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BIOLOGICAL ACTIVITIES OF LONG TERM TREATMENTS WITH *HALOXYLON SALICORNICUM* AND GLIBENCLAMIDE IN NORMAL AND ALLOXAN HYPERGLYCEMIC DIABETIC RABBITS

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ABSTRACT

Hypoglycemic activity of *Haloxylon salicornicum* and Glibenclamide were studied in normal and diabetic rabbits. The drugs were adminstered once daily at dose of 1g / Kg at *H.salicornicum* and 10 mg / Kg at Glibenclamide by gavage for one month. Serum glucose level, creatinine level, total bilirubine and total protein were reduced significantly in 15-30 days. The serum Transaminase (AST and ALT activities) had effect on the treated groups.

INTRODUCTION

A new wave of research interest in traditional practices which might be used to alleviate the symptoms of diabetes mellitus has been stimulated by the renewed attention to natural therapies, and by the recommendation of the world health organization expert committee which stated that traditional methods for treatment of diabetes mellitus should be thoroughly investigated (22).

The relevant literature on plants which are known to be used in folk-medicine of different culture for their hypoglycemic properties (i.e., decreasing blood glucose concentration) and therefore used in the treatment of diabetes mellitus have been reviewed extensively (3).

Haloxylon salicornicum is adesert plant which belongs to *Chenopodiaceae* family The plant is typical of aride regions and represents xerophytic species adapted to extremes of salinity, temperature, moisture fluctuations and wide variety of endemic factors. A wide variety of nutrition and multiple stress to learns shrubs. This plant species, though slow growing, respond very well to the favorable climatic condition particularly the timely rains and produce anple fodder (1,2). This plant grows in sandy soil (23). It contains various chemical constituents such as sodium (28.48 %), potassium (9.49 %) carbonate (61.06 %) and it's distributed in Iraq, Syria, Kuwait, Iran, Afghanistan and Pakistan (8,23).

In the traditional system of medicine, it is recommended in a wide range of ailments including antibacterial (5), diuretics activity (21) and antinuclear (8). In addition, the powder of *Haloxylon salicornicum* used forms a common house hold remedy for disease treatment.

However, no scientific studies of this preparation have been undertaken so far.

In the present investigation, an attempt has been made to study the effect of this herbal powder preparation on blood glucose level, serum AST,ALT, total bilirobine, total protein and creatinine levels in normal and diabetic rabbits. The doses chosen for this study were emoirically based on that used by local diabetic subjects and compared with Glibenclamide.

MATERIALS AND METHODS

Plant Material :

Dried plant of *Haloxylon salicornicum* was purchased from local market of Basrah and identified. Plant was kept in a sealed container at room temperature until used. The

aqueous extract prepared by infusion 50g of the plant was extracted with (500 ml) of distilled water over night till complete exhaustion and the filtrate was combined and concentrated under reduced pressure on a rotary evaporator below 40°c (10).

Animals :

A (36) adult rabbits of both sexes weighting 1.5-2 Kg respectively, obtained from local market. The animals were maintained on a 12 hour light – dark cycle with given regular laboratory stock diet and evaluated after over night fast for the pre-dose blood analyses. Biochemical analysis of serum was done in normal and diabetic rabbits.

Following a single i.p. dose of alloxan monohydrate (England BDH) 150 mg / Kg as 10 % saline solution, rabbits with blood glucose levels above 400 mg / 100ml were included in the experiment.

Experimental design :

Normal and alloxan diabetic rabbits were divided into 3 groups (6) rabbits for each. The first group was kept as a control and given 3ml of normal saline for 30 successive days. The Glibenclamide was given to the 2nd group (10mg/ Kg) dissolved in 3ml of normal saline. Third group was administered (1g / Kg) of aqueous extract of plant dissolved in 3ml of normal saline.

