Antidiabetic activity of *Convolvulus microphyllus* Sieb. root extracts in alloxan & dexamethasone induced diabetic rats

Khan N. I.*, Dr. N. S. Naikwade.
Department of Pharmacology, A. B. C. P. Sangli.

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**ABSTRACT**

The hypoglycemic effect of the aqueous and ethanolic extract of roots of *Convolvulus microphyllus* Sieb. was examined in alloxan and dexamethasone induced diabetic rats. Extracts at 500 and 300 mg/kg produced a significant decrease in plasma glucose levels when compared with diabetic control group in alloxan induced diabetes and dexamethasone induced insulin resistance in rats. Photochemical group tests were also accomplished and presence of alkaloids, tannins, flavonoids, saponins and glycosides were found in extracts.

**Keywords:** Diabetes mellitus, Alloxan, Dexamethasone, *Convolvulus microphyllus* Sieb.

**INTRODUCTION**

"Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia; is associated with abnormality in carbohydrate, fat and protein metabolism; and results in the chronic complications, microvascular and macrovascular disorders". Criteria for the diagnosis of DM have been proposed by several medical organizations. The American Diabetes Association (ADA) criteria include symptoms of DM (e.g., polyuria, polydipsia, and unexplained weight loss) and a random plasma glucose concentration of greater than 200 mg/dl, a fasting plasma glucose concentration of greater than 126 mg/dl, or a plasma glucose concentration of greater than 200 mg/dl 2 hours after the ingestion of an oral glucose load. Diabetes mellitus is one of the main threats to human health in the 21st century. The World Health Organization (WHO) estimated that there were 135 million diabetics in 1995 and this number would increase to 300 million by the year 2025. India leads the world today with the largest number of diabetics in any given country.

For the present study *Convolvulus microphyllus* Sieb. belonging to the family Convolvulaceae was chosen. The plant has been suggested in the Indian system of medicine for a number of diseases. There is no systematic and scientific investigation for antidiabetic activity has been conducted on this plant.

Hence, this study has been conducted to evaluate hypoglycemic activity of aqueous and ethanolic extracts of *Convolvulus microphyllus* Sieb. in alloxan induced diabetes and dexamethasone induced insulin resistance in rats.

**MATERIALS AND METHODS:**

**Plant materials:**

Plant materials used in this study consisted of the powder of roots of *Convolvulus microphyllus* Sieb., was collected from Bavadekar Ayurvedic shop, Kolhapur and authenticated by powder characters.

**Preparation of plant extracts:**

The powdered drug was defatted by petroleum ether. Extract was filtered and marc was dried in hot air oven below 50°C.

1) Ethanollic extract:

Then the plant material (marc) was extracted in soxhlet assembly using 95% ethanol. Each extract is filtered and concentrated by evaporating to dryness on water-bath.

2) Aqueous extract:

The drug is macerated with chloroform-water(1:10). Here the drug is shaken for first 6 hrs and then stand for next 18 hrs. Filter the residue and evaporate the filtrate to dryness on water bath.

**Animals:**

Male/female Wistar albino rats weighing 200-250 g, were procured from the animal house of Pharmacology Department of Appasheb Birnale College of Pharmacy, Sangli, and used after getting approval of the Institute Animal Ethics Committee. On arrival, the animals were distributed at random and housed in polypropylene cages (43 X 23 X 15 cm) according to sex so that each cage contains maximum 4 animals of the same sex. All the animals were subjected to a period of observation and acclimatization of at least two weeks between the date of issue and the start of treatment. All the rats were fed on the standard rat diet (Amrut laboratory animal feed, Sangli, Maharashtra).

**Test extracts:**

The aqueous and ethanolic extracts were administered orally using sterile oral feeding needle. The quantity of extracts administered to each animal was calculated daily from their body weights.

**Acute oral toxicity study:**

The acute oral toxicity study of aqueous and ethanolic extracts of roots of *Convolvulus microphyllus* Sieb. was studied as per the OECD guideline 425 in Wistar rats maintained under standard condition. The animal was fasted at overnight prior to the experiment. The initial dose of 5,000 mg/kg was administered orally to a single rat and observed for behavioral changes. Then the dose was fixed such as one tenth (1/10th) of safe treatment dose.

