



STUDY OF HISTOPATHOLOGICAL CHANGES AND CARBOHYDRATE METABOLIC PROFILES IN DIABETIC TESTIS TISSUE TREATED WITH *ALOE VERA* LEAF GEL EXTRACT IN BLACK RAT (*RATTUS RATTUS*).

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ABSTRACT

Several herbal preparations are use to get rid of diabetes, but their reported hypoglycaemic effects are complex. The plant *Aloe vera* is used in Ayurvedic, Homoeopathic and Allopathic streams of medicine, and not only tribal com-munity but also most of the people for food and medicine. The plant leaves contains numerous vitamins, minerals, enzymes, amino acids, natural sugars and other bioactive compounds with emollient, purgative, antimicrobial, anti inflammatory, anti-oxidant, aphrodisiac, antihelmenthic, antifungal, antiseptic and cosmetic values for health care. This plant has potential to cure sunburns, burns and minor cuts, and even skin cancer In this studies the histological changes and carbohydrate profile in Alloxan – induced diabetes in rat treated with the leaf gel of Aloe vera ethanol extract on was evaluated. Twenty eight male rats were used in the study by allocating them into four groups, each of seven rats. Control rats, control rats + *Aloe vera*, diabeticblack rats, diabetic + *Aloe vera*. Diabetes was induced intra peritoneally using 50 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 25days. 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 black rat(*Rattus rattus*,).

Key words:- Aloe vera, black rat (*Rattus rattus*), Alloxan, testis, carbohydrate metabolic profiles and histopathology

INTRODUCTION

Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources. The genus Aloe belonging to family Alliaceae is a succulent herb of 80 - 100 cm in height which matures in 4 - 6 years and survives for nearly 50 years under favorable conditions.

Diabetes is a complex disease where the carbohydrate and fat metabolism are impaired.^[24] for long time many causes many complications such as diabetic nephropathy, retinopathy, neuropathy, cardiomyopathy and hyper glycemia.^[3,35] According to world health organization, diabetes mellitus (DM) is one of the most common metabolic disorders all over the world.^[46,50] It has been well known that suffering from diabetes

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.^[35] *Aloe vera* is a perennial plant belonging to the family of liliaceae, which includes about 360 species.^[26] Taxonomists now refer to *Aloe barbadensis* as *Aloe vera*.^[12] *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 mucilaginous pulp 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.^[40] Hence the present study was carried out; the purpose of this investigation was to evaluate the effect of *Aloe vera* extract on Alloxan induced diabetes by measuring blood glucose levels and assaying the carbohydrates metabolic and profiles in testis of black rat.

MATERIALS AND METHODS

Selection of Animals

Black (*Rattus rattus*) (180 \pm 20g) were obtained from locally collected. The black rats were

housed in clean polypropylene cages having seven rats cage and maintained under temperature controlled room (25 ± 2 °C) with a photo period of 12 hours light and 12 hours dark cycle. The black rats were fed with a standard rat pellet diet and water ad libitum. 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 Angul Mahahila Mahavidyalaya, Angul, Odisha, India.

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 (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Induction of Diabetes

The black rats (*Rattus rattus*) were injected intraperitoneal with Alloxan monohydrate (Span chemical Co. Mumbai) 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 25 days.

Preparation of *Aloe vera* extract

The fresh *Aloe vera* was locally and authenticated by botanist in the department of Botany, Angul Mahila Mahavidyalaya, Angul, Odisha. *Aloe vera* solid gel in the center of the leaf was collected and homogenized resulting, mucilaginous, thick and straw coloured 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

Black Rats (*Rattus rattus*) were randomly divided into four groups of seven animals in each group. Group-1: Control black rats (*Rattus rattus*)
Group-2: Control + *Aloe vera* (300mg/kg body weight of *Aloe vera*)
Group-3: Diabetic black rats (*Rattus rattus*) (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 intragastric tube for 25 days)

After completion of 25 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.^[36] 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 resolubilized 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 5^{μm} Thick, and stained with hematoxylin 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 black rats (*Rattus rattus*) 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 in of glucose /gm wet weight of tissue. In group-IV where the diabetic black rats (*Rattus rattus*) were subjected to *Aloe vera* extract, increased levels were found when compared to control black rats (*Rattus rattus*).

Glucose

Control black rats (*Rattus rattus*) the amount glucose was found to be in testis 1.32 mg of glucose/gm wet weight of tissue. In group-II, where the control black rats (*Rattus rattus*) were treated with *Aloe vera* extract the levels were decreased. Group-III had showed a significantly increased to testis 1.46 mg of glucose /gm wet weight of tissue. In group-IV where the diabetic black rats (*Rattus rattus*) 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 29.00 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 19.00 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 black rats (*Rattus rattus*).

