Evaluation of Antihyperglycemic and Hypolipidemic Action of Euphorbia Caducifolia Latex Powder on Alloxan-Induced Diabetic Rats

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Abstract—

Background: The study of Plants having antihyperglycaemic and hypolipidaemic activities may give new approach in the treatment of diabetes with lesser side effects.

Objective: The study was intended to evaluate the antihyperglycemic and hypolipidemic action of Euphorbia caducifolia latex powder in alloxan-induced Diabetic Rats.

Materials and Methods: Diabetes was induced in albino rats by using alloxan monohydrate (150 mg/kg). Rats were divided into five groups of six animals each. First group served as non-diabetic control, second group as diabetic control, third group as standard and was treated with Nopal. Group 4 and 5 received 100 and 200 mg/kg body weight of ECLP. Blood samples were analyzed for blood glucose on day 1, 7, 14 and lipid profile on day 21.

Results: The ECLP showed significant reduction (P<0.01) in blood glucose level and serum lipid profile levels with 200 mg/kg body weight in alloxan-induced diabetic rats as compared with the control.

Conclusion: It is concluded that ECLP is effective in controlling blood glucose levels and in improving lipid profile in diabetic rats.

Keywords— Alloxan, Antihyperglycemic, Diabetes Mellitus, Hypolipidemic.

I. INTRODUCTION

Diabetes Mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid, and protein metabolism which causes hyperglycemia resulting from insufficient insulin secretion, insulin action or both. The increasing incidence of the disease worldwide may be due to sedentary life style, unhealthy diet, obesity and other predisposing risk factors. It is projected to become of the world’s main disablers and killers, as the number of people with diabetes multiplies worldwide. It is one of the refractory diseases identified by Indian Council of Medical Research for which an alternative medicine is a need for the treatment. Diabetes has become a growing problem in the contemporary world.

Due to the etiopathogenesis of diabetes mellitus, harmful side effects with synthetic drugs, the inability of existing modern therapies to control all the pathological aspects of the diabetic disorder, enormous cost of modern drugs as well as the poor availability of the advanced therapies for many rural populations in developing countries. Alternative strategies to current pharmacotherapy of diabetes mellitus are urgently needed. The use of medicinal plants is, therefore, going to be stepped up at primary health care in diabetic mellitus to make a breakthrough of diabetic treatment. Recent experiences are proving the natural drugs as relatively non-toxic, safe and even free from serious side effects.

The main objective of the study was to assess the antihyperglycemic action and hypolipidemic action of Euphorbia caducifolia Latex powder belonging to the family Euphorbiaceae. It is known as Thor, Danda-thor in Hindi and Kattejemudu in Telugu. Euphorbia caducifolia is a major species in rocky desert areas of western and central India and Pakistan. Euphorbia caducifolia grows in stony ground on barren coastal plains and in the hilly tracts of the Indian desert on well-drained limestone soils as well on sandy soil, and therefore the substrate varies from slightly alkaline to slightly acidic. Euphorbia caducifolia (a.k.a. Leafless Milk Hedge) is a great looking, sparsely spiny columnar, many-stemmed, cactus-like, shrub forming dense, Leaves are having leaf blades, oval, fleshy, variable size, 2.5-8 cm long and 2-5 cm broad. Flowers are yellowish rarely reddish about 5mm in diameter with oblong and joined nectar glands. Many herbaceous Euphorbia species have traditionally been used as a purgative or laxative. The roots of the plant are used as antidote and germicidal. The latex of this plant is used as Anti-gout, Anti-asthmatic, Anti-rheumatic, Counter-Irritant, Emetic, Expectorant, Rubefacient, Wound healing. Although many compounds have been reported from the genus, Euphorbia, Previous phytochemical investigations revealed the occurrences of ephul, tirucallol, cycloartenol, methyl octadecenoate, and 3,7,11,15-tetramethyl-2-hexadecene-1-ol (GCMS Analysis).

In the current literature, there is not much data concerning the effect of Euphorbia caducifolia on the blood glucose level and parameters used in lipid profile. Therefore, the present study has been planned to investigate the antihyperglycemic action and hypolipidemic action of latex powder in alloxan induced diabetic rats.
II. MATERIALS AND METHODS

Plant Materials

The plant of *Euphorbia caducifolia* was obtained from the college, where the plant specimen was maintained and authenticated by Dr. V.S. Raju, Professor, botany department, Kakatiya University, Warangal, Telangana. A voucher specimen was submitted at Botany Department, Kakatiya University, Warangal.