**Selection of Doses:**

Two doses of each extract were selected.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Extract Dose mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous 300</td>
</tr>
<tr>
<td>2</td>
<td>Aqueous 500</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic 300</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic 500</td>
</tr>
</tbody>
</table>

**Preparation of the formulation:**

The aqueous extract was prepared daily in distilled water. The ethanolic extract was suspended in 0.5%w/v CMC in distilled water.

**Reference drug - Selection of dose and administration route:**

The hypoglycemic effect of extract was compared with that of the standard antidiabetic drug glibizide, 4mg/kg single dose in alloxan induced diabetes. Pioglitazone was suspended in 0.5%w/v CMC and administered orally at a dose of 20 mg/kg b.w. using oral feeding needle and used as standard drug in dexamethasone induced diabetes. The quantity of drug administered to each animal was calculated daily from its body weight.
RESULTS:

Table No: 1: Effect of Aqueous and Ethanolic extracts of roots of Convolvulus microphyllus Sieb. on serum glucose level and glycogen content of Alloxan induced diabetic animals during chronic study.

<table>
<thead>
<tr>
<th>Days</th>
<th>Group</th>
<th>Days</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum glucose concentration (mg/dl)</td>
<td>Mean ± SEM</td>
<td>Glycogen content (mg/g)</td>
</tr>
<tr>
<td>0</td>
<td>NC</td>
<td>7</td>
<td>DC</td>
</tr>
<tr>
<td>14</td>
<td>Glimipride</td>
<td>21</td>
<td>AE 300</td>
</tr>
<tr>
<td>21</td>
<td>AE 500</td>
<td></td>
<td>EE 500</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fasting Blood Sugar</th>
<th>Mean ± SEM</th>
<th>Serum Total Cholesterol</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>NC</td>
<td>7</td>
<td>DC</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>Glimipride</td>
<td>21</td>
<td>AE 300</td>
<td>EE 500</td>
</tr>
<tr>
<td>21</td>
<td>AE 500</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, n = 5 in each group * P < 0.05, compared with the vehicle treated normal control group at respective hour. Values in brackets indicate percent increase or decrease vs. respective initial fasting value (0 day).

Table No: 2: Effect of Aqueous and Ethanolic extracts of roots of Convolvulus microphyllus Sieb. on Serum Triglyceride and Serum Total Cholesterol level of Alloxan induced diabetic animals during chronic study.

<table>
<thead>
<tr>
<th>Days</th>
<th>Group</th>
<th>Serum Triglyceride concentration (mg/dl)</th>
<th>Mean ± SEM</th>
<th>Serum Total Cholesterol concentration (mg/dl)</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>NC</td>
<td>7</td>
<td>DC</td>
<td>14</td>
<td>AE 300</td>
</tr>
<tr>
<td>14</td>
<td>Glimipride</td>
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<td>AE 500</td>
<td>EE 500</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>AE 500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, n = 5 in each group * P < 0.05, compared with the vehicle treated normal control group at respective hour. Values in brackets indicate percent increase or decrease vs. respective initial fasting value (0 day).

Diabetes induction:

Alloxan induced diabetes mellitus:
The study was carried out for 21 days to access the effect of various treatments on biochemical parameters and glycosgen content of different tissues in alloxan induced diabetic rats. The rats were divided into 7 groups, consisting five animals each. Rats in the first group received vehicle (2 ml/kg) and served as normal control group while the second group of rats received vehicle plus dexamethasone (10 mg/kg s.c.) and served as diabetic control group (positive control group). Rats in the third group were treated with pioglitazone (20 mg/kg, p.o.) plus dexamethasone (10 mg/kg). Rats in experimental groups 4 and 5 were treated with aqueous extract (300 and 500 mg/kg p.o. respectively) and dexamethasone (10 mg/kg, s.c.). Rats in the group 6 and 7 received ethanolic extract (300 and 500 mg/kg p.o. respectively) along with dexamethasone (10 mg/kg, s.c.).

