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REGULATION OF TYPE-1 DIABETES AND ANTIOXIDANT ENZYMES TREATMENT WITH *ALOE VERA* IN ALLOXAN INDUCED DIABETIC RAT TESTIS

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ABSTRACT

To examine the effects of *Aloe vera* treatment on the activities of antioxidant enzymes in the testis tissue of Alloxan-induced diabetic rats. *Aloe vera* leaf gel extract administered orally to different groups of rat at a dose of 300 mg/kg body weight for three weeks. Diabetes was induced intraperitoneally using 40 mg/kg body weight of Alloxan. Three months old male wistar rats were divided into four groups (n=6) namely: Normal, Normal + *Aloe vera*, diabetic, diabetic + *Aloe vera*. The experimental period was 21days. The aim of present study was evaluate the Antioxidant effects of *Aloe vera* leaf Extract in Alloxan induced diabetic rats. Antioxidants associated with a Superoxide dismutase - SOD, Catalase - CAT, Glutathione peroxidase - GPx, Glutathione Reductase - GR and Glutathione-S-transferase- GST statuses were observed in Alloxan induced diabetic rats. The diabetic rates exhibited lower activity levels of SOD, GPx, CAT, GST, and GR in testicular tissue as compared with normal rats. Whereas CAT activity was increased. The activities of SOD, GPx, CAT, GR, and GST were significantly increased in testis tissue of *Aloe vera* extract treated diabetic rats and CAT activity was decreased. As a results, *Aloe vera* leaf gel significantly reduced Alloxan induced testicular toxicity for both diabetic and normal rats.

KEYWORDS: Diabetes, *Aloe vera*, Alloxan, testis, Rat and antioxidant.

INTRODUCTION

Diabetes is a group of metabolic disorders characterized by hyperglycemia, where alterations in the carbohydrate, fat and protein metabolisms accompanied by absolute or relative

deficiencies in insulin secretion and / or its action. Moreover, basal hyperglycemia occurs irrespective of whether insulin deficiency or insulin resistance is the dominant defect.[1] It has been well known that suffering from diabetes for long time may cause many complications such as diabetic nephropathy, retinopathy neuropathy and cardiomyopathy and hyperglycemia.[2, 3]

Diabetes testicular dysfunction might be transient or permanent depending on the degree and duration of the disease. Erectile dysfunction (E.D) is a well-recognized complication of diabetes millions infertility among diabetic men is a less well-examined problem and the evaluation of the gonadal state in these cases in not clearly established. The low incidence of diabetes in infertile patients might be the reason for the limit amount of current research.[4]
Recent scientific investigations explore on traditional medicinal plays significant importance in the last few decades in the treatment of diabetes in worldwide.[5, 6] Plants often contain substantial amounts of antioxidants including Alfa-tocopherol (Vit-E), carotenoids, ascorbic acid (Vit-C), flavonoids and tannis.[7]

Aloe vera is a perennial plant belonging to the family of Liciaceae, which includes about 360 species.[8] Toxonomists, now refer to *Aloe* barbadensis as *Aloe vera*.[9] *Aloe vera* is the one of the few medical plants that has maintained its popularity for a long period of time. Clinical evaluations have revealed that the pharmacologically active ingredients are concentrated in both the gel and rind of *Aloe vera* leaves. The present investigation was undertaken to assess the effect of an aqueous extract from the *Aloe vera* on testis tissue enzymatic antioxidant in Alloxan induced diabetic rats.

MATERIALS AND METHODS

Selection of Animal

Wistar strain albino rats (180±20g) were obtained from Indian Institute of science, Bangalore. The rats were housed in clean polypropylene cages having six rats cage and maintained under temperature controlled room (26±20C) with a photo period of 12 hours light and 12 hours dark cycle. The rats were fed with a standard rat pellet diet and water adlibitum. The study was carried out according to guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupathi, India. (Regd. No.438/01a/CPCSEA, Dt: 17-07-2001, and its resolution no. 08/2012-2013/ (i)/a/ CPCSEA/IAEC/SVU/MBR-MRN/dt. 02-07-2012).

Chemicals

The entire chemical used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (ST. Louis, MO, USA), Fischer (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Induction of Diabetes

The rats were injected intraperitonial with Alloxan monohydrate (Span chemical Co.Mimbai) dissolved in sterile normal saline at a dose of 40 mg/kg body weight. After injection, they had a free access to food and water was given 5% glucose solution to drink, overnight to counter hypoglycemic shock. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day After Alloxan injection the treatment was continued for 21 days.

Preparation of *Aloe vera* extract

The fresh *Aloe vera* was locally and authenticated by botanist in the department of Botany, S.V.University, Tirupathi. *Aloe vera* solid gel in the center of the leaf was collected and homogenized resulting, mucilaginous, thick and straw colored homogenate was obtained and lyophilized. Then the lyophilized sample was extracted using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator at 60°C. The residue was stored in dry sterilized small containers at 4°C until further use. A Suspension which is the form customarily usual in folk medicine was prepared by dissolving suitable amount of ethanol free extra of *Aloe vera* leaf gel to get the desired concentration. The dosing schedule used was once per day. The extracts were administered orally, daily to different groups of rat at a dose of 300 mg/kg body weight.

