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Anti-Diabetic Effect of Aqueous and Ethanolic Extract of Dried Leaves of *Phoenix Dactylifera* in Alloxan Induced Hyperglycaemia Albino Rat

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Original Research Article

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Abstract: Diabetes mellitus (DM) is commonly referred to as a "sugar" and it is the most common endocrine disorder and usually occurs when there is deficiency or absence of insulin or rarely, impairment of insulin activity (insulin resistance). The powdered leaves phoenix dactylifera was extracted with 400ml of 70 % v/v of ethanol and water for 5 hour in soxhelet. Alloxan monohydrate at a dose of 150 mg/kg injected intraperitonally for induction of diabetes mellitus. The extract of *Phoenix dactylifera* leaves was subjected to anti-diabetic activity in rats. Group-IV & Group-V received the ethanolic & aqueous extract of phoenix dactylifera leaves(500mg/kg) shown a marked reduction in blood glucose level on day 4th i.e. in ethanolic extract it was 284.76±mg/dl while in aqueous extract it was 264.76±6.65. On 10th day of study the glucose level falls up to 264.40±7.72 in ethanolic extract rat & 254.05±9.18 in aqueous extract. The results of the present study indicate that *Phoenix dactylifera* leaf extract was found to reduce the glucose level in animals made diabetic with alloxan. In the present investigation ethanolic extract of *Phoenix dactylifera* leaf showed significant anti-diabetic activity.
Keywords: Diabetes mellitus, insulin, date palm, alloxan, antidiabetic, phoenix dactylifera.

INTRODUCTION

Diabetes mellitus is a chronic disorder of carbohydrates, fats, and protein metabolism. A defective or deficient insulin secretary response, which translates into impaired carbohydrates (glucose) use, is a characteristic feature of diabetes mellitus, as is the resulting hyperglycemias [1].

The insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure is found in type 2 diabetes mellitus. This leads to a decrease in glucose transport into the liver, muscle cells and fat cells. There is an increase in the breakdown of fat with hyperglycaemia [2, 3]. The hyperglycaemia of diabetes is indicated with long-term defects, and failure of many organs, such as eyes, kidneys, nerves, heart, and blood vessels [4]. Pharmacological treatment of DM is based on oral hypoglycaemic agents and insulin. The most important adverse effect of insulin are weight gain and hypoglycaemia when inappropriate dose of insulin is taken and when there is mismatch between meals and insulin injection [5,6]. Therefore, there is a need for natural and non-toxic antidiabetic drugs for diabetic therapy. Some studies have demonstrated the hypoglycaemic effects of different parts of various species of palm.

Date palms are loaded with various medicinal importances for ailment of certain diseases. Because of its high nutritional value and its long life the date palm has been known as the 'tree of life'. All part of date palms are useful for various purposes [7]. The leaves of Phoenix dactylifera show antidiabetic activity [8]. It has been shown that an aqueous extract from the leaves of the European fan palm, *Chamaerops humilis*, decreased plasma glucose levels as well as total cholesterol and triglycerides levels [9]. The alcoholic extract of palm seeds decreased the blood glucose and lipid concentration in male diabetic rats [10, 11].This research work is focused on understanding the effects of the aqueous & alcoholic extract of *Phoenix dactylifera* on some hyperglycaemia induced albino rat.

MATERIALS AND METHODS

The leaves of Phoenix dactylifera were collected in the month of April from the surrounding fields of Bhelara, Sultanpur and aunthenthicated by G. P. Singh (Scientist-E / Head Of Office) Botanical Survey Of India, CRC, 10 Chatham Line, Allahabad-211002.Then it was dried and coarsely powdered using hand grinding machine and were passed through sieve n. 60 and stored in air tight containers before extraction.

Preparation of Ethanolic extract

The leaves of phoenix dactylifera was thoroughly washed and reduced into small pieces before being ground and powdered into particles (about 1 mm in size). Then the powder was put into a hot air oven at 60°C until complete drying. 100g of the leaves was extracted with 400ml of 70 % v/v of ethanol and water for 5 hour in soxhelet. The extracts were filtered and evaporated under vacuum at 45°C before being dried [12].