Sampling blood was carried out only on the day 30th immediatly following administration of the last dose of the extract to overnight fasted rabbits. Blood samples were collected from the heart at 0,1,4,6,24h,15 & 30days after administration of the last dose. Glucose was determined in serum obtained from collected blood samples by the glucose oxidase method (7, 20), using liquid glucose GOD-PAP Kit. For revealing the changes in the activity of some enzymes in normal and diabetic, after treatments enzymes AST and ALT were determined photometrically as described by (16). The level of total bilirubin, total protein and creatinine in serum was measured according to the method explained by (19, 11) and (12) respectively.

Statistical Analysis :

Significant differences between different treatment were calculated using ANOVA test as appropriate. The results were reported as mean ± SD mean with N= number of animals used in the experiments.

RESULTS

Hypoglycemic Activity:

In normal rabbits, the glucose level was reduced significantly ($P< 0.05$) after 4-6 hour treatments (table 1). Highly significant decrease ($P< 0.01$) in glucose levels was noticed in both treatment aqueous extract and glibenclamide after 24 hour- 30 days.

In alloxan diabetic rabbits, the aqueous extract of plant ameliorated the diabetic condition. The aqueous extract dose (1g / Kg) reduced the glucose level (50%) after 6hour ($P< 0.05$). Also after 30 days the reduction was upto (50 %) ($P< 0.01$) almost to the same extent as did glibenclamie. In addition, aqueous exract reduce the glucose level better than the other drugs. Hence it can be summrized that the beneficial effect of aqueous extract could be due to hypoglycemic action.

Biochemical Analysis of Serum in Normal and Diabetic Rabbits :

The tested aqueous extract plant had effect on ALT and AST activities in all treated groups (table 2). Total billirubine and total protein were not altered in both normal and diabetic rabbits during the 1st 24 hour, while it's value was reduced between significant ($P< 0.05$) and highly significant ($P< 0.01$) after 15- 30 days (table 3). Creatinic concentration was significantly reduced in normal treated groups after 1 hour till the end of treatments ($P< 0.01$). On the contrary, the creatinine level was increased significantly in treated diabetic rabbits at the same time (table 3). However, these values decreased significantly after 30 days of treatments.

Table 1 : The effect of aqueous extract of plant and Glibenclamide on blood serum glucose level (mg / 100 ml) in normal diabetic rabbits (Mean ± SD), N = 6, N.S* = Normal saline.

Condition	0 time	1 hour	4 hour	6 hour	24 hour	15 days	30 days
Normal control 3ml N.S	140.18 ± 0.85 a	140.17 ± 1.30 a	140 ± 1.20 a	138 ± 0.79 a	136.23 ± 0.41 a	136 ± 0.36 a	138.32 ± 1.80 a
Normal 10mg/ Kg Glibenclamide	142.23 ± 2.10 a	139.32 ± 1.42 a	115.33 ± 1.09 A	101.67 ± 1.03 A	91.31 ± 1.05 B	90.33 ± 1.05 B	79.50 ± 1.51 B
Normal 1g /Kg aqueous extract	141.30 ± 1.30 a	130.33 ± 1.02 a	110.12 ± 1.70 A	92.15 ± 1.51 A	80.36 ± 1.78 B	72.30 ± 1.76 B	60.30 ± 1.76 B
Diabetic control 3ml N.S*	390 ± 0.82 a	386.19 ± 0.80 a	385 ± 1.16 a	385.20 ± 2.11 a	326.27 ± 2.90 a	360.27 ± 2.80 a	335.24 ± 3.10 a
Diabetic 10mg/Kg Glibenclamide	385.67 ± 3.61	300.17 ± 3.11	278.28 ± 0.9 a	253.16 ± 2.13 a	173.60 ± 4.10 b	170.60 ± 4.12 b	100.43 ± 4.15 b
Diabetic 1 g /Kg aqueous extract	355.10 ± 3.59 a	293.45 ± 3.39 a	272.40 ± 2.09 a	240.20 ± 1.80 a	165.12 ± 1.0 b	161.40 ± 1.70 b	94.81 ± 2.33 b

A: $p < 0.05$, B: $p < 0.01$ vs corresponding control.
a : (non significant)
b : $p < 0.01$ vs corresponding diabetic control.

