Hypoglycaemic activity of *Coccinia indica* Wight & Arn. fruits in Alloxan-induced diabetic rats

M Ramakrishnan, R Bhuvaneshwari, V Duraipandiyan and R Dhandapani*

1Department of Biotechnology, Thanthai Hans Roever College, Perambalur-621 212, Tamil Nadu, India
2Division of Plant Biotechnology, Entomology Research Institute, Loyola College, Chennai-600 034, Tamil Nadu
3Department of Botany, AVC College, Manapandal, Mayladurai-609 305, Tamil Nadu
4Department of Botany, Aringer Anna Government Arts College, Nammakal-637 002, Tamil Nadu

Received 21 September 2010; Accepted 31 March 2011

Fruit extracts of *Coccinia indica* Wight & Arn. were evaluated for their antidiabetic activity in Alloxan-induced diabetic rats. Diabetes was induced in experimental rats by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body wt). Ethanol, chloroform and aqueous extracts of *C. indica* fruits were administered orally at a dose of 250 mg/kg body wt to diabetic rats. Blood glucose was analysed using glucose oxidase-peroxidase reactive strips. Significant antidiabetic activity was observed in ethanolic extract in terms of reduction of fasting blood glucose level in diabetic rats. After 7 h blood glucose was depressed by 8.2% (*P* < 0.05) and 10.06% (*P* < 0.01) in alloxan-induced diabetic rats. The effect of the ethanolic extract particularly at 250 mg/kg was comparable to that of standard drug Glibenclamide (1 mg/kg body wt).

**Keywords**: Alloxan, *Coccinia indica*, Diabetes, Fruits, Glibenclamide, Hypoglycaemic.

**IPC code; Int. cl. (2011.01)**—A61K 36/42, A61K 131/00, A61P 3/10

**Introduction**

Diabetes mellitus is a heterogeneous metabolism disorder characterized by altered carbohydrate, lipid and protein metabolism. The incidence of diabetes is very high all over the world and particularly many Indians are suffering from this disease and its complication in liver, heart, kidneys and lungs. Many Indian medicinal plants have been used successfully for the treatment of diabetes.

*Coccinia indica* Wight & Arn. (Plate 1) is found throughout India in warm and humid conditions and more commonly seen in areas like Bengal, Bihar and Orissa. It is found in southern Asian islands, West Indies and Hawaiian islands. It is slender scandent or prostrate herb often with tuberous root. Fruits contain β-amyrin acetate lupeol and cucurbitacin B. Aerial parts contain heptacosane, cephalandrol, β-sitosterol and alkaloids cephalandrine A and B. Its root contains resins, alkaloids, starch, fatty acids and carbonic acid. The roots, stems, leaves and fruits are used in indigenous system of medicine for treating diabetes. Previous studies have reported that ethanolic extract of leaves possess hypoglycaemic and antioxidant properties. Root and leaves have antilipidemic effects. Aqueous extract of fresh leaves had anti-inflammatory, analgesic, antipyretic and anti-nociceptive activities. Hepatoprotective effects of diethyl ether extract of leaves and antimicrobial effects of aqueous and organic solvent extracts of fruits and leaves have also been reported. Aqueous, methanolic and ethanolic extracts of aerial parts showed antihyperglycaemic and hypolipidemic, antitussive, antilithitic and antimutagenic activities. It is also used to cure ring worm, psoriasis, small pox, scabies, other itchy skin eruptions and ulcers. The present study was undertaken to evaluate the hypoglycaemic activity in alloxan-induced diabetic rats using aqueous, ethanol and chloroform extracts of *C. indica* fruits as no studies have been carried out on its fruits for hypoglycaemic activity.

**Materials and Methods**

**Plant material**

Fresh fruits of *C. indica* were collected in the month of June 2009 from Vilamuthur village, Perambalur, Tamil Nadu, India. The plant was identified with the help of available Indian literature.
A voucher specimen was deposited in the Rapinet Herbarium, St. Joseph’s College, Tiruchirapalli, Tamil Nadu (Voucher Nos. RHT 24691 and RHT 28217).