Preparation of Aqueous extract

The leaves of phoenix dactylifera was thoroughly washed and reduced into small pieces before being ground and powdered into particles (about 1 mm in size). Then the powder was put into a hot air oven at 60°C until complete drying. 100g of the leaves was extracted with 400ml of distilled-water for 5 hour in soxhelet. The extracts were filtered and evaporated under vacuum at 45°C before being dried [12].

Animals

Adult Albino rats of wistar strain (150-200g) of either sex were procured from Hyderabad And were housed in the animal house of Shambhunath Institute of Pharmacy Jhalwa Allahabad, with 12 h light and 12 h dark cycle, standard pellets obtained from Goldmohar rat feed, Mumbai, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water *ad libitum.*

Preliminary phytochemical screening

Phoenix dactylifera leaves extract was subjected to qualitative chemical screening to identify the various major classes of active chemical constituents, namely tannins, steroid, terpenoids, saponins, flavonoids, and alkaloid[13].

Test for Tannins

500 mg of extract was stirred with about 10 mL of distilled water and then filtered. Four drops (0.3 ml) of 1% ferric chloride solution were added to 2 mL of the filtrate. The occurrence of a blue–black, green, or blue–green precipitate indicated the presence presence of tannins[14].

Test for Steroids

To 0.2 g of extract, 2 mL of acetic acid was added, the solution was cooled in ice, and then concentrated H_2SO_4 was carefully added. Colour development from violet to blue or bluish green indicated the presence of a steroidal ring, i.e., a glycone portion of cardiac glycoside [15].

Test for Terpenoids

100 mg of extract was dissolved in ethanol. Then, 1 mL of acetic anhydride was added, followed by the addition of concentrated hydrogen sulphate. A change in colour from pink to violet showed the presence of terpenoids[15].

Test for Saponins

1 g of extract was boiled with 5 mL of distilled water and filtered. Then, 3 mL of distilled water was added to the filtrate, and the mixture was shaken vigorously for about 5 minutes. Frothing that persisted upon warming was taken as evidence of the presence of saponins[12].

Shinodas Test for flavonoids

500 mg of extract was dissolved in ethanol, warmed, and then filtered. Three magnesium chips were then added to the filtrate followed by few drops of concentrated hydrochloric acid. Colour changing from pink, orange, or red to purple indicated the presence of flavonoids [12].

Ferric Chloride Test for Flavonoids

500 mg of extract was boiled with distilled water and then filtered. Four drops (0.3 ml) of 10% ferric chloride solution were then added to 2 mL of the filtrate. A green-blue or violet colour indicated the presence of a phenolic hydroxyl group[12].

Test for Alkaloids

100 mg of the extract was stirred with 5 mL of 1% aqueous hydrochloric acid in a water bath and subsequently filtered. Then, 1 mL filtrate was taken individually into 2 test tubes. To the first portion, 4 drops of Dragendroffs reagent were added; the occurrence of an orange–red precipitate was taken as positive. To the second portion, Mayer's reagent was added, and the appearance of a buff-coloured precipitate indicated the presence of alkaloids.

Acute toxicity assessment

To assess acute toxicity, the extract was orally administered in graded doses (1, 2, 4, 6, and 8 g/kg) to 5 treatment groups, while the control group received saline (5 mL/kg). All treated animals were closely observed for any abnormal or toxic manifestations and mortality up to 48 hours.

Assessment of Oral glucose tolerance test

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight (18 h) fasted rats were fed glucose (2 gm/kg) orally and blood samples were collected from tail puncturing of each rat at 0 minute, 30 minute, 60 minute and 120 minute and blood glucose was estimated using glucometer. Percent reduction in blood glucose was calculated with respect to the initial level[16].

Induction of diabetes

Alloxan monohydrate was first weighed individually for each animal according to their weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg b. wt. intraperitonally. After 1 hour of alloxan administration, the animals were given feed *ad libitum*, and 5% dextrose solution was also given in a feeding bottle for a day to overcome the early hypoglycaemic phase. The animals were kept under observation and after 48 hour blood glucose was measured by glucometer. The diabetic rats (glucose level >300 mg/dl) were separated and divided into six different groups for experimental study, each group containing six animals.

