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ETHANOLIC EXTRACT OF *ALOE VERA* TESTIS TISSUE IMPROVES LIPID METABOLISM PROFILES IN ALLOXAN INDUCED DIABETIC ALBINO MALE RATS

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ABSTRACT

The present investigate aims to examine the influence of *Aloe vera* in testis of Alloxan induced diabetic rats. Diabetes was induced by a single injection of Alloxan (40 mg/kg body weight). *Aloe vera* (300 mg/kg body weight) was treated to normal and diabetic rats for three weeks. Three month old male wistar were divided into 4 groups (n=6) namely: normal rats, normal + *Aloe vera*, diabetic, diabetic + *Aloe vera*. The present study, the protective use of *Aloe vera* on cholesterol, lipid peroxidation, Triglycerides levels in diabetic male albino-rats. The levels of cholesterol, lipid peroxidation, triglycerides in testis were increased significantly in diabetic rats. Oral administration of the *Aloe vera* leaf extract reduced significantly the levels of lipid metablolic profiles (cholesterol, lipid peroxidation, triglycerides). The Result of the study indicates that *Aloe vera* extract was effective in improving testis damage for diabetic rats. In conclusion, the *Aloe vera* extract played a key role in reduction of diabetic patients.

KEYWORDS: Diabetes, *Aloe vera*, Alloxan, Lipid profiles, testis, male rats.

INTRODUCTION

Diabetes mellitus, a life threatening as well as life style modifying metabolic disorder is characterized by hyperglycemia. Complications of diabetes mellitus include polyuria, polydipsia, weight loss, polyphagia and blurred vision.[1] Diabetes mellitus, a metabolic disorder, is characterized by fasting hyperglycemia, deficient insulin secretion or insulin receptor insensitivity.[2] It is known that diabetic mellitus affects more than 100 million

people worldwide and is considered as one of the five leading causes of death in the world.[3] Male reproductive alterations have been widely reported in diabetic induced animal models.

Diabetic testicular dysfunction might to transient or permanent depending on the degree and duration of the disease. Erectile dysfunction is a well-recognised complication of diabetes mellitus. Infertility among diabetic men is a less well-examined problem and the evaluation of the gonadal state in these cases is not clearly established: the low incidence of diabetes in infertile patients might be the reason for the limited amount of current research.[5] Some chemical drugs such as biguanides and sulfonylureas are currently available to reduce hyperglycemia in diabetes mellitus.[6] These drugs have side effect and thus search for new drug / compound is essential.[7, 8] Many herbs and plant products have been shown to have hypoglycemic action. This leads to increasing demand for herbal products plant with antidiabetic activity and lower side effects.[9, 10] Polysaccharide containing plants which *Aloe* barbadensis is also among are used in various diseases as anti-imflammatory, antiulcer, antieoplastic and in wound healing and against hepatitis.[11] In some studies it is shown that *Aloe vera* has an antioxidative effect. Its antigen toxic and chemo preventive effectives are also proven.[12, 13] The aim of present study was evaluation of protect effect of *Aloe vera* gel on testis damage of diabetic rats.

MATERIALS AND METHODS

Selection of Animals

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

Triglycerides (TG - Triacylglycerol)

Triglycerides were estimated by the method of[14] with slight modifications as given below. Triglycerides were assayed by hydrolyzing them to glycerol and the liberated glycerol was determined.

Tissue homogenates were prepared in $1NH_2SO_4$ and to it 4 ml of chloroform was added 0.5 ml of tissue homogenate was taken. To it 0.5 ml of $1NH_2SO_4$ and 4 ml of chloroform were added. The contents were centrifuged at 1000 rpm for 15 min 0.5 ml of chloroform layer was taken and to it 0.4 ml of methanol and 0.1 ml of alkaline barium solution were added and the contents were heated for 30 min at 80°C, the total volume was made up to 1 ml with $2NH_2SO_4$ and centrifuged for 10 min at 1000 rpm 0.5 ml of this supernatant was taken and to it 0.1 ml of sodium periodate was added and shaken well for 1 min, 0.1 ml of sodium arsenate and 5 ml of chromotrophic acid reagent was added and heated for 30 min and cooled. The samples were read at 575 nm in Spectrophotometer against the reagent blank. The results were finally expressed in mg of triglycerides / gram wet weight of the tissue.