Experimental design

Rats were randomly divided into four groups of six animals in each group.

Group-1: Control rats

Group-2: Control + *Aloe vera* (300mg/kg body weight of *Aloe vera*)

Group-3: Diabetic rats (40mg/kg body weight of Alloxan)

Group-4: Diabetic + *Aloe vera* extract (300mg/kg body weight in ethanol solution daily. Once in a day by an intragastic tube for three weeks)

After completion of three weeks treatment the animals were sacrificed by cervical dislocation and the testis tissue was excised at 4°C. The tissue was washed with ice-cold saline, and immediately stored in deep freeze at 80° C for further biochemical analysis.

Biochemical analysis and Enzymatic assays

Superoxide dismutase (SOD - EC: 1.15.1.6)

Superoxide dismutase activity was determined according to the method of[10] at room temperature. The Testis tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenate was centrifuged at 10,000 rpm for 10 min at 0°C in cold centrifuge. The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min 01 Hitachi U-2000 Spectrophotometer. Activity expressed as the amount µ enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit.

Catalase (CAT - EC: 1.11.1.6)

Catalase activity was measured by a slightly modified version of[11] at room temperature. The Testis tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenate was centrifuged at 10,000 rpm for 10 min at 0°C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 µl of 100% EtOH was added to 100 µ1 of tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 10 µ1 of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer and 50 µl of tissue extract was added 250 µl of 0.066 M H_2O_2 (in phosphate buffer) and decreases in optical density was measured at 240 nm for 60 s in a UV Spectrophotometer. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded / mg protein / min.

GLUTATHIONE PEROXIDASE (GPx - EC: 1.11.1.9)

Glutathione peroxidase (GPx) was determined by a modified version of[12] at 37°C. 5% (w/v) of Testis tissues of homogenate was prepared in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenate was centrifuged at 10,000 rpm for 10 min at 0°C in cold centrifuge. The resulting supernatant was used as enzyme source. The reaction mixture consist 500 µl of

phosphate buffer, 100 µl of 0.01 M GSH (reduced form), 100 µl of 1.5 mM NADPH and 100 µl of GR (0.24 units). The 100 µl of tissue extract was added to the reaction mixture and incubated at 37°C for 10 min. Then 50 µl of 12 mM t-butyl hydro peroxide was added to 450 µ1 of tissue reaction mixture and measured at 340 nm for 180s. The molar extinction coefficient of 6.22 X 10^3 M cm^{-1} was used to determine the activity. One unit of activity is equal to the mM of HADPH oxidized / mg protein / min. The enzyme activity was expressed in moles of NADPH oxidized / mg protein / min.

Glutathione Reductase (GR - EC: 1.6.4.2)

Glutathione reductase activity was determined by a slightly modified method of[13] at 37°C. The Testis tissue was homogenized (5% - w/v) in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 0°C in cold centrifuge. The separated supernatant part was used as enzyme source. NADPH (50 µl, *2* mM) in 10 mM Tris buffer (pH 7.0) was added to the cuvette containing 50 (µl of GSSG (20 mM) in phosphate buffer (0.5 M, pH 7.0 containing 0.1 mM EDTA) and 800 µl of phosphate buffer. The tissue extract (100 µl*)* was added to the NADPH - GSSG buffered solution and measured at 340 nm for 3 min. the molar extinction coefficient of 6.22 X 10 M cm^{-1} used to determine GR activity is equal to the mM of NADPH oxidized / mg protein / min. The enzyme activity was expressed in µ moles of NADPH oxidized / mg protein / min.

Glutathione - S - tranferase (GST - EC: 2.5.1.18)

Glutathione-S-tranferase activity was measured with its conventional substrate, 1-Chloro 2, 4-Dinitro Benzene (CDNB) at 340 nm as per the method of.[14] The Testis tissues was homogenized in 50 mM ice cold Tris-HCl buffer (pH 7.4) containing 0.2 M sucrose and centrifuged at 16,000 g for 45 at 4°C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a total volume of 3 ml contained 2.4 ml of 0.3 M potassium phosphate buffer (pH 6.9), 0.1 ml of 30 mM CDNB, 0.1 ml of 30mM GSH and 0.4 ml of enzyme source. The reaction was initiated by the addition of glutathione and the absorbance was read at 340 nm against the reagent blank and the activity was expressed in µ moles of thioether formed / mg protein / min.

Statistical Analysis

The data were statistically analysed using one-way analysis of variance (ANOVA) followed by Dunnets t-test and 'p' value <0.001 was considered significant. The data were presented as mean ± S.D. and analysis was carried at by using SPSS 16.01 program.

RESULTS

SUPEROXIDE DISMUTASE (SOD)

The activity of SOD in control rats was found to be testis 8.10 superoxide anion reduced mg protein/minute. In the group-II, where rats were non diabetic but treated with *Aloe vera* extract the activity was increased testis 9.01 superoxide anion reduced mg protein/minute in group-III, diabetic untreated the activity was found testis 4.13 superoxide anion reduced mg protein/minute. Group-IV has showed increased levels of activity when compared to diabetic rats.

