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STUDY OF HISTOPATHOLOGICAL CHANGES AND CARBOHYDRATE METABOLIC PROFILES IN DIABETIC TESTIS TISSUE TREATED WITH *ALOE VERA* LEAF GEL EXTRACT

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ABSTRACT

Several herbal preparations are used to treat diabetes, but their reported hypoglycemic effects are complex. In this study, histopathological changes and the carbohydrate profiles in Alloxan-induced diabetes in rats treated with the leaf gel of *Aloe vera* ethanol extract on was evaluated. Twenty four male rats were used in the study by allocating them into four groups, each of six rats. Control rats, control rats + *Aloe vera*, diabetic rats, diabetic + *Aloe vera*. Diabetes was induced intraperitoneally using 40 mg/kg body weight Alloxan. *Aloe vera* leaf gel extract administered orally to different groups of rat at a dose of 300 mg mg/kg body weight. The experimental period was 21days. The parameters studied are total carbohydrates, glycogen and glucose and histopathological changes of testis were investigated compared to the control group. These metabolic profiles were decreased in diabetic rats, expect glucose. Whereas, with *Aloe vera* extract treatment in diabetic rats these carbohydrate metabolic profiles were increased and glucose decreased. The observed reductions in carbohydrate metabolic profiles during diabetic condition in testis tissue may be due to the alterations in the carbohydrate metabolism. *Aloe vera* is capable of producing free radicles, which in turn cause damage to the cellular compartment system of rat.

KEYWORDS: Diabetes, *Aloe vera*, Alloxan, testis, carbohydrate metabolic profiles and histopathology.

INTRODUCTION

Diabetes is a complex disease where the carbohydrate and fat metabolism are impaired.[1] According to world health organization, diabetes mellitus (DM) is one of the most common metabolic disorders all over the world.[2,3] It has been well known that suffering from diabetes

for long time many causes many complications such as diabetic nephropathy, retinopathy, neuropathy, cardiomyopathy and hyper glycemia.[4,5]

Recent decades have shown a resurgent interest in traditional plant treatments for diabetes. Plants often contain substantial amounts of antioxidants including α-tocopherol (Vitamin-E), carotenoids, ascorbic acid (Vitamin-C), flavonoids and tannins.[5] *Aloe vera* is a perennial plant belonging to the family of liliaceae, which includes about 360 species.[6] Toxonomists now refer to *Aloe* barbadensis as *Aloe vera*.[7] *Aloe vera* is a one of the few medicinal plants that has maintained its popularity for a long period of time. The plant has stiff gray-green lance-shaped leaves containing clear gel in central mucil aginous pulp. Clinical evaluations have releaved that the pharmacologically active ingredients are concentrated in both the gel and rind of *Aloe vera* leaves. Our previous experimental results were lightly encouraging as they revealed that level of blood glucose was significantly lower after oral administration of ethanolic extract of *Aloe vera* gel in glucose load condition and in Alloxan induced diabetes.[8] Hence the present study was carried out; the purpose of this investigation was to evaluate the effect of *Aloe vera* extract on Alloxan induces diabetes by measuring blood glucose levels and assaying the carbohydrates metabolic and profiles in testis.

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 21 days)

After completion of 21 days 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.

Estimation of Blood glucose

Estimation of Blood glucose was carried out by using Accu Chek glucometer (Manufacture: Johnson and Johnson).

Biochemical analysis and Enzymatic assays

Total Carbohydrates

The total carbohydrates content was estimated by the method of (10). The testis tissue was homogenized in 10% Trichloro acetic acid to prepare 1% (W/V) homogenate. The precipitated were removed by centrifuging the homogenate for 15 minutes at 3000g. The clear supernatant was taken for the estimation of total carbohydrates. To 0.1 ml of the supernatant, 5ml of anthrone reagent was added and kept in a boiling water bath for 15 minutes. Then the contents were cooled and read at 620 nm against the reagent blank. The total carbohydrate content was expressed as mg of glucose/gm wet weight of the tissue.