Experimental Design: [17]
Each Group contains 6 rats.
Group-I: Normal control
Group-II: Normal rat treated with Alloxan
Group-III: Diabetic rat treated with Aq. Extract of Phoenix dactylifera (500mg/kg b. wt. p.o.)
Group-IV: Diabetic rat treated with ethanolic extract of Phoenix dactylifera (500mg/kg b.wt.)
Group-V: Diabetic rat treated with Glibenclamide (Std. Drug) 10mg/kg (aq. Suspension)

Measurement of blood glucose level

Blood glucose levels were measured by the Accu-chek Active glucometer (Dr. Morepan), blood and glucose strips (Roche Diagnostic Pvt Ltd, Mumbai) blood glucose testing system (glucose strip method). The tip of the tail was snipped with sharp scissors and gently squeezed for a drop of blood. The strip was inserted into the machine, and the drop of blood was placed on the strip. Within 20 seconds, the instrument measured and displayed the blood glucose level. The blood glucose level of the rats were taken just before the administration of alloxan (for the group that received alloxan) to induce mild and severe hyperglycaemia. The treatment was started on the same day except normal control and diabetic control groups for the period of 10 days. During this period animals in all groups had free access to standard diet and water. Blood glucose level of all group were estimated on 4^{th}, 7^{th} and 10^{th} day of the treatment.

RESULT

Preliminary phytochemical screening of the leaves of Phoenix dactylifera

Preliminary study was performed on the various extracts of the leaves of Phoenix dactylifera and the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, and tannins were determined and the results were presented in the Table1.

Table-1: Phytochemical analysis of the date palm leaves extracts

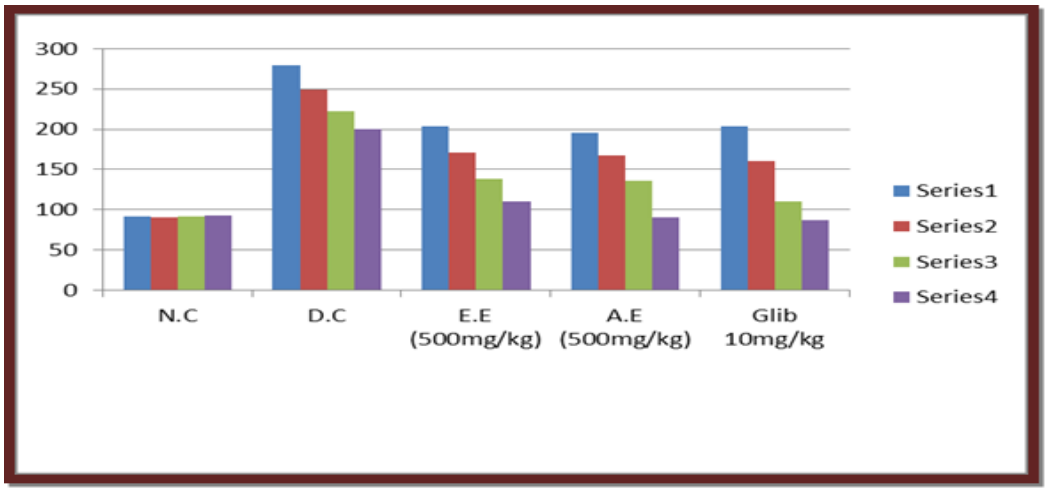
Chemical Group	Results
Tannins	+
Alkaloids	-
Terpenoids	-
Saponins	+
flavonoids:	+
Steroids	+
Phenols	+

Positive sign (+) indicates the presence and negative sign (-) indicates the absence

Oral glucose tolerance test (OGTT)

Table-2: Effect of aqueous and ethanol extract of Phoenix dactylifera on OGT of diabetic rats

Group	Treatment	Blood glucose levels (mg/dl)			
		0 min	30min	60 min	120 min
I	Normal control	92±1.3	90±1.2	91±1.4	93±0.9
II	Diabetic control, 0.5% Tween 80	279.2±1.9	248.9±1.6	222±1.02	200.1±0.8
III	Ethanolic extract (500mg/kg)	203.3±0.6	170.6±1.05	138.3±0.76	109.9±1.5
IV	Aqueous extract (500mg/kg)	195.5±2.1	167.7±0.76	136±0.11	90.1±0.7
V	Glibenclamide, 10mg/kg	204±1.9	160±2.26	110±1.5	87.4±2.6



N.C= Normal control, D.C= Diabetic control, E.E= Ethanolic extract, A.E= Aqueous extract, Glib= Glibenclamide.

