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Biochemical Studies on Hypoglycemic Agents (I) Effect of *Azadirachta indica* leaf extract

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Abstract□It was confirmed that extracts of some plants were used in folklore medicine as hypoglycemic agents. Of these plants is *Azadirachta indica* ("neem"), which grows in tropical regions. The present study deals with biochemical effects of the "neem" leaf water extract given orally to experimental animals, especially the hypoglycemic characteristics. Normals as well as alloxan diabetic rats have been used in this work. The results showed that the "neem" leaf extract produced some hypoglycemia in normal rats when given in two doses, while in diabetic rats there was a decrease in blood sugar, but it could not alleviate the diabetic state. However, the "neem" leaf extract produced some toxic effects in rats, as observed in body weight loss and high percentage mortality, especially with a high dose. It was observed that the clotting time of blood was higher than that of the normal. Serum cholesterol level increased with concomitant decrease in the liver fat as compared to normal levels. There was also a drop in liver proteins which was dose-related. The results were compared with those obtained with an oral hypoglycemic drug (Glibenclamide).

Keywords□Diabetes, hypoglycemia, *Azadirachta indica.*

Azadirachta indica trees grow in tropical regions known as "neem", the bark, leaves and fruits of which have been used for medicinal and other purposes. It was used to reduce arterial blood pressure in animals[1]. "Neem" bark extracts were shown to posses an antipyretic action in the treatment of fever, such as malaria.

As early as 1857, Cornish[2] discovered a bitter crystalline substance in the bark which he called margosine. Other important constituents such as nimbin, nimbinin, nimbidin, nimbiol, quercetin, nimbolin A, nimbolin B, nimbolide, azadirone, azadiradione and azadirachtin were discovered[3]. Patel and Bhanu[4] reported that leaves of "neem" are rich in protein, calcium, phosphorus, carbohydrates, fats, nitrogen and other mineral constituents.

"Neem" has also dermatological uses with antibacterial, antifungal activity. There are early and recent records of the plant being toxic to man[5-7]. The leaves, bark and flowers have been shown to be toxic, but the great majority of intoxication cases occur from ingestion of the fruits.

The leaves have not generally been used internally although in Saudi Arabia an aqueous infusion of fresh tender leaves has long been used in treatment of diabetes mellitus[8]. In a comparative study, a crude aqueous leaf extract of *Azadirachta indica,* has been demonstrated to be about one half as potent as tolbutamide in reducing blood glucose concentration. Various extraction processes have been performed on the leaves an a relatively pure extract was obtained[8]. It was suggested that the antidiabetic activity is dependent on the presence of functioning B-cells of the pancreas, since leaf extracts do not produce hypoglycemia in totally pancreatectomized rats, or in animals made serverely diabetic by alloxan.

The present study was undertaken to examine biochemical effects and particularly for antidiabetic activity of *Azadirachta indica.* It was hoped that this might yield informations about its use as a naturally occurring oral hypoglycemic agent.

MATERIALS AND METHODS

The materials of this study comprise the preparation of "neem" leaf extract which was tested on experimental animals. The leaves were dried in shade, crushed and ground to fine powder. A 10% w/v suspension of the powder was boiled for 30 minutes,

cooled, and then filtered. The filtrate was then tested for its hypoglycemic activity in rats. The dose of the leaf extract administered to each rat, represents the amount of extract by dry weight which the animal received.

The experimental animals were albino rats of 100-350g body weight and were classified into five groups: 9 normal rats of body weight ranging from 100-164g were taken as control group. Seventeen rats of body weight ranging from 159-220g were rendered diabetic by intraperitoneal injection of 125 mg/kg body weight of alloxan monohydrate[9]. Aqueous 5% solution of alloxan was freshly prepared by dissolving 1g of alloxan monohydrate in 20 m*l* distilled water. Groups 3 and 4 comprised 13 and 16 normal rats of body weights 210-350 and 245-330g. These two groups of rats have received *Azadirachta indica* leaf extract in doses of 200 and 300 mg/kg. The oral administration of the extract was given by gastric intubation after slightly anaethesized by ether once a day, for 7 days. Blood samples were taken before and 7 days after treatment, for analysis of blood glucose. Group 5 comprised 21 rats of body weight 140-270g, which were rendered diabetic. Blood glucose was estimated before and after alloxan injection. These diabetic rats were treated with 300 mg/kg body weight of "neem" leaf extract for 7 days. Blood glucose was determined before and after "neem" treatment. Group 6 comprised 9 diabetic rats of body weight 290-340g. This group was treated with oral hypoglycemic drug, which is glibenclamide (Euglucon) in a dose of 0.2 mg/kg body weight for 7 days. This group was used for comparison.

