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Effect of leaf extract of *Aegle marmelose* in diabetic rats

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Alloxan induced animal model was used to evaluate the potential antidiabetic effect of *A. marmelose* leaf extract. The diabetic animals were given insulin injection and another group *A. marmelose* leaf extract orally. It maintained the weight of the animals near to the control rats but a significant decrease in weight was noted in diabetic animals without any treatment. The blood glucose level in treated animals were near to that of control ones. Also a significantly increased glucose tolerance was observed in animals orally given the leaf extract prior to the experiment. A significant decrease in liver glycogen (1.24 ± .07 g/100 g of wet tissue) was observed in diabetic rats which was brought to almost the normal level (1.84 ± .14 g/100 g) with leaf extract treatment. Blood urea and serum cholesterol increased (62.66 ± 3.50 and 192.67 ± 13.64 mg/dl) significantly in alloxan diabetic rats. The leaf extract treatment decreased the blood urea and serum cholesterol (37.83 ± 3.97 and 99.20 ± 8.43 mg/dl) to that of control ones. A similar effect was seen with insulin treatment. The results indicate that the active principle in *A. marmelose* leaf extract has similar hypoglycaemic activity to insulin treatment.

Long before the use of insulin indigenous remedies have been used for the treatment of diabetes mellitus. There is an increasing demand by patients to use the natural products with antidiabetic activity. This is because insulin cannot be used orally and continuous insulin injection have many side effects and toxicity[1,2]. Besides certain oral hypoglycaemic agents are not effective in lowering the blood sugar in chronic diabetic patients[3].

Plant extracts have been used by various investigators as hypoglycaemic agents[4-12]. Recently Chandrasekhar *et al.*[13,14] showed hypoglycaemic activity in selected Curcurbitaceae plants of Indian origin and in *Swertia chirayita*. In the present study we have investigated the antidiabetic effect of *Aegle marmelose* leaf extract in the alloxan diabetic rats.

Materials and Methods

Chemicals used—All chemicals and reagents used in the study were of analytical grade. Glucose estimation was done by using glucose assay kit. Blood urea was estimated by the Urease method using Berthelot Reaction[15]. Serum cholesterol was estimated by using Liebermann-Burchard Reagent[16]. Isolation and estimation of liver glycogen was done according to Plummer[17].

Methods of preparing crude extract from A. marmelose leaves—Fresh tender leaves were collected, dried in shade and powdered. 10 g of leaf powder was mixed with 100 ml of distilled water and stirred for 2 hr. It was kept overnight at 4°C and the supernatant was collected. This was used as the crude leaf extract to study the antidiabetic effect in alloxan induced diabetes. The effective dose (the quantity of extract that can bring down the glucose level in the blood to the normal level) was 1 g/kg weight of the animal. The extract showed antidiabetic activity if kept at 4°C for 2 weeks.

Animals used for experiment—Albino rats (Wistar strain) of 2-3 months old were selected for all the experiments. Rats were divided into 6 groups of 6 each:

Group I, kept as normal group.

Group II, given physiological saline through the femoral vein and was taken as the control group.

Group III, alloxan injection (60 mg/kg body wt) was given through the femoral vein and kept without any treatment to study the diabetic nature.

Group IV, alloxan injected and one unit of insulin given on alternate days after 5 days.

Group V (a), alloxan injected and leaf extract given orally after 24 hr and the traetment continued for 30 days.

Group V (b), alloxan injected and leaf extract given orally after 5 days and the treatment continued for 30 days.

On every 5th day from the start of the experiment, body weight was taken and the blood glucose estimated. All animals were sacrificed after 30 days of the experiment. The blood urea, serum cholesterol

and glycogen was estimated from the samples of these animals.

Results and Discussion

A decreasing trend in the body weight was noted in alloxan induced diabetic rats. On treatment of such rats with insulin and leaf powder extract the body weight was brought back to the initial level (Table 1). This indicates that the leaf powder extract is having an action similar to that of insulin.

A significant increase ($P<0.01$) was observed in the blood glucose after 5 days of alloxan injection and a steady increase in the group of animals given no treatment (Fig. 1). But when group IV was given insulin a gradual decrease in blood glucose was observed which was kept almost to the control ones. A similar effect was observed when leaf extract was administerd to group Vb. But a daily administration of the leaf extract in group Va maintained the glucose level near to that of control ones. A hypoglycaemic effect of *A. marmelose* was first reported by Dhar *et al.*[18]. In the present study the antidiabetic effect of *Aegle marmelose* is substantiated by using alloxan induced diabetic animal model. A similar study was carired out by Akhtar *et al.*[19] in normal and alloxan diabetic rabbits using *Momordica charantia* fruits, which significantly decreased the blood glucose level.

Table 2 presents he effect of glucose tolerance test carried out in control and experimental rats treated with leaf powder extract 30 min prior to the start of the

Table 3—Liver glycogen in normal, control and experimental rats

[Values are mean ± SE of 6-8 separate determination in each group]

Group no. & animal status	g/100 g wet wt of tissue
I Normal group	2.11 ± .04
II Saline injected (control)	2.11 ± .07
III Diabetic (alloxan injected)	1.24* ± .05
IV Diabetic and insulin treated	1.75 ± .08
V Diabetic and leaf extract treated	1.84 ± .14

*$P<0.05$ compared to groups I, II, IV & V.

