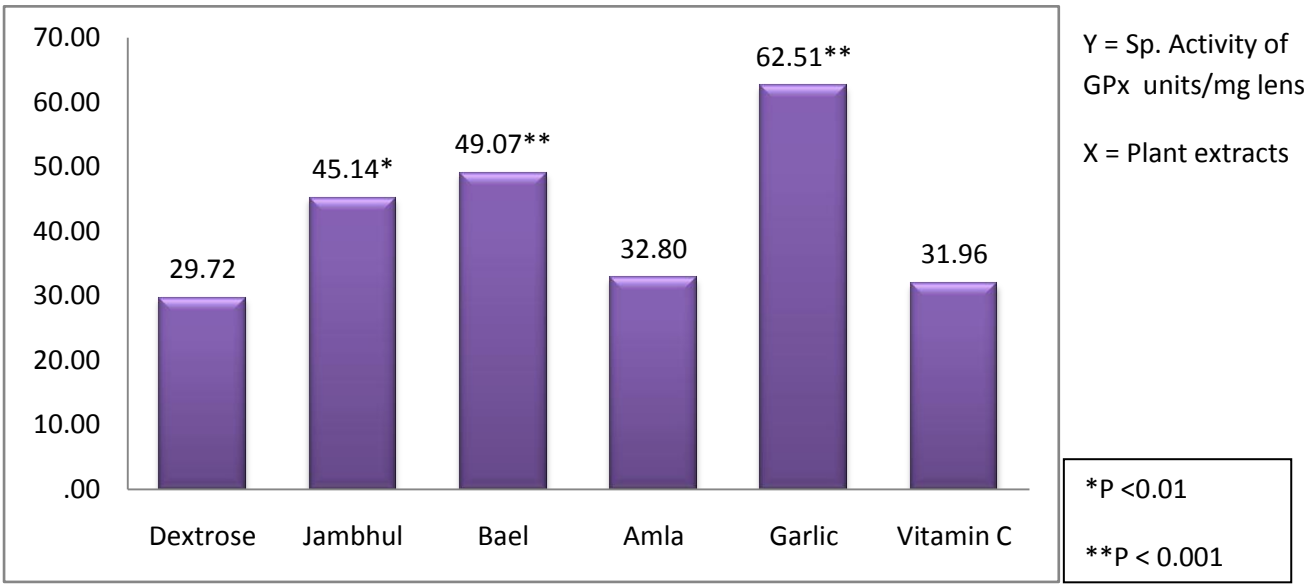
Group	N	Mean (units/mg lens)	SD ±
Dextrose	30	29.72	13.9
Jambhul	30	45.14	14.2
Bael	30	49.07	22.4
Amla	30	32.80	8.6
Garlic	30	62.51	17.9
Vitamin C	30	31.96	10.9
ANOVA: $F(5, 174) = 20.79$, $p < 0.001$			

A one-way ANOVA was conducted to compare the effect of the jambhul, bael, garlic, amla aqueous extracts and vitamin C on specific activity of Lens Glutathione Peroxidase enzyme in the presence of dextrose.

There was a significant effect of these medicinal plant extracts and vitamin C.

[F (5, 174) = 20.79, p < 0.001].

Figure 9: **Specific activity of Glutathione Peroxidase (GPx)(units/mg lens) in all groups**



Post hoc comparisons using the Tukey HSD test indicated that the mean score for the Glutathione peroxidase activity in groups with garlic extract (M= 62.51, SD = 17.90) ,bael (M= 49.07, SD = 22.4) and jambhul extract (M = 45.14, SD = 14.2) was significantly different than the dextrose induced cataract group (M = 29.72, SD = 13.92).The difference in the amla extract group and vitamin C group was not statistically significant.

