Evaluation of antihyperglycemic and antinociceptive properties of leaves of *Calotropis gigantea* R.Br. (Asclepiadaceae) – a medicinal plant of Bangladesh


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**ABSTRACT**

*Calotropis gigantea* is a well-known medicinal plant of Bangladesh. Various parts of the plant are used for treatment of a number of diseases in folk medicines of the country, including pain and lowering of blood sugar level in diabetic patients. Since scientific research on plants used in indigenous medicinal practices has led to the discovery of many important allopathic drugs, it was of interest to scientifically evaluate the antihyperglycemic and antinociceptive properties of the plant. Leaves were selected, because traditionally this is the plant part used in Bangladesh by folk medicinal practitioners for treatment of high blood sugar and pain. Antihyperglycemic activity was evaluated through oral glucose tolerance tests (OGTT) in glucose-loaded Swiss albino mice, while antinociceptive activity was evaluated in acetic acid-induced gastric pain model in Swiss albino mice. Administration of methanolic extract of leaves to glucose-loaded mice led to dose-dependent significant reductions in blood glucose levels at doses of 100, 200 and 400 mg extract per kg body weight of mice. At these three doses, the percent lowering of sugar in blood was 21.35, 25.39 and 28.54, respectively. In comparison, a standard antihyperglycemic drug, glibenclamide, lowered blood glucose level by 46.07%, when administered at a dose of 10 mg per kg body weight. Although the methanolic extract of leaves was not as potent as glibenclamide in lowering blood sugar levels, the results still validate the folk medicinal practitioner’s use of the leaves of this plant to lower blood sugar levels in diabetic patients. In intraperitoneally injected acetic acid-induced gastric pain model (as measured by the number of writhings), prior oral administration of the methanolic extract of leaves significantly and dose-dependently reduced the number of writhings by 51.04, 53.12, 61.20 and 63.28%, respectively, when administered at doses of 50, 100, 200 and 400 mg per kg body weight. The results compare favorably with that of a standard drug, aspirin, which at doses of 200 and 400 mg per kg body weight, reduced the number of acetic acid-induced writhings by 51.04 and 67.32%, respectively. The obtained results validate the folk-medicinal use of leaves of *Calotropis gigantea* for treatment of pain and high blood sugar levels in diabetic patients, and strongly indicate the potential of this plant in obtaining newer antihyperglycemic and antinociceptive drugs.

**Key words:** *Calotropis gigantea*, antihyperglycemic, antinociceptive, Asclepiadaceae

**Introduction**

*Calotropis gigantea* R.Br. (Asclepiadaceae) (English: Crown flower, Giant milkweed, Bengali: Dudh akond) is a species of *Calotropis* native to Bangladesh, Cambodia, Indonesia, Malaysia, Pakistan, Philippines, Thailand, Sri Lanka, India and China. The plant grows to a height of 8-10 feet, the leaf arrangement is opposite, and the flowers are white to purple-colored and not scented. The plant is often found by the roadsides of rural areas and in fallow lands of Bangladesh, where multiple plants can cluster together and form a hedge. When leaves or stems are broken, white-colored latex oozes out. Various parts of the plant like leaves are used in the folk medicinal system of Bangladesh for treatment of ailments like high blood sugar in diabetic patients or to reduce pain.

The flowers of the plant are reported to possess analgesic activity (Pathak and Argal, 2007), as well as antimicrobial and cytotoxic activity (Habib and Karim, 2009). Anti-diarrheal, anti-*Candida* (active against *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*), anti-bacterial (active against...
Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Micrococcus luteus and Klebsiella pneumoniae), and anti-oxidant activities have been reported for leaves and other aerial parts of the plant (Chitme et al., 2004; Kumar et al., 2010a,b; Singh et al., 2010). Anti-pyretic activity, cytotoxic activity, anti-microbial activity, insecticidal activity, wound healing activity, CNS activity, and pregnancy interceptive properties have been reported for roots (Chitme et al., 2005; Wang et al., 2008; Alam et al., 2008; Alam et al., 2009; Deshmukh et al., 2009; Argal and Pathak, 2006; Srivastava et al., 2007). Stem of this plant reportedly possess hepatoprotective effects as demonstrated by protection of liver of rats against carbon tetrachloride-induced liver injury (Lodhi et al., 2009). The latex of this plant contain purgative properties, pro-coagulant activity, wound healing activity, as well as anti-microbial activity (Rajesh et al., 2005; Nalwaya et al., 2009; Kumar et al., 2010c). Various pharmacological activities and phytochemical profile of the plant has been reviewed (Kumar et al., 2011).