Table: 1. showing glucose, glycogen, and total carbohydrate levels in testis the Control and Experimental animals black rat (*Rattus rattus*)

Parameter	Group I (non diabetic black rats)	Group II (non Diabetic black rats + <i>Aloe Vera</i>)	Group III (diabetic black rats)	Group IV (diabetic black rats + <i>Aloe Vera</i>)
Glucose mg of glucose/gm wet weight of tissue	1.32±0.029	1.35±0.32 (+1.42)	1.46±0.029 (+25.59)	1.29±0.025 (+6.73)
Glycogen mg of glucose/gm wet weight of tissue	29.00±0.73	29.45±0.73 (+1.02)	19.00±0.69 (-56.95)	27.10±0.67 (-9.65)
Total carbohydrates mg of glucose/gm wet weight of tissue	32.00±0.89	31.78±0.85 (+1.43)	25.00±0.76 (-39.89)	31.15±0.89 (-19.29)

Values are mean, ± S.D. of 6 individual black rat (*Rattus rattus*).

Values in the parenthesis are % changes from that of control.

Values are significantly difference from control at P < 0.00

creased. Group-III had showed a significantly decreased to testis 25.00 mg

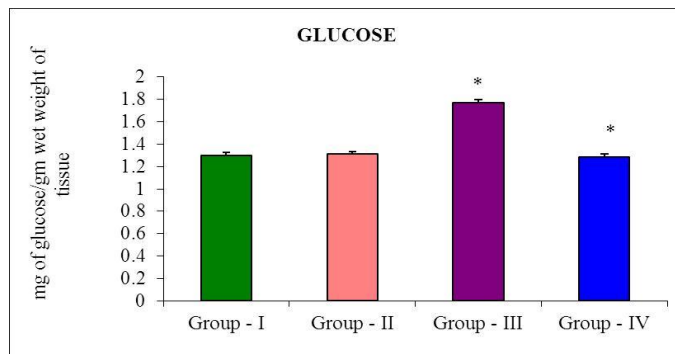
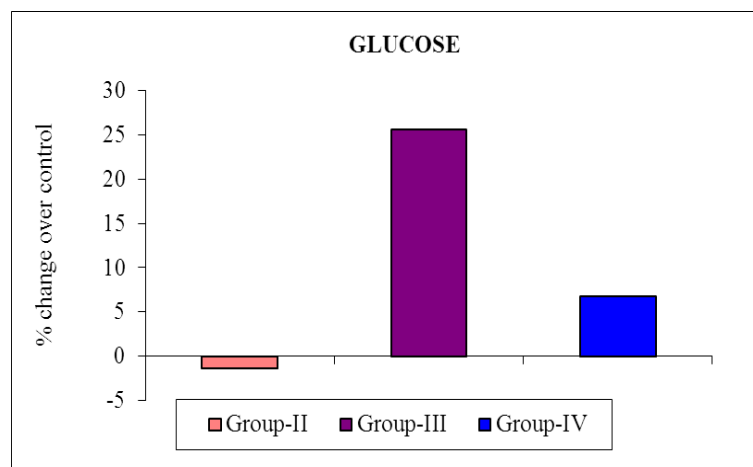


Fig: 1.1 showing glucose levels in testis tissue of control and experimental animals

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

Values are mean, SD: $n=7$



1.2 showing % change of glucose levels in testis tissue of control and experimental animals black rat (*Rattus rattus*)

Values in the parentheses are % change from Control

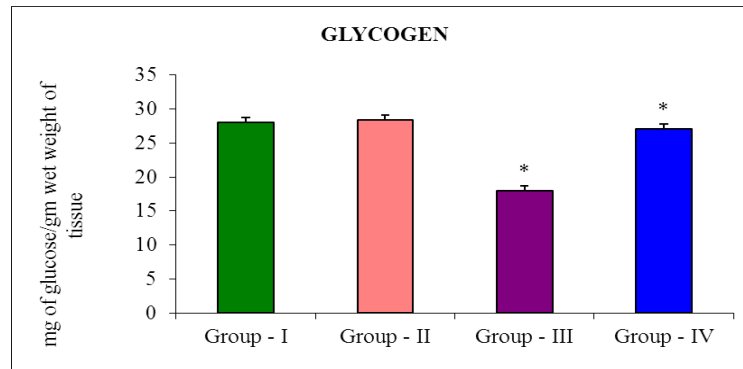


Fig:2. 1 Histological observations of testis of normal control rats, diabetic and *Aloe vera* extract treated diabetic black rat (*Rattus rattus*)