The comparison also showed that the mean score of GPx activity in Garlic group had a rise of high statistical significance ($p < 0.001$) over the GPx level of jambhul, amla extract ($M = 32.80$, $SD = 8.65$) and vitamin C group ($M = 31.96$, $SD = 10.91$). The rise in GPX activity over bael group was also significant ($p < 0.05$).

Moreover, the mean score of the jambhul group was seen to have a statistically significant increase ($p < 0.05$) over mean score of amla group ($M = 32.8$, $SD = 8.65$) and an increase of high statistical significance of $p < 0.01$ with vitamin C group.

The bael extract group was found to have an increase in GPx activity of high statistical significance over amla and vitamin C groups.

c iii) Specific activity of Glutathione Reductase:

Table 7: Glutathione Reductase specific activity (units/mg lens) in experimental diabetic cataract lenses incubated with S.cumini (Jambhul), A.marmelos (Bael), E.officinalis (Amla), A.sativum (Garlic) water extracts and Vitamin C respectively, compared with Dextrose group.

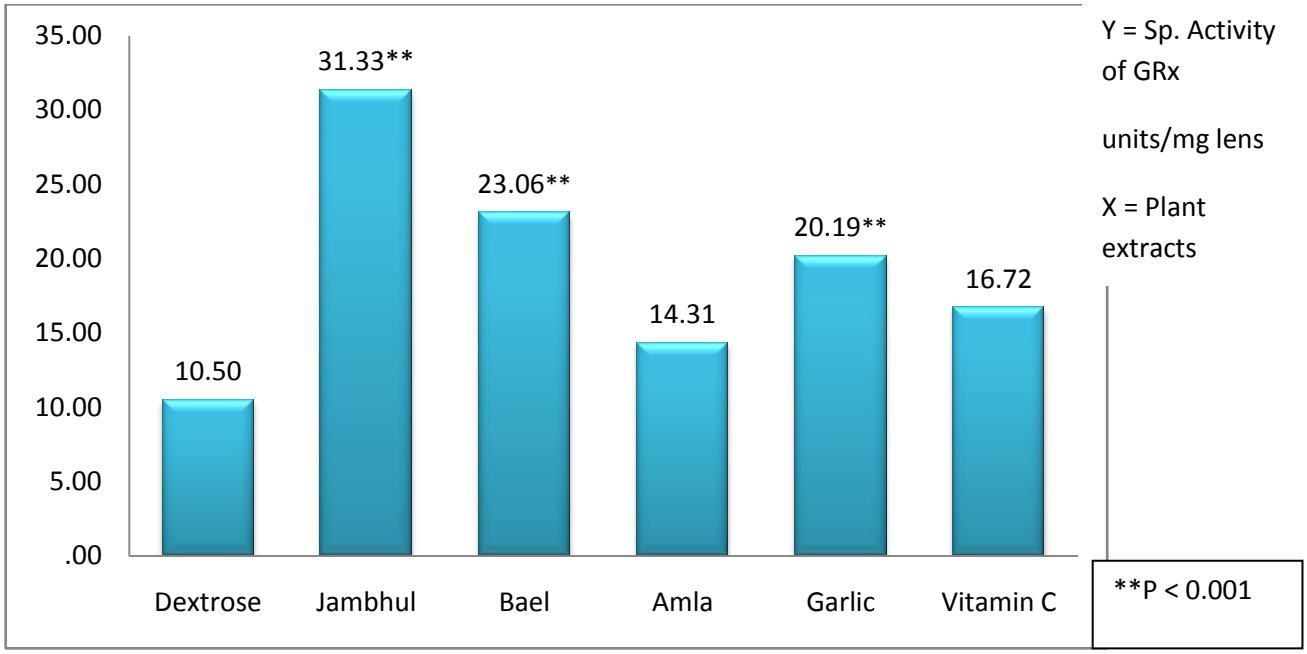
Group	N	Mean (units/mg lens)	SD ±
Dextrose	30	10.50	2.1
Jambhul	30	31.33	6.7
Bael	30	23.06	6.8
Amla	30	14.31	4.3
Garlic	30	20.19	4.8
Vitamin C	30	16.72	4.7
ANOVA: $F(5, 174) = 59.72$, $p < 0.001$			

A one-way ANOVA was conducted to compare the effect of the jambhul, bael, garlic, amla aqueous extracts and vitamin C on specific activity of Lens Glutathione Reductase enzyme in the presence of dextrose.

