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Evaluation of Antidiabetic And Antihyperlipidemic Activities of Alcoholic and Aqueous Extracts of *Balanites Aegyptiaca* (L.) Delile Leaves

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Abstract

Background:*Balanites aegyptiaca* (L.) Delilebelonging to family Zygophyllaceaeis a drought resistant plant which shows its antidiabetic profile.

Aim: The aim of the present work was to study the antidaibetic and antihyperlipidemic potential of aqueous and alcoholic extracts of *Balanites aegyptiaca*leaves.

Methods: Acute oral toxicity studies were carried out as per OECD guidelines-423. Antidiabetic along with antihyperlipidemic activities wasevaluated by serum biochemical analysis and blood sugar levels in STZ induced diabetic mice.

Results: The alcoholic extract (250 mg/kg) revealed significant activity in lowering blood glucose level in comparison to the aqueous extract (250 mg/kg) and Pet Ether Extract (250 mg/kg),when compared with standard drug glibenclamide (600µg/kg). On evaluation of different biochemical parameters, *i.e.* TGL (triglycerides), HDL, total cholesterol,LDL and VLDL level disclosed that both alcoholic and aqueous extracts has considerably reversed the diabetes including hyperlipidemia compared to standard drug. The raised levels of SGOT, SGPT got reduced by the treatment of alcoholic extract of leaves of *Balanites aegyptiaca* (L.) Delile. Alcoholicextractofleaves of *Balanites aegyptiaca* (L.) Delilerevealed noteworthy reduction in ALP andserum bilirubin, indicated a progress in the secretary mechanism.

Key words: *Balanites aegyptiaca* (L.) Delile,antidiabetic activity, antihyperlipidemic activity, alloxan, histopathology.

Introduction

Occurrence of diabetes mellitus is increasing all over the world especially in Asia.Different types of oral-hypoglycemic agents such as biguanides and sulfonylurea's are available along with insulin for the treatment of diabetes-mellitus but have side effects associated with their uses. Natural remedies from medicinal plants are considered to be useful and safe alternative treatment for diabetes mellitus. There is a growing attention in herbal remedies because of their efficiency and minimal side effects in clinical experience and comparatively low costs. Herbal drugs or their extracts are accepted

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widely, even when their biological active compounds are unidentified. Even the World-Health-Organization (WHO) approves utilization of plant drugs for different diseases, including diabetes. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed because of the incapability of existing modern therapies to control all the pathological aspects of the disorder.

It has been revealed from literature that extract of *Balanites aegyptiaca* (L.) Delile possess antifeedent, antidaibetic, molluscicide, antihelminthic and contraceptive activity [1-3].Pharmacological studies are very much important and only the means for evaluating any herbs/drug efficiency, therefore, keeping in mind its importance, stepwise evaluation of antidiabetic potential of pet ether, alcoholic and aqueous extracts of powdered leaves of *Balanites aegyptiaca* (L.) Delileincluding biochemical parameters has been carried out in the present study.

MATERIALS AND METHODS

Collection of Specimen:

Leaves of *Balanites aegyptiaca* (L.) Delile were collected from uncultivated fields in and around the Village Maroth of Nagaur District, Rajasthan, India during month of July-August. The Plant was identified from "Department of Botany, University of Rajasthan, Jaipur and confirmed on comparing with the help of herbarium maintained at the Department of Botany, University of Rajasthan, Jaipur. A voucher specimen(No. RVBL21073) was deposited and preserved in Herbarium Department of Botany, University of Rajasthan, Jaipur for further reference.

Preparation of extracts:

Preparation of the extracts of powdered leaves of *Balanites aegyptiaca* (L.) Delile and *Maytenus emarginata* (Willd)Ding Houwas carried out by using following solvents successively:

(a) Petroleum ether (60-80^{o}C)
(b) Benzene
(c) Chloroform
(d) Alcohol
(e) Distilled Water

The sample of leaves of *Balanites aegyptiaca* (L.) Delile (500 gm) were dried and grounded to a moderately coarse powder. The powdered Material was subjected to hot continuous extraction in a soxhlet extractor, successively with different known solvents in increasing order of polarity *viz*petroleum ether (60-80^{o}C), benzene, chloroform, alcohol. Finally, the powdered material was macerated with water for 24 hrs to obtain aqueous extract. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50^{0}C. Each extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely. It was finally dried and kept in desiccators[4-6].

Acute Oral Toxicity Study;

The procedure was followed by using OECD (Organization of Economic Co-operation and Development) guidelines 423(Acute toxic class method).

The acute toxic class method is a stepwise procedure with 3 animals of a single gender per step. Depending on the mortality and/or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment for the acute toxicity of the test substance [**Fig. 1**]. This procedure

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results in the use of a minimal number of animals while allowing for acceptable data-based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized system (GHS) for the classification of chemical which cause acute toxicity [7,8].

Procedure

Acute toxicity was determined by using *Swiss albino* mice as per Organization of Economic Co-operation and Development (OECD)guidelines 423 (Acute toxic class method)**.** The twelve *Swiss albino* mice of either gender of 30±5 gm weight were used for toxicity study. Initial dosing level of pet ether, alcoholic and aqueous extracts were 5, 50, 300, 2000 mg/kg body weight per oral (P.O.).The used crude extracts were found to possess LD_{50} value more than 2000 mg/kg and the route of administration was oral, 5 mg/kg was used as starting dose. Dose volume was administered 0.5 ml/kg body weight to the mice, which were fasted over night with water *ad libitum*. Food was withheld for next 3-4 hours after administration of drug[8-10].

Body weights of the mice before and after termination were noted and any change in skin, eyes, fur and mucous membrane along with circulatory, respiratory, autonomic, central nervous system and somatomotor activity behavior pattern were observed. Signs of tremors, convulsions, diarrhoea, salvation,lethargy, sleep and coma were also noted. The onset of toxicity and signs of toxicity werealso noted if any.

Experimental design

Method	**:**	Stz Induced (55mg/kg, i.p.)
Animal used	**:**	*Swissalbino* mice either sex
No. of group	**:**	Six
No. of animal per group	**:**	Six mice
Average weight	**:**	25-31 gm
Rout of Administration	**:**	I.P. and oral.
Standard drug used	**:**	Glibenclamide (600 µg/kg, orally)
Instrument used	**:**	Secomam semi auto analyzer.

The animals were housed in hygienic cages of polypropylene and maintained in a well-ventilated area of temperature 27±2°C, relative humidity 60-70%, controlled animal house with a regular 12 hour light/dark schedule. The animals were feed with standard rat pelleted diet (Hindustan Lever Ltd., Mumbai, India) and clean intake water was made available *ad libitum*. All procedures belonging to animals were performed after authorization from the ethics committee and in accordance with the recommendations for the proper care and use of laboratory animals (1283/C/09/CPCSEA). Animals were deprived of food initially for 16 hr but had free access to water[11-16].

Chemical used

Freshly prepared aqueous-solution of streptozotocin (streptozotocin taken from Sigma Aldrich, St. Louis, USA) Glibenclamide, carboxy methyl cellulose, Standard rat pelleted diet (Hindustan Lever Ltd., Mumbai, India) and test plant extracts (pet ether, alcoholic and aqueous).

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Induction of diabetes:

After overnight fasting (deprived of food for 16 hr but had been allowed free access to water), diabetes was induced in *Swiss albino* mice by I.P. injection of freshly prepared aqueous solution of streptozotocin(dissolved in 0.1M cold sodium citrate buffer (pH 4.5) at a dose of 55 mg/kg body weight).The animals were permitted to drink 5% glucose solution overnight to defeat the drug-induced hypoglycemia.Control mice were injected through citrate buffer alone. After a week of time for the development of diabetes, the mice among moderate diabetes having glycosuria and hyperglycemia (blood glucose level>200mg/dl) were measured as diabetic and used for the study[17-19].The animals which did not developed hyperglycemia i.e. glucose level<200mg/dl, were rejected/replaced through new animals. After induction of diabetes, immediately mice were classified into six groups of six mice each.

Study design

Evaluation of antidiabetic effect of test plant extracts was done by taking randomly six mice in each six groups as following.

Group I: Served as normal control (Received normal saline 0.5 ml/kg body weight).

