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## 8. Hypoglycaemic Effect of Alkaloids Preparation From Leaves of *Aegle Marmelose*

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### ABSTRACT

Alloxan induced diabetic animal model was used to evaluate the antidiabetic effect of alkaloids extracted from the leaves of *Aegle marmelose*. The alkaloid extract maintained the weight of animals near to that of control ones - whereas there was a decrease in the body weight of diabetic animals. A significant increase in blood glucose ( $342.14 \pm 14.89$  mg/dl) was seen in diabetic animals but in alkaloid treated group the blood glucose was lowered ( $90:12 \pm 5.81$  mg/dl). There was no decrease in blood urea and serum cholesterol in the alkaloid treated group of diabetic animals. The liver glycogen decreased in diabetic animals ( $1.27 \pm 12$  g/100g of wet tissue) and the treatment brought the glycogen level to that of control ones ( $2.51 \pm .75$  g/100 g of wet tissue). The result show that the alkaloid extract has hypoglycaemic activity.

### INTRODUCTION

Diabetes mellitus which affects about 10% of the population is due to deficiency of insulin produced by the pancreatic islets, insensitivity of target cell to insulin, obese condition or genetic factors. The disease may be manifested due to stress which act either by cellular utilization of glucose or by eliciting sympathetic discharges from the central nervous system. Treatment of this, disorder follows three pattern viz., diet and exercise, insulin replacement therapy and the use of oral hypoglycaemic agents. But long before the said modalities of treatment was carried out in patients 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<sup>1,2</sup>.

Nagarajan et al.<sup>3</sup>, Atta-Ur-Rahman and Khurshid Zaman<sup>4</sup> and Ivorra et al.<sup>5</sup> prepared a comprehensive review of the materials used in the treatment of diabetes mellitus. Plant extracts have been used by various investigators in different animal models as hypoglycaemic agents.<sup>6-13</sup> Recently we<sup>14</sup> have reported that the aqueous extract of *Aegle marmelose* can bring down the blood glucose, serum cholesterol and blood urea in 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 purchased from Ortho Diagnostic systems. Blood urea was estimated by the urease method using Berthelot Reaction<sup>15</sup>. Serum cholesterol was estimated using Liebermann - Burchard Reagent.<sup>16</sup> Isolation and estimation of liver glycogen was done according to Plummer.<sup>17</sup>

#### Method of isolating the alkaloids from *Aegle marmelose* leaves

Fresh tender leaves were collected, dried in shade and powdered. 25g of the dried leaf powder was mixed with 100ml of petroleum ether to remove the fatty material. It was followed by extraction with methanol. After the removal of the solvent, water was added to the residue and the mixture was acidified to pH 2.0 with 1.0N HCl. It was kept for 10 days at 4°C to obtain a clear solution which was then decanted. Water soluble organic substances were then separated by extraction with chloroform. The solution was adjusted to pH 9.0 using 5% sodium carbonate. It was then extracted with chloroform twice to extract the free bases. The solvent was removed and the aqueous mixture of alkaloids was lyophilized. A sample containing

1g/100ml/ was prepared and this was used to study the antidiabetic activity. The effective dose (the quantity of the extract that can bring down the glucose level in the blood to normal level) was 100mg/kg weight of the animal. The extract showed antidiabetic activity for 2 months if kept at 4°C.

#### Animals used for the experiment

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

Group I, kept as control group.

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

Group III, alloxan injected and the alkaloid 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 liver glycogen were estimated in the samples taken from these animals.

#### Results and Discussion

A decrease in body weight ( $P < 0.05$ ) was noted in alloxan induced diabetic animals kept without any treatment. But when the diabetic rats were treated with the alkaloid extract the body weight was almost maintained to the normal level (Table 1). This indicates that the abnormal metabolic activities in the body of diabetic animal can be brought to normal pattern with the alkaloid treatment.