There was a significant effect of these medicinal plant extracts and vitamin C.

[F (5, 174) = 6.44, p < 0.001].

Figure 10: Specific activity of Glutathione Reductase (GRx)(units/mg lens) in all groups



Post hoc comparisons using the Tukey HSD test indicated that the mean score for the Glutathione reductase activity in groups with jambhul (M= 31.33, SD = 6.7), bael (M= 23.06, SD = 6.8), garlic extract (M = 20.19, SD = 4.8) and vitamin C (M = 16.72, SD = 4.7) was significantly different ($p < 0.001$) than the dextrose induced cataract group (M =

10.50, SD = 2.1).The difference in the amla extract group (M = 14.31, SD = 4.3) was not statistically significant.

The comparison also showed that the mean score of GRx activity in Jambhul group had a rise of high statistical significance ($p < 0.001$) over the GRx level of bael, amla, garlic extracts (M = 32.80, SD = 8.65) and vitamin C group (M = 16.72 , SD = 4.7).

Tukey test also showed that the mean score of GRx in the bael group had a high statistically significant increase ($p < 0.001$) over mean score of amla and vitamin C group, but the rise over garlic extract group was not statistically significant.

The garlic extract group was found to have an increase in GRx activity of high statistical significance ($p < 0.001$) over amla extract group but not over vitamin C group.

c iv) Specific activity of Aldose Reductase:

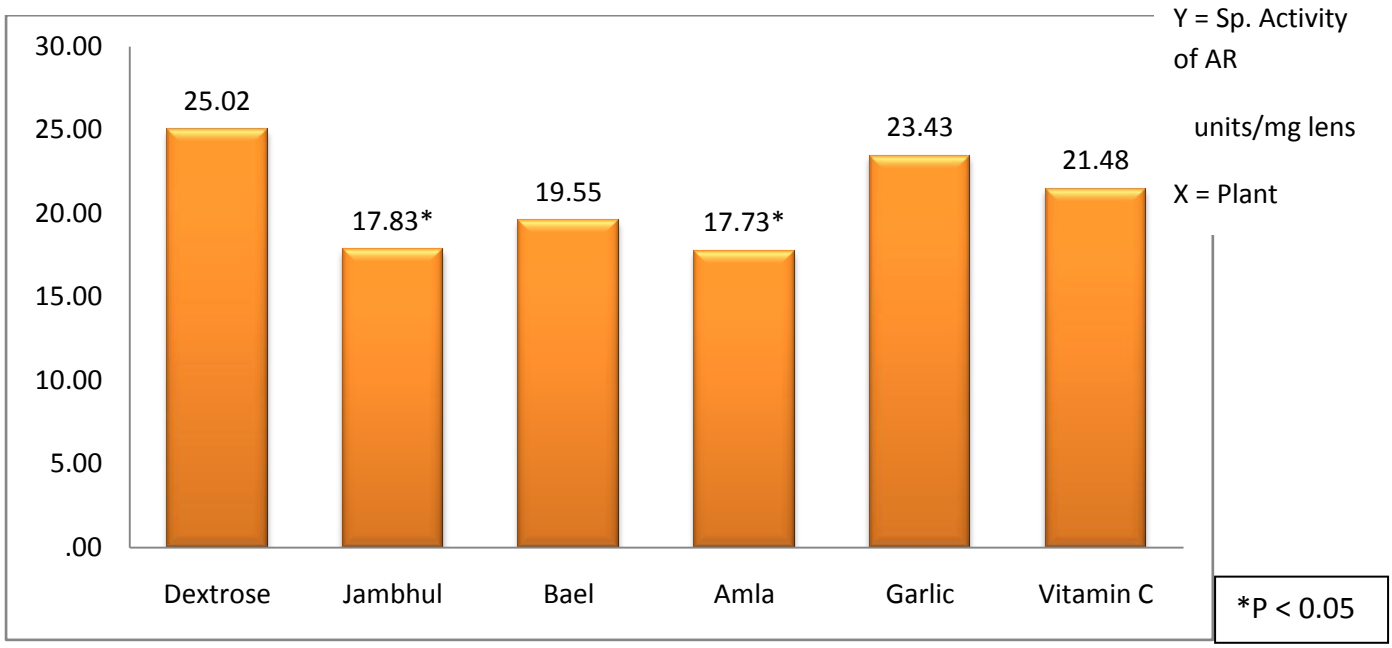
Table 8: Aldose Reductase specific activity (units/mg lens) in experimental diabetic cataract lenses incubated with S.cumini (Jambhul), A.marmelos (Bael), E.officinalis (Amla), A.sativum (Garlic) water extracts and Vitamin C respectively, compared with Dextrose group.