Group II: Served as diabetic control (Treated with STZ dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 55 mg/kg body weight).

Group III: Received pet ether extract, 250 mg/kg body weight which was prepared in 1% carboxy methyl cellulose (CMC) was given orally.

Group IV: Received alcoholic extract, 250mg/kg body weight which wasprepared in 1%carboxy methyl cellulose (CMC) was given orally.

Group V: Received aqueous extract, 250mg/kg body weight which was preparedin 1%carboxy methyl cellulose (CMC) was given orally.

Group VI: Served as reference standards (Glibenclamide, 600 µg/kg body weight orally).

The various treatments were started after induction of diabetes and measured as day 0 of diabetes. Drugs were given orally and treatment was continued for 21 consecutive days, with twice a day dose (morning and evening). The doses employed for all drugs were inside therapeutic range to suit the experimental animal used i.e. the mice.

Recording of body weight

The change body weight was recorded during the study period. Body weight was measured after and before the streptozotocin administration on the 0, 7^{th}, 14^{th} and 21^{st} reading days throughout the treatment in normal control, diabetic control, standard glibenclamide, pet ether, alcoholic and aqueous extracts[20].

Sample collection:

Blood samples for estimation of blood glucose was collected before the treatment (0 day) and at the end of 7^{th}, 14^{th} and 21^{st} day(during treatment).Blood samples were collected from the tip of the tail of each mice under mild-ether anesthesia in Eppendrof(1ml) tubes containing 50µl of anticoagulant (heparin) and serum was separated by centrifuge at 3000 rpm[17-20].Mice were sacrificed by cervical disruption under mild ether anesthesia after 21 daysand tissues were used for histopathological studies.

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Estimationof biochemical parameters:

Blood sugar estimation was doneby means of a glucometer (Accu-check® sensor, Roche Diagnostics GmbH, Mannheim) and strips. Blood glucose level was checked on the 0^{th}, 7^{th}, 14^{th} and 21^{st} study days during the treatment in mice of normal control, diabetic control, standard glibenclamide, pet ether, alcoholic and aqueous extracts[9,21,22]**.**

Serum was subjected for the estimation of lipid profile (Total cholesterol, Triglyceride, LDL, HDLand VLDL) using Star 21 bio auto analyzer (E114947) at 505 nm by standard kits (Span diagnostics Ltd. India) following manufacturers instructions.

Histopathological Studies:

Mice were sacrificed by cervical disruption under mild ether anesthesia after day 21. The whole pancreas of each animal was removed and placed in formalin solution (10%) and immediately processed by the paraffin technique. Sections of 5 µm width were cut and stained by eosin (H & E) and haematoxylin for histological examination[16,22,23].

RESULTS AND DISCUSSION:

Taxonomical Identification:

The species for the proposed study was identified and authentified as *Balanites aegyptiaca* (L.) Delile(Certificate no. PARC/2013/2064.) by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai.

Acute oral toxicity study:

The mice were treated with graded dose of powered leaves extracts of *Balanites aegyptiaca* (5, 50, 300, 2000 mg/kg body wt./mice/day) to find out any possible toxic effects and/or changes in behavioral pattern, and were kept under close observation. All symptoms including changes in awareness, mood, motor activity, posture activity & mortality were recorded and no changes were observed in behavior and mortality as well as no toxicity or death was observed for these given dose levels in the selected and treated animals **(Table 1,2).** The LD_{50} of the pet ether, alcoholic and aqueous extracts of the leaves of *Balanites aegyptiaca* (L.) Delile as per OECD guidelines-423 is greater than 2000mg/kg(LD_{50}>2000mg/kg). Hence the biological dose was fixed 250 mg/kg body weight for all the extracts for further treatment.

Antidiabetic studyof *Balanites aegyptiaca* (l.) Delile:

Effect of extracts on body weight:

The body weight of each animal was recorded before streptozotocin and during the period of treatment. The change in body weight gain/loss of control and experimental groups of mice has been tabulated in **Table no.3** and graphically represented in **Figure no. 2.**

A significant decrease was observed in the body weight of diabetic mice compared with control mice. On treatment with various extracts of leaves of *Balanites aegyptiaca* (L.) Delile and glibenclamide, the body weight gain was improved but the effect was more pronounced in alcoholic and aqueous extracts of leaves of *Balanites aegyptiaca* (L.) Delile treated mice than glibenclamide on $14^{th}$and 21^{st} day of study.

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Estimation of blood glucose level:

The blood glucose level was measured on the 0^{th}, 7^{th}, 14^{th} and 21^{st} day of study during the treatment in normal control, standard glibenclamide, diabetic mice and different extracts of leaves of *Balanites aegyptiaca* (L.) Delile. The change in blood glucose level of control and experimental groups of mice were observed has been represented in **Table no. 4** and in **Figure no. 3**. Treatment with oral glibenclamide& various extracts of leaves of *Balanites aegyptiaca* (L.) Delilereduced blood glucose level on day 0, 7^{th}, $14^{th}$and 21^{st}.

In STZ induced diabetic rats the blood glucose levels were in the range of 259-358 mg/kg, which was considered as sever diabetes. In the standard drug (Glibenclamide 600µg/kg) and alcoholic extract (250 mg/kg) treated groups the peak values of blood sugar significantly decreased to 142.7 mg/kg and 174.9 mg/kg simultaneously on the 21^{st} day. Thus the alcoholic extract was found to be almost significant as standard drug in lowering blood glucose level. Whereas the Pet Ether Extract (250 mg/kg) and aqueous extracts (250 mg/kg) treated group showed 283.3 kg/mg and 204.6 kg/mg blood glucose level, which is comparatively less to alcoholic extract and standard drug.

Estimation of lipid profile of different extracts of leaves of *Balanites aegyptiaca* (L.) Delile:

Lipid profiles (Total cholesterol, TG, LDL, HDL & VLDL) were estimated on 21^{st} day of study after treating with Pet Ether, alcoholic and aqueous extract of the leaves of *Balanites aegyptiaca* (L.) Delile. Results were tabulated in **table no. 5** and graphically represented in **figure no. 4** from which it was revealed that alcoholic extracts significantly ($p<0.001$) reversed the diabetes-included hyperlipidemia compared to standard drug. Alcoholic extracts with 250mg/kg produced significant percentage reduction of total cholesterol level 98.7 mg/dl TGL-74.8mg/dl, HDL- 35.6mg/dl VLDL-14.96 mg/dl, and LDL-43.3mg/dl. Alcoholic extract was found to be comparative to standard drug treated groups (Glibenclamide 600µg/kg) total cholesterol 96.1 mg/dl, TGL 56.1 mg/dl, HDL-38.9 mg/dl, VLDL- 11.22 mg/dl LDL- 43.1 mg/dl. Pet Ether extract (250mg/kg) treated group was observed with total cholesterol 116.0mg/dl,TGL-82.5mg/dl,HDL-26.1 mg/dl, VLDL-16.5 mg/dl, LDL-63.0 mg/dl and aqueous extract (250mg/kg) treated group total cholesterol 104.0mg/dl,TGL-84.2mg/dl,HDL-34.3 mg/dl, VLDL-16.84 mg/dl, LDL-45.2 mg/dl, which was comparatively less than the observed ratio of TGL, HDL, VLDL, LDL and cholesterol.

So these results showed that alcoholic extract was more significant than other extracts. The HDL cholesterol level was found to improve significantly in alcoholic extract than other extracts.

The several plant constituents were known to reduce TC, TG, LDL and VLDL which is usually increased in the serum of diabetic such a significant increase in TC, TG, LDL and VLDL might be due to the lack of insulin under diabetic condition.

Histological Examination of Pancreas:

Section of normal control mice:

Histological examination of pancreas of different group revealed the presence of intralobular duct, interlobular duct, connective tissue, blood vessels, islet of langerhans, acinar cells and intercalated ducts. Histology of islet of langarhans was observed specifically. It was observed that structure and arrangement of islet of langarhans was normal. None of the cells in pancreas were observed to be inflamed. The cells of islet were tightly arranged and there were not any significant gaps between these cells. The presence of islet of lengarhans was uneven throughout the lobules **(Fig. no. 5)**.

