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

P T C Ponnachan, C S Paulose & K R Panikkar
Amala Cancer Research Centre. Amala Nagar, Thrissur 680 553, India
*Centre for Biotechnology, Cochin University of Science & Technology, Cochin 682 022, India
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Alloxan induced animal model was used to evaluate the potential antidiabetic effect of *A. marmelos* leaf extract. The diabetic animals were given insulin injection and another group *A. marmelos* 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 ± 0.07 g/100 g of wet tissue) was observed in diabetic rats which was brought to almost the normal level (1.84 ± 0.14 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. marmelos* 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. Besides certain oral hypoglycaemic agents are not effective in lowering the blood sugar in chronic diabetic patients.

Plant extracts have been used by various investigators as hypoglycaemic agents. Recently Chandrasekhar et al. showed hypoglycaemic activity in selected Cucurbitaceae plants of Indian origin and in *Swertia chirayita*. In the present study we have investigated the antidiabetic effect of *Aegle marmelos* 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. Serum cholesterol was estimated by using Liebermann-Burchard Reagent. Isolation and estimation of liver glycogen was done according to Plummer.

**Methods of preparing crude extract from *A. marmelos* 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 treatment 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 administered 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 hypoglycemic effect of A. marmelos was first reported by Dhar et al. 18. In the present study the antidiabetic effect of A. marmelos is substantiated by using alloxan induced diabetic animal model. A similar study was carried out by Akhtar et al. 11 in normal and alloxan diabetic rabbits using Momordica charantia fruits, which significantly decreased the blood glucose level.

Table 2 presents the 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 test.

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

<table>
<thead>
<tr>
<th>Group no. &amp; animal status</th>
<th>g/100 g wet wt of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Normal group</td>
<td>2.11±0.04</td>
</tr>
<tr>
<td>II Saline injected (control)</td>
<td>2.11±0.07</td>
</tr>
<tr>
<td>III Diabetic (alloxan injected)</td>
<td>1.24±0.05</td>
</tr>
<tr>
<td>IV Diabetic and insulin treated</td>
<td>1.75±0.08</td>
</tr>
<tr>
<td>V Diabetic and leaf extract treated</td>
<td>1.84±0.14</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Group no. &amp; animal status</th>
<th>Blood urea mg/dl</th>
<th>Serum cholesterol, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Normal</td>
<td>22.66±2.30</td>
<td>84.16±2.78</td>
</tr>
<tr>
<td>II Saline (control) injected</td>
<td>24.00±2.09</td>
<td>84.83±4.53</td>
</tr>
<tr>
<td>III Diabetic (alloxan injected)</td>
<td>62.66±3.50</td>
<td>192.67±13.64</td>
</tr>
<tr>
<td>IV Diabetic and insulin treated</td>
<td>34.33±2.06</td>
<td>108.17±9.47</td>
</tr>
<tr>
<td>V Diabetic and leaf extract treated</td>
<td>37.83±3.97</td>
<td>99.20±8.43</td>
</tr>
</tbody>
</table>

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

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

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

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

Table 2—Glucose tolerance test in control and treated rats

<table>
<thead>
<tr>
<th>Wt (g) of animals</th>
<th>Blood glucose in mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>I Control</td>
<td>268±7</td>
</tr>
<tr>
<td>II Experimental</td>
<td>276±5</td>
</tr>
</tbody>
</table>

*P<0.05 compared to control.
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.\textsuperscript{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.

References