**Preparation of plant powder and extracts**

Aqueous, ethanol and chloroform extracts of fruits were prepared following standard procedures. Matured unripe fruits were dried in an incubator for two days at 40°C, crushed in a mechanical grinder into fine powder. The powder (500 g) was extracted sequentially with 2.5 litres of water, 2.5 litres of 70% ethanol and 2.5 litres of 60% chloroform in a Soxhlet apparatus at 65°C until the powder became exhausted totally. The resulting extracts were filtered, concentrated and dried in vacuo (yield 7.60, 8.25 and 8.75% w/w, respectively). The extracts were stored in desiccators for use in subsequent experiments.

**Phytochemical analysis**

Preliminary phytochemical analysis was done following the method of Harbone.

**Animals**

Healthy adult wistar albino rats weighing 180-240 g were used for this study. Animals were allowed to acclimatize for a period of 15 days in the laboratory environment prior to the experiment. Rats were housed in standard polypropylene cages (three animals per cage), maintained under standard laboratory conditions (i.e. 12:12 h light and dark cycle; at an ambient temperature of 25±5°C; 35-60% of relative humidity); the animals were fed with standard rat pellet diet (Hindustan Lever Ltd. Mumbai) and water ad libitum. Animal House of Periyar Pharmacy, Trichirappalli was used for the study after prior scrutinization and approval from Institutional Animal Ethical Committee (No. PPCG–IAEC-19/2003-2004).

**Chemicals**

Alloxan monohydrate was procured from Loba Chemie, Mumbai. Other reagents used in the experiment were of analytical grade. Glibenclamide (Batch No.029057) a standard antidiabetic agent, was purchased from Aventis Pharma Ltd., Goa.

**Antihyperglycaemic studies**

Induction of diabetes, hyperglycaemia was induced in overnight fasted adult rats weighing 180-240 g by a single intraperitoneal injection of freshly prepared alloxan monohydrate in normal saline (150 mg/kg body wt) in a volume of 2 ml/kg body wt. Hyperglycaemia was confirmed by the elevated glucose level in plasma determined at 48 h after injection. The hyperglycaemic rats were used for antihyperglycaemic study.

**Experimental design**

Animals were divided into six groups of six rats per group. Test groups were administered aqueous, ethanol and chloroform extracts at a dose of 250 mg/kg body wt, respectively by oral route. Positive control group animals were treated with standard drug Glibenclamide at an oral dose of 1 mg/kg body wt. All doses were started 48 h after alloxan injection. The experimental designs were as follows:

- **Group I** – Control (2 ml/kg body wt)
- **Group II** – Diabetic + Alloxan (2 ml/kg body wt)
- **Group III** – Diabetic + aqueous extracts of *C. indica* fruit extracts (250 mg/kg body wt)
- **Group IV** – Diabetic + ethanol extracts of *C. indica* fruit (250 mg/kg body wt)
- **Group V** – Diabetic + chloroform extracts of *C. indica* fruit (250 mg/kg body wt)
- **Group VI** – Diabetic + Glibenclamide (1 mg/kg body wt).

Fasting blood glucose levels were estimated at 1, 3, 5 and 7 h after administration of treated and control drugs.
Table 1—Effect of *Coccinia indica* fruits on blood glucose level of alloxan-induced diabetic albino rats

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Dose</th>
<th>Blood glucose level (mg/100 ml) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Normal control</td>
<td>2 ml saline</td>
<td>98.56 ± 0.874</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>2 ml saline</td>
<td>203.6 ± 3.850</td>
</tr>
<tr>
<td>(Alloxan monohydrate)</td>
<td>150 mg/kg b. wt.</td>
<td></td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>250 mg/kg b. wt.</td>
<td>204.6 ± 4.162</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>250 mg/kg b. wt.</td>
<td>203.3 ± 3.697</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>250 mg/kg b. wt.</td>
<td>205.7 ± 3.042</td>
</tr>
<tr>
<td>Standard drug</td>
<td>1 mg/kg b. wt.</td>
<td>286.33 ± 10.84</td>
</tr>
<tr>
<td>(Glibenclamide)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05—Significant, ** P < 0.01—Highly significant vs Diabetic; SEM—Standard Error of Mean, n—Number of animals in each group (6)

Collection of blood and determination of serum glucose

Blood was withdrawn from the tail vein and glucose levels were estimated using glucose oxidase-peroxidase reactive strips and a glucometer (Ascensia Entrust, Bayer Health Care, USA).