CATALASE (CAT)

CAT activities in control rats were found to be 79.62 mg protein/minute. In the group-II, the activity was slightly increased. In group-III it was found significantly decreased to 59.02 mg protein/minute. Group-IV has showed decreased levels of activity when compared to control rats whereas they had showed increased activity when compared to group-III rats.

GLUTATHIONE PEROXIDASE (GPx)

The activity of GPx in control rats was found to be testis 9.13 mg protein/minute. In the group-II, where rats were non diabetic but treated with *Aloe vera* extract the activity was increased testis 9.45 mg protein/minute in group-III, diabetic untreated the activity was found in testis significantly decreased to 6.86 mg protein/minute. Group-IV had showed decreased levels of activity when compared to control rats.

GLUTATHIONE REDUCTASE (GR)

The activity of GR in control rats was found to be testis 1.15 mg protein/minute. In the group-II, where rats were non diabetic but treated with *Aloe vera* extract the activity was increased 5.99 mg protein/minute in testis 1.30 mg protein/minute in group-III, diabetic untreated the activity was found in testis significantly decreased to 0.800 mg protein/minute. Group-IV had showed decreased levels of activity when compared to control rats.

GLUTATHIONE-S-TRANSFERASE (GST)

The activities of GR in control rats were found to be testis 7.00 mg protein/minute. In the group-II, where rats were non diabetic but treated with *Aloe vera* extract the activity was increased testis 7.96 mg protein/minute in group-III, diabetic untreated the activity was found in testis significantly decreased 3.98 mg protein/minute. Group-IV had showed decreased levels of activity when compared to control rats.

Table: Showing Antioxidant Enzymes Activities in testis tissue of Control and Experimental rats.

Parameter	Group I(non diabetic rats)	Group II(non diabetic rats + *Aloe Vera*)	Group III(diabetic rats)	Group IV (diabetic rats + *Aloe Vera*)
SOD Superoxide anion reduced/mg protein/ minute	8.10±1.05	9.01±1.08 (+6.03)	4.13±0.62 (-46.15)	7.78±0.71 (-8.74)
CAT µ moles of H_2O_2/ mg of protein/minute	28.01±2.04	29.20±2.08 (+3.13)	18.10±1.29 (-26.07)	26.81±2.82 (-5.78)
GPx µ moles of NADPH oxidised/ mg protein/ minute	9.13±0.80	9.45±0.88 (+3.50)	6.86±0.51 (-24.86)	8.42±0.421 (-7.77)
GR µ moles of NADPH oxidised/ mg protein/ minute	1.15±0.007	1.30±0.003 (+13.04)	0.800±0.004 (-30.43)	0.980±0.006 (-14.78)
GST µ moles of thiourea/mg of protein/ hour	7.00±0.11	7.96±0.14 (+13.71)	3.98±0.15 (-43.14)	5.67±0.16 (-19.00)

Values are mean ± S.D. of 6 individual rats

VALUES in the parenthesis are % change from that of control

Values are significantly different from control at $P < 0.001$.

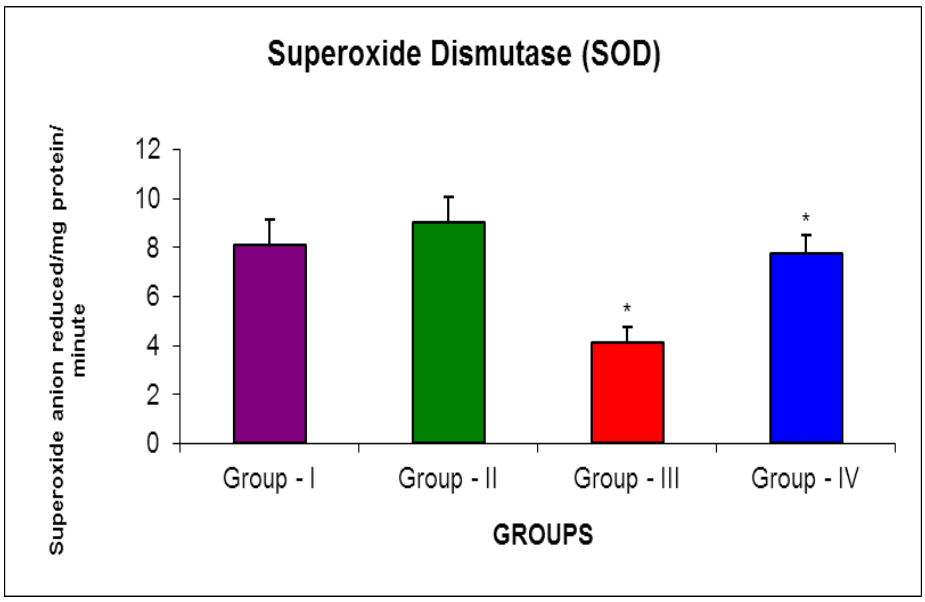

Fig: 1.1 showing SOD levels in testis tissue of control and experimental animals.