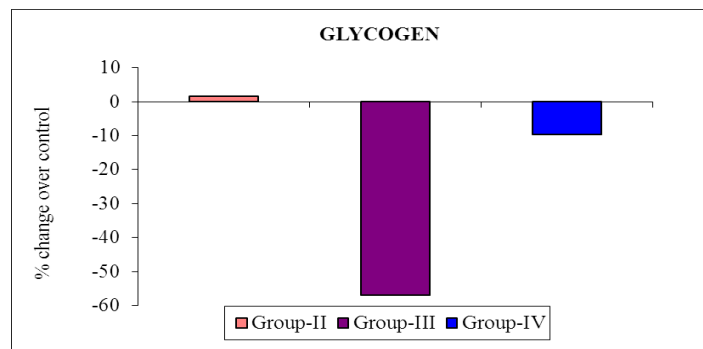
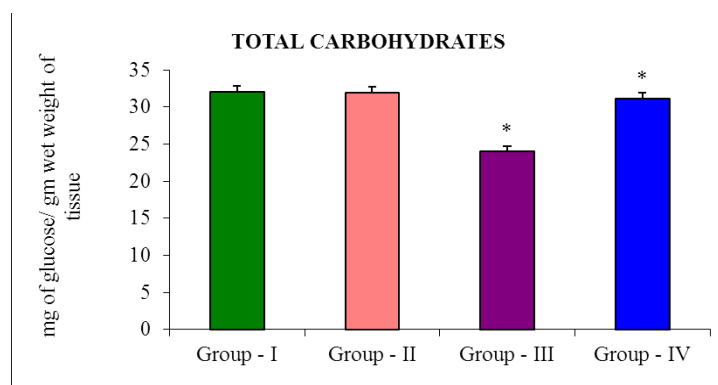


Fig: 2.2 showing % change of glycogen levels in testis tissue of control and experimental animals blackrat (*Rattus rattus*)

Values in the parentheses are % change from Control



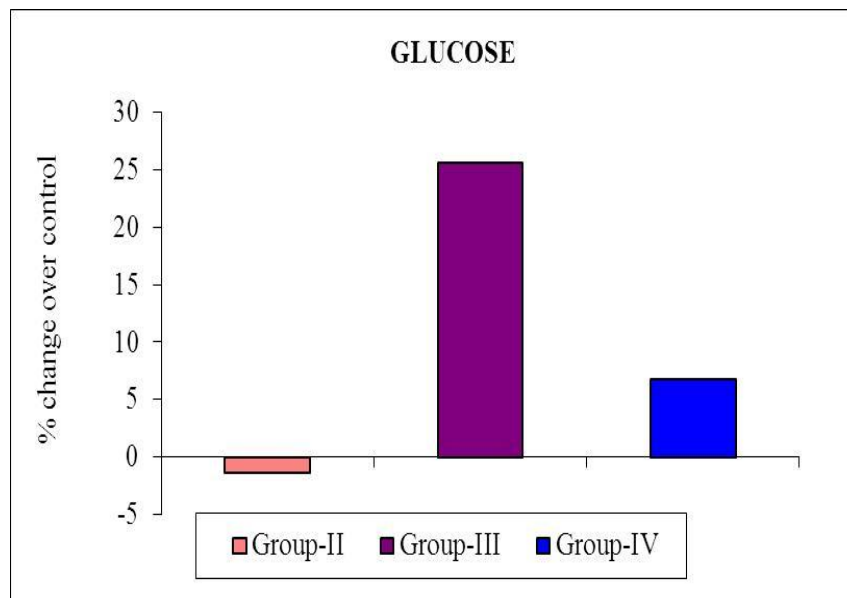


Fig: 3.1 showing total carbohydrate levels in testis tissue of control and experimental animals black rat (*Rattus rattus*)

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

Values are mean, SD: $n=7$.

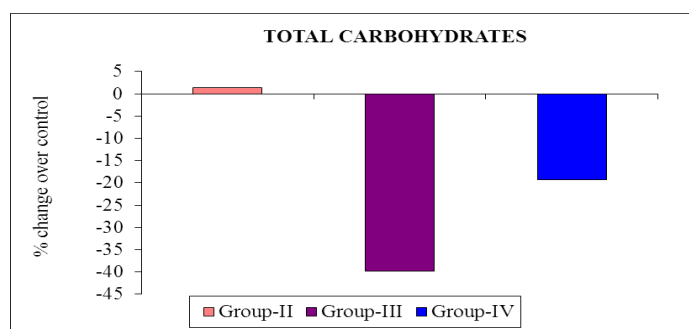


Fig: 3.2 showing % change of total carbohydrate levels in testis tissue of control and experimental animals black rat (*Rattus rattus*)

Values in the parentheses are % change from Control.