Glycogen

Glycogen content was determined as described by.[11] Weighed amounts testis tissue was homogenized in 10 volume of ice cold 30% KOH and boiled at 100°C for 30 min. glycogen was precipitated with ethanol pelleted, washed with and resolubizilized in distilled water. Glycogen content was determined by treatment with anthrone reagent and measured at 625 nm.

Glucose

Glucose levels were estimated by a commercially available glucose kit based on the glucose oxidase method (Sigma Diagnostics, St. Louis, MO).

Histopathological Studies

Small portion of testis was fixed in 10% formalin for histopathological studies. Testis section taken with 5μm Thick, and stained with hemotoxylin and eosin[9] section was observed under microscope for histopathological changes.

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

Total carbohydrates

In control rats the amount total carbohydrate was found to be testis 32.00 mg of glucose /gm wet weight of tissue. In group-II, where the control rats were treated with *Aloe vera* extract the levels were increased. Group-III had showed a significantly decreased to testis 24.00 mg

of glucose /gm wet weight of tissue. In group-IV where the diabetic rats were subjected to *Aloe vera* extract, increased levels were found when compared to control rats.

Glucose

Control rats the amount glucose was found to be in testis 1.30 mg of glucose/gm wet weight of tissue. In group-II, where the control rats were treated with *Aloe vera* extract the levels were decreased. Group-III had showed a significantly increased to testis 1.44 mg of glucose /gm wet weight of tissue. In group-IV where the diabetic rats were subjected to *Aloe vera* extract, decreased levels were found when compared to control rats.

Glycogen

In control rats the amount glycogen was found to testis 28.00 mg of glucose /gm wet weight of tissue. In group-II, where the control rats were treated with *Aloe vera* extract the levels were increased. Group-III had showed a significantly decreased to testis 18.00 mg of glucose/gm wet weight of tissue. In group-IV where the diabetic rats were subjected to *Aloe vera* extract, increased levels were found when compared to control rats.

Table: Table: showing glucose, glycogen, and total carbohydrate levels in testis the 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*)
Glucose mg of glucose/gm wet weight of tissue	1.30±0.028	1.31±0.028 (+1.42)	1.44±0.027 (+25.58)	1.29±0.024 (+6.71)
Glycogen mg of glucose/gm wet weight of tissue	28.00±0.71	28.44±0.72 (+1.01)	18.00±0.67 (-56.93)	27.10±0.65 (-9.60)
Total carbohydrates mg of glucose/gm wet weight of tissue	32.00±0.88	31.88±0.82 (+1.43)	24.00±0.74 (-39.88)	31.10±0.78 (-19.28)

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 $P < 0.00$.

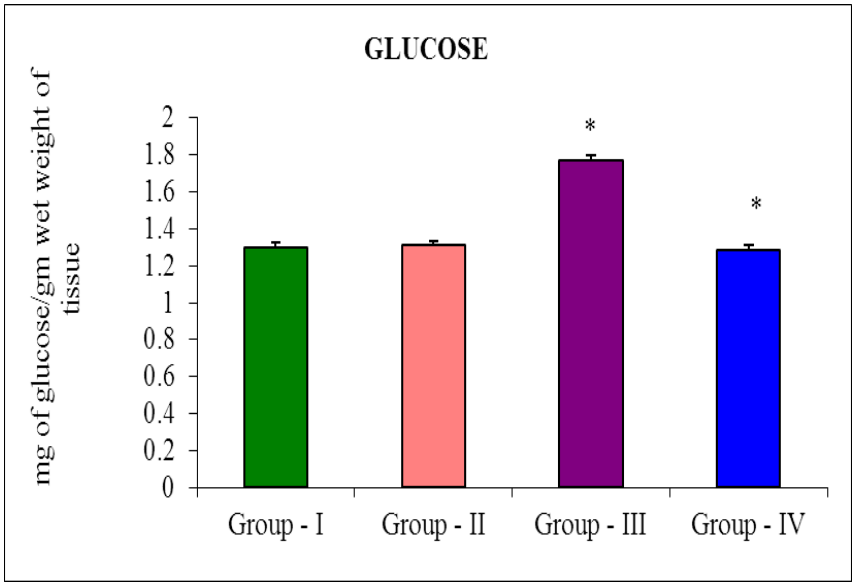

Fig: 1.1 showing glucose levels in testis tissue of control and experimental animals

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

Values are mean, SD: n=6.