MDA content [Lipid Peroxidation (LP)]

This assay is used to determine MDA levels as described by (15). 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 for the estimation. 200 µl of the tissue extract was added to 50 µl of 8.1% sodium dodecyl sulphate (SDS), vortexed and incubated for 10 min at room temperature. 375 µl of 20% acetic acid (pH 3.5) and 375 µl of thiobarbituric acid (0.6%) were added and placed in a boiling water bath for 60 min. the samples were allowed cool at room temperature. A mixture of 1.25 ml of butanol: phyridine (15:1) was added, vortexed and centrifuged at 1000 rpm for 5 min. The colored layer (500 µl) was measured at 532 nm using 1, 1, 3, 3-tetraethoxypropane as a standard. The values were expressed in µ moles of malondialdehyde formed / gram wet weight of the tissue.

Total Cholesterol

The total cholesterol content was estimated using Liebermann Burchard reaction as described by (14). Testis tissue was homogenized in isopropanol. The contents were centrifuged at l000 rpm for 15 min. 0.5 ml of supernatant was taken and to it 4 ml of cholesterol reagent was added. Then the contents were heated at 90°C for 15 min. After cooling, the samples were read at 560nm in

spectrophotometer against the reagent blank. The results were finally expressed in mg of total cholesterol/gram wet weight of the tissue.

Statistical analysis

The data has been analyzed by using one-way Analysis of Variance (ANOVA) followed by Dunnet's-test and 'P' value < 0.001 was considered significant. The data were presented as Mean ± S.D. And analysis was carried out by using SPSS 16.0.1 program

RESULTS

LIPID PEROXIDATION

In control rats the amount of lipid peroxidation was found to 33.21 µ moles of malondialdehyde formed / gm wet weight tissue in testis. In group-II, the levels were decreased. In case of group-III the levels were increased, in group- IV where the diabetic rats were subjected to *Aloe vera* extract, decreased levels were found when compared to control rats.

TRIGLYCERIDES

In control rats the amount triglycerides was found to be testis 1.609 mg of triglycerides/g wet weight of tissue. In group-II, where the control rats were treated with *Aloe vera* plant extract the levels were decreased. Group-III had showed a significantly increased to testis 4.01mg of triglycerides/g wet weight of tissue. In the group-IV where the diabetic rats were subjected to *Aloe vera* extract, decreased levels were found when compared to control rats.

TOTAL CHOLESTEROL

In control rats the amount triglycerides was found to testis 50.12 mg of cholesterol /gm wet weight of tissue. In the group-II, where the control rats were treated with *Aloe vera* extract the levels were in decreased. Group-III had showed a significantly increased to 82.10 mg of cholesterol /g wet weight of tissue in testis 69.12 mg of cholesterol /gm wet weight of tissue. In the group-IV where the diabetic rats were subjected to *Aloe vera* extract, decreased levels were found when compared to control rats.

Table: showing Lipid peroxidation levels in testis of Control and Experimental animals.

Parameter	Group I (non diabetic rats)	Group II (non diabetic rats + *Aloe Vera*)	Group III(diabetic rats)	Group IV (diabetic rats + *Aloe Vera*)
Cholesterol µ moles of total cholestrol/gram wet weight of tissue	50.12±4.12	52. 30±8.20 (+-4.39)	69.12±7.18 (+24.92)	48.99±5.24 (-11.56)
Lipid peroxidation µ moles of lipid peroxidation formed/ gm wet weight of tissue	33.21±9.423	35.08±8.231 (+7.046)	50.02±8.79 (+51.97)	32.02±3.47 (-17.61)
Triglycerides mg of triglycerides/gm wet weight of tissue	1.619±0.161	1.519±0.154 (-36.80)	4.02±0.420 (+106.09)	1.20±0.329 (-36.90)