Discovery of new drugs often occur from close observations of traditional medicinal practices of indigenous communities, because such communities due to their long association with medicinal plants, which are used for medicinal purposes, has over the centuries accumulated considerable knowledge of plant species, particularly in their immediate vicinity. Close observations of indigenous medicinal practices involve extensive ethnomedicinal surveys, and we had been conducting such surveys for quite some time (Rahmatullah et al., 2009a-c; Rahmatullah et al., 2010a-g; Rahmatullah et al., 2011a,b; Rahmatullah et al., 2012a-d). In addition, based on the results of our ethnomedicinal surveys, we had been screening medicinal plants in our laboratory for any analgesic, antihyperglycemic and cytotoxic potential in these plants. A number of such analysis has also been documented (Anwar et al., 2010; Jahan et al., 2010; Khan et al., 2010; Mannan et al., 2010; Rahman et al., 2010; Rahmatullah et al., 2010h; Sutradhar et al., 2010; Ali et al., 2011; Barman et al., 2011; Hossan et al., 2011; Jahan et al., 2011; Rahman et al., 2011; Sutradhar et al., 2011). Such studies can give a clue as to which plants are effective against, respectively, pain, hyperglycemia (like arising from diabetes), and cancer. Cumulatively, these three diseases are prevalent throughout the world and afflict millions of people on a daily basis. While pain can be treated with a number of common over the counter medicines like aspirin or paracetamol, such medicines when used for prolonged time periods or during over-dosage can lead to gastric ulceration or hepatotoxicity. Diabetes cannot be cured by allopathic medicine, and while some forms of cancer can be treated in the early stages, other forms of cancer like pancreatic cancer have no known treatment in modern medicine. As such, it is essential to make continuous searches towards discovery of newer and more efficacious drugs against these diseases or symptoms. The objective of the present survey was to evaluate the antihyperglycemic and antinociceptive potential of leaves of Calotropis gigantea, which has a long history of usage by folk medicinal practitioners of Bangladesh for treatment of diabetes and pain.

Materials and Methods

Leaves of Calotropis gigantea were collected from Dinajpur district, Bangladesh during April, 2012. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 36,574). The sliced and air-dried leaves of Calotropis gigantea were grounded into a fine powder and 100g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract was 4.00g.

Chemicals:

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh.

Animals:

In the present study, Swiss albino mice (male), which weighed between 15-22 g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Antihyperglycemic activity:

Glucose tolerance property of methanol extract of Calotropis gigantea leaves was determined as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice were grouped into six groups of six mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, group 2 received
standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received methanol extract of Calotropis gigantea leaves at doses of 50, 100, 200 and 400 mg per kg body weight. Each mouse was weighed and doses adjusted accordingly prior to administration of vehicle, standard drug, and test samples. All substances were orally administered. Following a period of one hour, all mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method (Venkatesh et al., 2004).

Antinociceptive activity:

Antinociceptive activity of the methanol extract of Calotropis gigantea leaves was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of six mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic leaf extract of Calotropis gigantea at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

Statistical analysis:

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Results and Discussion

The methanolic extract of leaves of Calotropis gigantea exhibited dose-dependent significant reductions of blood glucose levels in oral glucose tolerance tests conducted with glucose-loaded mice. The antihyperglycemic activity was more pronounced and statistically significant at doses of 100, 200 and 400 mg extract administered per kg body weight. At these doses, the percent lowering of blood glucose levels were respectively, 21.35, 25.39 and 28.54 in comparison to control animals. In comparison, the standard antihyperglycemic drug glibenclamide, when administered orally at a dose of 10 mg per kg body weight, lowered blood glucose level in mice by 46.07%. Thus although the various doses of the leaf extract exhibited lesser antihyperglycemic activity than glibenclamide, yet the statistically significant reductions in blood glucose levels amounts to scientific validation of the use of leaves in folk medicine to lower high glucose levels in diabetic patients. At the same time, the obtained results suggest that the leaves of this plant merit further studies to isolate and identify the actual antihyperglycemic constituents.