Fig-1: Graph representing effect of phoenix dactylifera on OGTT

Anti -diabetic study

Ethanolic & Aqueous extract of Phoenix dactylifera leaves was subjected to anti-diabetic activity in rats where alloxan monohydrate (150mg/kg b.wt., p.o) used as the diabetogenic agent. A marked rise in fasting blood glucose level observed in diabetic control rats. Ethanolic extract of phoenix dactylifera (at 500mg/kg) exhibited a dose dependent significant anti-hyperglycemic activity on 4^{th}, 7^{th} and 10^{th} day post treatment. Aqueous extract of phoenix dactylifera (500mg/kg) exhibited a dose dependent significant anti-hyperglycemic activity on 4^{th}, 7^{th} and 10^{th} day post treatment. The antihyperglycemic effect of ethanol extract was found less effective than the reference standard, Glibenclamide. The basal value of blood glucose level in normal control was 87.45±3.50 it remain almost same on 4^{th}, 7th, &10^{th} of study. While in diabetic control group (Group-II) where no treatment provided due to basal value was 295.6±5.25mg/dl which proves the induction of diabetes due to alloxan. Group-IV & Group-V received the ethanolic & aqueous extract of phoenix dactylifera leaves(500mg/kg) shown a marked reduction in blood glucose level on day 4^{th} i.e. in ethanolic extract it was 284.76±mg/dl while in aqueous extract it was 264.76±6.65. On 10^{th} day of study the glucose level falls up to 264.40±7.72 in ethanolic extract rat & 254.05±9.18 in aqueous extract.

The rat treated with standard drug glibenclamide shown a marked reduction in blood glucose level on 4^{th} day (204.26±7.05mg/dl) & 10^{th} day (172.12±6.21). Glibenclamide produced a significant reduction in blood glucose compare to diabetic control. The results are shown in the Table No.3.

Table-3: Effect of phoenix dactylifera leaf extract on fasting blood glucose level in alloxan induced diabetic rats

Group	Treatment	Fasting blood glucose level (mg/dl)			
		Basal value	4^{th} day	7^{th} day	10^{th} day
I	Normal Control	87.45±3.50	91.80±2.91	93.31±1.72	89.30±3.42
II	Diabetic Control (Vehicle)	295.6±5.25	288.90±5.04	292.5±5.40	290.42±9.74
III	Alloxan + Glibenclamide (10mg/kg)	282.8±6.91	204.26±7.05	182.17±6.32	172.12±6.21
IV	Alloxan + Ethanolic extract (500mg/kg)	293.75±4.77	284.76±5.66	272.22±8.18	264.40±7.72
V	Alloxan + Aqueous extract (500mg/kg)	285.46±5.34	264.24±6.65	259.86±9.96	254.05±9.18