Section of STZ diabetic control mice:

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In streptozotocin treated group it was observed that the islets were separated with gaps between them. Widening in intralobular and interlobular duct was also observed. The size of islets was also observed to be increased. No. of islets was also found to be decreased as compared to vehicle treated groups **(Fig. no. 6).**

Section of pancreatic tissue of Glibenclamide treated mice (Fig. no. 7):
In this group it was observed that still there were gaps between the islets but the number of islets were found to be in increased in numbers as compared to that of streptozotocin alone treated group and this was significantly different.

Section of pancreatic tissue of mice treated with pet ether, alcoholic and aqueous extract of leaves of *Balanites aegyptiaca* (L.) Delile. :
In pet ether, alcoholic and aqueous extracts of leaves of *Balanites aegyptiaca* (L.) Delile treated group it was observed that still there were gaps between the islets but the number of islets were found to be in increased in numbers as compared to that of streptozotocin alone treated group and this was significantly different. Thus alcoholic and aqueous extractof leaves of *Balanites aegyptiaca* (L.) Delileproduced protective effect against streptozotocin induced diabetes and this may be due to regeneration **(Fig. no. 09,10).** In Pet ether extract of leaves of *Balanites aegyptiaca* (L.) Delilethe extent of protection was not found significant on the basis of histological examination **(Fig. no. 8).** The damage to architect of islet of pancreas and reduction in its number was observed approximately similar in all groups.
Thus histological examination revealed that alcoholic extractsof leaves of *Balanites aegyptiaca* (L.) Delileand aqueous extractsof leaves of *Balanites aegyptiaca* (L.) Delile were more effective than other.

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Table 01: Acute toxicity class method (OECD-423)

S. No	Group	Dose	No. of animals	Average weight of animal in gms		Death	Signs of toxicity	Onset of toxicity	Reversible / Irreversible	Duration of observation
				Before test	After test (on4th day)					
1.	Pet. ether extract	5 mg/kg	03	26.50	27.00	00	No signs of toxicity	Nil	Nil	3 days
2.	Pet. ether extract	50 mg/kg	03	27.00	27.40	00	No signs of toxicity	Nil	Nil	3 days
3.	Pet. ether extract	300 mg/kg	03	25.00	25.50	00	No signs of toxicity	Nil	Nil	3 days
4.	Pet. ether extract	2000 mg/kg	03	25.80	26.75	00	No signs of toxicity	Nil	Nil	3 days
5.	Alcoholic extract	5 mg/kg	03	26.00	26.50	00	No signs of toxicity	Nil	Nil	3 days
6.	Alcoholi	50	03	26.50	27.8	00	No	Nil	Nil	3 days

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	c extract	mg/kg			0		signs of toxicity			
7.	Alcoholic extract	300 mg/kg	03	25.50	26.70	00	No signs of toxicity	Nil	Nil	3 days
8.	Alcoholic extract	2000 mg/kg	03	26.70	28.10	00	No signs of toxicity	Nil	Nil	3 days
9.	Aqueous extract	5 mg/kg	03	28.00	28.40	00	No signs of toxicity	Nil	Nil	3 days
10.	Aqueous extract	50 mg/kg	03	26.70	27.40	00	No signs of toxicity	Nil	Nil	3 days
11.	Aqueous extract	300 mg/kg	03	27.50	28.70	00	No signs of toxicity	Nil	Nil	3 days
12.	Aqueous extract	2000 mg/kg	03	26.10	27.50	00	No signs of toxicity	Nil	Nil	3 days

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Table02:Gross behavioral studies of the animal

time	Alert	Passive	Groo-ming	Restles s-ness	Aggre-ssive	Touch espons e	Pain respon se	Convu-lsion	Gripping strengt h	Pin reflex	Corneal reflex	Writ-hing	Pupil size	Saliv-ation	Skin colour	Lacri-matio	Resp-iratio n
1st hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2nd Hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3rd Hour	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
4th Hour	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
24th hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48th hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72th hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = Normal ++ = Moderate effect
+ = Mild +++ = Marked effect

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Table03: Effect of various extracts of leaves of balanites aegyptiaca on body weight

Groups	Before STZ	Body weight afterstreptozotocin (gm)			
		0^{th}	7^{th}	14^{th}	21^{st}
Control	27.8±0.590	27.4±0.585	28.1±0.585	28.7±0.585	29.6±0.578
Diabetic control	30.8±0.880	30.3±1.562	29.1±0.880	26.2±1.523	24.4±1.452
GLB	27.9±0.997	27.5±0.601*	28.4±0.998*	28.9±0.5989*	29.4±0.594*
Pet. ether extract	27.2±1.956	26.8±1.065	27.1±1.312*	27.4±1.423*	28.2±1.265*
Alcoholic extract	28.7±1.420	28.2±1.523*	28.9±1.563*	29.6±1.956*	30.5±1.864*
Aqueous extract	28.1±1.130	27.6±1.263*	28.2±1.235*	28.9±1.125*	29.6±1.152*

The results are expressed as mean ± SEM; n=6 animals in each group; Values are statistically significant at *P<0.05; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One wayANOVA was used, followed by Bonferroni multiple comparison tests; GLB = Glibenclamide

Table 04:Effect of various extracts of leaves of *balanites aegyptiaca* on blood glucose level

Drug treatment	Dose mg/kg	Blood glucose (mg/dl)			
		0 day	7^{th} day	14^{th} day	21 day
Control (Normal Saline)	0.5 ml/kg	92.5 ± 1.373	94.6 ± 0.5907	95.6 ± 0.586	96.1 ± 0.716
Diabetic control (STZ)	55	283.8 ± 1.561	291.6 ± 0.999	297.1 ± 0.601	301.8 ± 1.653
Pet ether Extract	250	290.9 ± 4.659	285.6 ± 2.234*	281.7 ± 6.067**	283.3 ± 4.29
Alcoholic Extract	250	287.3 ± 3.833	267.0 ± 3.659**	236.7 ± 4.134**	174.9 ± 5.13

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Aqueous Extract	250	288.3 ± 3.791	271.7 ± 3.851**	243.7 ± 6.193**	204.6 ± 5.735
Glibenclamide	600µg/kg	289.6 ± 2.266*	208.3 ± 0.880*	151.9 ± 1.978*	142.7 ± 1.391*

- Data are expressed as mean ± SEM; n=6 animals in each group.
- Values are statistically significant at *P<0.01 Vs Control and **P<0.001 Vs Control by Student't' test.
- Diabetic control was compared with control mices. Diabetic + pet ether extract, Diabetic + alcoholic extract, Diabetic + aqueous extract and diabetic + glibenclamide were compared with diabetic control.

Table 05: Effect of various extracts on levels of TGL, HDL, LDL and VLDL

Drug treatment	**Total cholesterol (mg/dl)**	**TGL (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
Control (Normal saline 0.5 ml/kg)	93.5 ± 1.49	54.2 ± 1.101	36.6 ± 0.884	44.6 ± 1.182	10.84 ± 0.220
Diabetic control (STZ 55 mg/kg)	126.4 ± 0.979	88.5 ± 2.195	20.0 ± 1.142	80.5 ± 2.252	17.7 ± 0.439
Pet Ether Extract (250 mg/kg)	116.0 ± 3.396	82.5* ± 4.256	26.1* ± 2.983	63.0** ± 4.63	16.5 ± 2.24
Alcoholic Extract (250 mg/kg)	98.7 ± 3.955	74.8* ± 4.042	35.6* ± 2.113	43.3** ± 3.112	14.96 ± 1.255
Aqueous Extract (250 mg/kg)	104.0 ± 3.972	84.2* ± 2.763	34.3* ± 3.24	45.2** ± 2.921	16.84 ± 1.112

