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Biochemical Studies on Some Hypoglycemic Agents (II) Effect of *Calligonum comosum* extract

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Abstract□People in some tropical countries used to use some plant extracts in folklore medicine as treatment of diabetes mellitus. Of these plants is *Calligonum comsum* ("orta"). The present work deals with the biochemical effects of the whole plant water extract given orally to the albino rats, normals and alloxan diabetics, as to fulfil its hypoglycemic effects on rats. The results showed that "orta" extract produced a hypoglycemic effect indicated by the decrease in blood sugar level. It was observed that the diabetic state in rats treated with 300 mg/kg body weight of "orta" extract, was alleviated, showing normal levels of blood glucose, liver fat and cholesterol contents. Liver proteins were still below the normal level in the rats. Gibenclamide, a hypoglycemic drug, was used for comparison with "orta" extract. Although it did alleviate the diabetic state, yet the liver fat and cholesterol contents were still higher than those of normal. Also the liver proteins were lower than the control levels.

Keywords□Diabetes, hypoglycemia, *Calligonum comosum*

The literature search about the antidiabetic or even the biological activity, failed to reveal any informations. However, a phytochemical study on *Calligonum comosum* plant was conducted by El-Sayyad and Wanger[1]. From the herb of *Calligonum comosum,* the flavonoids kaempferol, quercetin, quercetin-2-O-beta-D-glucoside (isoquercetin) and kaempferol-3-O-beta-D-glucuronide, procyanidines and the carotenoids violaxanthin and neoxanthin, were isolated and identified by chemical and spectro scopic methods.

However, many workers studies the hypoglycemic effects of some plants as naturally occurring antidiabetic agents. Augusti[2, 3] reported on the effect of allicin (diallyl disulphide oxide) from *Allium cepa,* in lowering the blood glucose levels of alloxan treated rabbits. He found that oral administration of 0.25 g/kg body weight was shown to produce an equivalent lowering of blood glucose as the same dose of tolbutamide. These studies were extended in humans where a significant drop in fasting blood glucose levels, with a concomitant rise in serum insulin levels were noted following oral administration of allyl propyl disulfide[4, 5]

Some plant are hypoglycemic agents such as *Trigonella foenum graecum* which have been used as an oral insulin substitute in Israel[6]. The authors found that nicotinic acid, isolated from the seeds lowered blood glucose level in normal as well as alloxan treated rats, but the effect was of short duration.

A review of hypoglycemic plants reported by Fornsworth and Segelman[7] listed about 50 plants which give hypoglycemic activity. But many crude extracts produce toxic effects. For this reason they recommended not to use any of the plants cited in their article in any form for the treatment of diabetes mellitus.

The aim of the present study is to examine biochemical effects and particularly for antidiabetic activity of *Calligonum comosum* plant. It was compared also with other plant extracts, for example "neem" leaft extract, especially from the hypoglycemic and toxicity effects, and one of the hypoglycemic drugs which is euglucon.

MATERIALS AND METHODS

The materials of the present study comprise the preparation of *Calligonum comosum* plant water extract which was tested on albino rats. The dry herb was crushed and ground to fine powder. A 10% w/v suspension of the powder was boiled for 30 minutes, cooled, and filtered. The filtrate was then tested for its hypoglycemic activity in rats. The dose of the ex-

tract represents 200 and 300 mg/kg body weight from the dry powder, which the animal received orally by gastric intubation.

The experimental animals were albino rats of 6 groups. Nine rats were taken as controls (group 1). Seventeen rats were rendered diabetic by intraperitoneal injection of 125 mg/kg alloxan monohydrate (5% aqueous solution) (group 2). Groups 3 and 4 are 10 and 13 normal rats given *Calligonum comosum* ("orta") extract at oral doses of 200 and 300 mg/kg. Group 5 comprised 18 diabetic rats, treated with oral dose of 300 mg/kg of the "orta" extract daily for 7 days. Group 6 comprised 9 diabetic rats treated with one of the oral hypoglycemic drugs which is glibenclamide (Euglucon). A daily oral dose of 0.2 mg/kg suspended in water was induced for 7 days. This group of rats was taken of comparison.

Blood samples by heparinized capillary tubes from the occular vein of the eye were examined for blood glucose analysis by Haselewood and Strookman[8]. At the end of the experiment, rats were sacrificed and blood was collected, serum was separated for estimation of cholesterol by MacIntyre and Ralston[9]. Livers were taken for determination of protein by Lowery *et al.* method[10]. Water and fat contents were estimated as described by Osborne and Voogt[11]. Statistical analysis was conducted according to Duncan[12].