Values are in mean ±S.E.M: n=6

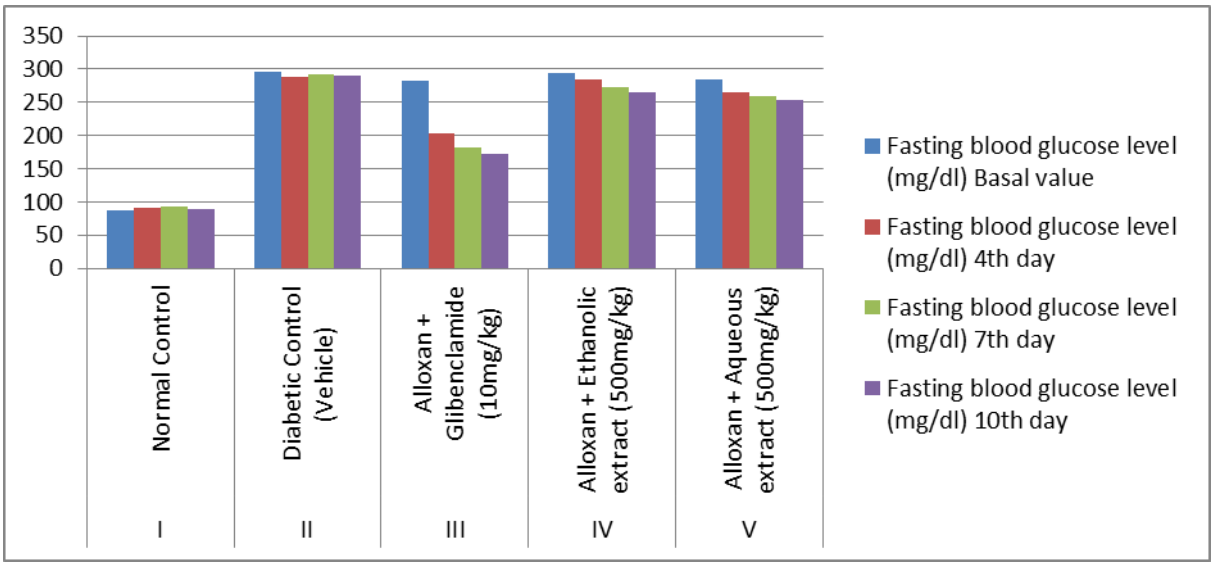


Fig-2: Graph representing effect of *phoenix dactylifera* leaf extract on fasting blood glucose level in diabetic rat

Effect of *phoenix dactylifera* leaf extract on body weight in diabetic rat:

Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 10 days. Alloxan mediated body weight reduction was significantly reversed by the ethanolic extract in dose dependent fashion (500mg/kg). Alloxan mediated body weight reduction was significantly reversed by the aqueous extract in dose dependent fashion (500mg/kg). The results are shown in table 4.

Table-4: Effect of phoenix dactylifera leaf extract on body weight of alloxan induced rat

Group	Treatment	Body weight of the animal (g)			
		Initial	4th day	7th day	10th day
A	Normal Control	210.52±2.72	212.00±2.76	216.58±2.82	215.92±2.84
B	Diabetic Control (Vehicle)	204.52±2.88	175.02±2.58	156.04±2.50	142.02±1.70
C	Standard (Alloxan + glibenclamide 10mg/kg)	212.52±2.82	208.01±2.62	186.52±2.00	180.02±1.92
D	Alloxan + Ethanolic extract (500mg/kg)	208.22±2.75	170.04±2.42	162.02±2.30	150.03±1.70
E	Alloxan + Aqueous extract (500mg/kg)	206.52±2.36	200.22±2.12	190.54±1.86	182.52±1.22

Values are in mean ±S.E.M: n=6

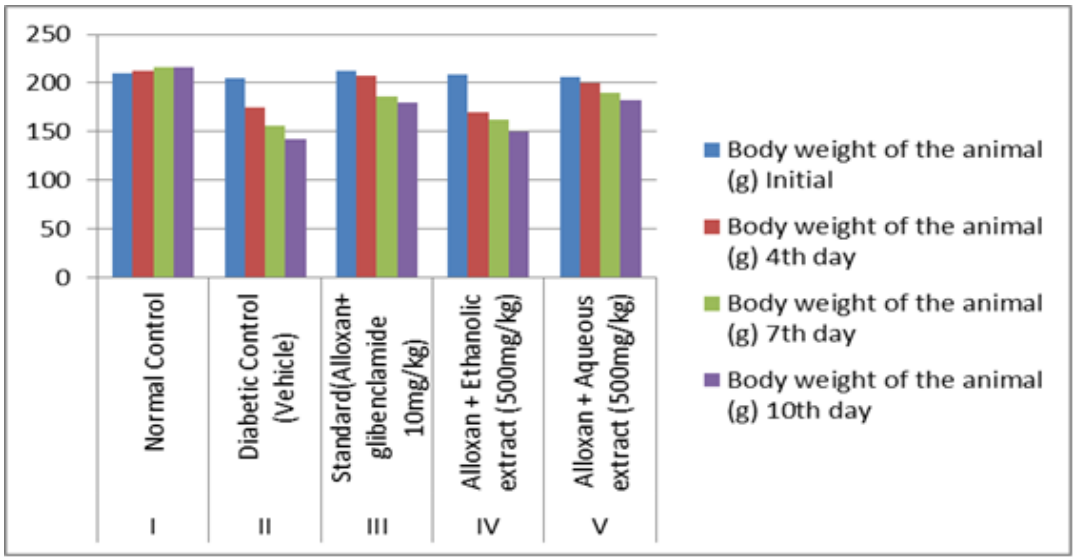
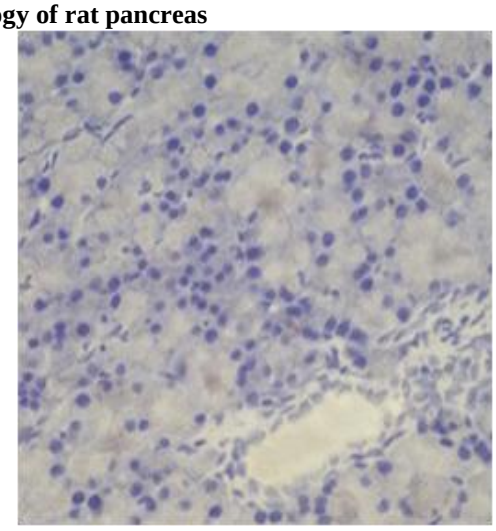
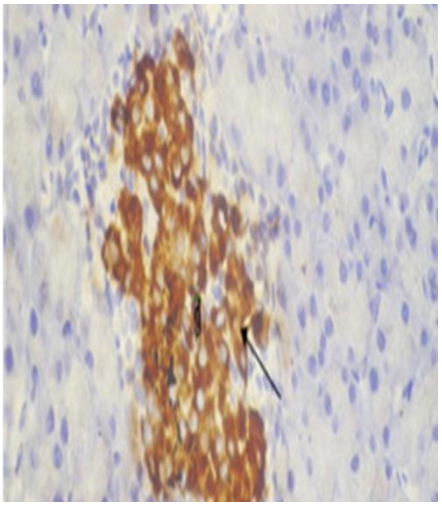