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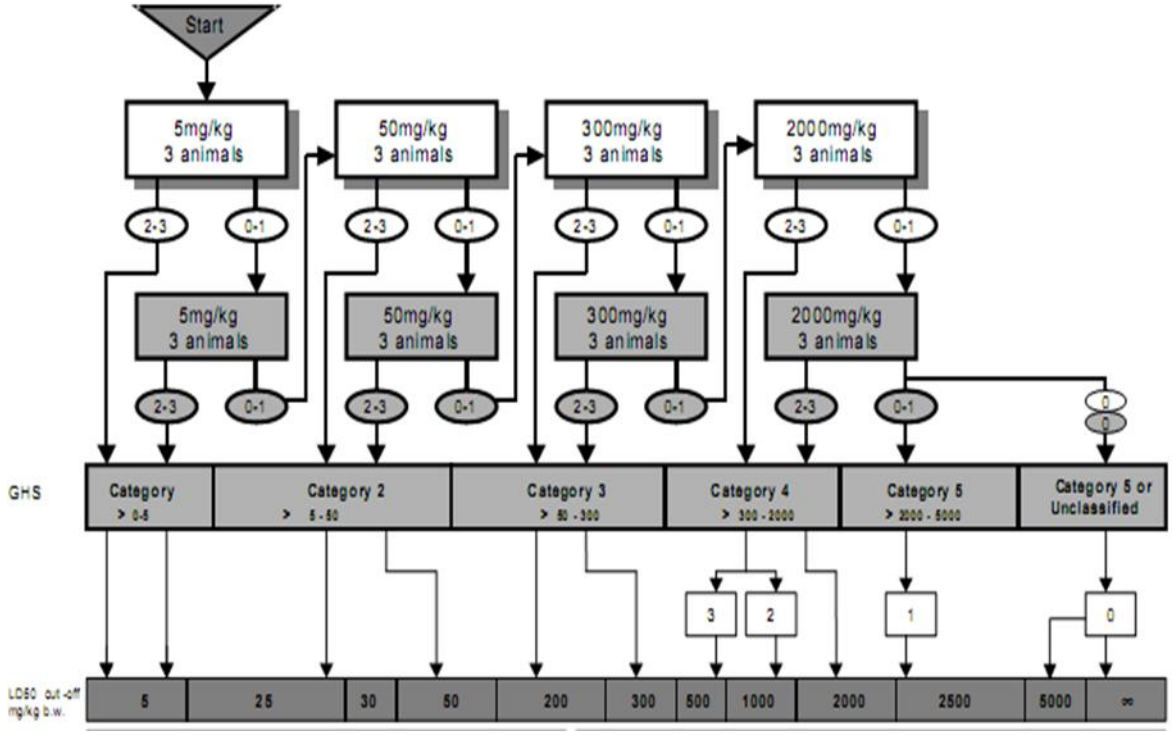
Glibenclamide (600µg/kg)	96.1 ± 2.695*	56.1 ± 1.452*	38.9 ± 2.112*	43.1 ± 2.462*	11.22 ± 0.118*

- Data are expressed as mean ± SEM; n=6 animals in each group by student't' test.
- Values are statistically significant at * P<0.01 and ** P<0.001 Vs Control.
- Diabetic control was compared with control mices. Diabetic + pet ether extract, Diabetic + alcoholic extract, Diabetic + aqueous extract, and diabetic + glibenclamide were compared with diabetic control.



Per step 3 animals of a single sex (normally females) are used
0,1,2,3: Number of moribund or dead animals at each step
GHS: Globally harmonized **classification** system (mg/kg b.w.)
∞: Unclassified

Fig. 01: Flow chart for acute toxic class method (OECD Guideline 423).

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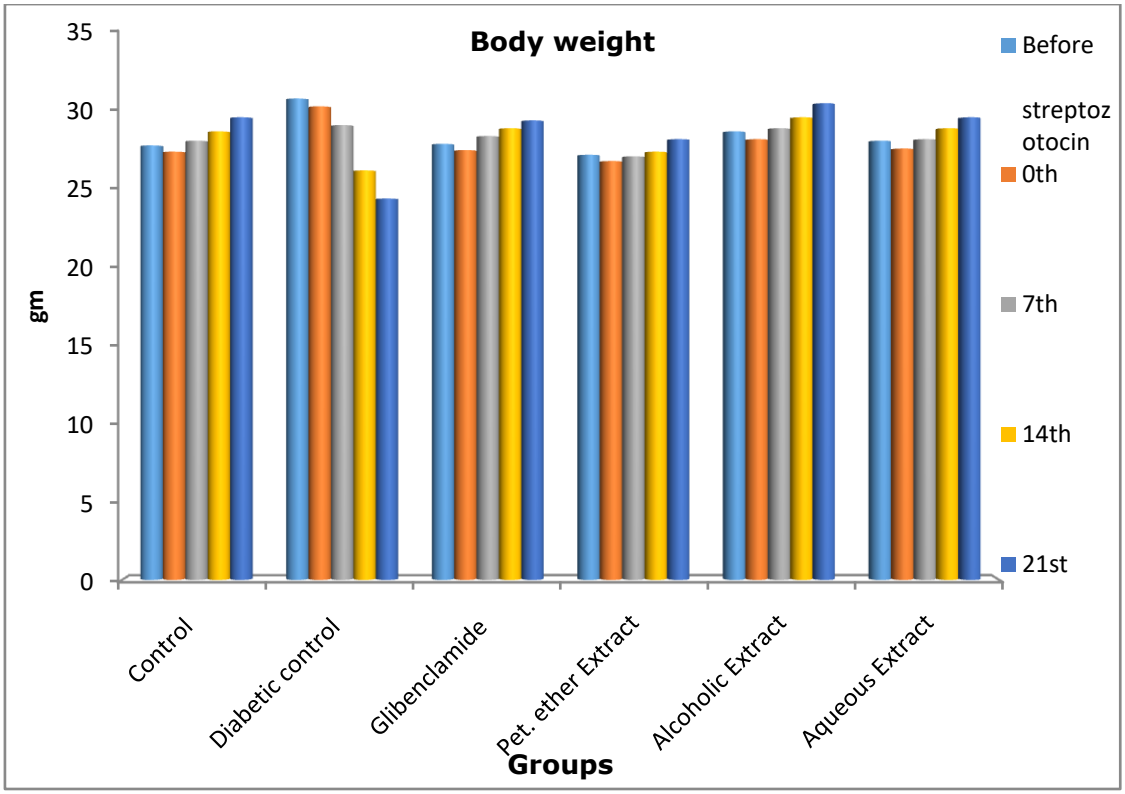

Fig. 02: Graphical representation of effect of various extracts of leaves of *Balanites aegyptiaca* on body weight

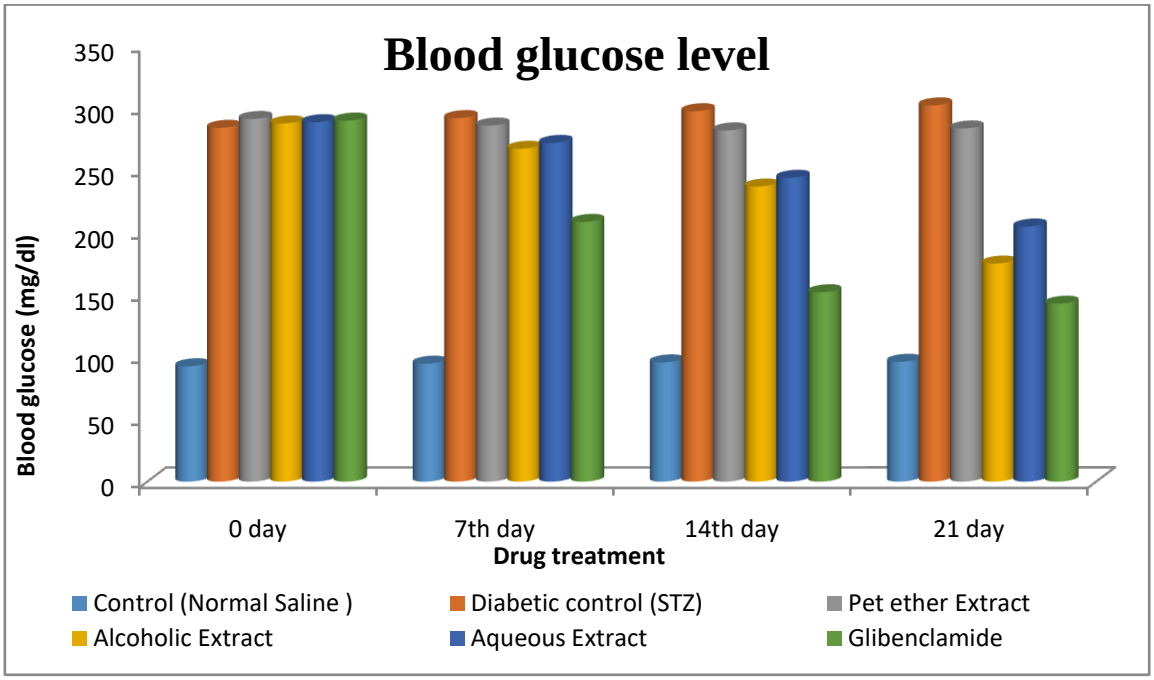

Fig. 03:Graphical representation of effect of various extracts of leaf of *Balanites Aegyptiaca* on blood glucose level