Preparation of Extract

*Euphorbia caducifolia* plant was air dried and powdered then it was extracted with 95% Ethanol by hot continuous percolation method in a Soxhlet apparatus. The extract was evaporated to dryness under vacuum desiccator to obtain a dried ethanol extract and that was stored at 4°C for further experimental study.

Procurement of Chemicals and Animals

Alloxan and Nopal are obtained as gift samples. Carboxy methyl cellulose is obtained from Vaagdevi College, Hanamkonda. GOD-POD Kit, Total Cholesterol, HDL, LDL and Triglyceride kits were procured from Biologicals Pvt. Ltd., Bangalore. Accuchek strips were purchased from Care Pharmacy, Hanamkonda.

Healthy Wistar albino rats of either sex weighing 150-200g were procured from Mahaveera Enterprises, Hyderabad. Housed in polypropylene cages, maintained under standard laboratory conditions (27 ± 2 °C) 12 hrs light-dark cycle throughout the period of acclimatization and experimentation. Animals were fed with standard diet and water *ad libitum*. The experiments were planned after the approval of Institutional Animal Ethical Committee (IAEC), (1047/ac/07/CPCEA, dated 24-04-2007) at Vaagdevi College of Pharmacy, Warangal, Telangana.

Acute Toxicity Studies

The acute oral toxicity study was carried out according to OECD guidelines, method described in the literature. The ethanolic extracts of *Euphorbia caducifolia* (ECEE) were suspended in 0.5% of carboxy methyl cellulose in doses of 100, 200, 400, 800, 1000, 1200, 1400, 1800 and 2000 mg/kg were administered orally to albino mice. The treated groups were observed continuously for any change in autonomic or behavioral responses for first few hours and later at 24 hrs intervals for a period of 48 hrs. At the end of this period, the mortality rates in all groups were noted. Mortality was noticed in the dose of 2000 mg/kg. The LD50 of the extracts was found to be 200 mg/kg body weight.

Invitro Glucose Absorption Studies

In this study 3 animals were taken for performing *in vitro* studies.

Animal -1: Glucose 0.2ml of 50mg/ml
Animal -2: Glucose (0.2ml of 50mg/ml) + Test Extract (15mg in 0.3ml solution)
Animal -3: Glucose (0.2ml of 50mg/ml) + Standard (15mg in 0.3ml solution)

The rats were fasted overnight, sacrificed and the intestine was removed. Duodenum, jejunum and ileum were cut separately. Each part was then divided into 3 smaller pieces. The sacs were tied at 1 end, filled with the solution and then the other end was also tied. These were placed in organ bath containing 25ml Dulbeccos buffer and maintained temperature at 35°C, upto 2 hrs. The samples were collected for every 30min and the glucose concentration was estimated using GOD-POD method.[8,9]

\[
\text{Conc of Glucose (mg/dl)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times \text{Conc of std}
\]

Induction of Diabetes in Rats: [10,11]

Selected group rats were made diabetic by injected with a single dose of alloxan (150mg/kg) by Intraperitoneal according to body weight of each animal. Alloxan was dissolved in citrate buffer (0.1 mol/Lt) before administration. After 48hrs of alloxan injection those rats blood glucose levels >150mg/dl were considered for this study.

Assessment of Anti-Diabetic Activity in Rats by using Alloxan-Induced Method: [10,11]

In the present study, the albino wistar rats (150-200 mg/kg) were taken, the rats were divided into five groups (I–V), each group consisting of six animals.

- **Group 1** – Normal control, administered with vehicle (Normal saline)
- **Group 2** – Diabetic control administered with vehicle (Without any drug)
- **Group 3** – Diabetic rats administered with Standard drug Nopal (120 mg/kg b.w.) p.o
- **Group 4** – Diabetic rats administered with ECEE 100mg/kg b.wt. p.o
- **Group 5** – Diabetic rats administered with ECEE 200mg/kg b.wt. p.o

Group 1 animals served as normal control Group II-V animals are diabetic control rats, which received as mentioned above. The animals were allowed food and water *ad libitum*. The blood samples were taken on 1st, 7th and 14th day of the start of the treatment for every 2hrs upto 10 hrs after the administration of the drug.[12,13]

Blood was collected from retro-orbital vein of each rat and was obtained by centrifuge each blood sample at 3000 rpm and 25°C temperature for 10 minutes. The specific enzymatic kit was used to assess the serum glucose levels of rats using spectrophotometer.