Blood was taken by heparinized capillary tubes from the occular vein of the eye. Blood glucose was estimated by the method of Haslewood and Strookman[9]. Rats were sacrificed and blood was collected, sera were separated for estimation of cholesterol by Lieberman-Burchard reaction and modified by Macintyre and Ralston[10]. Rat livers were separated immediately. Liver proteins were determined after homogenization of a known weight of liver samples using a glass homogenizer and a mechanical stirrer by the method of Lowery *et al.*[11]. Water and fat contents were determined as described by Osborne and Voogt[12] on known weights of liver samples. Statistical analysis was con-

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	210 – 350	165 – 320	3.2 – 21.4	90.7 – 121.2	85.7 – 120.9	88.3 – 115.2	491.2 – 825.7	
Mean ± SE	287.2 ± 13.1	263.9 ± 15.7	8.5 ± 1.8	108.8 ± 2.6	102.2 ± 3.5	105.2 ± 3.2	609.8 ± 35.5	30.8
p>	–	>0.1	–	–	>0.1	>0.1	>0.001	
(4) Range	245 – 330	220 – 310	6.1 – 10.2	102.4 – 121.8	92.7 – 120.0	79.2 – 122.0	474.6 – 969.8	
Mean ± SE	307.5 ± 31.3	283.3 ± 13.5	8.0 ± 0.6	115.0 ± 18.9	103.7 ± 4.4	101.5 ± 8.0	670.3 ± 83.2	62.5
p>	–	>0.25	–	–	>0.01	>0.01	>0.001	
(5) Range	140 – 270	110 – 250	0.0 – 34.6	93.0 – 124.1	139.5 – 488.5	96.3 – 288.1	218.1 – 594.6	
Mean ± SE	205 ± 11.3	168.8 ± 10.6	17.3 ± 3.3	110.6 ± 2.6	246.4 ± 33.8	151.0 ± 15.7	467.6 ± 30.8	38.1
p>	–	>0.05	–	–	>0.001	>0.02	>0.001	
(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 weight extract; (5) Diabetics treated with 300 mg/kg body weight extract; (6) Diabetics treated with 0.2 mg/kg body weight Euglucon.

ducted according to Duncan[13].

RESULTS

Body weights, blood glucose, serum cholesterol and percentage mortality before and after *Azadirachta indica* leaf extract, for normal controls, alloxan diabeties, normal rats treated with 200 and 300 mg "neem" extract, alloxan diabetics treated with 300 mg of "neem" extract and alloxan diabetic rats treated with glibendamide (0.2 mg/kg body weight) are shown in Table I. All the liver analyses for the previously mentioned rat groups are shown in Table II.

DISCUSSION

For normal rats treated with 200 and 300 mg/kg of "neem" extract, the results showed a drop in blood sugar after 7 days amounting to 3.3 and 11.7%, respectively. These results mean that there was hypoglycaemic effect for the extract which might be due to either the effect of certain compounds present in the extract that can release excessive insulin from the pancreatic cells and/or decreased food intake in these groups of rats, which was observed, and which participate in lowering of blood sugar. These suggestions were supported by evidence from the present finding of losses in body weights amounting to 30.8 (Table I, groups 3 and 4).

For alloxan diabetic rats, which were treated with 300 mg/kg of "neem" leaf extract for 7 days, blood glucose was lowered by 38.7%, but it was still higher than that of the normal. In comparison with the oral hypoglycemic drug glibenclamide, the latter did alleviate the diabetic state, while the present "neem" extract did not relief diabetes in these rats. However, the above mentioned proposals may be applied as the causes for the present phenomenon.