A significant increase ( $P < 0.01$ ) in blood glucose level was noted in animals given alloxan injection and there was a steady increase if no

treatment was given (Table 2). In the alkaloid treated group of animals the blood glucose level was maintained to that of control ones. Mohapatra et al.<sup>18</sup> reported antidiabetic activity in the alkaloid extracts of *Zizyphus mauritiana*. Undie and Akubor reported that the alkaloid containing fraction *Dioscorea dumetorum* shows hypoglycaemic activity in fasting normal mice and alloxan diabetic rabbits.

Table 3 presents the blood urea, serum cholesterol and liver glycogen in different experimental groups of animals. In alkaloid extract treated group of diabetic animals the blood urea and serum cholesterol levels were not reversed to the control levels. But the liver glycogen was almost the same in the treated and control group of animals. It was reported by Boyadzhieva<sup>19</sup> that an alkaloid extract from the aerial parts of *Lepidium ruderale* increased liver glycogen in alloxan induced diabetic animal models.

In the diabetic condition the normal function of the kidney is disturbed and so there is an increase in blood urea. The alkaloid treatment cannot rectify this and it can be assumed that during the extraction of alkaloids the active principle involved in this may be lost. The alkaloid extract may be promoting glycogenesis and thereby decreasing blood glucose in the treated group. So this alkaloid can be given for the treatment of diabetes only where there is no hypercholesteremia and uraemia.

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TABLE 1

Weight (g) of normal, control and experimental rats

(Values are mean  $\pm$  SD 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	140 $\pm$ 12	147 $\pm$ 7	150 $\pm$ 9	152 $\pm$ 8	161 $\pm$ 8	163 $\pm$ 9	168 $\pm$ 4
II. Diabetic (alloxan injected)	152 $\pm$ 7	150 $\pm$ 9	142 $\pm$ 7	138 $\pm$ 6	135 $\pm$ 4	132 $\pm$ 4	133* $\pm$ 7
III. Diabetic and alkaloid fraction treated	160 $\pm$ 6	156 $\pm$ 7	152 $\pm$ 7	144 $\pm$ 10	148 $\pm$ 9	151 $\pm$ 9	154 $\pm$ 6

\* P &lt; 0.05 compared to zero day of the experiment.

TABLE II

Blood glucose levels (mg/ dl) in normal, control and experimental rats  
(Values are mean  $\pm$  SD of 6-8 rats in each group)

Group & animals status	Initial	5 days	10 days	15 days	20 days	25 days	30 days
I. Normal group	84.76 $\pm$ 1.86	80.72 $\pm$ 2.62	82.11 $\pm$ 0.97	82.62 $\pm$ 1.27	80.12 $\pm$ 0.87	81.71 $\pm$ 1.27	84.34 $\pm$ 2.17
II. Diabetic (alloxan injection)	82.79 $\pm$ 1.97	162.87 $\pm$ 8.44	210.81 $\pm$ 9.47	243.82 $\pm$ 8.94	256.84 $\pm$ 7.72	302.72 $\pm$ 12.74	342.14 $\pm$ 14.89*
III. Diabetic and alkaloid	79.97 $\pm$ 2.66	158.47 $\pm$ 8.79	138.91 $\pm$ 6.27	109.11 $\pm$ 11.89	98.27 $\pm$ 3.87	90.84 $\pm$ 4.88	90.12 $\pm$ 5.81

\*P < 0.01 compared to zero day of the experiment.

TABLE III

Blood urea serum cholesterol and liver glycogen levels in normal, control and experimental rats  
(Values are mean  $\pm$  SD of 6-8 rats in each group)

Group & animal status	Blood urea mg/dl	Serum cholesterol mg/dl	Liver glycogen g/100g wet weight of tissue
I. Normal group	23.84 $\pm$ 4.32	89.92 $\pm$ 6.27	2.67 $\pm$ 0.74
II. Diabetic (alloxan injected)	56.82 $\pm$ 8.37	182.92 $\pm$ 11.27	1.27 $\pm$ 0.12*
III. Diabetic and alkaloid fraction treated	43.76 $\pm$ 5.89	176.89 $\pm$ 14.39	2.51 $\pm$ 0.75

\*P < 0.05 compared to group I & III