* Significant different from that of Diabetic Control animals $P < 0.001$.

Values are mean SD: n=6

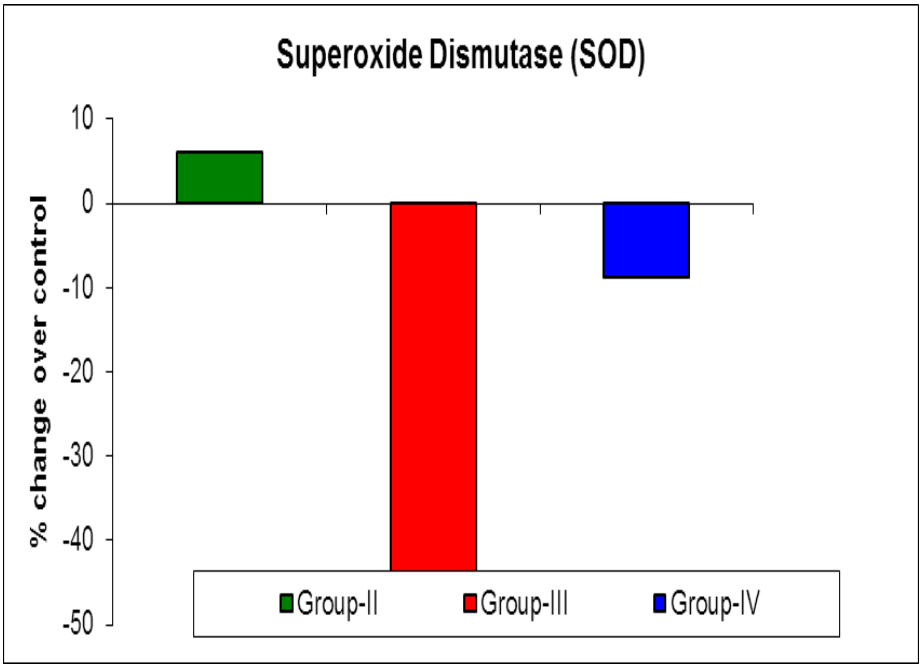

Fig: 1.2 showing % change of SOD levels in testis tissue of control and experimental animal.

Values in the parentheses are % change from Control.

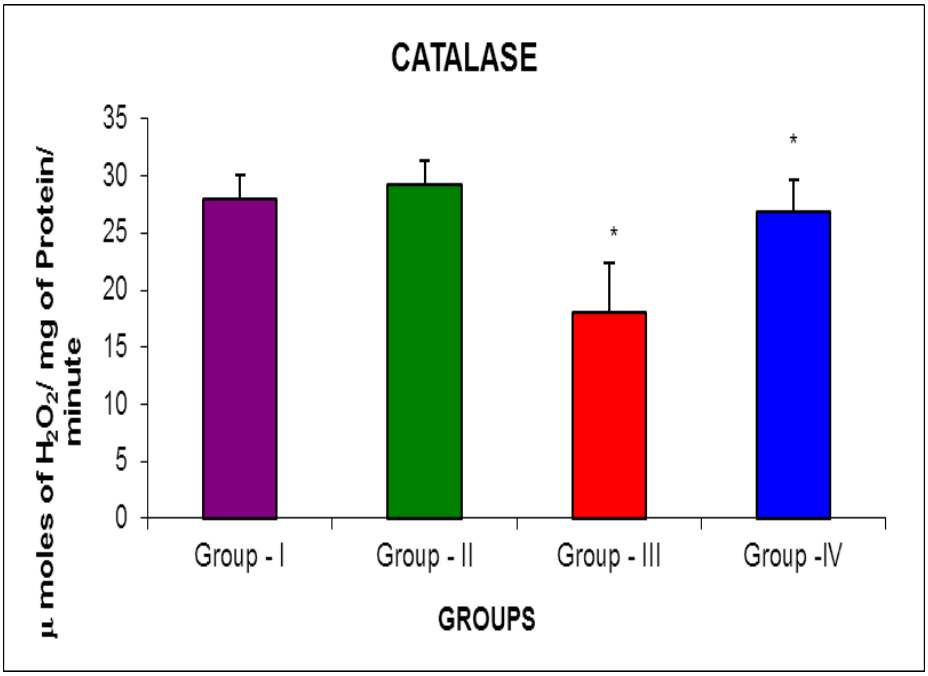

Fig: 2.1 showing Catalase levels in testis tissue of control and experimental animals

* Significant different from that of Diabetic Control animals $P < 0.001$.

