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HYPOGLYCEMIC EFFECT OF BIOPHYTUM SENSITIVUM IN THE ALLOXAN DIABETIC RABBITS

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Abstract : Hypoglycaemic effect of a Neapalese plant Biophytum sensitivum was investigated in the alloxan diabetic male rabbits of different severities: subdiabetic (Alloxan recovered; AR), mild diabetic (MD) and severely diabetic (SD). Assessment of activity of the extract, prepared from the plant leaves, was done by fall in fasting plasma glucose (FPG) and improvement in the oral glucose tolerance test (OGTT), following single dose and prolonged administrations. Following single dose administration there was fall in 1 hour and 2.5 hour glucose values by 25.9% and 27.4% respectively in the subdiabetic rabbits, and by 36.9% and 37.7% in the mild diabetic rabbits. Improved GTT response is shown by fall in area under curve (AUC) from 16138 mg/dl to 12355 mg/dl (23.4%) in the subdiabetic rabbits, and from 19258 to 12238 mg/dl in the MD rabbits. More significant improvements occurred following one week of above treatment. The results prove that the plant material has significant hypoglycaemic effect, which is possibly due to pancreatic beta-cell stimulating action. To investigate its possible role in correction of other metabolic abnormalities of diabetes further long term studies are required.

Key words : hypoglycaemia, alloxan-diabetic, biophytum sensitivum, glucose tolerance test

INTRODUCTION

Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times there has been a renewed interest in the plant remedies (1, 2, 3). In an attempt to discover natural hypoglycaemic compounds, various plants having "folk-medicine reputation" have been screened by biochemical tests for their hypoglycaemic effects (4, 5). Nepal has a rich heritage of medicinal plants of wide diversity which were used by the traditional healers for the treatment of many diseases, including diabetes (6). Biophytum sensitivitum D. C. syn Oxalis sensitiva L (family Oxalidaceae; local name Nagbeli) is one such plant that is traditionally used for the treatment of "Madhumeha". It is an annual herb that grows in the inner Tarai region, east of Koshi river. In spite of its widespread use by the traditional healers, there are no systematic scientific studies to validate effects of this potentially useful plant. The present study was undertaken to scientifically investigate its possible

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usefulness in diabetes mellitus.

METHODS

Plant material: Biophytum sensitivum was collected during August-September, 1996 on the bank of Koshi river, in the inner Tarai region (Sunsari, Nepal). For reliable identification a sample was sent to Mahender Morang Campus (Botany Department, P. G. Institute, Biratnagar). Leaves were separated and dried, and were soaked in water for about 16 hours. Thereafter they were homogenized and centrifuged at 3000 g for 15 minutes. The extract was force fed to the rabbits using feeding tubes, number seven.

Animals: Inbred male Angora rabbits of body weight 700 to 1000 gm, and age 2-2.5 months were purchased from the Pakhribas Agricultural Centre, Dhankuta district. They were given pallet diet and water *ad-libitum* and were acclimatized to the laboratory conditions and handling for at least two weeks before carrying out any experimental work. The oral glucose tolerance test (OGTT) was performed in them to rule out diabetes and other forms of carbohydrate intolerance.

Induction of alloxan diabetes: Following an overnight fast of 14-16 hrs, alloxan was injected intravenously at a dose of 80 mg/kg body weight for the induction of diabetes.

During next few days the fasting plasma glucose (FPG) levels were estimated at intervals of five days for one month, in order to follow the course of hyperglycaemic state. The animals were arbitrarily divided into the following three types, depending on the response :

1. Alloxan Recovered (AR; Subdiabetic) : In such rabbits initial rise in the FPG level was observed for about 15 days followed by a period of recovery when it gradually came down. By one month the FPG returned to normal or slightly elevated (upto 120 mg%) but still on performance of oral glucose tolerance test (OGTT), the glucose tolerance was found abnormal and the OGTT curve was substantially elevated. In the earlier studies, conducted in our lab. with the male albino rabbits, the AR animals were found to be better animal models for the experimental evaluation of the hypoglycaemic effect of compounds (7).

2. Mild diabetic (MD) : The hyperglycaemic state persisted in this group of animals and FPG levels stabilized between 120–250 mg/dl.

3. Severely diabetic (SD) : The hyperglycaemic rabbits with FPG levels above 250 mg/dl were included in this group.

Subsequently, before carrying out any experiments, a period of 45 days was allowed after the day of alloxan injection for the stabilization of rabbits into one of the three diabetic states (7, 8).

Assessment of the hypoglycaemic activity

The hypoglycaemic effect of the plant material was assessed by evaluating the fasting plasma glucose and/or glucose tolerance test.

1. Glucose tolerance test : Basal GTT was done in all the animals with 3 gm/kg body weight glucose load, following an over-night fast of 14-16 hours. Next morning the fasting, 1 hour and 2.5 hour samples were collected, as recommended by some earlier workers (9). Five days later, fasting blood sample was drawn and immediately the plant material was given orally at a dose found optimum in preliminary trials

(200 mg/kg body weight). Ninety minutes later, GTT was performed (on the same rabbit) with same oral glucose load. The mean GTT curve obtained after the treatment was compared with the pretreatment basal curve. The activity was assessed quantatively by the extent of fall in area under curve (AUC). This method had earlier been reported to be more sensitive and meaningful because the animals served as their own control, thus minimizing the biological variations (7, 8).