Statistical analysis

Data were statistically analysed using one-way ANOVA and expressed as mean ± S.E.M. followed by Dunnett’s t-test using computerized Graph pad instate version 3.05, Graph pad software, U.S.A.

Results and Discussion

Alloxan (β-cytotoxin chemical) induced diabetes in a wide variety of animal species including rats, damaged the insulin-secreting β-cells. In the present study, hypoglycaemic activity of *C. indica* fruit extracts was evaluated in alloxan-induced diabetic rats. The preliminary phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids and triterpenoids. The effect of chloroform, ethanol and aqueous extracts of fruits on blood glucose levels of alloxan-induced diabetic rats are shown in Table 1. The blood glucose level was reduced maximum in ethanol extract at 5th and 7th h after treatment. Blood glucose was depressed by 8.2% (*P < 0.05*) and 10.06% (*P < 0.01*) in alloxan-induced diabetic rats after treatment which was comparable to the standard drug, Glibenclamide. This may be due to the activation of the existing pancreatic cells in diabetic rats by the ethanolic extract. Earlier the pectin isolated from the fruits of *C. indica* showed a significant reduction in blood glucose levels by decreasing the absorption level of glucose from the intestine, increasing the rate of liver glycogen and decreasing the glycogen phosphorylase. Also in some previous studies the leaves showed hypoglycaemic and antihyperglycaemic effect in Streptozotocin (STZ) induced diabetic animals, oral administration of 200 mg/kg body wt of ethanol extract of leaves to diabetic animals for 45 days resulted in a significant reduction in blood glucose, glycosylated haemoglobin and an increase in total haemoglobin and plasma insulin. The activities of lipogenic enzyme and hexokinase were significantly decreased, whereas the activities of gluconeogenic enzyme were significantly increased in the diabetic animals and *C. indica* leaves showed hypoglycaemic activity in STZ induced male diabetic rats, with 3 liters of 60% ethanol extract suspension was administered orally at a dose of 200 mg/kg body wt, after 90 min the blood glucose, liver glucose-6-phosphatase, liver fructose-1,6-bisphosphatase and liver arginase significantly decreased, blood glucose was depressed by 23% (*P < 0.01*) and 27% (*P < 0.001*), whereas the activities of liver G6PDH and red cell GDPH significantly increased by 34% (*P < 0.01*) and 39% (*P < 0.001*). In the present study, the activity of fruit showed less activity compared to leaves which were carried out by earlier researchers. The ethanolic extract of fruit also has activity in blood glucose level in diabetic rats compare to ethanolic extract of its leaves. Further, studies on its mechanism of action by studying the effect of extracts in insulin, lipids, free radicals and antioxidant potential and also to compare the alloxan-induced results with Streptozotocin-induced diabetic rats with respect to fruit and other parts of the plant are to be taken up by us.

Conclusion

The present study suggested that the ethanolic extract of *C. indica* fruit possesses hypoglycaemic activity and therefore further studies can be taken up for drug discovery.

Acknowledgement

The authors are thankful to Dr A. Jaswanth, Head, Department of Pharmacology, Periyar Pharmacy College for Girls, Trichirappalli, Tamil Nadu for providing infrastructural facilities for this work.
References

5 Kar Ajit, Choudhary BK and Bandyopadhyay NG, Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats, J Ethnopharmacol, 2003, 84, 105-108.
20 Behl PN, Arora RB, Srivastava G and Malhotra BN, Herbs useful in Dermatological therapy, CBS Publishers and Distributor, Delhi, 1993.
23 PHS (Public Health Service) Policy on Human Care and use of Laboratory animals, available from office for protection from research risks, Washington DC, U S Department of Health Service (Bethesda, NIII), 1986.