12. Effect of Aegle Marmelose Leaf Extract on Alloxan Induced Diabetic Rats

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BSTRACT

Alloxan induced diabetic animal model was sed to evaluate the potential antidiabetic effect of ne aqueous leaf extract of *Aegle marmelose*. Insun injection and oral treatment of *Aegle marmelose* af extract to the alloxan induced rats maintained ne animal weight near to the control rats whereas here was a significant decrease in weight in alloxan duced rats. Insulin injection and oral treatment the leaf extract reversed the decrease of protein ontent in all the organs studied except the pancas. Glucose level was maintained near to the corol levels in the insulin injected and leaf extract eated alloxan diabetic rats. A significant increased ucose tolerance was observed in animals orally ven the leaf extract prior to the experiment. The sults indicate that there is an increased utilization glucose in the *Aegle marmelose* leaf extract tred rats.

TRODUCTION

Diabetes is a disorder in which there is an imlance between nutritional energy source and eney expenditure. It is caused either by a deficiency insulin or by insensitivity of the target cells to sulin. Diabetes is clearly influenced by multiple d complex env ronmental and genetic factors nich interact. Diabetes may also be induced by a mber of toxic substances and stressful stimuli, nich act either by interference with cellular utilizon of glucose or by eliciting sympathetic dischges from the central nervons system. Treatment this disorder follows three patterns viz. diet and ercise, insulin replacement therapy and the use of l hypoglycaemic agents such as sulfonylureas d biguanides.

Long before the use of insulin, indigenous nedies have been used for the treatment of diabetes mellitus. There is an increasing demand of patients to use the natural antidiabetic drugs. This is because insulin cannot be used orally and oral hypoglycaemic agents have many side effects and toxicity. Besides that certain synthetic oral hypoglycaemic agents do not remain effective in lowering the blood sugar in chronic diabetic patients (Nagarajan *et al* (1).

Gupta and Variyar (2) reported that alcoholic extract of the leaves of *Gymnema Sylvestre* has beneficial effects in mild diabetic animals. Plant extracts have been used by various investigators as hypoglycaemic agents after the above study (Teodosio *et al* (3), Brahmachary and Augusti. (4) Gupta *et al* (5) Padmini and Chakraborthy, [6] Sharma *et al*, [7], Singh *et al*, [8] Giri *et al*, (9), Narayanan et al., [10] Recently Tarza *et al* [11] showed cabbage oil prepared from fresh cabbage *(Brassia* Var. *Capitata* Lin*)* given orally 100 mg/kg to diabetic rats have profound hypoglycaemic activity. Also, Vinod kumar and Augusti [12] reported that a demethoxy derivative of leucocyanidin 3 - 0 - beta-D-galactosyl cellobioside isolated from the bark of *Ficus bengalensis* demonstrated antidiabetic action.

In the present investigation we have used aqueous leaf extract of *Aegle marmelose* to study the antidiabetic activity in alloxan treated rats.

MATERIALS AND METHODS

Chemicals used:

All chemicals and reagents used in the study were of analytical grade. Glucose assay kit was purcha sed from Ortho Diagnostic Systems.

Choice of plant:

To study the antidiabetic principle the plant selected for our work was the leaves of *Aegle marmelose*. Its leaves contain an active antidiabetic principle as reported by Dhar *et. al*, (13)

Method of preparing the crude extract:

Fresh tender leaves of *Aegle marmelose* were collected after identification on comparison with authentic species. The leaves were dried and powdered.5gms of the leaf powder was dissolved in 20ml of distilled water and stirred for 30 minutes. It was kept overnight and the supernatant collected was centrifuged to remove the suspended debris. This was made upto 20ml which remained stable for about 1 week at 4°c and it was used as the crude drug for alloxan induced diabetes and the effective dose was 1gm/kg weight of the animal.

Animals used for the experiment:

Albino rats *(Rattus norvegicus)* of about 2-3 months old and weighing about 200gms were selected for all the experiments. Rats were divided into 5 groups. One group received physiological saline through the femoral vein which was treated as the control group. A normal group of animals were kept to compare with the saline treated group. Adequate number of animals were injected with alloxan (intrafemoral 60mg/kg body weight) to use for other experimental groups. One group of alloxan treated rat was kept untreated all through to study the diabetic nature. Second group of alloxan treated animals were injected with one unit of insulin after two days of the starting of the experiment on alternate days. Third group of rat was given aqueous leaf powder extract orally (1 gm/kg body weight) 24 hrs. after alloxan injection. Weight of the animals were taken and blood sugar was estimated on all animals after every 5 days of starting the experiment using glucose assay kit following glucose oxidase peroxidase method for quantitative estimation of glucose. All animals were sacrificed after 28 days of the experiment. Organs-heart, liver, kidney, pancreas and brain were removed and were used for protein estimation (Lowry *et al* 14)

RESULTS

A decreasing trend in weight was noted in alloxan treated diabetic rats without any treatment (Table 1). Treatment with insulin and leaf power extract to the alloxan treated rats maintained the weight to the near control weights.