At the end of experimental period, i.e. on day 11, the overnight fasted animals were anaesthetized with ether and blood was collected by retro-orbital puncture method. Serum was separated by using centrifuge machine at 5000 rpm for 5 min and stored at 4-8°C until use.

Biochemical analysis:
Blood Sugar level, Serum Triglyceride, Total Cholesterol and Glycogen content were estimated.

Phytochemical analysis:
Phytochemical group tests showed the presence of alkaloids, tannins, sapoinis, flavonoids and glycosides were recognized in extracts.

Statistical analysis:
All the results are expressed as mean ± SEM. The statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison post-test using Graph-pad software. P-values < 0.05 were considered significant.

Change in Serum Glucose (alloxan)

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Table No: 3:- Effect of Aqueous and Ethanolic extracts of roots of *Convolvulus microphyllus* Sieb. on Serum glucose, Serum Triglyceride, Total Cholesterol level and Glycogen content of Dexamethasone induced insulin resistance in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum glucose concentration (mg/dl) Mean ± SEM</th>
<th>Serum Triglyceride concentration (mg/dl) Mean ± SEM</th>
<th>Serum Total Cholesterol concentration (mg/dl) Mean ± SEM</th>
<th>Glycogen content (mg/g) (Mean ± SEM)</th>
<th>Liver (mg/dl) Mean ± SEM</th>
<th>Muscle (mg/dl) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>88.49±2.072</td>
<td>123.72±7.131</td>
<td>121.56±4.29</td>
<td>20.12±0.230</td>
<td>2.82±0.05</td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>182.92±3.716</td>
<td>180.64±9.92</td>
<td>162.98±6.004</td>
<td>8.99±0.353</td>
<td>1.31±0.031</td>
<td></td>
</tr>
<tr>
<td>Glimipride 115.34±2.74*</td>
<td>(436.947)</td>
<td>(415.611)</td>
<td>(420.18)</td>
<td>(1107.99)</td>
<td>(1107.63)</td>
<td></td>
</tr>
<tr>
<td>AE 300</td>
<td>150.37±4.09</td>
<td>164.91±6.42</td>
<td>176.82±6.7</td>
<td>14.95±0.208</td>
<td>2.35±0.057</td>
<td></td>
</tr>
<tr>
<td>AE 500</td>
<td>146.18±5.314</td>
<td>151.01±5.24</td>
<td>160.12±10.16</td>
<td>15.66±0.18</td>
<td>2.48±0.069</td>
<td></td>
</tr>
<tr>
<td>EE 300</td>
<td>146.18±5.314</td>
<td>158.78±12.57</td>
<td>174.76±8.98</td>
<td>15.56±0.16</td>
<td>2.43±0.065</td>
<td></td>
</tr>
<tr>
<td>EE 500</td>
<td>149.47±2.96</td>
<td>166.2±15.32</td>
<td>176.2±15.32</td>
<td>16.38±0.269</td>
<td>2.53±0.057</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, n = 5 in each group, * P < 0.05 compared with the vehicle treated normal control group at respective hour. Values in brackets indicate percent increase or decrease vs. respective initial fasting value (0 day).

**DISCUSSION:**

Diabetes mellitus is the most common endocrine disorder that affects more than 194 million people worldwide. If nothing is done to control this disease, the number will exceed 333 million by 2025 (6.3% of population). In addition to the primary effects of diabetes, diabetes is accompanied by increased risk factors such as...
as hyperglycaemia, dyslipidaemia, hypertension, decreased fibrinolytic activity, increased platelet aggregation, and severe atherosclerosis.

In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, dithiothreitol, and anti-insulin serum. Alloxan causes massive destruction of the B cells of islets of langerhans. Dexamethasone causes insulin resistance as measured by several markers, including a reduction in insulin-stimulated glucose uptake and a decrease in glucose oxidation.