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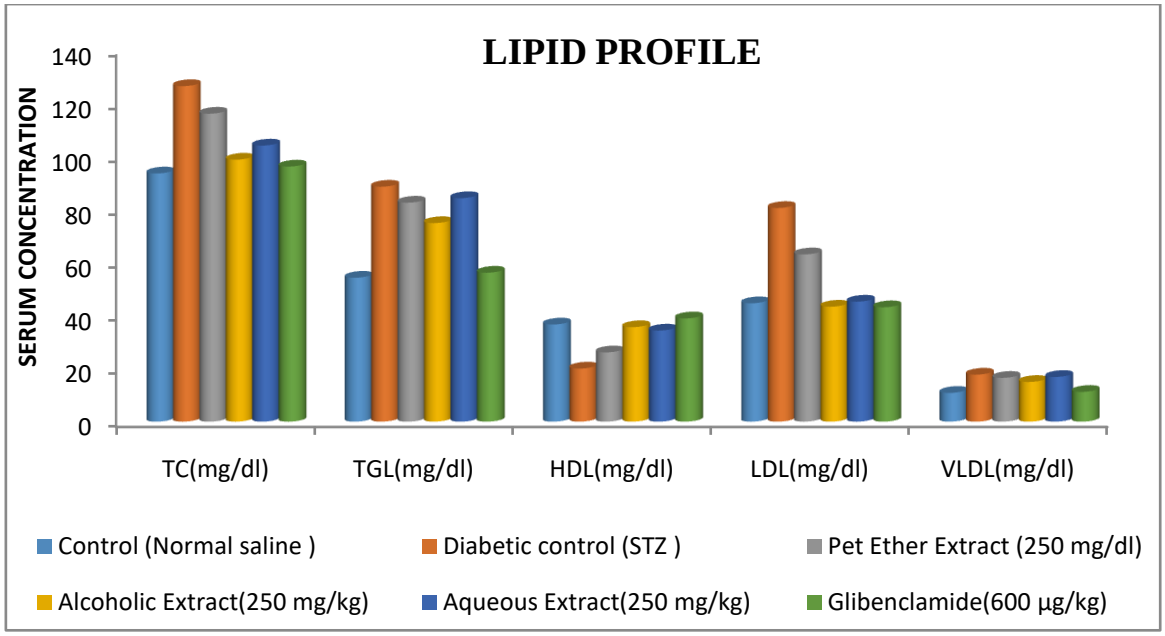
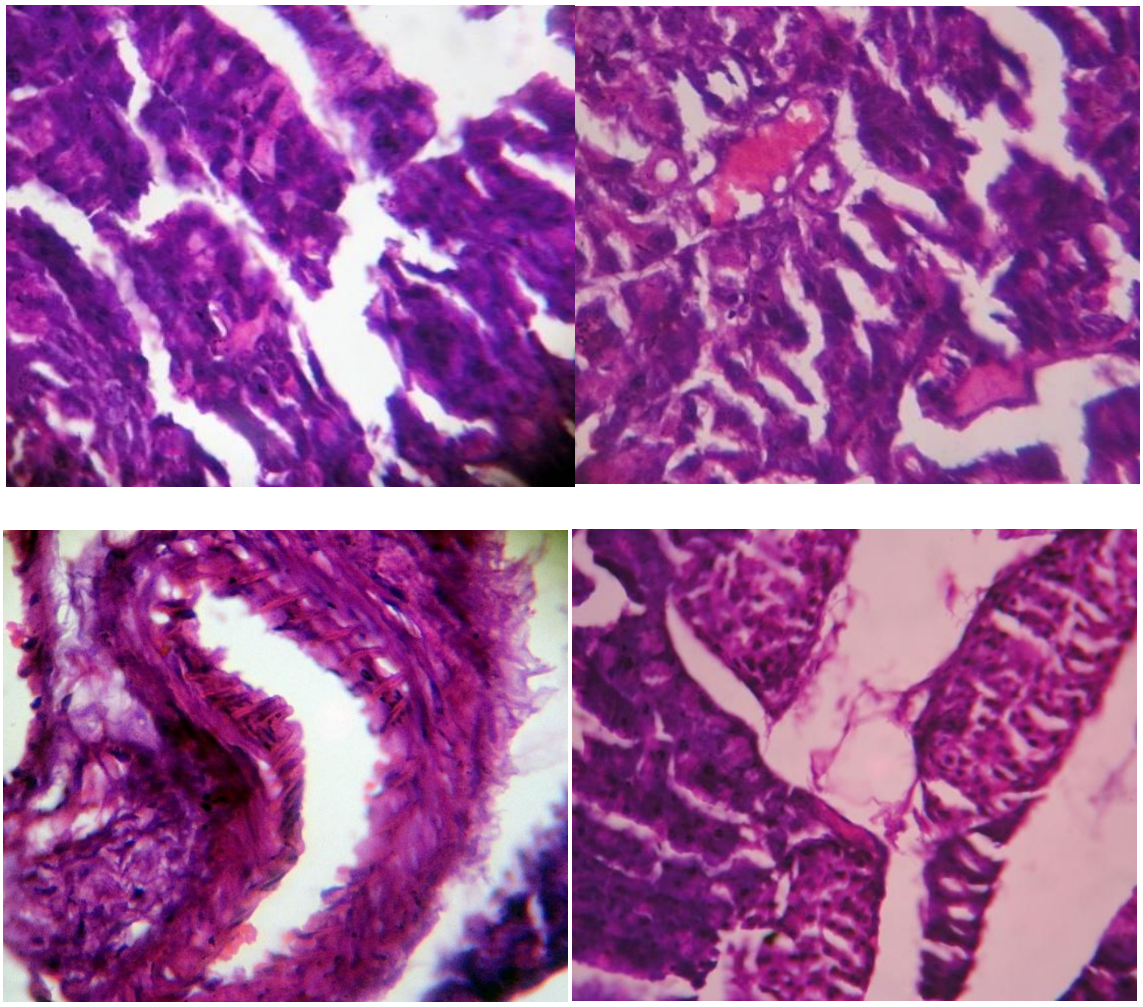


Fig. 04: Graphical representation of effect of various extracts of leaf of *Balanites aegyptiaca* on levels of Total Cholesterol, TGL, HDL, LDL and VLDL.