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

Values in the parenthesis are % change from that of control

Values are significantly difference from control at P < 0.001

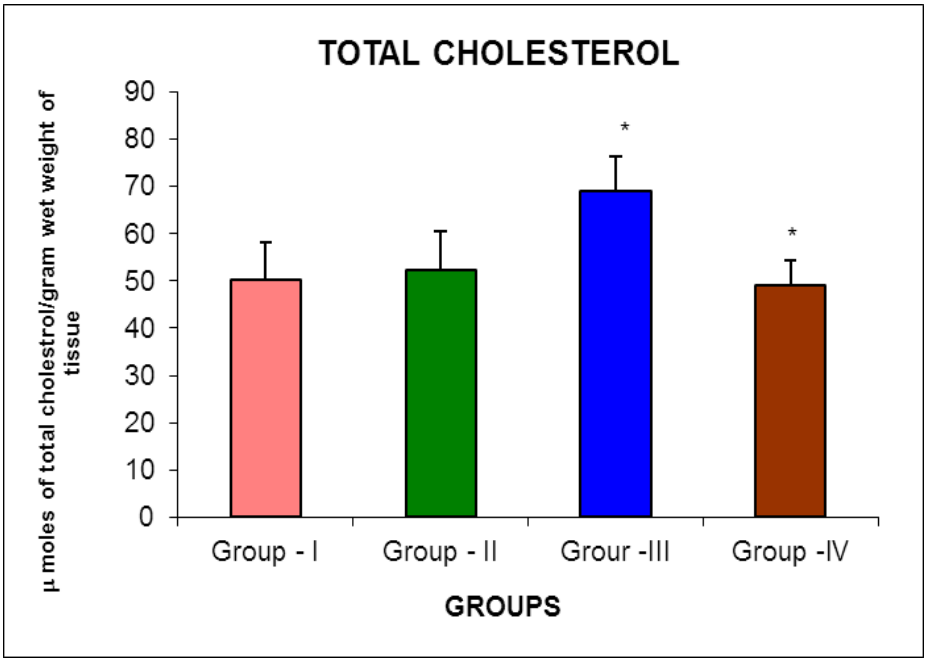

Fig: 1.1 Showing Total Cholesterol levels in testis tissue of control and experimental animals.

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

Values are mean, SD: n=6

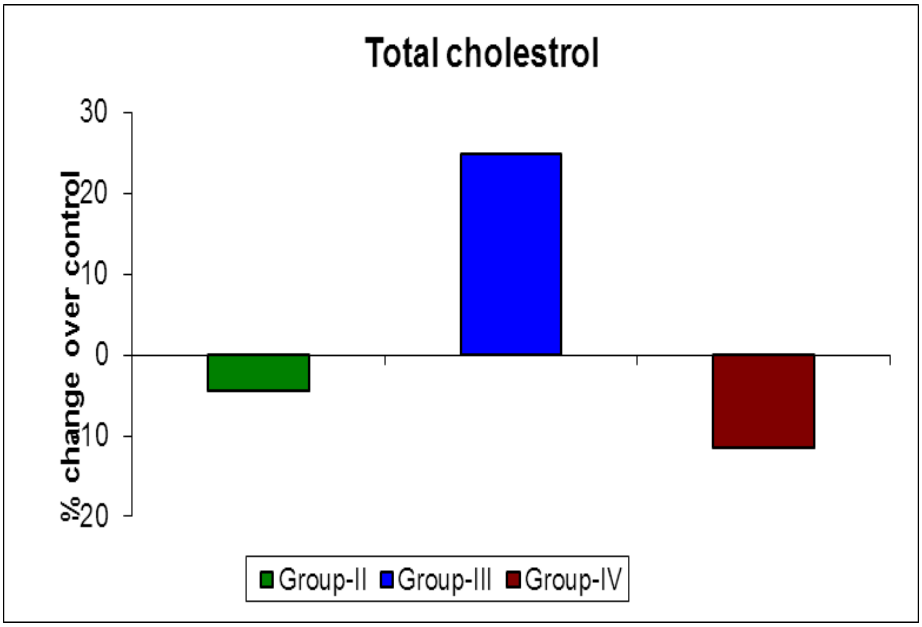

Fig: 1.2 showing % change of Total Cholesterol levels in testis tissue of control and experimental animals.