Values are mean SD: n=6

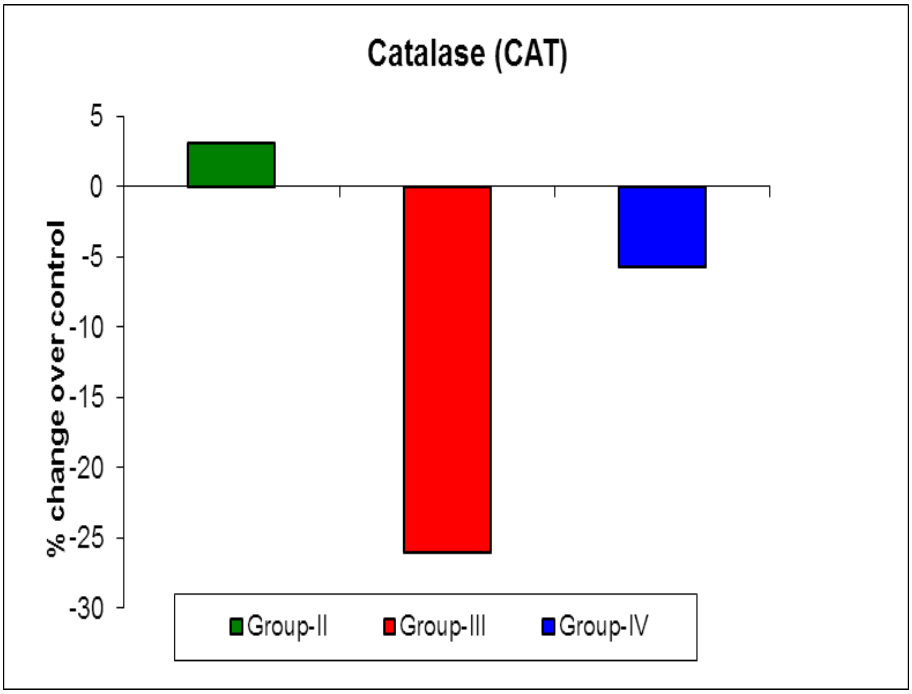

Fig: 2.2 showing % change of Catalase levels in testis tissue of control and experimental animals.

Values in the parentheses are % change from Control.

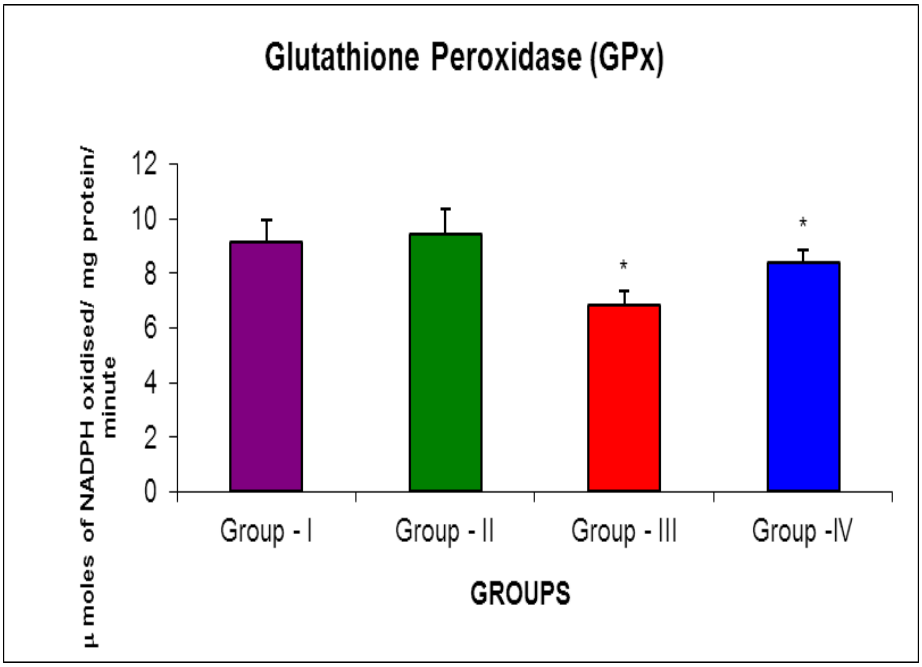

Fig: 3.1 showing GPx levels levels in testis tissue of control and experimental animals

* Significant different from that of Diabetic Control animals $P < 0.001$.

Values are mean SD: n=6.

