A study on hypoglycemic activity of methanolic root extract of *Curculigo orchioides*

Anandakirouchenane Elumalai 1, Dr. Sarath Chandiran Irisappan 2, Dr. Kadalmani Balamuthu 3
1 Research scholar, PRIST University, Vallam, Thanjavur-613403, Tamilnadu, India.
2 Professor & Principal, Gokula Krishna College of Pharmacy, Sullurpet-524121, Nellore dist, A.P, India.
3 Department of animal science, Bharathidasan University, Trichirapalli-620024, Tamilnadu, India.

Received on:21-07-2013; Revised on:18-08-2013; Accepted on:28-09-2013

**ABSTRACT**

**Objective:** The aim of the present study was to investigate the hypoglycemic effect of methanolic root extract of *Curculigo orchioides* (MECO) on normal and glucose loaded hyperglycemic rats. Further the study was carried out to study the single dose treatment effect of the extract on glycemia of alloxan-induced hyperglycemic rats. **Method:** Diabetes was induced by intraperitoneal injection of alloxan (150 mg/kg, b.wt). The hypoglycemic activity was evaluated by normal glucose and oral glucose tolerance test (NG-OGTT) method and by acute reduction in glucose levels in normal rats and glucose loaded hyperglycemic rats and in alloxan-induced hyperglycemic rats. **Conclusion:** In conclusion, the methanolic root extract of *Curculigo orchioides* was found to exhibit a hypoglycemic activity and further investigation is under progress to evaluate it for the treatment of diabetes mellitus.

**Keywords:** Hypoglycemic effect, Glibenclamide, *Curculigo orchioides*, Alloxan, Diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. It affects more than 100 million people worldwide and its incidence is increasing steadily with changes in life styles.1 Hyperglycemia result from an absolute deficiency of insulin caused by pancreatic β-cell destruction or by a combination of peripheral resistance to insulin action and an inadequate secretory response by the pancreatic β-cells.2 Insulin has proved to be effective to some extent in increasing the life expectancy of diabetic patients, but is not a permanent solution science there are many drawbacks of this therapy. Also the therapy with oral hypoglycemic agents is not satisfactory. Thus, the search for a new therapeutic agent devoid of adverse effect originating from plants used in traditional medicine would be of interest.

Plants have been used in traditional medicine since ancient times for the treatment of various diseases of man and animals. However, still a large number of local herbs claimed to be useful in the treatment of many diseases including diabetes have not been screened in addition increased awareness to the unwanted effects of allopathic drugs has encouraged people to look alternative drugs.3

One such ethno botanically important plant, *C. orchioides* Gaerth, is a well known medicinal plant belonging to the family *Hypoxidaceae* (*Amaryllidaceae*). It is distributed widely in the southern parts of Japan, China and Australia, generally used as a tonic in traditional Chinese medicine to treat decline in physical strength.4 Its rhizomes are used as an alternative for demulcent, diuretic, restorative and for the treatment of jaundice.5 Curculigoside, an active compound isolated from *C. orchioides* can improve cognitive function and is developed as a new drug for the treatment of Alzheimer’s disease.6,7 Despite the use of the plant in traditional, so far no scientific evaluation was carried out on this plant. Hence the present study was undertaken to evaluate the possible hypoglycemic activity of the methanolic root extract of *C. orchioides* in normal, glucose loaded hyperglycemic and alloxan induced hyperglycemic rats.

2. MATERIALS AND METHODS:

2.1. Preparation of Methanolic extract of Curculigo orchioides

The root parts of *C. orchioides* were collected, shade-dried and then finely powdered (collected from the Bharathidasan University, Tamil Nadu). 500 gm of powder was extracted with methanol using a Soxhlet apparatus. The solvent was then evaporated under reduced pressure at 40°C and dried in vacuum dessicator.

2.2. Experimental Animals

Adult male wistar albino rats (170 - 190 grams) were used in the present study and were obtained from Madras Veterinary College, TANUVAS, Chennai, India. The animals were housed in clean polypro-
pylene cages under conditions of controlled temperature (25±2°C) with a 12/12-h day–night cycle, they had free access to food and water ad libitum. Animal experimentation was carried out as per the rules and protocols approved by the Institutional Animal Ethical Committee (IAEC).