2. Fasting plasma glucose (FPG) : After an overnight fast, the fasting blood sample was collected and immediately thereafter the plant material was administered, as discussed above. Ninety minutes later next blood sample was withdrawn. The FPG values before and after the administration of the plant material reflected its hypoglycemic effect. As above, the animals served as their own controls.

In the sub-diabetic (AR) animals the activity was assessed by improvement in the OGTT response but not by FPG since it was nearly normal or only slightly elevated even before treatment; in the severely diabetic animals by a fall in FPG and in the mild diabetics by both FPG and GTT. OGTT was rarely performed in the severely diabetic rabbits as they show high mortality with it.

RESULTS AND DISCUSSION

Effect of single dose administration of the plant material on fasting plasma glucose levels and GTT pattern in diabetic rabbits :

The effect of a single administration of the plant material in a dose of 200 mg/kg body weight was assessed in the alloxanated subdiabetic, mild diabetic and severely diabetic rabbits. Five animals in each group were taken.

In the subdiabetic rabbits (Table I), the one hour peak value in the treated animals showed a significant fall of 25.9% from 268.1 ± 11 mg/dl to 198.6 ± 6 mg/dl. The two and half hour value in the treated animals (160.4 ± 4 mg/dl) was 27.4% lower than the corresponding pre-treated value of 220.8 ± 8 mg/dl. These were significant (P<0.01). The

TABLE I : Effect of single dose administration of the plant material on GTT in the subdiabetic and mild diabetic rabbits (n=5).

	Plasma glucose (mg/dl)			
	Fasting	*1-hour*	*2.5-hours*	*AUC (mg/dl)*
Normal-Pretreated	808 ± 6	152.2 ± 5	130.4 ± 6	
Treated	84.6 ± 11 (NS)	143.7 ± 10 (NS)	121.2 ± 7 (NS)	
Subdiabetic-Pretreated	90.8 ± 4	268.1 ± 11	220.8 ± 8	16138
Subdiabetic-Treated	92.1 ± 4	198.6 ± 6	160.4 ± 4	12355
% Change	–1.43	–25.9	–27.4	–23.4
Significance	NS	P<0.01	P<0.01	
Mild diabetic-pretreated	140.4 ± 10	298.8 ± 12	272.8 ± 9	19258
Mild diabetic-Treated	140.8 ± 6	188.4 ± 12	169.8 ± 6	12238
%Change	–0.3	–36.9	–37.7	–36.4
Significance	NS	P<0.01	P<0.01	

NS – Not significant

mean area under curve (AUC) in the treated animals (12355 mg/dl) was lower than that before treatment (16138 mg/dl) by 23%. However, no significant alteration in the FPG occurred either in this group of the AR or the MD rabbits, in course of performing the GTT. In normal controls no change in FPG and a slight attenuation of the glucose induced hypoglycemic response was observed. In the mild diabetic treated animals significant reductions were observed in the one hour peak value from 298.8 ± 12 mg/dl to 188.4 ± 12 mg/dl (36.9% fall), two and half hour value from 272.8 ± 9 mg/dl to 169.8 ± 6 mg/dl (37.7% fall) and AUC from 19258 mg/dl to 12238 mg/dl (36.4% fall). These were significant ($P<0.01$).

Fasting plasma glucose levels in the subdiabetic, mild diabetic and severely diabetic rabbits were separately estimated before and after single dose administration of the plant material. No significant fall was, however, observed in any of the three groups (Table II).

Effect of one week administration of the plant material on fasting plasma glucose levels and GTT pattern : The plant material was daily administered to the subdiabetic, mild diabetic and severely diabetic rabbits in a daily oral dose of 200 mg/kg/day for a period of one week. The experiment was aimed at finding out whether such treatment would improve the GTT curve any further, and whether it would produce any fall in the FPG also, where single dose was found inadequate. At the end of one week treatment, the GTT curves obtained in the subdiabetic and the MD rabbits were evaluated in relation to the corresponding pre-treatment curves. GTT was not repeated in the severely diabetic rabbits as many animals had died with it initially.

In the subdiabetic rabbits there was no change in FPG values which were normal even in untreated rabbits. GTT returned to normal after 7 days (Table III), as judged by the post glucose load plasma glucose values and the AUCs, which were comparable with those of the normal animals (Table I). In the mild diabetic rabbits fasting plasma glucose returned to 98.6 ± 6 mg/dl from the pretreatment value of 153.1 ± 14 mg/dl after the above treatment, whereas the post treatment GTT response, though significantly attenuated, was still impaired compared to a normal response (Table III). When the above treatment was given to the severely diabetic rabbits with FPG level of 438.9 ± 41 mg/dl, the reduction after the third and the seventh days were 0.09% and 7.2% respectively. However, the post treatment values were still markedly elevated (Table IV).