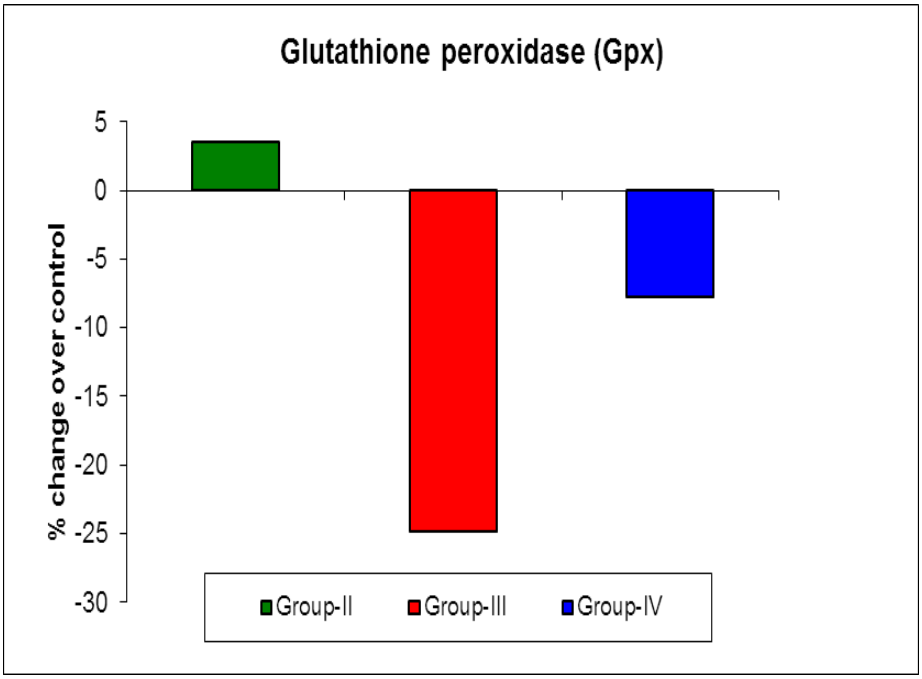

Fig: 3.2 showing % change of GPx levels in testis tissue of control and experimental animals.

Values in the parentheses are % change from Control

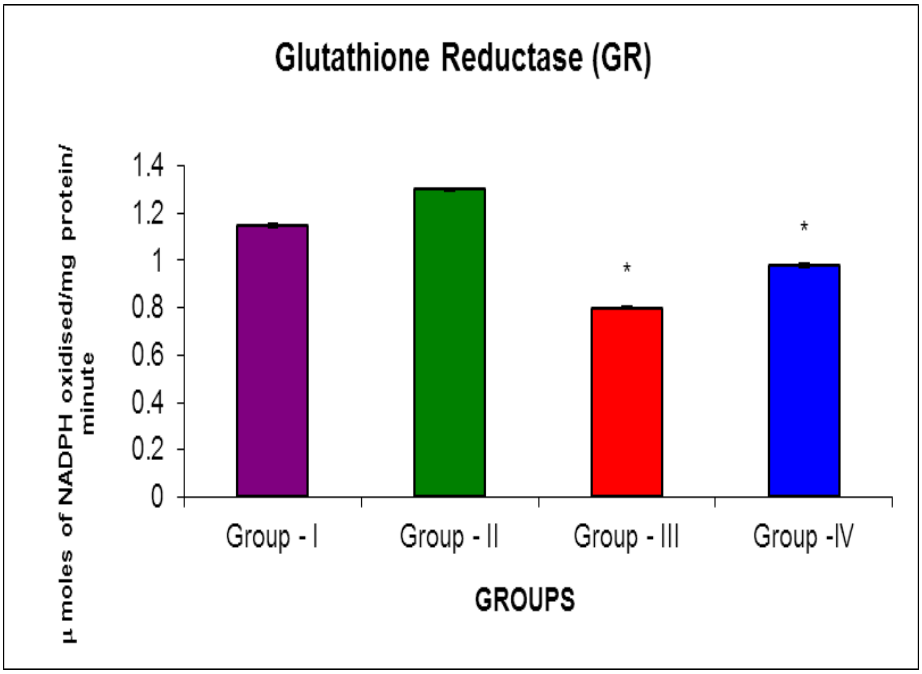

Fig: 4.1 showing GR levels in testis tissue of control and experimental animals

* Significant different from that of Diabetic Control animals $P < 0.001$.

Values are mean SD: n=6.

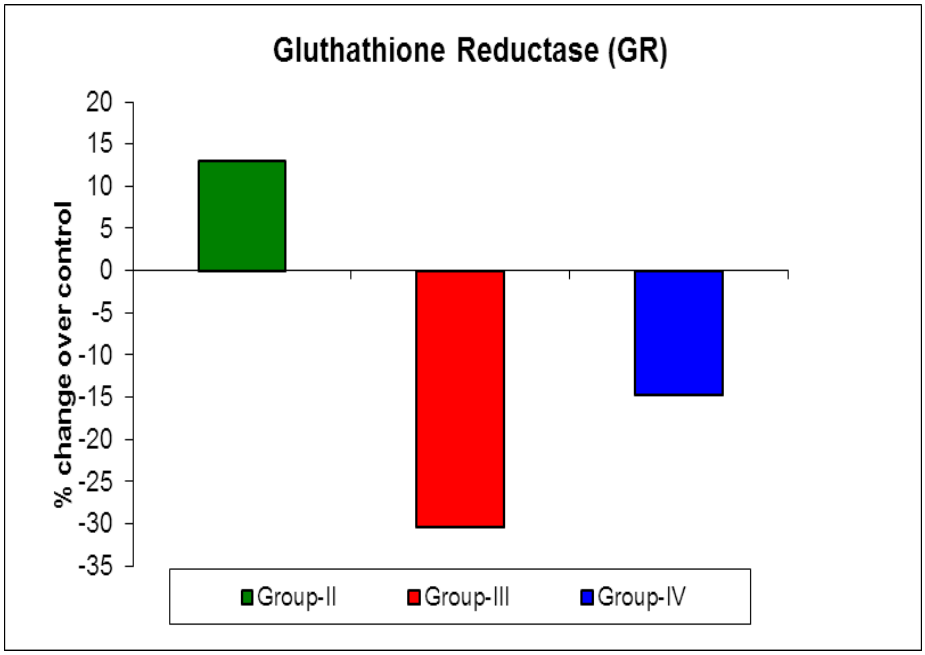

Fig: 4.2 showing % change of GR levels in testis tissue of control and experimental animals.