Statistical Analysis

All the values were expressed as Mean±SEM. The results were analyzed for statistical significantly by using one-way ANOVA followed by Dunnett’s test. P<0.05 was considered significant.

III. RESULTS

Acute Toxicity Study

In the acute toxicity study, *Euphorbia caducifolia* latex at the dose of 2000mg/kg (b.w) rats was found to be safe. No lethality was observed upto 2000mg/kg and no behavioral,
neurological and autonomic profiles and was found to be safe. Hence, as 5% or 10% of the dose i.e 100mg/kg and 200mg/kg can be taken for the studies.

**In-vitro Glucose Absorption Studies:**

Amount of glucose absorption through the intestine is shown in Table 1. 2. 3. Glucose absorption through the intestine increases with time. When given along with the standard drug Nopal, the absorption is inhibited and hence the concentration of glucose absorbed by the intestine is decreased. This could be one of the reason for the anti-diabetic activity. The same mechanism is supposed to be expressed even for Euphorbia caducifolia. It has shown its effect in the duodenum and jejunum but the same is not observed in the ileum.

**TABLE 1. In-vitro glucose absorption in Duodenum of Rat.**

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>0 (Hr)</th>
<th>0.5 (Hr)</th>
<th>1 (Hr)</th>
<th>1.5 (Hr)</th>
<th>2 (Hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (µg/dl)</td>
<td>8.63</td>
<td>9.03</td>
<td>9.29</td>
<td>29.69</td>
<td>12.21</td>
</tr>
<tr>
<td>Glucose+Nopal (µg/dl)</td>
<td>6.64</td>
<td>6.77</td>
<td>6.77</td>
<td>8.49</td>
<td>9.03</td>
</tr>
<tr>
<td>Glucose+ECEE</td>
<td>7.03</td>
<td>8.36</td>
<td>8.49</td>
<td>9.16</td>
<td>10.09</td>
</tr>
</tbody>
</table>

**TABLE 2. In-vitro glucose absorption in Jejunum.**

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>0 (Hr)</th>
<th>0.5 (Hr)</th>
<th>1 (Hr)</th>
<th>1.5 (Hr)</th>
<th>2 (Hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (µg/dl)</td>
<td>6.24</td>
<td>6.37</td>
<td>6.5</td>
<td>6.57</td>
<td>8.1</td>
</tr>
<tr>
<td>Glucose+Nopal (µg/dl)</td>
<td>6.77</td>
<td>7.83</td>
<td>8.1</td>
<td>9.03</td>
<td>9.56</td>
</tr>
</tbody>
</table>

Glucose absorption in Ileum is more when compared to absorption in duodenum and jejunum. Glucose absorption in Ileum increased with time. When glucose was administered along with Nopal, the amount of glucose absorbed was decreased, to a lesser extent. When glucose was given with ECEE, in ileum the amount of glucose absorption was increased tremendously.

**TABLE 3. In-vitro Glucose Absorption in Ileum.**

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>0 (Hr)</th>
<th>0.5 (Hr)</th>
<th>1 (Hr)</th>
<th>1.5 (Hr)</th>
<th>2 (Hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (µg/dl)</td>
<td>9.16</td>
<td>14.74</td>
<td>14.87</td>
<td>15</td>
<td>16.33</td>
</tr>
<tr>
<td>Glucose+Nopal (µg/dl)</td>
<td>8.63</td>
<td>11.15</td>
<td>11.3</td>
<td>11.68</td>
<td>15.67</td>
</tr>
<tr>
<td>Glucose+ECEE</td>
<td>18.32</td>
<td>21.3</td>
<td>21.24</td>
<td>21.38</td>
<td>23.1</td>
</tr>
</tbody>
</table>

Glucose absorption in Ileum was increased when compared to absorption in duodenum and jejunum. Glucose absorbtion through the intestine is decreased, to a lesser extent. When glucose was given with ECEE, in ileum the amount of glucose absorption was increased tremendously.