Preliminary phytochemical analysis of extracts of Convolvulus microphyllus Sieb. shows presence of the alkaloids, tannins, saponins, flavonoids and glycosides which may be responsible for antidiabetic activity of plant.

The acute toxicity study of aqueous and ethanolic extract of extracts of Convolvulus microphyllus Sieb. shows that the LD50 values are more than 5000 mg/kg, over the period of 14 days, so 1/10th dose was selected to study antidiabetic activity. There was no alteration between the initial and final blood glucose levels of different extracts treated normoglycemic rats in short term and long term studies which indicates that Convolvulus microphyllus Sieb. has no hypoglycemic activity.

Both aqueous and ethanolic extracts improved the conditions of diabetes mellitus indicated by parameters like body weight, food intake, water intake and urine output.

The results of oral glucose tolerance test and acute study in alloxan induced diabetic rats shows that both aqueous and ethanolic extracts produced significantly (p<0.05) lower fasting blood glucose levels, compared to diabetic control group (dose dependent manner).

While in chronic administration for 21 days of aqueous and ethanolic extract produced significant (p<0.05) decrease in fasting serum glucose level in diabetic rats at 7, 14 and 21st day of treatment. Aqueous extract reduces fasting glucose by 43.76% and ethanolic extract by 48.61% and extracts were causing dose dependent reduction in fasting blood glucose level.

Further the effect on lipid profile indicates that, the aqueous and ethanolic extracts produced significantly beneficial effect on lipid profile in diabetic rats since level of triglyceride and cholesterol were maintained near to normal. The aqueous extract reduces serum triglyceride by 42.32% and ethanolic extract by 44.40% while cholesterol level reduced by 40.44% and 38.87% for aqueous and ethanolic extract respectively when compared with diabetic control at 21st day.

In diabetes the stored glycogen in the liver and muscle is converted to the glucose due to absence or insensitive insulin. Hence the liver and the muscle glycogen level decreased in diabetic individual as compared to normal. Glycogen content of various tissues (liver and muscle) was estimated on the 21st day. In diabetic control, hepatic and skeletal muscle glycogen content decreased significantly by 52.16% and 62%, respectively when compared with non diabetic control. Treatment with aqueous and ethanolic extract leads to significant (p<0.05) increase in liver and muscle glycogen. The aqueous extract increases liver glycogen and muscle glycogen by 87.28% and 137.72% and ethanolic extract by 71.31% and 97.37% respectively at 500mg/kg dose. This prevention of depletion of glycogen in the liver and muscle is possibly due to either stimulation of insulin release or due to insulominetic activity of some component of extract resulting in direct peripheral glucose uptake.

In dexamethasone induced insulin resistant model there was significant reduction in elevated level of blood glucose level by aqueous extract at 500mg/kg (20.08%) in comparison with control and pioglitazone showed reduction in elevated level of blood glucose level by (36.94%).

The dexamethasone significantly increased serum triglyceride and total cholesterol level (46.01 and 34.07%) compared to normal control group. Both aqueous and ethanolic extracts caused no significant (p>0.05) reduction in blood triglyceride and total cholesterol level.

Further effect on glycogen content of various tissues (liver and muscle) in dexamethasone induced insulin resistance was estimated and results showed that both the extracts increases the liver and muscle glycogen. The aqueous extract increases liver glycogen and muscle glycogen by 76.15 % and 89.31% and ethanolic extract by 84.25% and 93.13% respectively at 500mg/kg dose.

CONCLUSION:
The aqueous and ethanolic extract of this plant is an attractive material for the development of the good phytotherapy for the diabetes. However the drug cannot be substituted to present allopathic drugs but can at least act as an adjuvant in antidiabetic therapy. Thus the claim made by the Indian traditional systems of medicine regarding the use of roots of this plant in the treatment of diabetes stands confirms. Present efforts are directed to isolate the active constituents from plant and elucidation of mechanism of action.

The results of this preclinical study will provide the necessary data for phase II clinical trials in type 1 and 2 diabetes. Finally further studies are required to disclose the lead chemical constituents and mechanism of the antidiabetic action.

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