Values in the parentheses are % change from Control.

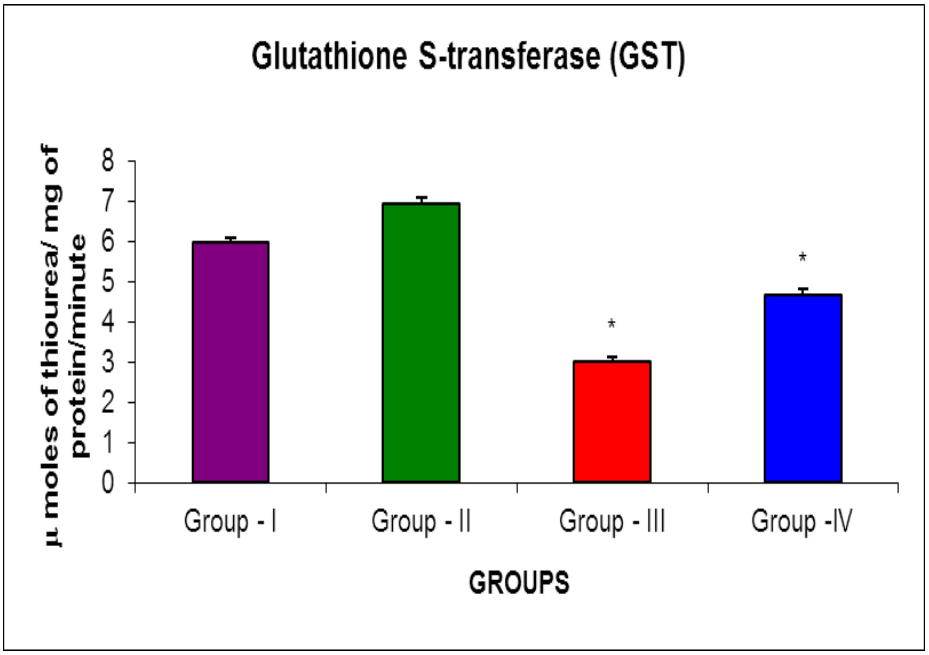

Fig: 5.1 showing GST levels in testis tissue of control and experimental animals

* Significant different from that of Diabetic Control animals $P < 0.001$.

Values are mean SD: n=6

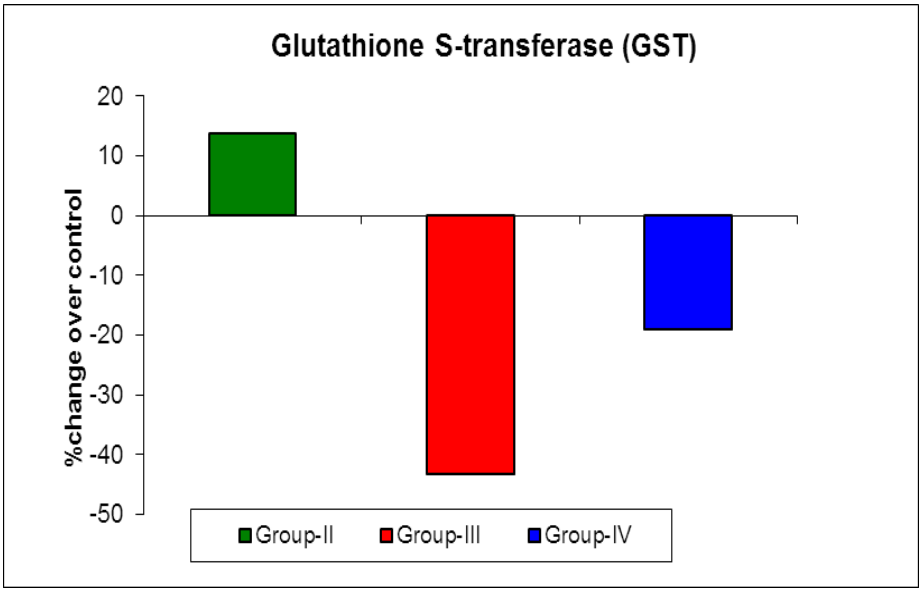

Fig: 5.2 showing % change of GST levels in testis tissue of control and experimental animals.

Values in the parentheses are % change from Control.

DISCUSSION

In the normal conditions its activity would be proportional to the amount of superoxide radicals produced.The increase in its activity in group-II, where rats were non-diabetic and treated with *Aloe vera* extract was due to the neutralization of reduction potential developed by the over produced super oxide radicals. In case of group-III the significant decrease in its activity was due to the Alloxan treatment. Alloxan treatment induces hyperglycemia through the generation of free radicals in high concentration. This free radical stress gets amplified gradually and is propagated by an autocatalytic cycle of metabolic stress, testis tissue damage and cell death, leading to further increase in free radical production and depletion of antioxidants[15] including SOD.