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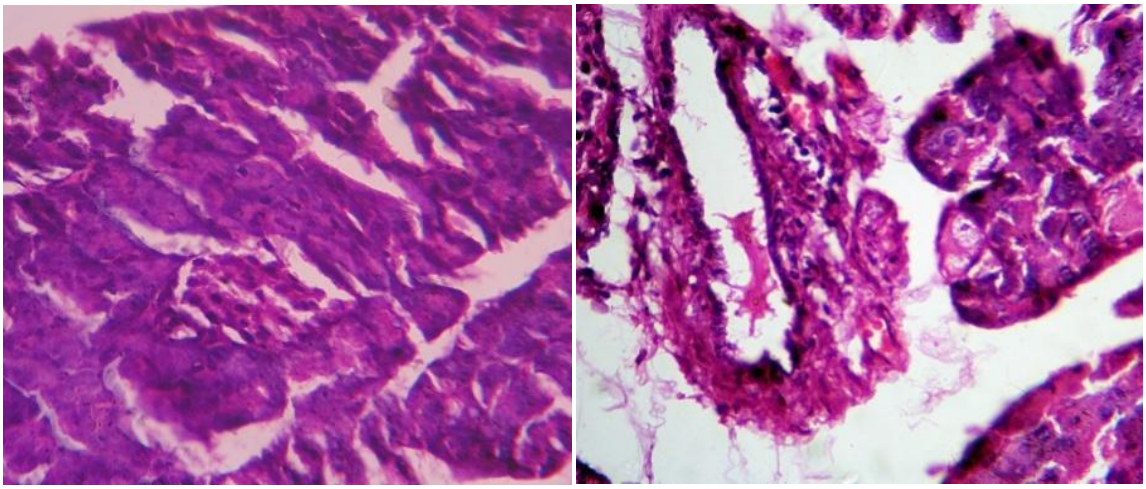
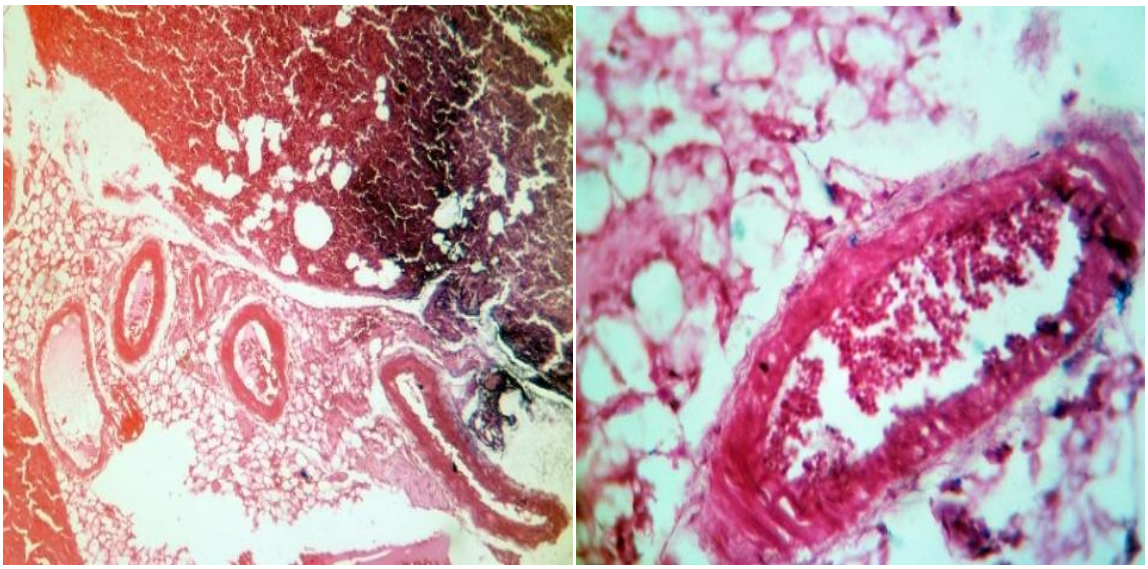
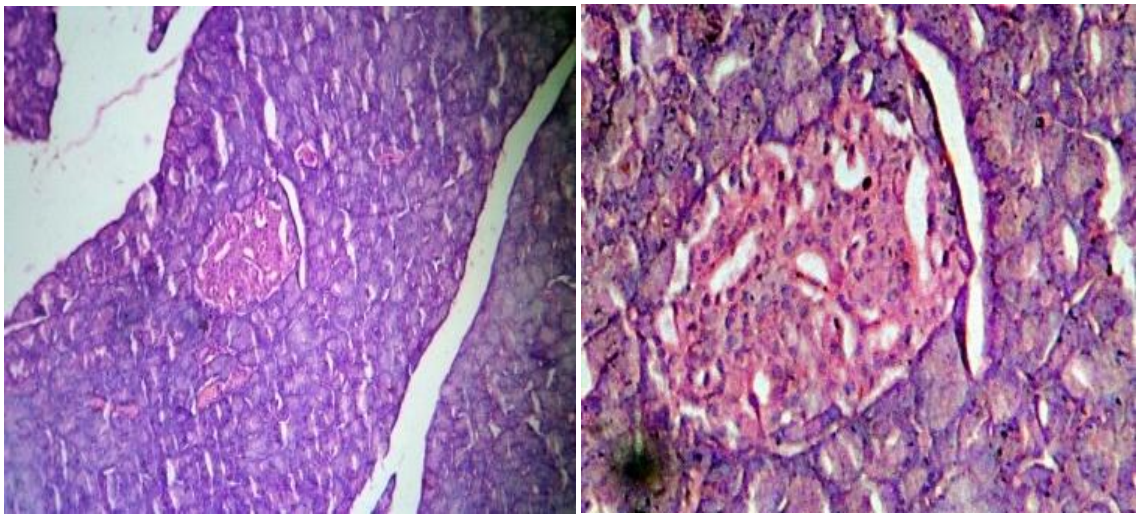


Fig. 05:Section ofpancreatic tissue of normal control mice.





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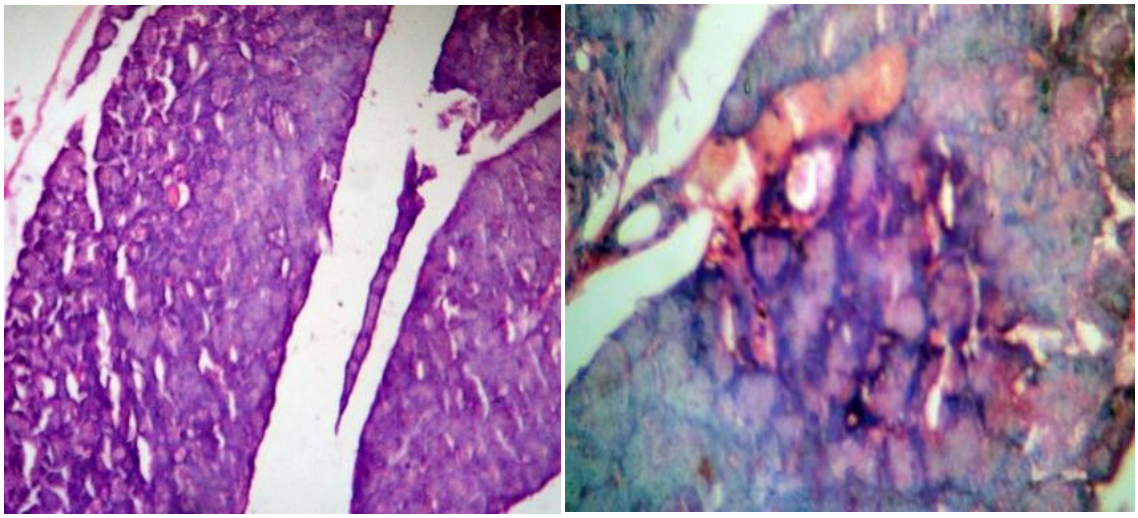
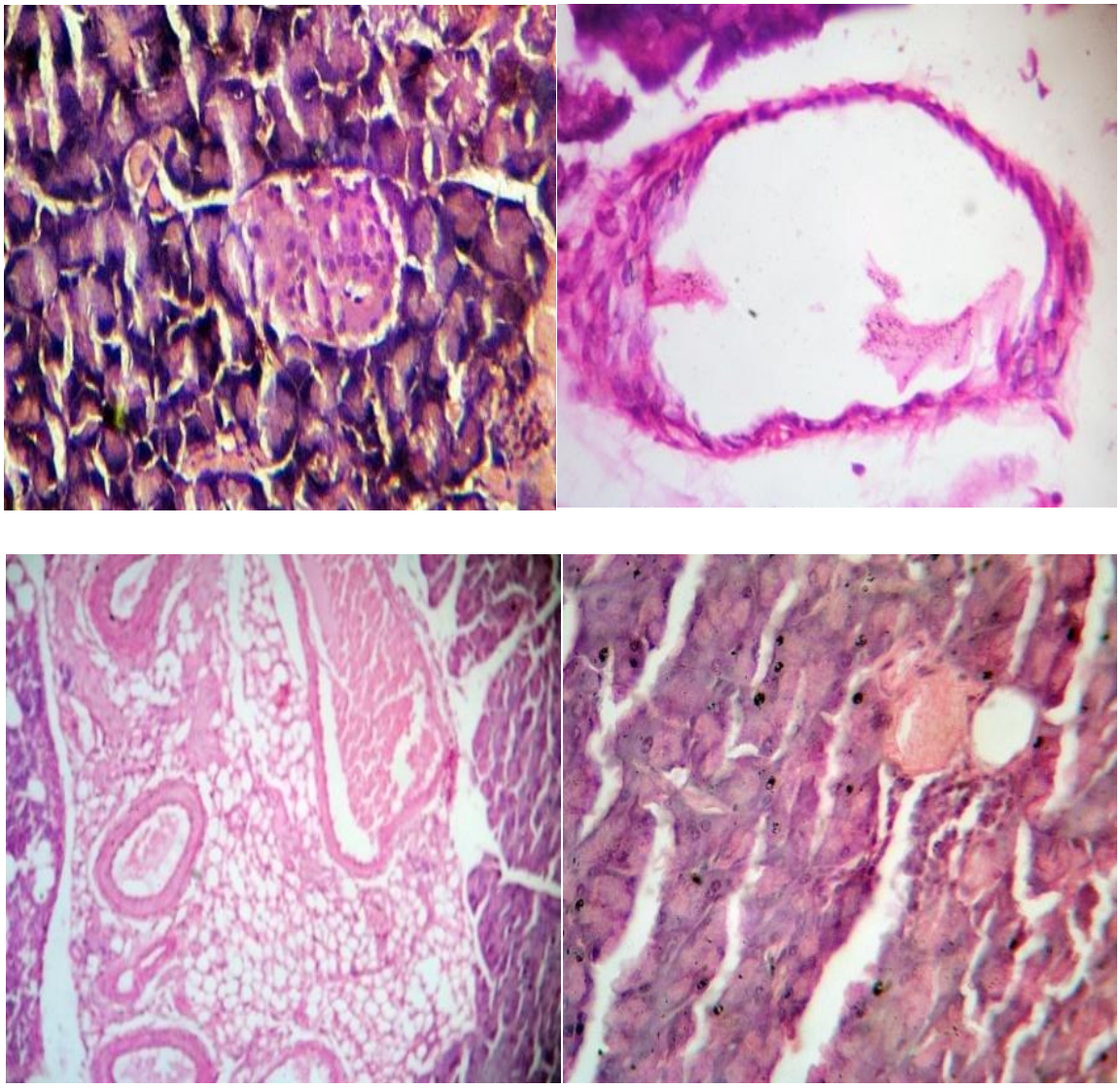