Table (2) : The effect of aqueous extract of plant and Glibenclamide on serum AST and ALT activities unit / L in normal and diabetic rabbits. (Mean ± SD , N = 6. N.S = normal saline).

Condition	0 time	1 hour	4 hour	6 hour	24 hour	15 days	30 days	
Normal control 3ml N.S	10.3 ± 2.6	10.2 ± 1.7	10 ± 2.3	9.8 ± 2.5	10.8 ± 1.3	10.4 ± 1.2	11 ± 2.1	AST
	13 ± 3.2	13.4 ± 1.8	12.7 ± 1.7	13.2 ± 1.5	14.5 ± 1.4	13.8 ± 1.2	14 ± 2.5	ALT
Normal 10mg/ Kg Glibenclamide	14 ± 2.1 a	14.2 ± 1.3 a	10.5 ± 2.4 a	14.2 1.6 a	12.5 ± 1.7 a	13.7 ± 1.8 a	14 ± 1.7 a	AST
	11.00 ± 0.7 a	9.2 ± 0.9 a	8.5 ± 1.3 a	9.7 ± 0.4 a	9.4 ± 3.1 a	10.00 ± 1.4 a	10.4 ± 1.7 a	ALT
Normal 1g /Kg aqueous extract	12.1 ± 0.8 a	13.7 ± 0.9 a	12.3 ± 1.9 a	12.7 ± 0.3 a	14.2 ± 0.4 a	11.9 ± 2.3 a	12.8 ± 3.4 a	AST
	8.4 ± 1.3 a	9.6 ± 0.5 a	10.2 ± 0.9 a	11.7 ± 1.4 a	12.1 ± 0.3 a	12.3 ± 1.2 a	11.9 ± 0.8 a	ALT
Diabetic control 3ml N.S	47.7 ± 1.3 a	47.1 ± 1.1 a	44.3 ± 1.7 a	44.5 ± 0.7 a	51.3 ± 1.9 a	62.3 ± 1.1 a	80.5 ± 0.1 a	AST
	30.4 ± 1.8 a	33.2 ± 0.6 a	34.00 ± 1.2 a	33.1 ± 0.7 a	30.7 ± 1.6 a	36.1 ± 0.3 a	32.1 ± 2.1 a	ALT
Diabetic 10 mg /Kg Glibenclamide	53.3 ± 2.00 a	51.0 ± 0.7 a	38.2 ± 1.9 A	30.3 ± 1.2 A	24.4 ± 2.5 B	16.2 ± 2.8 B	14.3 ± 1.0 B	AST
	33.1 ± 2.00a	30.00 ± 0.3 a	25.2 ± 0.6 A	19.2 ± 0.4 A	14.8 ± 0.1 A	12.00 ± 0.3 B	10.2 ± 1.5 B	ALT
Diabetic 1 g/Kg aqueous extract	44.00 ± 1.6 a	40.5 ± 0.7 a	31.00 ± 0.2 A	26.2 ± 0.4 A	20.00 ± 0.8 B	15.3 ± 1.6 B	10.00 ± 1.4 B	AST
	26.2 ± 0.4 a	24.7 ± 1.2 a	21.00 ± 0.5 A	18.1 ± 1.3 A	12.4 ± 0.2 B	10.00 ± 0.5 B	8.5 ± 1.1 B	ALT

A : P< 0.05 . B : P< 0.01 vs corresponding control .
a : (non significant)

Table (3) : The effect of Aqueous extract of plant on serum total bilirubine (mg / dl), total protein (g / dl) and creatinine concentration (mg %) in normal and alloxan diabetic rabbits (Mean ± SD) , N=6 , N.S = normal saline.