Values in the parentheses are % change from Control.

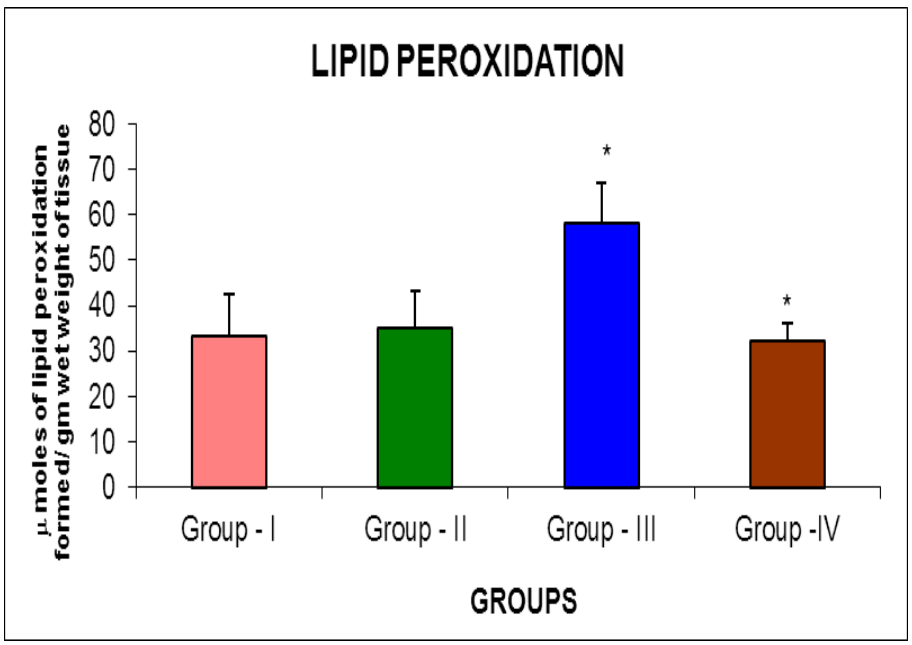

Fig: 2.1 showing Lipid peroxidation levels in testis tissue of control and experimental animals.