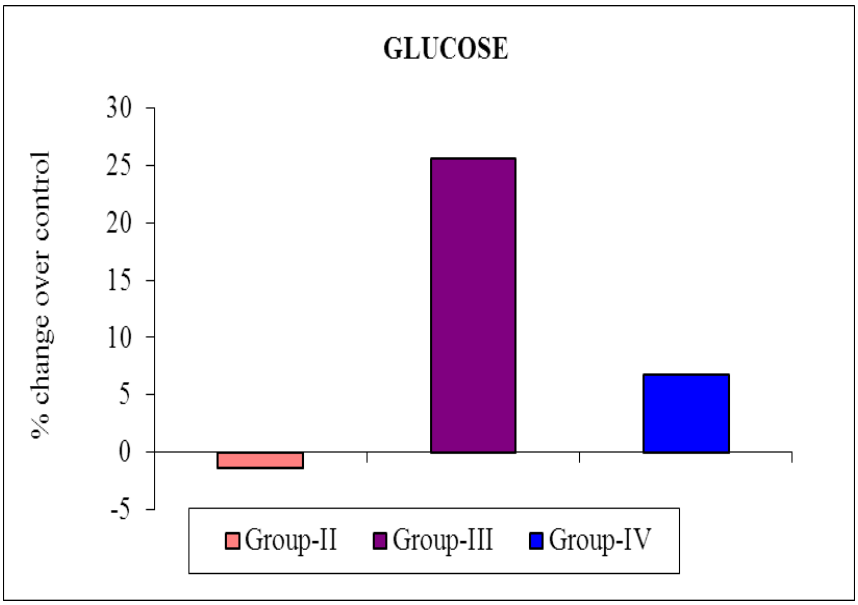

Fig: 1.2 showing % change of glucose levels in testis tissue of control and experimental animals

Values in the parentheses are % change from Control.

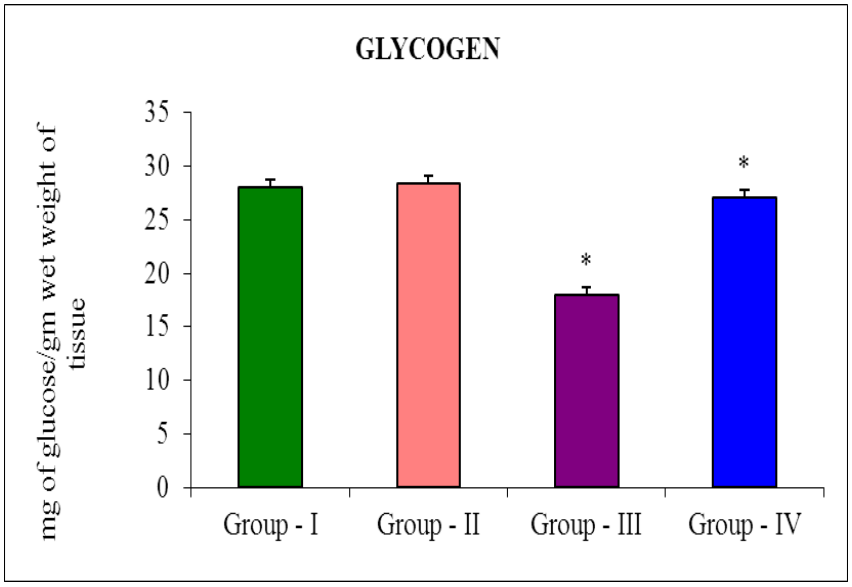

Fig: 2.1 showing glycogen levels in testis tissue of control and experimental animals

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

Values are mean, SD: n=6.