Group	N	Mean (units/mg lens)	SD ±
Dextrose	30	25.02	11.6
Jambhul	30	17.83	10.0
Bael	30	19.55	9.4
Amla	30	17.73	7.1
Garlic	30	23.43	8.5
Vitamin C	30	21.48	9.8
ANOVA: $F(5, 174) = 2.95, p < 0.05$			

A one-way ANOVA conducted to compare the effect of the jambhul, bael, garlic, amla aqueous extracts and vitamin C on specific activity of Aldose reductase in the presence of dextrose showed a significant effect of these medicinal plant extracts and vitamin C.

$[F(5, 174) = 2.95, p < 0.05]$.

Figure 11: **Specific activity of Aldose Reductase (GRx)(units/mg lens) in all groups**

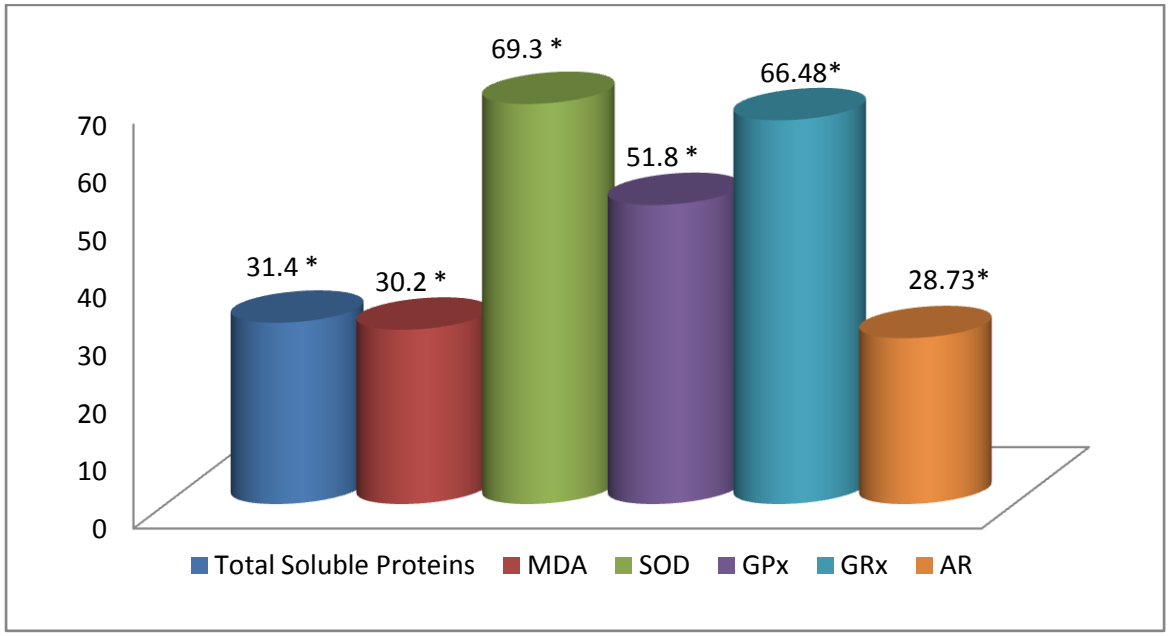


Post hoc comparisons using the Tukey HSD test indicated that the mean score for specific activity of Aldose reductase in groups with Jambhul extract (M = 17.83, SD = 10.0) and amla extract (M= 17.73, SD = 7.1) was significantly different ($p < 0.05$) than the dextrose induced cataract group (M = 25.02, SD = 11.6). The difference in the Bael extract (M = 19.55, SD = 9.4), Garlic extract (M = 23.43, SD = 8.5) and vitamin C group (M = 21.48, SD = 9.8) was not statistically significant when compared with dextrose groups.

Moreover, the difference in the mean score of aldose reductase activity when compared among the different medicinal plant extract and vitamin C groups did not show any statistical significance.

C] Percentage difference in the mean scores of Total soluble proteins, MDA and specific activities of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase was calculated between dextrose induced cataract group and each of the medicinal plant extract group and vitamin C.

Figure 12: Percentage difference caused by Jambhul extract on Total soluble proteins, MDA and specific activities of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase compared to dextrose induced cataract group