Fig. 06:Section of pancreatic tissue ofSTZ diabetic control mice.



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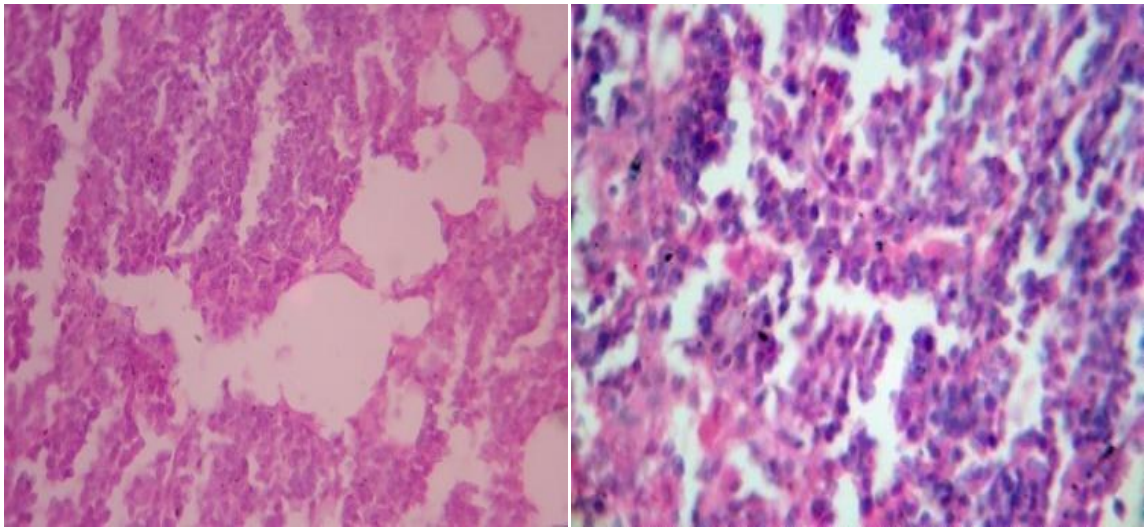

Fig. 07:Section of pancreatic tissue of glibenclamide treated mice.

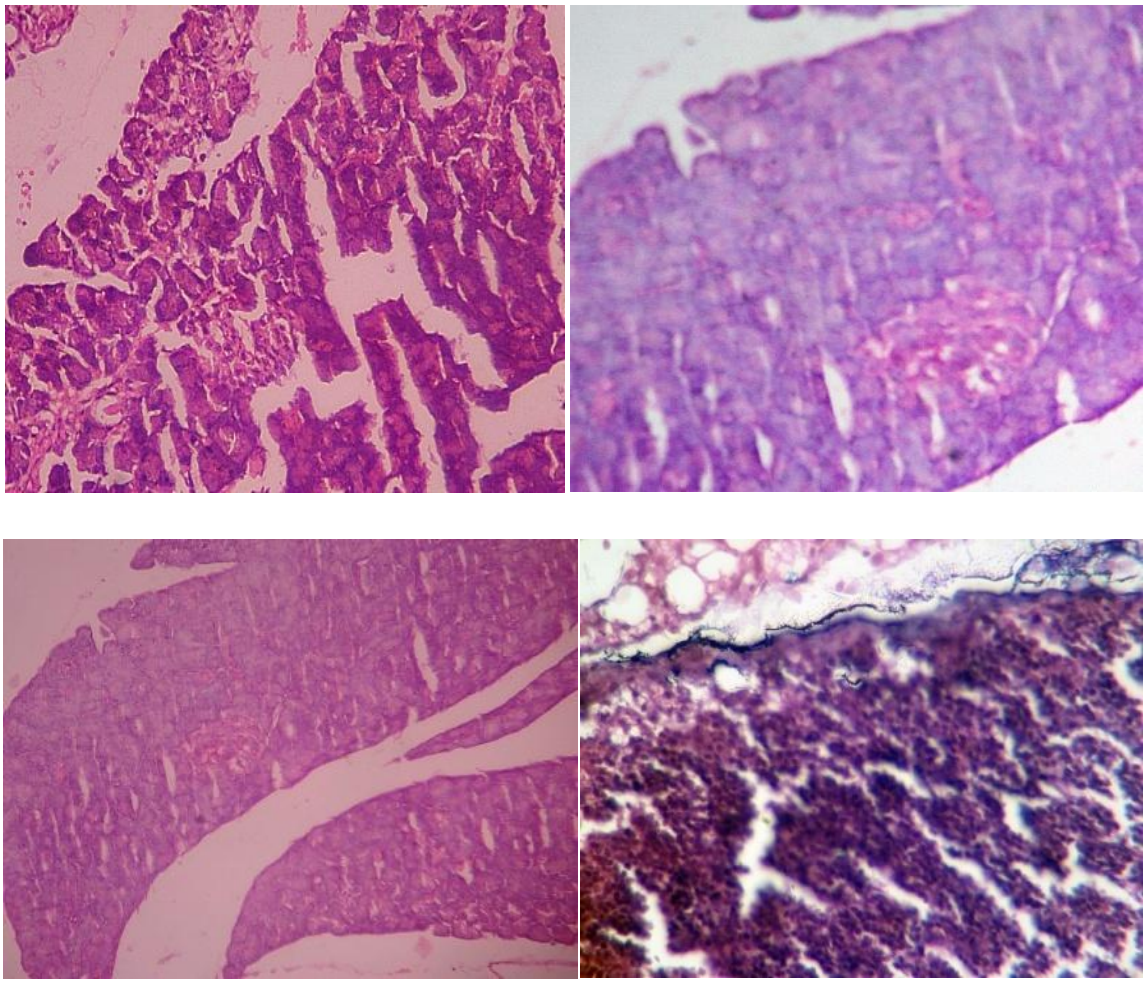

Fig. 08:Section of pancreatic tissue of mice treated with pet ether extract of leaves of *Balanites aegyptiaca* (L.) Delile