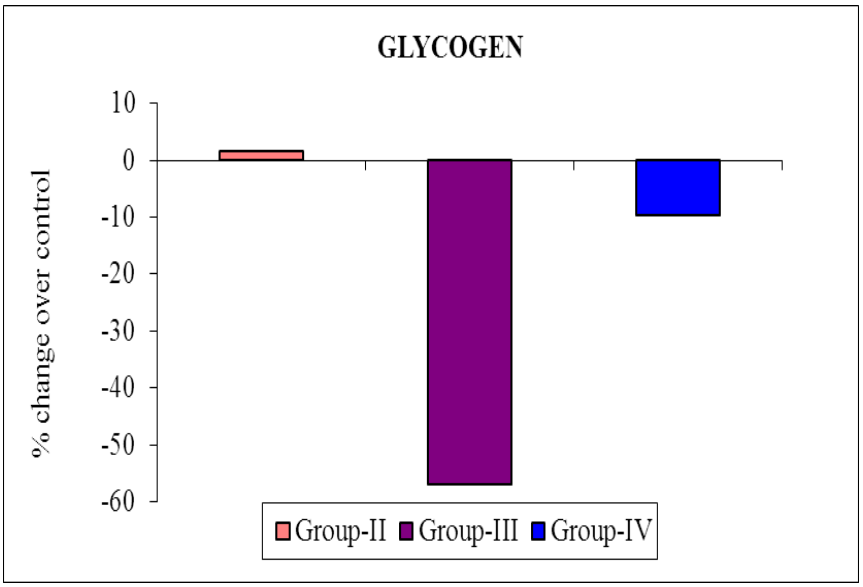

Fig: 2.2 showing % change of glycogen levels in testis tissue of control and experimental animals

Values in the parentheses are % change from Control.

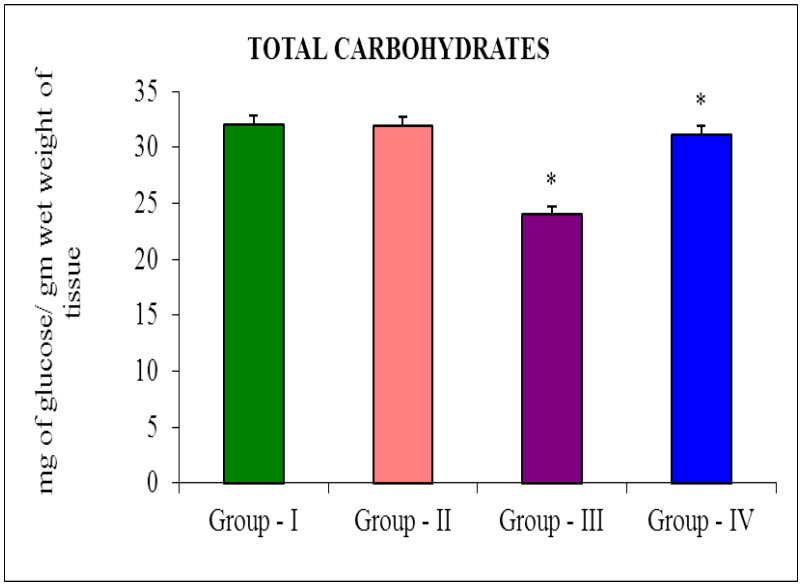

Fig: 3.1 showing total carbohydrate levels in testis tissue of control and experimental animals

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

Values are mean, SD: n=6.