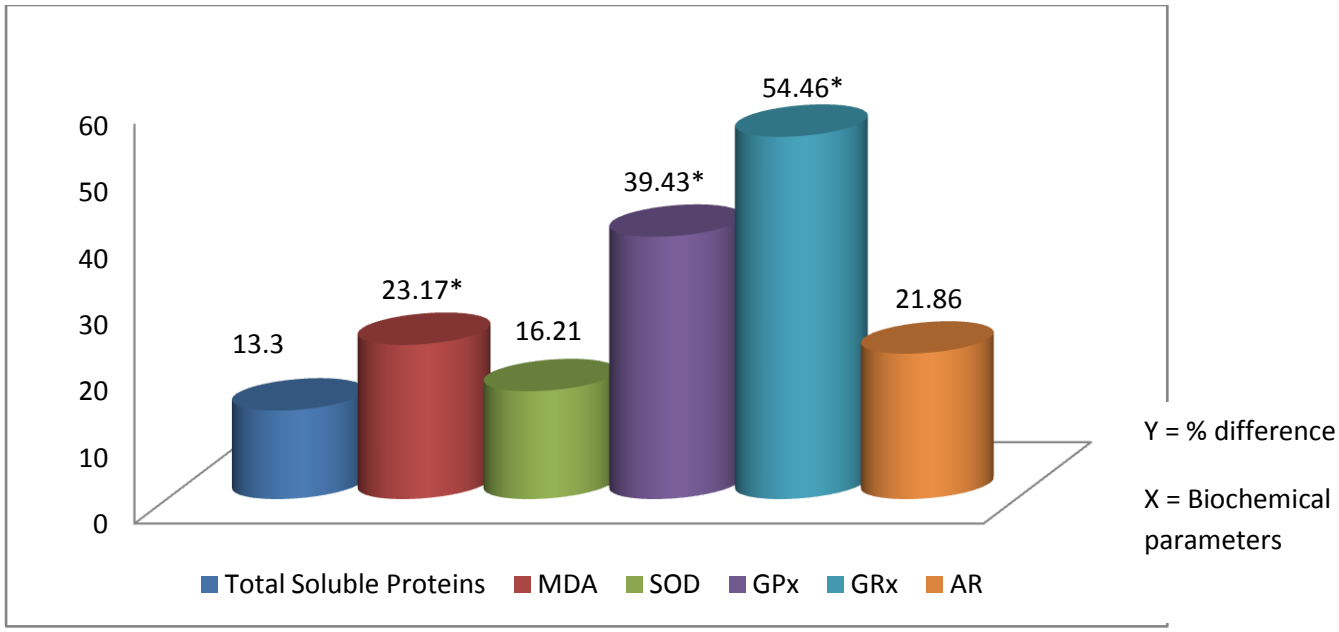


Jambhul extract was found to greatly affect all lens parameters under study. It caused an increase in Total soluble lens proteins by 31.4% and decreased the MDA levels by 30.2 %.

Maximum beneficial effect was observed on the antioxidant enzymes SOD, GPx and GRx where the activities were increased by 69.3 %, 51.8% and 66.4% respectively.

The aldose reductase activity was also lowered by 28.73 % as compared to the dextrose induced cataract group.