Condition	0 time	1 hour	4 hour	6 hour	24 hour	15 days	30 days	
Normal control 3ml N.S	0.37 ± 0.035	0.38 ± 0.024	0.39 ± 0.029	0.37 ± 0.023	0.24 ± 0.012	0.20 ± 0.010	0.18 ± 0.014	Total bilirubine
	7.40 ± 0.80	7.42 ± 0.71	7.30 ± 0.71	7.21 ± 0.90	7.00 ± 0.65	7.11 ± 0.30	6.79 ± 0.8	Total protein
	1.47 ± 0.066	1.65 ± 0.025	1.65 ± 0.022	1.58 ± 0.028	1.53 ± 0.020	1.46 ± 0.020	1.40 ± 0.021	Creatinin
Normal 10 mg / Kg Glibenclamide	0.034 ± 0.021	0.40 ± 0.027	0.42 ± 0.026	0.39 ± 0.022	0.23 ± 0.017	0.19 ± 0.10	0.15 ± 0.009	Total bilirubine
	7.00 ± 0.74	7.17 ± 0.63	7.00 ± 0.50	6.92 ± 0.42	6.11 ± 0.31	5.21 ± 0.21 A	4.5 ± 0.10A	Total protein
	1.38 ± 0.009	1.43 ± 0.018A	1.44 ± 0.005A	1.40 ± 0.019A	1.43 ± 0.019A	1.23 ± 0.023B	1.04 ± 0.14B	Creatinin
Normal 1g /Kg aqueous extract	0.33 ± 0.020	0.13 ± 0.021	0.34 ± 0.023	0.32 ± 0.020	0.20 ± 0.011	0.15 ± 0.008A	0.11 ± 0.005A	Total bilirubine
	7.15 ± 0.16	7.05 ± 0.21	7.00 ± 0.32	6.57 ± 0.25	6.00 ± 0.16	5.12 ± 0.11A	4.00 ± 0.13B	Total protein
	1.29 ± 0.018A	1.25 ± 0.019B	1.27 ± 0.023B	1.36 ± 0.021	1.36 ± 0.021B	1.29 ± 0.030A	1.25 ± 0.028B	Creatinin
Diabetic control 3ml N.S	0.25 ± 0.013	0.27 ± 0.014	0.30 ± 0.020	0.36 ± 0.023	0.31 ± 0.025	0.25 ± 0.014	0.19 ± 0.010	Total bilirubine
	7.19 ± 0.76	7.06 ± 0.51	6.98 ± 0.43	6.81 ± 0.31	6.51 ± 0.20	6.00 ± 0.12	5.7 ± 0.16	Total protein
	1.45 ± 0.025	1.47 ± 0.023	1.50 ± 0.027	1.52 ± 0.014	1.42 ± 0.023	1.44 ± 0.24	1.45 ± 0.025	Creatinin
Diabetic 10 mg /Kg Glibenclamide	0.29 ± 0.016	0.32 ± 0.013	0.22 ± 0.012	0.21 ± 0.015	0.19 ± 0.0010	0.10 ± 0.007A	0.08 ± 0.005A	Total bilirubine
	7.12 ± 0.61	7.02 ± 0.41	6.90 ± 0.71	6.72 ± 0.45	6.41 ± 0.18	6.00 ± 0.12A	5.2 ± 0.72B	Total protein
	1.52 ± 0.025	1.79 ± 0.025B	2.50 ± 0.046B	1.88 ± 0.013B	1.78 ± 0.034A	0.21 ± 0.023B	1.18 ± 0.20B	Creatinin
Diabetic 1 g/Kg aqueous extract	0.27 ± 0.011	0.26 ± 0.017	0.21 ± 0.014	0.30 ± 0.013	0.20 ± 0.011	0.08 ± 0.006B	0.066±0.010B	Total bilirubine
	7.00 ± 0.41	7.02 ± 0.32	7.00 ± 0.15	6.92 ± 0.52	6.32 ± 0.30	5.9 ± 0.10A	5.00 ± 0.08B	Total protein
	1.83 ± 0.021B	2.14 ± 0.029B	2.16 ± 0.031	3.32 ± 0.051B	1.48 ± 0.43	1.22 ± 0.025B	1.14 ± 0.012B	Creatinin

A : $p < 0.05$, B : $p < 0.01$ vs corresponding control and diabetic control.