There was a significant decrease of protein content in the brain and kidney of the diabetic rat (Table II). A decreased trend was observed in liver and pancreas. Insulin injection or *Aegle marmelos* leaf extract treatment reversed the protein status in all the organs except the pancreas.

Alloxan treated rats showed significant increase in blood sugar levels compared to the controls after 5 days (Table III) which increased continuously till 20 days of the experiment. Insulin treatment on alternate days after 5 days of experimental animal injected with alloxan reversed the glucose level close to the control level. Oral feeding of the aqueous extract of the leaf powder after 24 hrs of alloxan injection daily maintained the glucose level near to the control level.

Glucose Tolerance Test (GTT) was carried out in control and experimental animals treated with leaf powder extract half an hour prior to the test (Table IV), A significant increased glucose tolerance was observed in the experimental animals given orally the leaf powder extract prior to the experiment.

DISCUSSION

Hypoglycaemic effect of leaf extract of *Aegle marmelose* was shown by Dhar *et al* in 1968. In the present study we used alloxan injected diabetic animal modal for substantiating antidiabetic effect of *Aegle marmelose* leaf extract. From our data it is clear that in different organs protein contents varied differently with alloxan injection and insulin treatment. Similar results are reported by Giri *et al*(9) with the administration of red gram seed (*Cajanus cajan*) aqueous extract on alloxan diabetic rats. They also reported a decrease in blood urea and serum cholesterol values with the treatment.

Tiangda *et al* (15) reported that unripe fruits of *Momordica charantia* administered orally to alloxan diabetic rabbits significantly decreased the blood glucose levels which supports our finding. This indicates that changes can occur in different organs if the system is disturbed Maintenance of weight close to the control level in diabetic rats orally treated with leaf powder extract indicates that metabolic pathways are functioning normally. Also there is proper utilization of glucose in the experimental animals orally given the leaf powder extract

as shown in table III. It is clearly evident from Glucose Tolerance Test (Table IV) that *Aegle marmelose* leaf powder extract increases the glucose utilization. Active compound in the aqueous extract of *Aegle marmelose* may be used as an effective treatment for diabetes.

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TABLE—I

Percentage waight in control and experimental animals (percentage compared with Saline treated control as 100 percent)

	0 day	5 days	10 days	15 days	20 days	28 days
Animals injected with Saline (control)	100	100	100	100	100	100
Normal animals	115	110	111	106	100	100
Animals injected with alloxan (60 mg/kg)	120	110	101	93	85	75*
Animals injected with alloxan + Insulin treated 1 unit on alternate days after 10 days of the experiment.	105	100	103	100	96	94
Animals injected with alloxan + leaf powder treated 1 gm/kg daily.	105	104	108	109	101	95

* Significantly different from 0 day of the experiment.

TABLE II

Percentage protein contents in the different organs of control and experimental animals after 28 days of treatment (Percentage compared with saline treated control as 100 per cent)

	Brain	Liver	Pancreas	Kidney	Heart
Animal injected with saline (Control)	100	100	100	100	100
Normal animals	111	104	106	92	103
Animals injected with alloxan (60mg./kg)	74*	88	90	67*	96
Animals injected with alloxan + insulin treated 1 unit on alternate days	90	117	87	88	103
Animals injected with alloxan + leaf powder treated 1g/kg daily	98	104	90	85	100

*Significantly different from saline treated control.

TABLE—III

Percentage Blood Sugar levels in control and experimentl animals (Percentage compared with saline treated control as 100 per cent)

	0 day	5 days	10 days	15 days	20 days	28 days
Animals injected with saline (control) Group 1	100	100	100	100	100	100
Normal animals Group II	100	101	100	96	103	105
Animals injected with alloxan (60mg/kg) Group III	102	161*	239*	320*	377*	377*
Animals injected with alloxan + Insulin treated 1 unit on alternate days after 10 days of the experiment. Group IV	98	164*	254*	133+	138+	138+
Animals injected with alloxoan + leaf powder treated 1gm/kg daily Group V	100	115+	107+	92+	105+	90+

* Significantly different compared to Group 1 (Control)

\+ Significantly different compared to the Group II (Alloxan-diabetic)

TABLE—IV

Glucose Tolerance Test in Control and experimental rats Blood sugar in mg/100ml ± SEM

	0 hr.	30 Mts.	60 Mts.	90 Mts.
Control 1.5g/kg of glucose solution given orally	78 ± 1.5	130 ± 1.5	114 ± 1.0	98 ± 1.7
Experimental Leaf powder extract 1g/kg given orally half an hour prior to 1.5g/kg glucose given	78 ± 1.8	115 ± 1.4*	99. ± 1.3*	84 ± 1.8*

* P 0.05 compared to the control rats.