Figure 13: Percentage difference caused by Bael extract on Total soluble proteins, MDA and specific activities of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase compared to dextrose induced cataract group

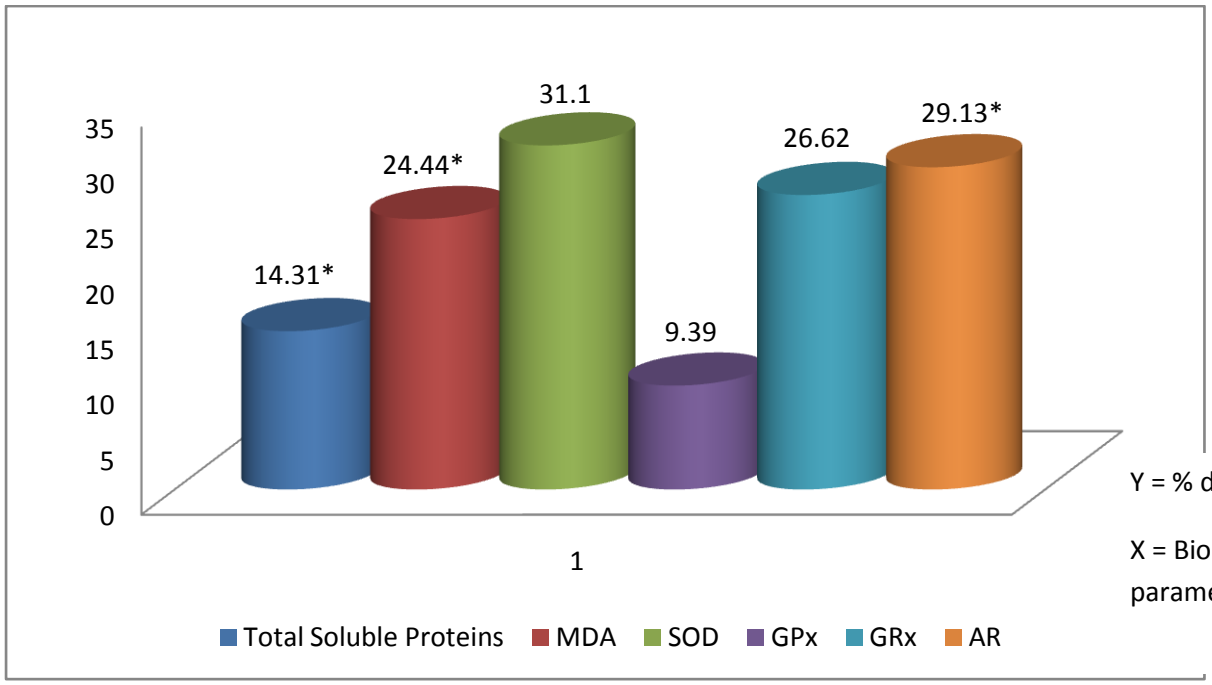


Bael extract was found to have maximum effect on the activities of enzymes GPx and GRx . It increased the activities of both enzymes by 39.43 % and 54.46 % respectively.