Fig-3: Graph representing effect of *phoenix dactylifera* leaf extract on body weight in diabetic rat

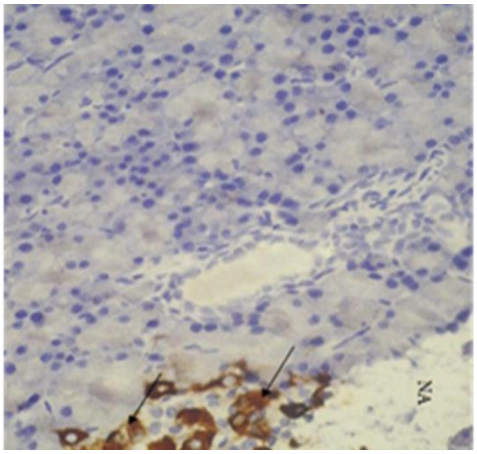
Histology of rat pancreas



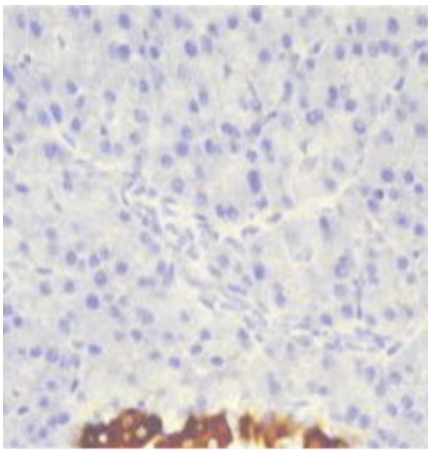
Normal rat pancreas



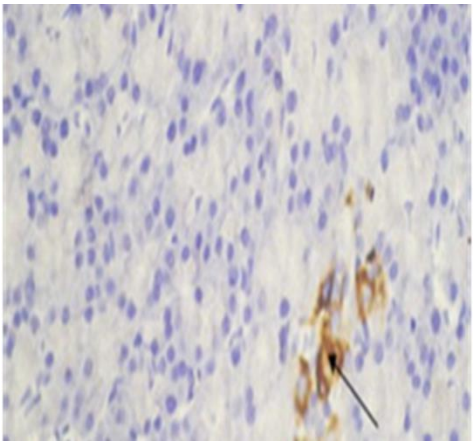
Diabetic control



Treatment with ethanolic extract



Treatment with aqueous extract



Treated with standard drug

CONCLUSION

Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the islets of Langerhans, thereby inducing hyperglycemia. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases. The results of the present study indicate that *Phoenix dactylifera* leaf extract was found to reduce the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is

especially susceptible to the action of alloxan induced free radical damage. In the present investigation ethanolic extract of *Phoenix dactylifera* leaf demonstrated the significant anti-diabetic activity.

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