RESULTS

Table I shows change in body weight, percentage loss in body weight; blood glucose level before experiment, at one day and 7 days of experimentation. The table shows also cholesterol levels and percentage mortality in groups of experimental rats. Table II illustrates liver analysis of the present groups of animals.

DISCUSSION

Administration of 200 mg/kg body weight of the "orta" extract lowered blood glucose by 12.7 and 11.1% after one day and 7 days, respectively, in normal rats (Table I). Treatment with doses of 300 mg/kg caused decrease of 7.6 and 15.4 in blood sugar level. The data show that the plant extract had

Table I. Comparison of body weight before and after, loss in body weight, blood glucose, serum cholesterol contents and % mortality in groups of rats used and those values and those values for normal controls

Rat Groups*	Body weight g		% Loss in Weight	Blood glucose (mg %)			Serum cholesterol mg%	Percentage mortality
	Before	After		Before	One day	7 days		
(1) Range	100 – 164	–	–	79.5 – 124.7	–	–	217.4 – 359.8	–
Mean ± SE	134.3 ± 6.8			91.6 ± 5.3			175.3 ± 15.2	
(2) Range	159 – 220	127 – 190	11.8 – 20.1	82.0 – 122.0		130.2 – 168.1	254.7 – 1070.1	
Mean ± SE	179 ± 5.3	152.7 ± 5.4	15.1 ± 0.7	100.8 ± 4.0	–	159.9 ± 3.8	542.2 ± 73.3	35.3
p>	–	>0.02	–	–		>0.001	>0.001	
(3) Range	230 – 300	215 – 280	4.3 – 13.7	104.4 – 128.0	89.9 – 113.5	87.0 – 126.2	285.0 – 444.5	
Mean ± SE	260 ± 8.2	237.5 ± 7.4	8.7 ± 1.2	118.0 ± 3.0	103.0 ± 3.2	104.9 ± 5.4	363.1 ± 24.0	20.0
p>	–	>0.05	–	–	>0.05	>0.05	>0.01	
(4) Range	225 – 280	195 – 265	3.8 – 18.5	90.2 – 117.4	80.9 – 100.5	75.5 – 96.8	290.4 – 586.6	
Mean ± SE	262.2 ± 6.0	237.2 ± 7.6	9.6 ± 1.7	100.4 ± 2.7	92.8 ± 2.4	84.9 ± 2.4	436.7 ± 32.8	30.8
p>	–	>0.05	–	–	>0.05	>0.001	>0.001	
(5) Range	215 – 315	185 – 285	8.3 – 19.7	80.4 – 128.6	151.7 – 234.6	105.9 – 131.5	252.0 – 533.2	
Mean ± SE	270.0 ± 10.8	239.4 ± 9.5	11.2 ± 1.3	108.8 ± 5.4	177.0 ± 8.5	120.2 ± 3.6	389.6 ± 28.6	50.0
p>	–	>0.02	–	–	>0.001	>0.05	>0.01	
(6) Range	290 – 340	230 – 295	6.9 – 30.3	100.2 – 125.7	132.4 – 195.7	90.5 – 123.3	385.2 – 486.8	
Mean ± SE	324.3 ± 6.1	260.7 ± 9.7	19.3 ± 3.8	111.6 ± 3.3	171.4 ± 8.2	108.4 ± 4.2	442.6 ± 14.2	22.2
p>	–	>0.001	–	–	>0.001	>0.1	>0.001	

*Rat group: (1) Normal controls; (2) Alloxan diabetic rats; (3) Normals treated with 200 mg/kg body weight extract; (4) Normals treated with 300 mg/kg bedy weight extract; (5) Diabetics treated with 300 mg/kg body weight extract; (6) Diabetics treated with 0.2 mg/kg body weight Euglucon.

hypoglycemic activity and this effect may be due to either the compounds found in the extract and its effects on the β-cells of islets of Lagerhans, thus affecting release of excess insulin and/or increased glucose utilization in the body.

The loss in body weight in the normal treated rats was found to be 8.7 and 9.6%, and the percentage mortality was 20.0 and 30.8% for the above groups 3 and 4. These effects were observed to be dose-related. These phenomena might be due either to the hypoglycemic effect of the plant and/or some toxic factors found in the plant extract.

Cholesterol levels in the two treated normal groups 3 and 4 showed highly significant increase from controls and is dose-related. This effect might be due to decreased lipolysis and oxidation of serum cholesterol. These results were evidenced here by the finding of the normal liver fat content in group 3 and increased liver fat in group 4 (Table II). The liver proteins in the two groups of rats showed slightly significant decrease from normals (Table II). These values indicate that there may be some factors or compounds that gave that phenomenon.