A 16.21 % increase in the activity of SOD was noted and total soluble proteins in lens were conserved by 13.3 %. The MDA levels were decreased by 23.17 % as compared to dextrose induced cataract lenses.

The Aldose reductase activity was decreased by 21.86 %.

Figure 14: Percentage difference caused by Amla extract on Total soluble proteins, MDA and specific activities of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase compared to dextrose induced cataract group.

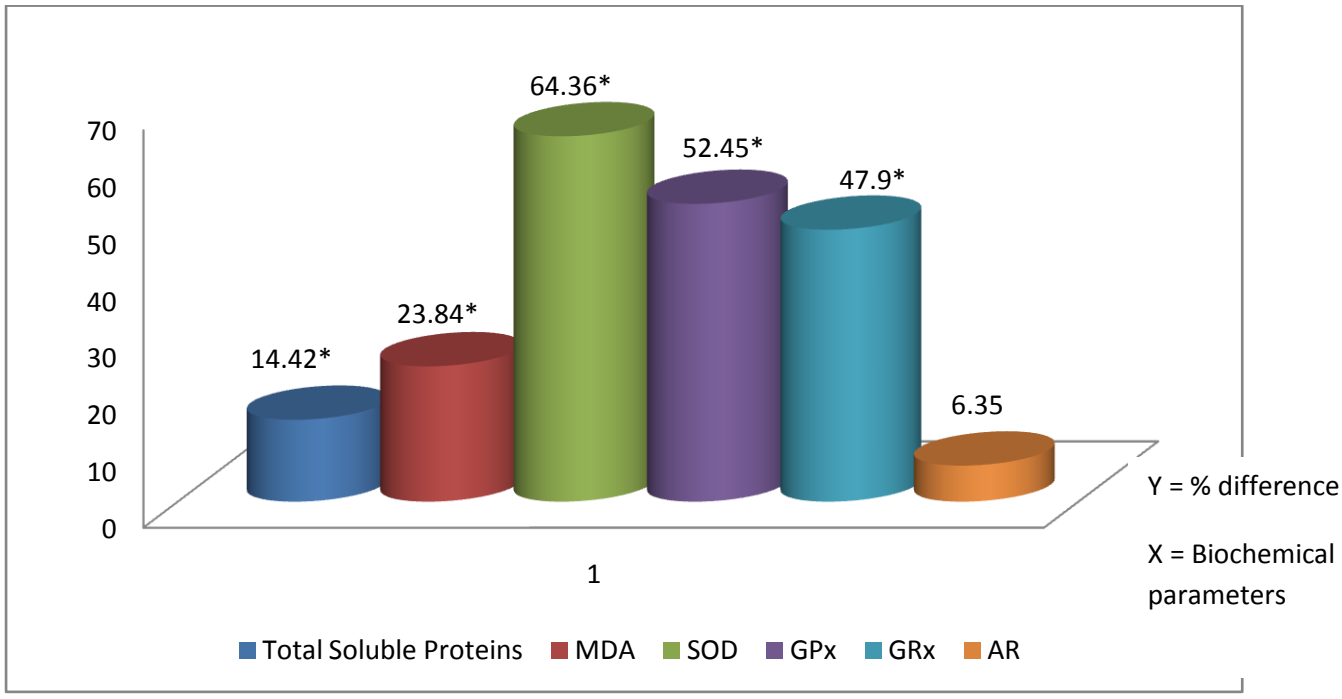


Amla extract was found to increase the total soluble proteins by 14.31 %.

The lipid peroxidation in terms of MDA was reduced by 24.44 % and the activity of antioxidant enzymes SOD and GRx were increased by 31.1 % and 26.62 % respectively. However the activity of GPx increased by only 9.39 %.

Amla extract was also found to cause a reduction in the activity of Aldose reductase by 29.13 %.