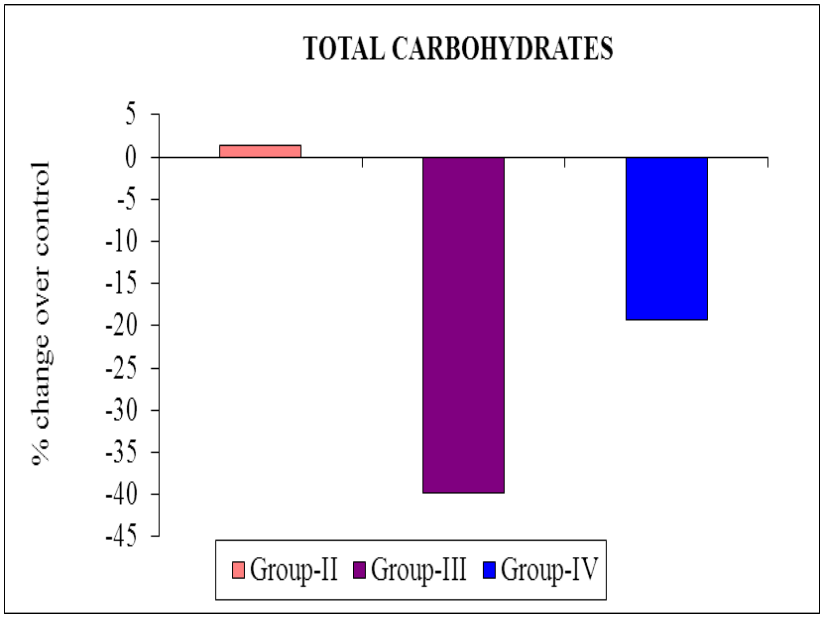

Fig: 3.2 showing % change of total carbohydrate levels in testis tissue of control and experimental animals

Values in the parentheses are % change from Control.

Effect of *Aloe vera* on Histopathological changes in testis of diabetic rats

In control rat testis contain Seminiferous Tubules contain number of Spermatid with central view. Whereas diabetic rats lumen of central view extensively filled with fibrous tissue.

However, in diabetic rats treated with *Aloe vera* extract the testis looked almost normal (Figure – 1.a, b, c.).

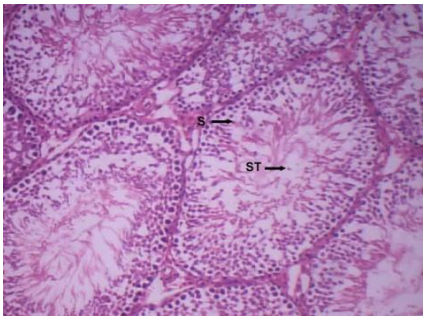

Fig: 1.a

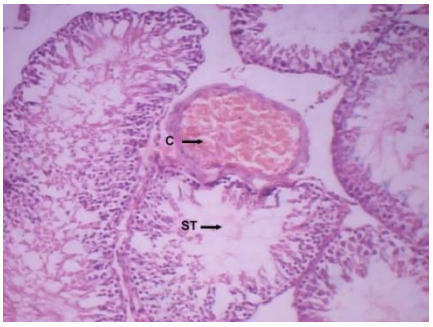

Fig: 1.b

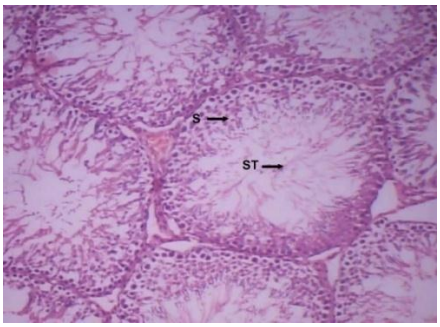

Fig: 1.c

Fig: 1 Histological observations of testis of normal control rats, diabetic and *Aloe vera* extract treated diabetic rats

a. Seminiferous Tubules and spermatids cells were observed in testis of control rats.
b. Seminiferous Tubules with hemarage was observed in testis of diabetic control rats.
c. Seminiferous Tubules mild degenerative change and spermatids was observed in testis of *Aloe vera* extract treated diabetic rats.