For alloxan diabetic rats of group 5, treated with 300 mg/kg of the "orta" extract for 7 days revealed that blood glucose level returned to the normal values. This indicates that the diabetic state which was observed after one day from alloxan injection was alleviated by administration of the "orta" extract. This also proves its hypoglycemic activity, that the loss in body weight and percentage mortality were highly significant which may be due to its hypoglycemic effect and/or some toxic factors in the crude plant extract, and that serum cholesterol level showed moderately significant increase than that of the normals, while comparing it with the glibenclamide treated group, there was a decrease amounting to 11.8%. There were decreases in liver fat and protein contents than those of the normal and drug treated groups. The ratio between liver weights to body weights gave normal values.

These forementioned effects may be due to some factors or compounds that were present in the extract.

However, in conclusion, we found that the "orta" extract was better than "neem" leaf extract in alleviating the diabetic state. The biochemical parameters used were found to change to the normal level. When comparing the percentage mortality, it was found to be higler in the "orta" treated group than the "neem" and the drug treated groups. The drug treated diabetic rats, although showing

Table II. Comparison of liver weight, water content, liver fat and protein content for different groups rats used and those values for normal rates

Rat Groups*	Liver weight (g)	Ratio $\frac{\text{Liver weight}}{\text{Body weight}}$	Water content (g%)	Liver fat (g%)		Liver proteins (g%)	
				Fresh	Dry	Fresh	Dry
(1) Range	3.5 – 7.5	0.035 – 0.050	68.5 – 72.9	4.3 – 6.1	15.5 – 21.7	18.1 – 20.7	61.2 – 74.2
Mean ± SE	5.8 ± 0.4	0.043 ± 0.0015	70.6 ± 0.5	5.6 ± 0.9	19.0 ± 0.7	19.2 ± 0.3	65.8 ± 1.6
(2) Range	7.0 – 12.0	0.043 – 0.071	69.8 – 75.5	6.8 – 11.6	24.9 – 48.5	7.5 – 9.2	25.9 – 41.7
Mean ± SE	8.7 ± 0.5	0.057 ± 0.0023	72.5 ± 0.8	9.5 ± 0.5	34.8 ± 2.1	8.4 ± 0.2	30.8 ± 1.5
p >	–	> 0.001	> 0.1	> 0.002	> 0.001	> 0.001	> 0.001
(3) Range	7.0 – 11.0	0.032 – 0.048	69.8 – 73.4	4.5 – 7.0	15.1 – 23.2	15.1 – 19.3	55.5 – 67.7
Mean ± SE	9.2 – 0.5	0.039 ± 0.0018	71.2 ± 0.4	5.3 ± 0.3	18.4 ± 1.1	17.4 ± 0.5	60.6 ± 1.4
p >	–	> 1.0	> 0.5	> 0.5	> 0.5	> 0.01	> 0.05
(4) Range	7.5 – 12.5	0.035 – 0.049	68.9 – 72.5	4.2 – 9.6	14.5 – 30.7	15.4 – 19.3	49.5 – 67.2
Mean ± SE	10.0 ± 0.5	0.042 ± 0.0017	70.6 ± 0.4	6.7 ± 0.6	22.5 ± 2.0	17.2 ± 0.5	58.4 ± 1.9
p >	–	> 0.8	> 0.5	> 0.5	> 0.25	> 0.01	> 0.01
(5) Range	9.0 – 12.0	0.036 – 0.049	66.5 – 70.5	3.1 – 4.6	9.2 – 13.8	11.3 – 16.4	34.5 – 54.1
Mean ± SE	9.7 ± 0.4	0.041 ± 0.0015	68.6 ± 0.5	3.6 ± 0.2	11.4 ± 0.5	14.2 ± 0.7	45.1 ± 2.3
p >	–	> 0.5	> 0.02	> 0.05	> 0.001	> 0.001	> 0.001
(6) Range	6.0 – 11.5	0.026 – 0.040	65.4 – 72.0	5.7 – 8.5	18.7 – 27.4	12.8 – 18.5	39.6 – 60.1
Mean ± SE	8.8 ± 0.8	0.033 ± 0.0019	68.7 ± 0.8	7.0 ± 0.3	22.3 ± 1.2	16.1 ± 8.0	51.4 ± 2.6
p >	–	> 0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

lower blood glucose level, the rest of the biochemical parameters did not return to the normal state.

From these findings it is recommended that the plant could not be used as crude extract for treating diabetes. It is suggested that separation and identification of the plant compounds to know active ingredients which affect and lower blood glucose and that separation of the toxic factors found in the plant before usage in the treatment. These suggestions need follow-up research by specialists in these programs.

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