**Effect of Euphorbia caducifolia Latex Powder on Blood glucose levels in Alloxan induced Diabetic Rats**

The anti-hyperglycemic effect of ECEE 100mg/kg, 200mg/kg and Nopal on serum glucose levels of diabetic rats was given in Table 4. Daily treatment with ECEE 100mg/kg, 200mg/kg and Nopal, a decrease in the serum glucose levels was found to be dose-dependent.

There was no change of blood glucose levels in the control group in all the days of measurement. The diabetic group experienced an increase in the levels of blood glucose due to lack of anti-hyperglycemic treatment. Group 3, received Nopal, had a decrease in blood glucose levels, but at the end of the study they reached normal. Blood glucose levels in animals of group 4 and 5 decreased as seen from day 1, day 7 and day 14, but that was not as efficient as that of Nopal. When the dose was increased to 200mg/kg b.w., the activity showed a dose dependent response, though its action was less than that of Nopal.

*p* value was found to be <0.0001 as compared with the diabetic group, indicating the result to be more significant.

**TABLE 4. Effect of ECEE on blood glucose levels in Alloxan induced Diabetic Rats.**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>1st day</th>
<th>7th day</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Control</td>
<td>81.91±1.83</td>
<td>81.91±1.83</td>
<td>81.91±1.83</td>
</tr>
<tr>
<td>Group 2: Diabetic</td>
<td>321.9±1.76</td>
<td>340±2.14</td>
<td>389.76±10.84</td>
</tr>
<tr>
<td>Group 3: Nopal</td>
<td>264.36±1.26***</td>
<td>254±2.07***</td>
<td>100.76±1.28***</td>
</tr>
<tr>
<td>Group 4: ECEE (100mg/kg)</td>
<td>332.13±0.95***</td>
<td>286.73±1.09***</td>
<td>264.36±1.26***</td>
</tr>
<tr>
<td>Group 5: ECEE (200mg/kg)</td>
<td>317.7±0.95***</td>
<td>264.36±1.26***</td>
<td>133.23±1.56***</td>
</tr>
</tbody>
</table>

Data represents Mean±S.D. (n=6), *P<0.05, **P<0.01, ***P<0.001 (<0.0001), Significant compared to diabetic control analysed by one-way ANOVA followed by Dunnett’s test.
The mean blood glucose level of control group was found to be 81.91±1.83, for diabetic group, it was 321.9±1.76. In Nopal group, it was 264.36±1.26, for E.c (100mg/kg) it was 332.13±0.95 and for ECEE (200mg/kg), it was 317.7±0.95. Group 3 which was treated with Nopal exhibited a decrease in the blood glucose levels and groups 4 and 5 exhibited a dose dependent decrease in the blood glucose levels.

### Effect of ECEE on Serum Lipids

Effect of ECEE on total cholesterol, triglycerides, HDL, LDL levels were shown in Table 5. The levels of total cholesterol, triglycerides and LDL increased and HDL levels decreased in diabetic rats as compared to the normal control rats. Treatment with ECEE 100mg/kg, 200mg/kg and Nopal reduced the total cholesterol, triglycerides and LDL while they increased HDL levels very significantly (P <0.0001). The results were more profound with ECEE 200mg/kg, but was lower as compared to that of Nopal.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol</th>
<th>HDL</th>
<th>Triglycerides</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>141.33±1.21</td>
<td>40.16±2.13</td>
<td>85.16±2.85</td>
<td>79.16±5.23</td>
</tr>
<tr>
<td>Diabetic</td>
<td>177.5±3.01</td>
<td>33.16±2.92</td>
<td>108.66±4.27</td>
<td>180.83±4.99</td>
</tr>
<tr>
<td>Nopal</td>
<td>158.16±6.43</td>
<td>70.33±6.03</td>
<td>98.16±2.63</td>
<td>71.16±2.48</td>
</tr>
<tr>
<td>ECEE (100mg/kg)</td>
<td>166.33±2.16</td>
<td>48.5±4.37</td>
<td>104.1±1.32</td>
<td>80.33±3.55</td>
</tr>
<tr>
<td>ECEE (200mg/kg)</td>
<td>162.16±1.47</td>
<td>50.33±4.47</td>
<td>101.66±1.63</td>
<td>77.33±3.55</td>
</tr>
</tbody>
</table>

Data represents Mean±S.D (n=6). *P<0.05 **P<0.01 ***P<0.001, Significant compared to diabetic control analyzed by one-way ANOVA followed by Dunnett’s test.