DISCUSSION

The observed hypoglycemia in normal rabbits may be due to effect of great portion of alkaloids and flavonoids presence in aqueous extract influncing hypoglycemia as reported (14). The administration of aqueous extract for 30 days at the dose (1g / kg) caused a significant reduction of serum glucose level in diabetic rabbits better than the other drugs and didn't show any side effect of plant as compared with Glibenclamide this result in agreement with (4) and (17) they said the extract of *H. salicornicum* persistent hypoglycemic effect and more than glibenclamide .These results may be justified by the presence of the inorganic sulphide in the extract which seem to be remove insuline inactivating compounds by competing with insuline, of flavonoids that improve the vascularization of pancreas (15). Some constituent of plant increase the an aerobic glycosis and decrease glyconeogenesis, therfore; increasing the rate of transfer of glucose from blood to tissue (18). The plant may be accelarate the passage of food through the upper gastrointestinal tract and due to it's viscosity, could delay access of glucose to the intestinal epithelium (6). Reported that sulfonyle urea including Glibenclamide produced hypoglycemic responce by stimulating beta pancreatic cells to release more insulin into the blood stream(13). Reported that the aqueous extract of plant reduce the elevation of blood glucose (5).

In normal rabbits, treatment had no effect on serum AST and ALT. In alloxan diabetic rabbits, serum AST and ALT levels were significantly higher than normal values (table 2). The gluconeogenic action of AST and ALT could represent a comp-ensatory responce by providing new supplies of glucose precursors. In this study, the treatment of alloxan diabetic rabbits with aqueous extract of this plant caused lower of serum AST and ALT levels. (9) found that the liver was necrotized in alloxan diabetic rabbits. This supports the view that the hypoglycemic phase of alloxan poisoning may be results of liver damage (9). Therefore the high levels of transaminase enzymes foud in the study may be due to hepatotoxic effect of alloxan. Total billirubin, total protein and creatinine levels were reduced significantly in all treated groups. In diabetic rabbits significant increase in creatinine levels was observed. These observation may be due to the effect of alloxan on liver and kidney which may irritate the renal tissue and excessive irritation may lead to inflammation which increased creatinine level. In alloxan diabetic rabbits, microscopic pictures of kidney tissues showed interstitial nephritis with degenerative changes in epithelial cells of the renal tubules (9).The aqueous extract of this plant(1g/kg)was administration daily observation of rabbits during the study had no toxic effect (did not show any visible signs of toxicity .i.e. no excitment, restlessness, respiratory distress, convultions or coma regardless of the dose used. Moreover ,the treated rabbits appeared to remain in good health.

دراسة الفعاليات البايولوجية ذات المدى الطويل للأرانب المعاملة مع نبات الشنآن *Haloxylon salicornicum* ودواء Glibenclamide الطبيعية والمستحدث بها داء السكر بواسطة الالوكسان

منى حميد محمود السعيد

فرع الفسلجة و الادوية,كلية الطب البيطري,جامعة البصرة

الخلاصة

في هذا البحث تمت دراسة الأثر الفعال لنبات الشنآن *Haloxylon salicornicum* ودواء Glibenclamide لخفض مستويات السكر في الدم للأرانب الطبيعية والمصابة بمرض السكر نتيجة لحقنها بجرعات من الالوكسان.

وتم إعطاء 1غم /كغم من نبات الشنان و10 ملغم / كغم من Glibenclamide يوميا" لمدة شهر واحد .

في هذه الدراسة لوحظ انخفاض مستوى السكر مابين معنوي وعالي المعنوية خلال فترة المعاملة لمدة شهر بالنسبة للأرانب الطبيعية والمصابة بالسكر و كذلك بالنسبة لتركيز الكريتين .

أما بالنسبة لمستوى البروتين الكلي و البايولوربين الكلي لوحظ الانخفاض فقط خلال فترة 15-30 يوم .

بالنسبة لتركيز أنزيمات AST و ALT لوحظ تغيير معنوي في المجاميع المعاملة للأرانب المصابة بداء السكر .

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