Effect of the plant product on body weight of the diabetic rabbits : Body weights of the subdiabetic, mildly diabetic and

TABLE II : Effect of single dose administration of the plant material on FPG in the subdiabetic, mild diabetic and SD rabbits (n=5).

	Plasma glucose (mg/dl)			
	Fasting	*90 minutes after administration*	*% Change*	*Significance*
Subdiabetic	88.4±5	89.4±9	+1.1	NS
Mild Diabetic	154.6±9	146.6±16	–5.2	NS
Severely Diabetic	488.1±46	456.4±44	–6.4	NS

NS = Not significant

TABLE III : Studies with one week administration of the plant material in the subdiabetic and mild diabetic rabbits. (n=5).

	Plasma glucose (mg/dl)			
	Fasting	*1-hour*	*2.5-hours*	*AUC (mg/dl)*
Subdiabetic				
Day 0	90.6 ± 6	266.6±36	218.4 ± 22	13446
Day 7	91.6 ± 5	150.4±13	132.4 ± 17	10352
% Change	+1.1	– 43.6	– 39.4	– 23.01
Significance	NS	P<0.01		<0.01
Mild Diabetic				
Day 0	153.1 ± 14	298.4± 41	217.5 ± 15	17838
Day 7	98.6 ± 6	184.7±12	156.6 ± 14	11844
% Change	–35.6	–38.1	–28.0	–33.6
Significance	P < 0.01	P < 0.01	P < 0.01	

NS – Not significant; (% Change compared with day 0 value)

TABLE IV : Studies with one week administration in the SD rabbits (n=5).

	Plasma glucose (mg/dl)		
	FPG	*% change*	*significance*
Day 0	438.9 ± 41		
Day 3	436.6 ± 42	–0.09	NS
Day 7	405.4 ± 28	–7.2	NS

NS = Not significant

severely diabetic animals were taken before and after the treatment. Weight gains in the AR, mild diabetic and severely diabetic rabbits were from 1.06 ± 0.10 kg to 1.12 ± 0.17 kg (5.6%), from 0.84 ± 0.08 kg to 0.90 ± 0.11 kg (7.1%) and from 0.76 ± 0.11 kg to 0.80 ± 0.12 kg (5.2%) respectively with 7 days of the treatment; whereas weight loss in the three groups without the treatment were 1.8%, 4.5% and 10.5% respectively in 7 days (Table V), possibly due to metabolic alterations associated with the diabetic state. In the AR and MD rabbits the weights reached the normal value (i.e., value before induction of diabetes). In the SD rabbits also there was considerable gain in the body weight, which reached almost normal value of 0.8 kg which is only slightly lower than the average normal (0.85 kg). The treatment, therefore, not only arrests loss of weight due to diabetic state but also results in weight gain, probably due to its role in initiating an improvement in the overall metabolic status.

The above results clearly show that the plant extract has a significant and sustained hypoglycaemic action. It is evident from the hypoglycaemic effect elicited in the subdiabetic and the MD rabbits. Furthermore, the above treatment possibly initiates improvement in the metabolic alterations associated with diabetes mellitus, as suggested by gain in weight in the treated group of the animals. However, no significant biochemical alteration was observed, such as in lipid profile with one week of treatment. This period is too short to bring about changes in the lipid profile which, once elevated, takes a much longer

TABLE V : Effect of the diabetic state and 15 days of the treatment on body weights (kg) of the AR, mild diabetic and severely diabetic rabbits.

	AR	*Mild diabetic*	*Severely diabetic*
Untreated (0 Week)	1.08 ± 0.10	0.88 ± 0.07	0.85 ± 0.08
Untreated (1 Week)	1.06 ± 0.10	0.84 ± 0.08	0.76 ± 0.11
%change	−1.8%	−4.5	−10.5
Treated (2 Week)	1.12 ± 0.17	0.90 ± 0.11	0.80 ± 0.12
%Change	+5.6	+7.1	+5.2
Significance	NS	P<.01	NS

time than one week to fall. Studies of longer duration are, therefore, necessary to delineate the metabolic alterations, if any. Weight gain in the treated rabbits is in contrast with the untreated diabetic animals which lost considerable weight during this period. However, in the SD rabbits, which are reasonable analogues of human IDDM, having near complete beta cell destruction, single dose as well as prolonged administrations were found inadequate to decrease the FPG. The results suggest a predominant insulinotropic (beta cell stimulating) effect, because improvement in the glycaemic state was observed in the subdiabetic and MD rabbits only, which have functional beta cell reserve. In the SD rabbits, where beta cell damage is extensive, this effect can not be elicited. However, extra-pancreatic mode of action can not be ruled out on the basis of study of this duration.

In view of the fact that the crude plant extract was administered in this preliminary study, the purified and homogenous material, if isolated from it, is expected to be effective at a much lower dose. Attempts towards purification of the extracts are being planned at present, and the above results are encouraging for carrying out more detailed biochemical studies in order to observe other beneficial effects and to elucidate the mode of action.

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