2.3. Pharmacological studies

2.3.1. Induction of diabetes mellitus in experimental animals*

Adult inbred male Wistar albino rats (32 numbers) were overnight fasted and received a freshly prepared solution of alloxan, [S.d.fine chemicals Ltd], (150 mg/kg) in distilled water injected intraperitoneally in a volume of 1 ml/kg. After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. Normal rats (6 numbers) received 1ml 1% Sodium carboxymethyl cellulose (SCMC) as vehicle. The development of diabetes was confirmed after 48 hours of the alloxan injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation. Out of 32 animals subjected for diabetes induction, 6 animals died before groupings and two animals were omitted from the study, because of sub diabetic condition (118mg/dl) and (122mg/dl). Of the remaining 24 animals, 4 groups of 6 animals were formed and used for the experimentation. In the present study, glibenclamide (650 µg/kg, b.wt) was used as the standard drug.

2.3.2. Determination of the blood glucose levels

Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer (One Touch Ltd) based on glucose oxidase method.

2.3.3. Effect of MECO on normoglycemic and glucose fed-hyperglycemic rats [NG-OGTT] *

A combined methodology is preferred for the activity assessment of extract in order to avoid wasting animals; there are some modifications incorporated in the time pattern for blood glucose level determination. After overnight fasting (16 h) the blood glucose level of rats were determined and then were given the test samples and standard. The animals were divided into three groups of 6 rats in each.

Group I - Animals received 1% Sodium Carboxy methyl cellulose (SCMC)
Group II - Animals received glibenclamide 650 µg/kg b.w/p.o.
Group III - Animals received MECO 200mg/kg b.w/ p.o.

Test samples and standard were given immediately after the collection of initial blood samples. The blood glucose levels were determined in the following pattern: 30 and 60 min to access the effect of test sample on normoglycemic animals. The rats were then loaded orally with 2g/kg glucose and the glucose concentrations were determined at 60, 90 and 210 min after glucose load.

2.3.4. Single dose treatment effect of the MECO on glycemia of Alloxan-induced hyperglycemic rats

Rats were fasted for 16 h and made hyperglycemic by intraperitoneal injection of Alloxan, at a dose of 150 mg/kg After 48 h their blood glucose level was estimated and rats having plasma glucose level above 200 mg/dL were selected and animals were divided in to 3 groups each constituting 6 Alloxan induced diabetic rats. Group I received 0.5% CMC 5ml/kg b.w/p.o, Group II received glibenclamide 650 µg/kg b.w/p.o. and Group III received MECO 200mg/kg b.wt/p.o. In a single dose treatment study, all surviving diabetic animals were fasted overnight. Blood samples were collected from the fasted animals prior to the treatment with above dosage schedule and after drug administration at 0, 2, 4, and 6 hour time interval to determine the blood glucose level by glucometer.10

2.3.5. Statistical analysis

Data was analysed statistically using graph pad prism 0.5 Version as Mean ± standard error using one way analysis of variance (ANNOVA) followed by Dunnett test.

3. RESULTS

3.1. Effect of MECO on blood glucose levels in normoglycaemic and glucose induced hyperglycemic rats. [NG-OGTT]

The MECO at a dose level 200mg/kg b.w/p.o exhibit hypoglycemic effect in fasted normal rats after 30 minutes of administration and reduced blood glucose levels in normal rats significantly after 60 min of drug administration (p<0.01). In the same group of rats which are loaded with glucose (2gm/kg b.w/p.o), after 60 min of MECO administration blood glucose levels reduced significantly (p<0.01). The standard drug glibenclamide (650 µg/kg b.w/p.o) treatment showed significant reduction in blood glucose levels in both normal and oral glucose loaded hyperglycemic rats (p<0.01). Results are shown in Table 1.