### Conclusion

The total cholesterol levels increased in diabetic rats as compared to that of the control. When Nopal was given, the levels decreased significantly (P < 0.0001). The administration of ECEE to the diabetic rats decreased the total cholesterol levels dose dependently, though not as much as that of Nopal.

The HDL levels decreased in diabetic rats as compared to that of the control. When Nopal was given, the levels increased significantly ($P < 0.0001$). The administration of ECEE to the diabetic rats increased the total cholesterol levels dose dependently, though not as much as that of Nopal.

ECEE to the diabetic rats decreased the LDL levels dose dependently, though not as much as that of Nopal.

**IV. DISCUSSION AND CONCLUSION**

The present study showed the Anti-hyperglycemic action and hypolipidemic action of *Euphorbia caducifolia* latex powder on Alloxan induced diabetic rats. On performing the Acute toxicity studies, it has been found that a dose of 2000mg/kg b.wt of *Euphorbia caducifolia* is not toxic, and so a dose of 100mg/kg and 200mg/kg b.wt is selected for activity.

In-vitro glucose absorption studies has shown that the absorption of glucose from the gut can be delayed by the drug. Even though its effect is lower than that of Nopal, it is found to establish the action. Hence, the mechanism behind the anti-hyperglycemic activity may be the inhibition of glucose absorption through the gut.

The anti-hyperglycemic activity of *Euphorbia caducifolia* is shown in alloxan induced diabetic rats. Blood glucose levels in animals of group 100mg/kg b.wt, decreased as seen from day 1, day 7 and day 14, but that was not as efficient as that of Nopal. When the dose was increased to 200 mg/kg b.w., the activity showed a dose dependent response, though its action was less than that of Nopal. The activity is found to be significant with $P$ value $<0.001$.

The hypolipidemic activity is studied for the plant. Administration of vehicle to alloxan-induced diabetic rats resulted in an increase in the level of triglycerides, total cholesterol, LDL, and VLDL, and decreased HDL, after 21 days. Continuous administration of the test drug lead to significant decrease ($P < 0.001$) in the level of triglycerides, total cholesterol, LDL, and VLDL in the diabetic rats, while it increased ($P < 0.01$) the level of HDL, that means after the administration of Nopal and *Euphorbia caducifolia*, the lipid levels were restored to normal.

Alloxan induces diabetes by destroying the insulin-producing beta cells of the pancreas. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to induction of cell necrosis. This action is mediated by reactive oxygen species with a simultaneous massive increase in calcium concentration leading to a rapid destruction of beta cells. The use of lower dose alloxan (120 mg/kg b.w.) produced partial destruction of pancreatic beta cells even though the animals became permanently diabetic. Thus these animals have surviving beta cells and regeneration is possible.

The antihyperglycemic effect of the test drug may be due to the enhanced secretion of insulin from the beta cells of pancreas or may be due to increased tissue uptake of glucose by enhancement of insulin sensitivity or due to inhibition of glucose absorption through the gut.

Prolonged administration of an test drug leads to significant reduction in blood glucose level, which is in agreement with other studies. The hypoglycemic activity of the drug was due to the regeneration of pancreatic cells that were partially destroyed by alloxan, and potentiation of insulin secretion from surviving b-cells of the islets of Langerhans.

Diabetic rats were observed to have increased plasma lipids, which are responsible for several cardiovascular
The higher lipid levels seen in diabetic rats was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones. The test drug leads to regeneration of the b-cells of the pancreas and potentiation of insulin secretion from surviving b-cells; the increase in insulin secretion and the consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this context, a number of other plants have also been reported to have antihyperglycemic, antihyperlipidemic, and insulin stimulatory effects.

It is well known that LDL plays an important role in atherosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The consequent decrease of cholesterol and LDL level with a defect relating to the lack of LDL receptors. The administration of the powder of Euphorbia caducifolia, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about. From the present study, it was concluded that the latex powder of Euphorbia caducifolia has anti-hyperglycemic and hypolipidaemic activity and also can restore the lipid levels to the normal, the dose being 200mg/kg b.w. Further studies are needed to identify the chemical constituents of the latex powder of Euphorbia caducifolia that may be responsible for the anti-hyperglycemic and hypolipidaemic activity.

REFERENCES