The loss in body weights and high mortality percentage for normal rats observed after administration of extract may be attributed to either loss of appetite and/or the toxic effects of *Azadirachta indica* leaf extract. However, this suggestion receives support from the previous findings. Many authors reported that "neem" extract is toxic and used as an insectiside [Teotia and Tiwari[14]; Oelvichs *et al.*,[7]]. These groups of rats suffered from increased blood clotting time. These results received evidence from the work of Bhat and Broker[15] and that of Thompson and Anderson[1]. The latter reported that these effects included profound

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 croups*	Liver weight (g)	Ratio Liver weight / 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 – 10.0	0.028 – 0.043	66.5 – 70.2	4.1 – 6.2	12.6 – 19.6	14.9 – 19.2	44.5 – 61.7
Mean ± SE	9.0 – 0.4	0.035 ± 0.0018	68.7 ± 0.4	4.9 ± 0.2	15.8 ± 0.8	17.0 ± 0.5	55.8 ± 1.9
p>	–	>0.005	>0.01	>0.25	>0.01	>0.005	>0.001
(4) Range	8.0 – 13.5	0.028 – 0.044	61.5 – 78.2	3.1 – 5.7	8.2 – 19.9	13.2 – 16.2	37.1 – 60.6
Mean ± SE	10.4 ± 0.7	0.037 ± 0.0025	70.2 ± 2.2	4.4 ± 0.4	15.3 ± 2.0	15.0 ± 0.4	50.9 ± 3.2
p>	–	>0.05	>0.5	>0.25	>0.1	>0.001	>0.001
(5) Range	5.5 – 11.0	0.038 – 0.053	61.5 – 70.5	4.9 – 10.6	13.3 – 33.6	9.2 – 18.2	29.6 – 60.9
Mean ± SE	7.5 ± 0.4	0.045 ± 0.0011	67.7 ± 0.8	7.8 ± 0.6	24.5 ± 2.0	12.9 ± 0.8	40.4 ± 2.7
p>	–	>0.5	>0.01	>0.05	>0.02	>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

hypotension and a minimal negative chronotropic effect which increased at higher doses.

On the other hand, alloxan diabetic rats receiving 300 mg/kg of "neem" extract showed higher rate of body weight loss and lower percentage mortality than those of the treated normal rats. That phenomenon may be due to the presence of some compounds in the water leaf extract of which are proteins and which may supress the effect of alloxan in this group of rats.

Serum cholesterol was found to be 609.8 and 670.3 mg % which gave very highly significant increase as compared to normal control and even when compared with the other groups of rats. The rise in cholesterol level was dose related. These results show that the leaf "neem" extract exerted biochemical changes in fat metabolism, namely increased lipolysis and low oxidation rate of serum cholesterol. The increased lipolysis can be evidenced in the present low level of liver fat (Table II, group 3 and 4). It is suggested that these may be due to presence of some compounds in the extract which exerted these effects. Serum cholesterol in alloxan diabetic and treated rats showed a highly significant increase from the normal level (Table I), as compared with alloxan diabetic rats it gave a decrease of 13.8%. Even when comparing these values with those obtained for the normals treated with the same dose of extract, it showed a decrease of 30.2%.

Considering the liver architecture (Table II), the liver fat of the normal rats treated with 200 and 300 mg/kg of the extract amounted to 4.9 and 4.4g%. These results show nonsignificant decrease from these obtained for the normal rats. The protein contents of livers were 17.0 and 15.0g% for the two groups, respectively, and which are significantly lower than those of the normals. This may be attributed to increased gluconeogenesis to meet the body demands for energy source, since for thse rats the food intake was low. This effect was also observed in the loss of body weight in thse treated normal rats.

For alloxan diabetic treated rats, liver fats were 7.8%, which is lower by 17.9% from the diabetics, but still was higher than the normal treated rats. However, in this case it is suggested that this phenomenon may be attributed to some compounds which might be present in the extract that could somewhat alleviate fatty infiltration in the liver than diabetics and could increase oxidation of cholesterol in the presence of the diabetic state. The liver protein in this group showed a decrease from the normal level, but it was higher than the diabetic rats.

Using an oral hypoglycaemic drug which is glibenclamide (Euglucon) in a dose of 0.2 mg/kg body weight, blood glucose for diabetic rats returned to the normal level after 7 days. Apparently, there was an alleviation of the diabetic state, but the other biochemical parameters showed some deteriorations shown in Table I and II, group 6.

In conclusion, the present study concerning the biochemical effect of *Azadirachta indica* leaf extract, showed that alloxan diabetes in albino rats resulted in some biochemical derangements in carbohydrate, fat and protein metabolism in rats and that the leaf "neem" extract, although it lowered the blood glucose level to some extent, exerted severe metabolic changes in the normal rats especially with high doses. These effects were attributed to its toxic and antifeedant properties of the plant From these findings, it is recommended that this 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 factors which affect and lower blood glucose. Aslo, separation of the toxic factors found in this plant may be of great benefit before its use in folklore medicine.

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