DISCUSSION

The present study was conducted to evaluated beneficial effects of *Aloe vera* extracts on carbohydrate metabolic profiles and histopathological changes in Alloxan induced diabetic rats. Diabetes mellitus is characterized by reduced capacity of the Beta-cells in the pancreas, whether the cells are destroyed as in type-1.diabetes, to release sufficient insulin to induce the activity of glucose metabolizing enzymes.[12] Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phospho fructokinase and pyruvatekinase.[13] One of the key enzymes in the catabolism of glucose is glucokinase, which phosphorylates glucose to glucose-6-Phosphate. The elevated blood glucose levels in diabetes are thought to lead to cell death through oxidative stress induction that occur as a common sequel of diabetes induced modification of sugar moieties on proteins and lipids.[14]

Carbohydrates are the major source of energy fuels for metabolic processes readily assimilable, though fats yield more energy. The carbohydrates serve as energy fuels for metabolic processes.[15] The abnormal regulation of glucose and impaired carbohydrate utilization that results from this defective and/or deficient insulin secretory response are the key pathogenic events in diabetes mellitus leading to the development and progression micro- and macro vascular complications which include neuropathy, nephropathy, cardio vascular and carebrovasular disease.[16] The significant decrease in total carbohydrate levels in the testis of diabetic rats suggests possible utilization of carbohydrates to meet the energy demand during Alloxan toxicity.

Similar pattern of Changes in carbohydrate levels has been reported in brain and other tissues of make albino-rats during Alloxan induce diabetic condition. Toxic compounds inhibit the formation of glucose from other compounds such as amino acids etc.[17] Glycogen is the primary intracellular storable from of glucose and its levels in various tissues, especially in liver, testis and skeletal muscles, are a direct reflection of insulin activity, which regulate glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. The amount of glycogen present in tissues varied widely with diet and physiological status.[18] Glycogen is the major storage form of Carbohydrate in animals for biological function and the maintenance of the glycogen reserves in a important feature of the normal metabolism.[19] The entry of glucose in testis tissue is not dependent on action of insulin and therefore, in the event of hyperglycemia there is an increase in the entry of

glucose.[20] In the present study oral administration *Aloe vera* extract to Alloxan induced diabetic rats regulated the activity of glycogen metabolizing enzymes by stimulating the remnant beta cells to secrete more insulin there by normalized the altered glycogen consent. Same results were observed in extract of seed of Tamarinds indica for 7 and 14 days in diabetic rats[21] observed graded and significant elevation in testis glycogen levels. The glycogen contact was increased in testis, treatment with *Aloe vera* in Alloxan induced rats. Thus the obtained results focus the one possible way of antidiabetogenic action of *Aloe vera* extract by the improvement of glycogenesis process in testis.

Histological observation under microscope, using eosin-haemotoxylin stain, clearly shows the defective architecture of seminiferous tubules within the testis of *Aloe vera* treated animals. The lumens of seminiferous tubules were also found to be reduced, indicating their compressed and bounded disorientation, whereas the same were radially oriented in control testis. Similarly,[22] demonstrated the histological damage s in the semiferous tubuls and Lyding cells of *Aloe vera* –treated mice. so, the altered architecture of semiferous tubuls in *Aloe vera* group of animals is very well correlated to the damaged membrane proteins and lipids. These finding suggest that *Aloe vera* extract has complimentary potency to develop an antihyperglycemic agent for the treatment of diabetes mellitus. Further studies are in progress to elicit the exact mechanism of antihypperglycemic action of *Aloe vera* extract in diabetes

CONCLUSION

Our data suggest that, the *Aloe vera* could inhibit the diabetes-induced damages in testicular tissue. Thus it could be suggested that simultaneous administration of *Aloe vera* could be considered as appropriate form of application, as the testes of *Aloe vera*-received groups were manifested with improved histological features. Moreover, *Aloe vera* could improve testicular endocrine activities partly by regulating gonadotropins levels.