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

Values are mean, SD: n=6

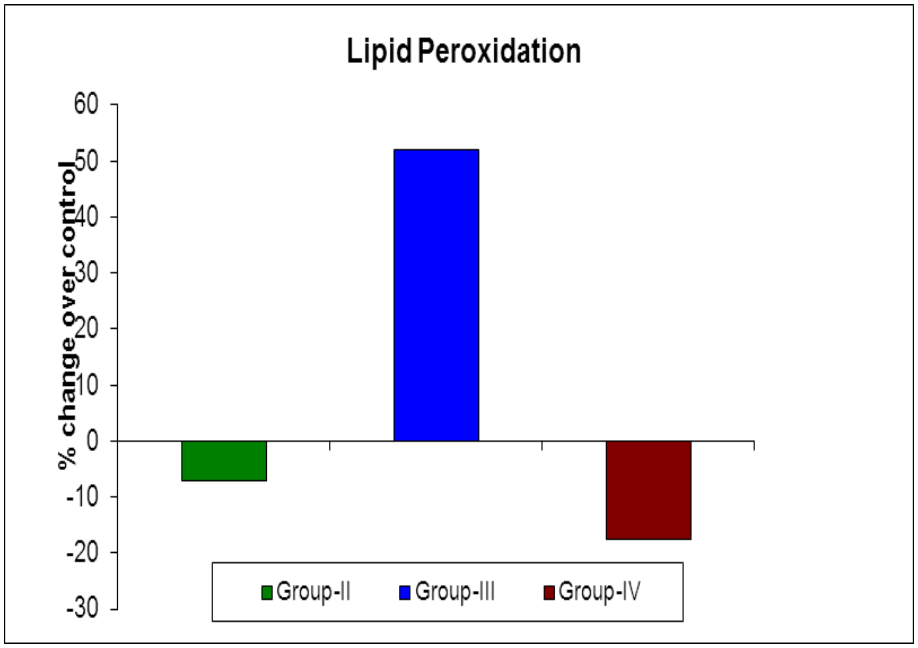

Fig: 2.2 showing % change of Lipid peroxidation levels in testis tissue of control and experimental animals

Values in the parentheses are % change from Control.

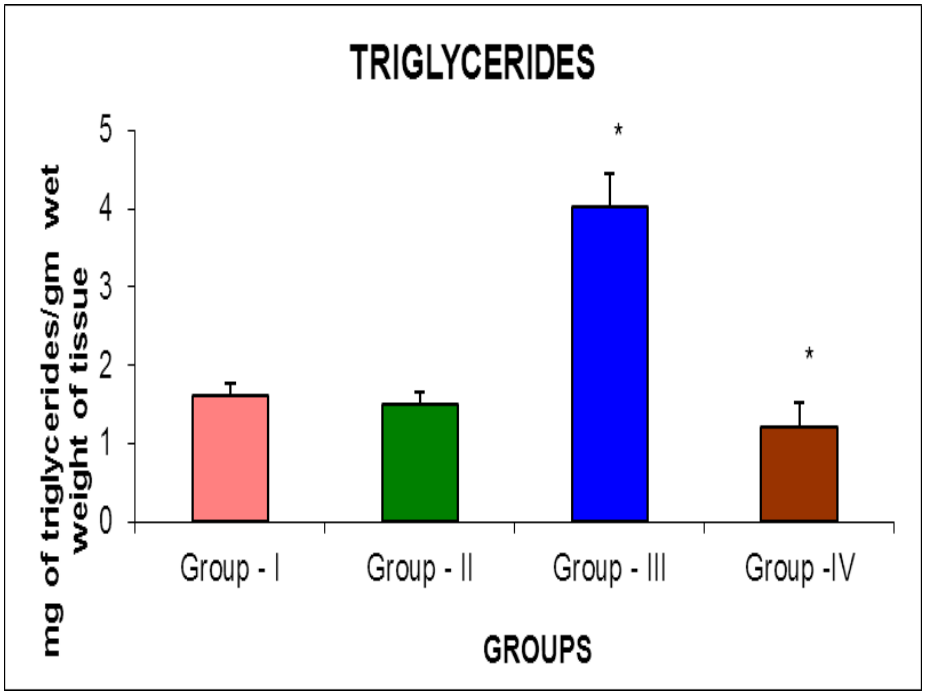

Fig: 3.1 Showing Triglycerides levels in testis tissue of control and experimental animals