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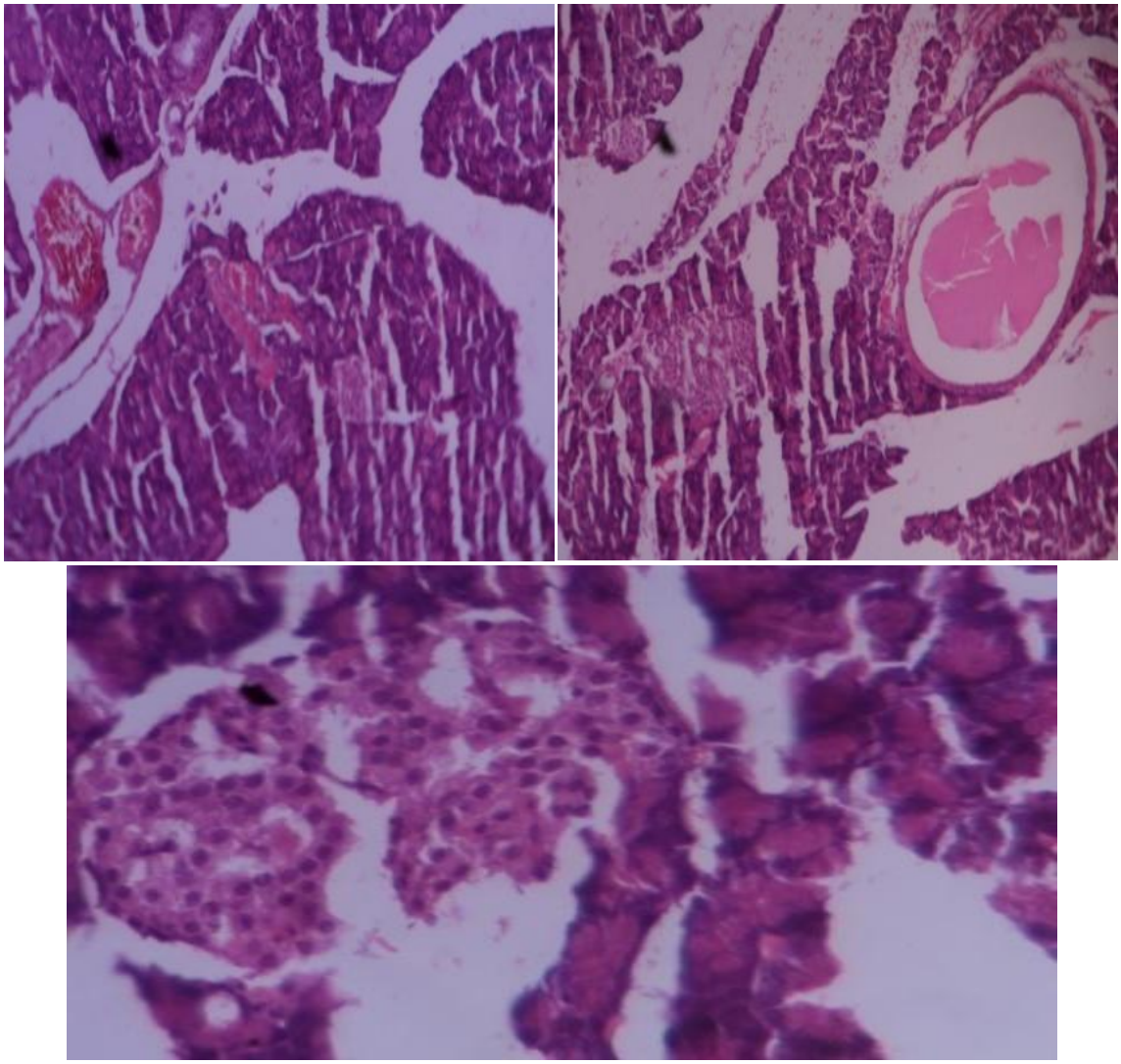
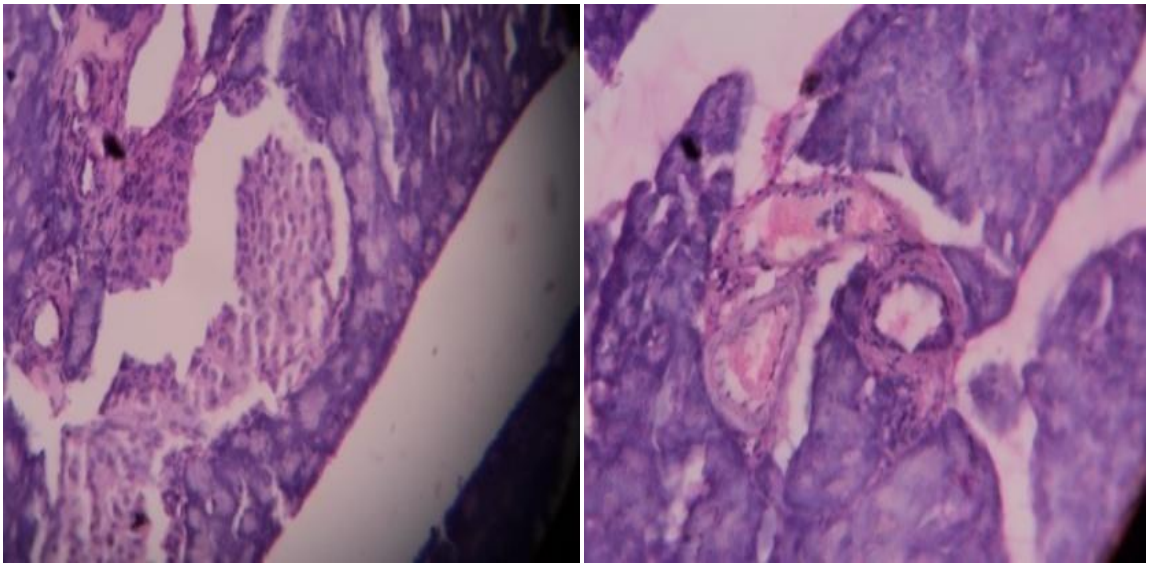


Fig. 09:Section of pancreatic tissue of mice treated with alcoholic extract of leaves of *Balanites aegyptiaca* (L.) Delile



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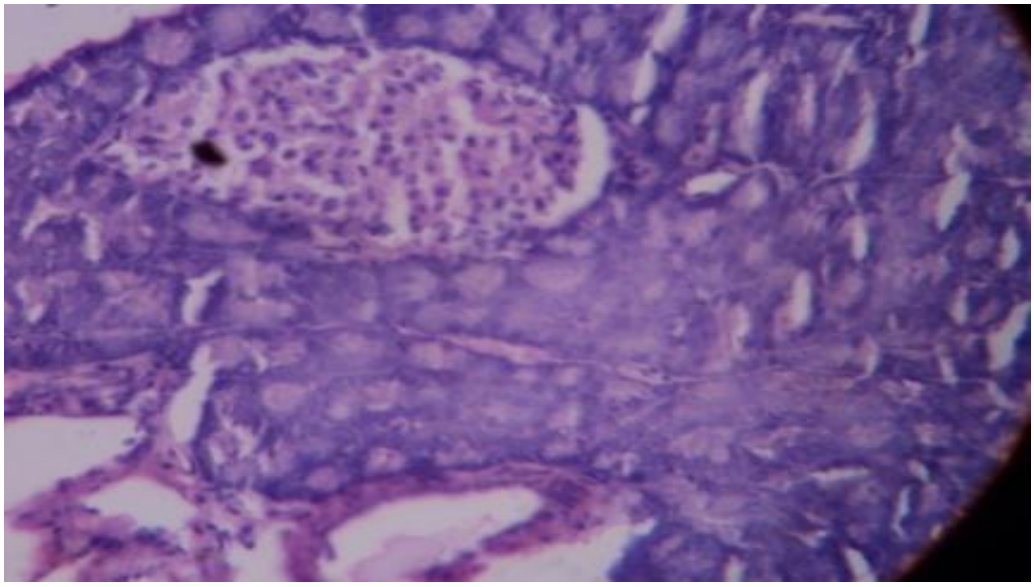

Fig. 10:Section of pancreatic tissue of mice treated with aqueous extract of leaves of *Balanites aegyptiaca* (L.) Delile