Effect of *Aloe vera* on Histopathological changes in testis of diabetic black rat (*Rattus rattus*)

In control black rat (*Rattus rattus*) 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 black rats (*Rattus rattus*) treated with *Aloe vera* extract the testis looked almost normal

(Figure – 1.a, b, c.).

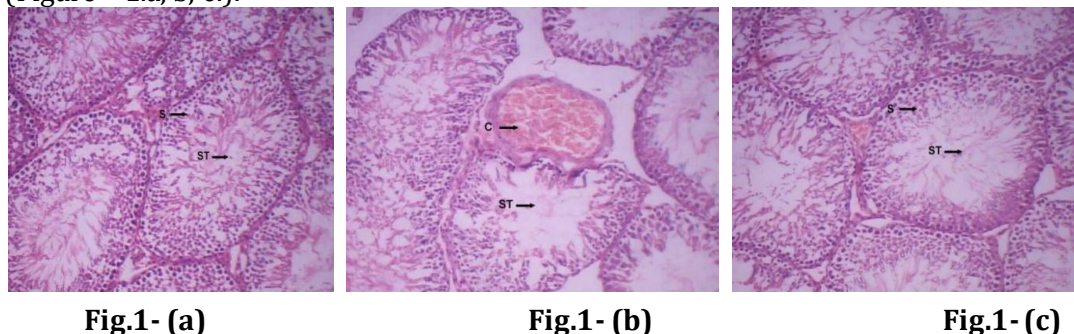


Fig: 1 Histological observations of testis of normal control rats, diabetic and *Aloe vera* extract treated diabetic black rats (*Rattus rattus*)

- a. Seminiferous Tubules and spermatids cells were observed in testis of control rats (*Rattus rattus*).
- b. Seminiferous Tubules with hemorrhage was observed in testis of diabetic control of rats (*Rattus rattus*).
- c. Seminiferous Tubules mild degenerative change and spermatids was observed in testis of *Aloe vera* extract treated diabetic rats (*Rattus rattus*).

DISCUSSION

The present study was conducted to evaluate beneficial effects of *Aloe vera* extracts on carbohydrate metabolic profiles and histopathological changes in Alloxan induced diabetic black rats (*Rattus rattus*). 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.^[11] Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phospho fructokinase and pyruvate kinase.^[29] 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 occurs as a common sequel of diabetes induced

modification of sugar moieties on proteins and lipids.^[15]

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.^[28] 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, cardiovascular and cerebrovascular disease.^[1] . The Significant decrease in total carbohydrate levels in the testis of diabetic rat suggests possible utilization of carbohydrate to meet the energy demand during Alloxan toxicity

Similar pattern of Changes in carbohydrate levels has been reported in brain and other tissues of male albino-rats during Alloxan induce diabetic condition. Toxic compounds inhibit the formation of glucose from other compounds such as amino acids etc.^[37] Glycogen is the primary intracellular storable form 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.^[31] Glycogen is the major storage form of Carbohydrate in animals for biological function and the maintenance of the glycogen reserves is an important feature of the normal metabolism.^[47] 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.^[6] In the present study oral administration *Aloe vera* extract to Alloxan induced diabetic black rats (*Rattus rattus*) regulated the activity of glycogen metabolizing enzymes by stimulating the remnant beta cells to secrete more insulin there by normalized the altered glycogen content. Same results were observed in extract of seed of Tamarinds indica for 10 and 15 days in diabetic black rats (*Rattus rattus*) ^[21] observed graded and significant elevation in testis glycogen levels. The glycogen content was increased in testis, treatment with *Aloe vera* in Alloxan induced black rats (*Rattus rattus*). 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-haematoxylin stain, clearly

shows the defective architecture of seminiferous tubules within the testis of *Aloe vera* treated animals (*Rattus rattus*). 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,^[32] demonstrated the histological damage s in the seminiferous tubules and Leydig cells of *Aloe vera* –treated black rat (*Rattus rattus*) s. so, the altered architecture of seminiferous tubules in *Aloe vera* group of animals is very well correlated to the damaged membrane proteins and lipids. These findings 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 antihyperglycemic action of *Aloe vera* extract in diabetes

CONCLUSION

The results of present study showed that *Aloe vera* brings back the blood glucose and body weight to normal in the diabetes black rats. After treatment with *Aloe vera*, the numbers of normal follicles were increased and atretic follicle reduced significantly. It is shown that *A. vera* could have a beneficial and supporting effects on testicular tissue if used as a hypoglycemic agent in diabetes.

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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BLACK RAT
Rattus rattus



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