In antinociceptive activity tests, the methanolic extract of leaves also demonstrated significant and dose-dependent reduction in the number of writhings in acetic acid-induced gastric pain models in mice. The percent reductions in the number of writhings at doses of 50, 100, 200 and 400 mg extract per kg body weight were, respectively, 51.04, 53.12, 61.20 and 63.28 in comparison to control animals. The standard antinociceptive drug, aspirin, at doses of 200 and 400 mg per kg body weight reduced the number of gastric writhings by 51.04 and 67.32%, respectively. The results strongly suggest that the methanolic extract of leaves possess antinociceptive constituents, which possess antinociceptive activity comparable to that of aspirin and so can be a source of novel and efficacious antinociceptive drugs.

The exact mechanism of antihyperglycemic action exerted by the methanolic extract of leaves remain to be elucidated. Essentially, there are three mechanisms through which an antihyperglycemic compound or extract may lower blood glucose concentrations. The extract may potentiate the pancreatic secretion of insulin or increase the glucose uptake (Farjou et al., 1987; Nyuain et al., 2009). Alternately, the extract may inhibit glucose absorption in gut (Bhowmik et al., 2009). Further isolation of the active antihyperglycemic constituent(s) and elucidation of the mechanism(s) responsible for the observed antihyperglycemic effect are at present being carried out in our laboratory.

Analgesia can be central or peripheral, and both central and peripheral analgesia can be detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005). Prostaglandins are considered to be responsible for the sensation of pain. Production of prostaglandins [mainly prostacyclines (PGI$_2$) and prostaglandin- (PG-E)$_2$] has been shown to be responsible for excitation of Adelta-nerve fibers, leading to the sensation of pain (Reynolds, 1982; Rang and Dale, 2003). As such, the antinociceptive activity exhibited by crude methanolic extract of the leaves may be due to the extract’s ability to block any synthesis of prostaglandins. This, in turn, may be mediated through inhibition of cyclooxygenase and/or lipooxygenase activities. It is to be noted that a similar mechanism has been proposed for antinociceptive activity of Ficus
deltoidea aqueous extract in acetic acid-induced gastric pain model (Sulaiman et al., 2008), and this may also be the mechanism operating in the present study.

Taken together, the results suggest two things. First, it validates the folk medicinal uses in Bangladesh of leaves of Calotrops gigantea to relieve pain, and lower blood sugar in diabetic patients. Second, this study serves as a pointer to further studies for isolation and identification of novel bioactive constituents, which can pave the way for discovery of novel and more efficacious antinociceptive and blood sugar lowering drugs. Furthermore, scientific validation of the folk medicinal uses makes it easier for potential patients in rural areas who lack access to modern medicines to use the leaves of this plant for getting necessary relief at substantially lower costs and with readily available materials.

Table 1: Effect of methanol extract of Calotrops gigantea leaves on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Blood glucose level (mmol/l)</th>
<th>% lowering of blood glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>4.45 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide (Group 2)</td>
<td>10 mg</td>
<td>2.40 ± 0.35</td>
<td>46.07*</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 3)</td>
<td>50 mg</td>
<td>3.67 ± 0.48</td>
<td>17.53</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 4)</td>
<td>100 mg</td>
<td>3.50 ± 0.25</td>
<td>21.35*</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 5)</td>
<td>200 mg</td>
<td>3.32 ± 0.43</td>
<td>25.39*</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 6)</td>
<td>400 mg</td>
<td>3.18 ± 0.21</td>
<td>28.54*</td>
</tr>
</tbody>
</table>

All administrations were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to hyperglycemic control animals.

Table 2: Antinociceptive effect of crude methanol extract of Calotrops gigantea leaves in the acetic acid-induced gastric pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of writhings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>8.17 ± 0.79</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>200 mg</td>
<td>4.00 ± 0.58</td>
<td>51.04*</td>
</tr>
<tr>
<td>Aspirin (Group 3)</td>
<td>400 mg</td>
<td>2.67 ± 0.88</td>
<td>67.32*</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 4)</td>
<td>50 mg</td>
<td>4.00 ± 0.52</td>
<td>51.04*</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 5)</td>
<td>100 mg</td>
<td>3.83 ± 0.70</td>
<td>53.12*</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 6)</td>
<td>200 mg</td>
<td>3.17 ± 0.70</td>
<td>61.20*</td>
</tr>
<tr>
<td>Calotrops gigantea (Group 7)</td>
<td>400 mg</td>
<td>3.00 ± 0.37</td>
<td>63.28*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to control.

References