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

Values are mean, SD: n=6

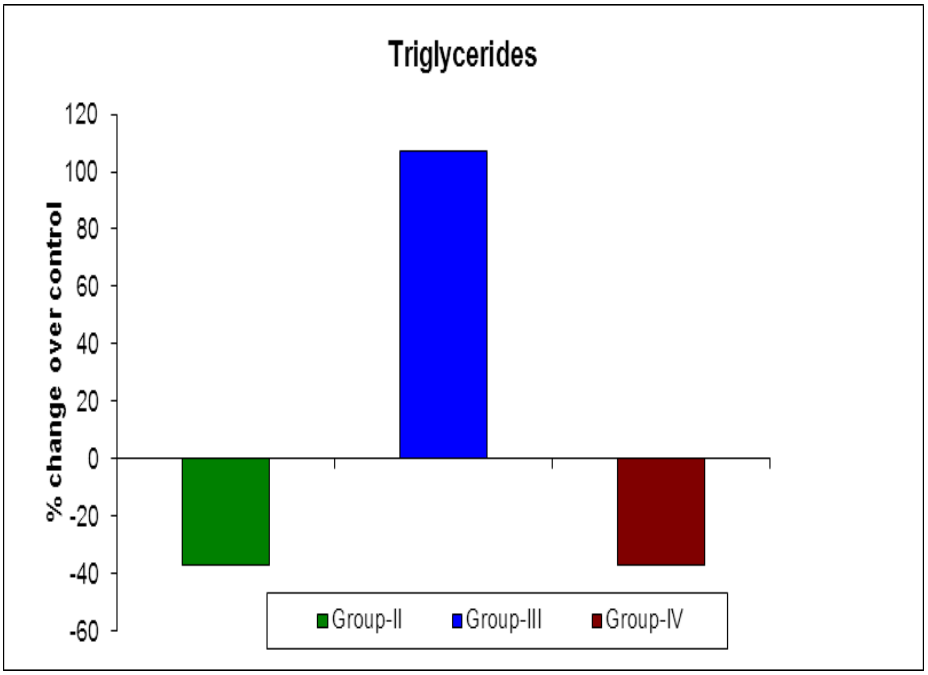

Fig: 3.2 showing % change of Triglycerides levels in testis tissue of control and experimental animals.

Values in the parentheses are % change from Control.

DISCUSSION

The present investigation demonstrated the protective potentials of *Aloe vera* against hyperglycemia mediated oxidative stress in Alloxan induced diabetic testicular damage. Diabetes mellitus is a chronic disease affecting many tissues and systems of the body. Oral administration of *Aloe vera*, daily to male rats for three weeks of duration, suppressed the activities of antioxidant enzymes in testis.

Cholesterol is an amphipatic lipid present in testis tissue and in plasma lipo proteins either as free cholesterol with a long chain fatty acid or cholesterol ester. It is synthesized in many tissues from acetyl co-A.[16] It forms a precursor for all steroids in the body such as corticosteroids, sex-hormones, bile acids and Vitamin-D.[17]

In normal rats cholesterol levels were well regulated as the insulin production is normal Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is potent inhibitor of lipolysis since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids.[18]

In *Aloe vera* extract treated rats the total cholesterol content in testis was decreased. This was due to the hypocholestemic effects of plant extract by the inhibition of cellular cholesterol synthesis.

Alloxan induced diabetic rats had showed increased total cholesterol levels. There were many reports on elevated levels of total cholesterol content in diabetic rats. During diabetes enhanced activity of lipase enzymes increases lipolysis and release more free acids in to the circulation.[19]

In diabetes, it is thought that hypo insulinemia increases the activity of the enzymes such as fatty acylcoenzyme-A oxidase, which initiates beta-oxidation of fatty acids, resulting in LPO.[20] In the present results the formation of TBARS, a product of lipid peroxidation reaction, was significantly increased in diabetic tissue as reported earlier.[21,22]

The increased testis MDA content of diabetic rats suggests that peroxidative injury may be involved in development of disease. In the present study triglycerides content was increased in testis tissue of diabetic rats. Several studies demonstrated that beneficial effects of exercise in a reducing and or minimizing these risk factors of many diseases.The increase in serum Triglycerides in diabetic controls is in conformation with previous reports documentary elevated serum triglycerides and lipid peroxide levels in diabetic subjects.[23]

The increase in testis cholesterol in diabetic rats observed in the present study could be due in testis triglycerides in diabetic rats after treatment with *Aloe vera* extract. This reduction may be attributed to increased clearance and decrease production of the major transporters of endogenously synthesized and triglycerides.

CONCLUSION

The results of the present study showed that *Aloe vera* gel extract possess potent antioxidant activity, which may be directly or indirectly responsible for its hypoglycemic property. Further studies are in progress to identify the active components in *Aloe vera* their role in controlling diabetes.

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