The present study revealed the decreased activity of SOD in diabetic rats, as reported previously, which could be due to increased consumption for free radicals' detoxification. Treatment with *Aloe vera* extract has increased the activities of SOD, which could be a result of decreased lipid peroxidation and/or decreased utilization. Super oxide Dismutases play an essential role as a defense system against oxidative stress. SOD activity has been measured in tissues of animals with chemically induced diabetes. The loss of SOD activity may be a function of the length[16] and severity of diabetes.[17, 18]

CAT is known to be involved in detoxification of high H_2O_2 concentrations. CAT has been regarded as a major determinant of testicular antioxidant status and catalyzes the reduction of hydrogen peroxides and protects the tissue from highly reactive hydroxyl radicals. Catalase performs a vital task. In addition to its enzymatic reactions, catalase binds four molecules of NADPH, which serve to prevent and reverse enzyme inactivation.[20]

Under the normal conditions its activity would be proportional to[19] the amount of hydrogen peroxide produced. The increase in its activity in group-II where rats were non-diabetic and treated with *Aloe vera* extract was due to the antioxidant scavenging activity of the plant extracts. In case of group-III the significant decrease in its activity was due to the Alloxan treatment. Decrease in CAT activity could result from inactivation by super oxide radical and glycation of the enzymes, Alloxan treatment induces hyperglycemia through the generation of free radicals in high concentration. This free radical stress gets amplified gradually and is propagated by an autocatalytic cycle of metabolic stress, tissue damage and cell death, leading to further increase in free radical production and depletion of antioxidants.[15] When Alloxan subjected rats were treated with *Aloe vera* extract the activity is increased or otherwise stress is relieved because of the neutralization of free radicals and increased catalase activity.

GPX is a cytosolic enzyme that is complementary to CAT to detoxify H_2O_2 and organic hydro peroxides.[21] Glutathione peroxides are a class of seleno proteins that use two molecules of GSH to facilitate the reduction of oxidants.In addition, our results showed that there was a generalized decrease in testis GPX activity in both the control and diabetic animals. Such alterations in GPX activities in testis of the animals as they age warrant further investigations as they indicate important changes in the body antioxidant defense system during aging. When Alloxan subjected rats were treated with *Aloe vera* as in the case of group-IV respectively, the activity was increased or otherwise stress was relieved because of the free radical neutralization by antioxidants present in plant extracts.

Reduced Glutathione, a non-enzymatic antioxidant is known to protection against reactive O_2 species by effectively scavenging free radicals and other ROS directly and indirectly through enzymatic reactions. GSH reductase uses NADPH to reduce oxidized GSH formed by the action of GSH peroxidase. In case of group-III the significant decrease in its activity was due the Alloxan treatment.[22] has reported that GR activity decreased in testis.

GST catalyzes the conjugation of GSH with a wide variety of organic compounds, including certain species of hydro peroxides thereby shares peroxidase activity with GSH-GPx.[14] In control rats its activity would be proportional to the concentration of reactive oxygen spices (ROS) produced as the ratio of GSH to GSSG is maintained high. The increase in its activity in group-II, where non-diabetic rats were treated with *Aloe vera* was due to the antioxidant scavenging activity of the plant extract. GST increased activity clearly indicate that enhanced oxidative stress present in the renal context at a very stage of diabetes. In case of group-III the significant decrease in its activity was due the Alloxan treatment. Alloxan treatment induces generation of free radicals in high concentration leading to a drop GSH to GSSG ratio. This drop inhibits the activity of GST. Hence, the free radical stress gets amplified gradually and is propagated by an autocatalytic cycle of metabolic stress, tissue damage and cell death, leading to further increase in free radical production and depletion of antioxidants.[14]

Glutathione-S-transferase, found in many tissues such as liver, lungs, erythrocytes and testis, performs many functions with a broad specificity for organic hydro peroxides but not for H_2O_2.[23] The cytosolic GST catalyzes the conjugation reaction of GSH and electrophillic substances and therefore, has an integral role in the detoxification of electrophillic toxicants.[24] Earlier reports indicated decreased activity of GST in liver and testis of experimentally induced diabetic rats[25], In the present study such decrease was observed in liver and erythrocytes which may be due to decreased levels of GSH in Alloxan-diabetic rats.[26] *Aloe vera* extract to Alloxan-diabetic rats enhanced the activities of both G-6-PDH and GST. The overall results related to antioxidant — detoxification system which is impaired in diabetes appeared to be restored to a major extent by *Aloe vera* extract in diabetic rats.

CONCLUSION

The effect of the ethanol extract of *Aloevera* on tistis tissue antioxidants due to reduction enzyme in activities diabetic rats. Further studies are in progress to identify the active components in *Aloe vera* and thick role in controlling diabetes.

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