Figure 15: Percentage difference caused by Garlic extract on Total soluble proteins, MDA and specific activities of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase compared to dextrose induced cataract group.



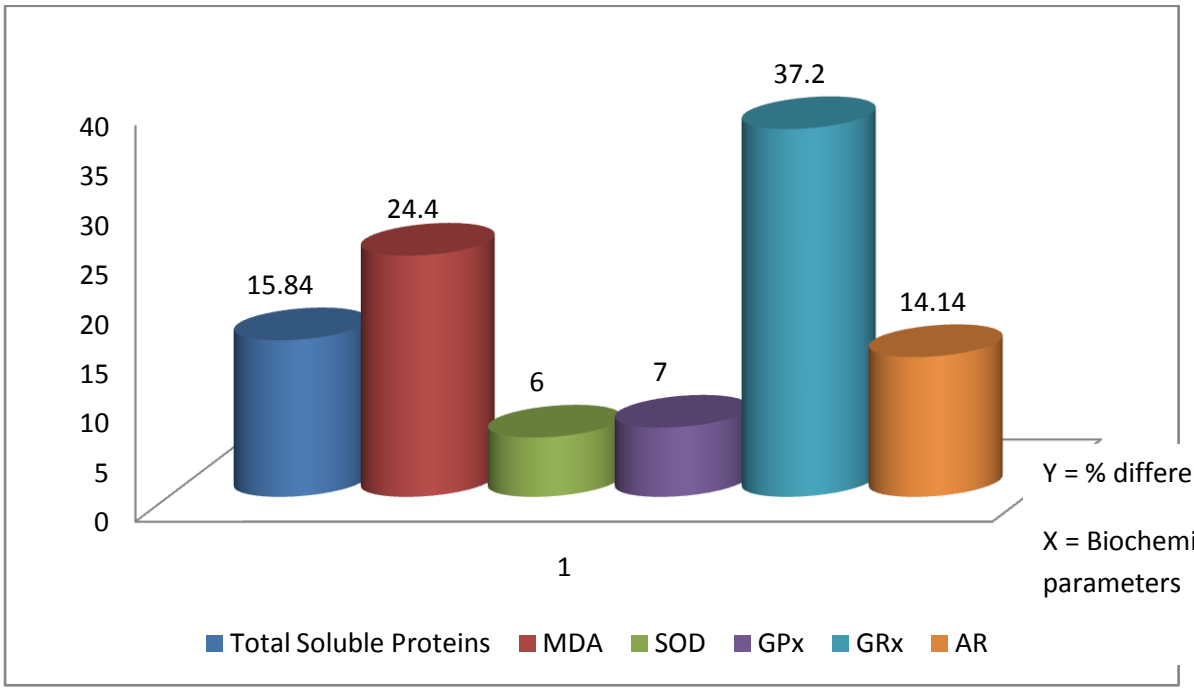
The total soluble proteins in the lenses incubated with garlic extract increased by 14.42 %.

Garlic extract showed a marked reduction in the lipid peroxidation as observed by the reduction in MDA levels by 23.84 %. The activities of all

antioxidant enzymes SOD, GPx and GRx were greatly increased by 64.3 %, 52.45 % and 47.9 % respectively.

The Aldose reductase activity was reduced by only 6.35 %.

Figure 16: Percentage difference caused by Vitamin C on Total soluble proteins, MDA and specific activities of enzymes Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Aldose reductase compared to dextrose induced cataract group.



Vitamin C caused an increase in the total lens soluble proteins by 15.84 % as compared to dextrose induced cataract lenses.

Lipid peroxidation measured in terms of MDA was lowered by 24.4 % by vitamin C; however there was very minimal effect on the activities of

antioxidant enzymes SOD and GPx which increased by only 6 % and 7% respectively. The activity of GRx was increased by 37.2 %.

Aldose reductase activity in the vitamin C group was found to decrease by 14.14%.