### Table 1. Effect of MECO on blood glucose in normal and glucose loaded hyperglycemic rats [NG-OGTT]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Test Sample (mg/kg)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>150 min</th>
<th>270 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (1% SCMC)</td>
<td>72.3±1.6</td>
<td>82.1±2.3</td>
<td>73.9±0.8</td>
<td>117.2±0.5</td>
<td>51.1±0.5</td>
<td>79.1±0.2</td>
</tr>
<tr>
<td>II</td>
<td>Std</td>
<td>73.1±2.7</td>
<td>50.9±1.7</td>
<td>41.8±0.6</td>
<td>90.8±0.3</td>
<td>71.5±0.5</td>
<td>55.8±0.5</td>
</tr>
<tr>
<td>III</td>
<td>MECO-200</td>
<td>74.7±1.9</td>
<td>72.0±0.8</td>
<td>59.0±0.2</td>
<td>110.1±0.5</td>
<td>75.9±0.5</td>
<td>60.6±0.5</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet’s test. The blood glucose values of group II and III are compared with control animal values. *-p< 0.05, **-p< 0.01, ns-non significant.

---

3.2. Single dose treatment effect of the extract on glycemia of Alloxan-induced hyperglycemic rats

MECO at a dose 200mg/kg b.w/p.o) did not produce significant reduction in the blood glucose levels in alloxan induced diabetic rats at 2nd hour of administration. MECO only at the 4th and 6th hour of administration shows significant difference in blood glucose levels in alloxan induced hyperglycemic rats (p<0.01 to p<0.001). The standard drug glibenclamide (650 µg/kg b.w/p.o) treatment showed significant reduction in blood glucose levels (p<0.001). Results are shown in Table 2.

Table 2: Hypoglycemic activity of methanolic root extract of C. orchioides in Alloxan-induced hyperglycemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Test sample (mg/kg)</th>
<th>0th hour</th>
<th>2nd hour</th>
<th>4th hour</th>
<th>6th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>262.52±11.42</td>
<td>262.46±9.65</td>
<td>263.20±10.09</td>
<td>266.41±9.27</td>
<td></td>
</tr>
<tr>
<td>II Std</td>
<td>264.89±11.99</td>
<td>202.84±11.68***</td>
<td>142.14±6.11***</td>
<td>136.75±5.05***</td>
<td></td>
</tr>
<tr>
<td>III MECO-200</td>
<td>264.02±18.64</td>
<td>255.92±8.75</td>
<td>239.17±6.33</td>
<td>236.30±1.67**</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± S.E.M. (n = 6); *p<0.05; **p<0.01; ***p<0.001, when compared with control. Statistical significance test was done by one way ANOVA followed by Dunnet’s test.

DISCUSSION

Plants have been used as source of drugs for the treatment of diabetes mellitus in developing countries where the cost of conventional medicines represents a burden to the population. Many species have been reported to present antidiabetic activity. Therefore, in the present study, the hypoglycemic activity of methanolic root extract of Curculigo orchioides was evaluated in normal, glucose loaded hyperglycemic and alloxan induced hyperglycemic rats. The MECO at a dose 200 mg/kg bw.p.o significantly suppress blood glucose levels in overnight fasted normoglycemic animals but showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in rate of intestinal glucose absorption, achieved by an extra pancreatic action including stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process.

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin dependent diabetes mellitus. There is an increasing evidence that alloxan causes diabetes by rapid depletion of β-cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. MECO at a dose of 200mg/kg/bwt showed significant hypoglycaemic action in alloxan induced hyperglycemic rats.

CONCLUSION

In conclusion, the methanolic root extract of Curculigo orchioides was found to exhibit a hypoglycemic activity in normal and hyperglycemic rats. Further pharmacological investigations are in progress to elucidate the mechanism of the observed hypoglycemic effect and its treatment for the treatment of diabetes mellitus.

REFERENCES

2. Vinay kumar, abbas K nelson F, Pathologic bases of disease; 2007; 1189-1207
10. Mustafa A, Didem D, Orhan N, Invivo antidiabetic and anti-


Source of support: Nil, Conflict of interest: None Declared