Table 4—Blood urea and serum cholesterol in normal, control and experimental rats

[Values are mean ± SEM of 6-8 separate determination in each group]

Group no. & animal status	Blood urea mg/dl	Serum cholesterol, mg/dl
I Normal	22.66 ± 2.80	84.16 ± 2.78
II Saline (control) injected	24.00 ± 2.09	84.83 ± 4.53
III Diabetic (alloxan injected)	62.66* ± 3.50	192.67* ± 13.64
IV Diabetic and insulin treated	34.33 ± 2.06	108.17 ± 9.47
V Diabetic and leaf extract treated	37.83 ± 3.97	99.20 ± 8.43

*$P<0.01$ compared to groups I, II, IV & V.

Table 1—Weight (g) of normal, control and experimental rats

[Values are mean ± SE of 6-8 rats in each group]

Group & animal status	Initial	5 days	10 days	15 days	20 days	25 days	30 days
I Normal Group	115 ± 5	120 ± 4	125 ± 6	131 ± 6	140 ± 8	147 ± 6	160 ± 7
II Saline injected (control)	120 ± 7	123 ± 4	127 ± 7	134 ± 7	139 ± 5	147 ± 6	155 ± 9
III Diabetic (alloxan injected)	124 ± 5	116 ± 8	110 ± 7	106 ± 5	100 ± 5	80 ± 8	80* ± 5
IV Diabetic and insulin treated	116 ± 7	107 ± 5	110 ± 4	100 ± 7	107 ± 5	103 ± 4	100 ± 6
V Diabetic and leaf powder treated	118 ± 6	114 ± 7	112 ± 5	110 ± 4	107 ± 6	107 ± 5	109 ± 8

*$P<0.05$ compared to the zero day of the experiment.

Table 2—Glucose tolerance test in control and treated rats

[Values are mean ± SE of 6-8 separate determination in each group]

	Wt (g) of animals	Blood glucose in mg/100 ml			
		0 hr	30 min	60 min	90 min
I Control 1.5 g/kg glucose solution given, po	268 ± 7	78.3 ± 1.2	130 ± 1.2	114 ± 0.9	98 ± 1.4
II Experimental: 1 g/kg leaf powder extract given (po) 1/2 hr before oral administration of 1.5 g/kg glucose solution	276 ± 5	78.3 ± 1.5	115.5 ± 1.2	99.3 ± 1.1	83.7* ± 1.5

*$P<0.05$ compared to control.

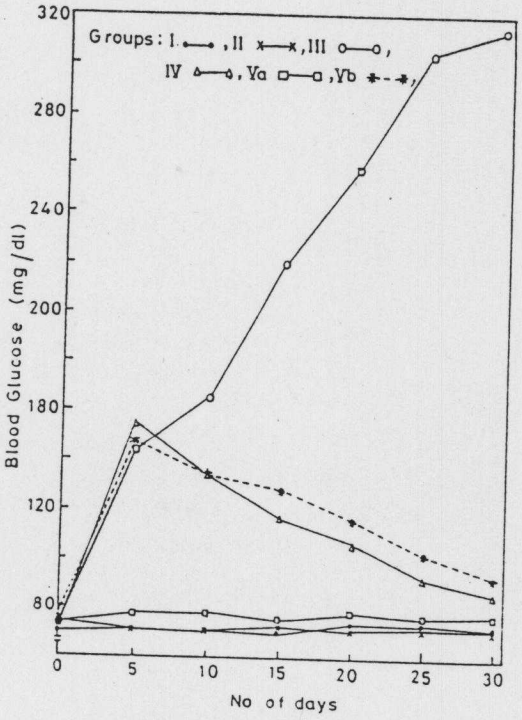

Fig. 1—Blood glucose levels is normal, control and experimental rats after varying intervals (group I—normal; group II—control (saline injected); group III—diabetic (alloxan injected); group IV—diabetic and insulin treated; group Va—diabetic and leaf extract treated 24 hr after alloxan injected and group Vb—diabetic and leaf extract treated 5 days after alloxan injection.

text. A significant increase ($P<0.05$) in glucose tolerance was observed. The liver glycogen level was significantly decreased ($P<0.05$) in diabetic rats, whereas, those treated with leaf powder extract maintained the glycogen in par with control rats (Table 3).

The blood urea and serum cholesterol levels increased significantly ($P<0.01$) in diabetic rats. In treated animals the blood urea and serum cholesterol were brought back to that of control rats. Giri *et al.*[9] reported a similar effect with the aqueous extract of *Cajanus cajan* is alloxan diabetic rats. It is a known fact that the kidney functioning is disturbed in diabetic condition. The treatment with leaf extract may have normalised the kidney function as indicated by the reversal of blood urea and cholesterol levels. Thus our results suggest that the active principle from *A. marmelose* leaf extract is effective for the treatment of diabetes.

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