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REFERENCES

1. Hunt J.V., R.T. Dean, S.P. Wolff, Biochem J., 1988; 256: 205-212.

2. Thorsby P, Undleien DE, Berg JP, Thorsby E, Birkeland KI. Tiolsskr Nor Laegeforen. 1998; 118: 2519-24.
3. Wild S, Roglic G, Green A, Sicree R, King H. Diabetes Care, 2004; 27: 1047-53.
4. Asbun J, Villarreal FJ. J Am Coll Cardiol., 2006; 47: 693-700.
5. Oluleye T.S. Afr J Med Med Sci, 2010; 39: 199-206.
6. Klein AD, Penneys N: *Aloevera*. J Am Acad Dermatos, 1988; 18: 714 – 720.
7. Coats BC, Ahola R: *Aloevera* the Salint Healer:a the modern study of *Aloe vera*. Dallas, 1979.
8. Rajasekaran S. Sivagnanam K. Ravi K, S. Subramaniam S: Hyoglycemic effect of *Aloe vera* gel on streptozocin induced diabetes in experimental rats. J Med food, 2004; 7: 61-66.
9. Chance B, Maechley A. Methods Enzymol, 1955; 2: 764-75.
10. Carrol, N. V., Longley, R. W. and Roe, J. H. Glycogen determination in liver and muscle by use of anthrone reagent. J. Biol. Chem. 1956; 220: 583-593.
11. Ong, K.C. & Khoo, H.E. Effects of myricetin on glycemia and glycogen metabolism in diabetic rats. *Life Sciences*, 2000; 67: 1695-1705.
12. Chattarjee, M.N. and Shinde, R. (2000) metabolism of carbohydrates. In Text book of medical Biochemistry. Jaypee Medical publishers, Delhi P. 421.
13. Murray, R.K. Granner, D.K. Mayes, P.A. and Rodwell, V.W. (2000) Harperls Biochemistry, 25th Ed., Appleton and Lange, Stanford PP. 610-617.
14. Donnini D., Zambito A.M. and Parella G. Glucose may induce cell death through a free radical-mediated mechanism. Biochem; Biophysic Res. Communications, 1996; 219: 412-417.
15. Martin F.C. and peters T.T. Assesment in vitro and in vivo of muscle degradation in chronic skeletal muscle myopathy of alcoholism. Clin. Science, 1985; 66: 693-700.
16. Adisakwattana S., Rosengsamran S. 9 HSU W.H. and Yibchok – anun S. Mechanisms of antihyperglycemic effect of P-methoxycinnamic acid in normal and streptozotocin – induced diabetic rats. Life Sci. 2005; 78: 406-12.
17. Patel P.J. Aging and antimicrobial immunity. Impaired production of mediator T cells as a basis for the decreased resistance of senescent micro to listeriosis, J. Exp. Med., 1981; 154: 821-831.
18. Nelson D.L. and Cox M.M. (2001): in: Lehninger principles of Biochemistry, 3rd edition, MacMillian press Ltd., Hampshire, UK, P-873.

19. Turner L.V. and Manchester K.L. Effects of denervation on the glycogen content and on the activities of Enzymes of the glucose and glycogen metabolism in rat disphragm muscle. Biochem. J., 1972; 128: 789.
20. Belfiore F., Rabuazzo A.M. and Iannello S. Anabolic response of some tissues to diabetes; Biochem, Med. Metabol. and Biology, 1986; 35: 149-155.
21. Anderson J.W. and Stowring I. Glycolytic and gluconeogenic enzyme activities in renel cortex of diabetic rats. American Journal of Physiology, 1973; 224: 930-936.
22. N Mehedi, S Ainad-Tabet, N Mokrane, S Addou, C Zaoui, O Kheroua and D Saidi, Reproductive Toxicology of Tartrazine (FD and C Yellow No. 5) in Swiss Albino Mice, *American Journal of Pharmacology and Toxicology, 2009; 4(4): 130-135.*