ORAL GLUCOSE TOLERANCE, PHYTOCHEMICAL SCREENING, ACUTE TOXICITY AND ANALGESIC ACTIVITY EVALUATION OF LEAVES OF PHRYNIUM CAPITATUM

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ABSTRACT
Oral glucose tolerance tests were conducted with methanolic extract of leaves of Phrynium capitatum in glucose-loaded Swiss albino mice. The extract, when administered at doses of 50, 100, 200 and 400 mg/kg, dose-dependently reduced blood glucose by 16.8, 26.3, 42.7, and 48.9%, respectively. Glibenclamide (a standard antihyperglycemic drug), when administered at a dose of 10 mg/kg reduced blood glucose level by 47.4%. In analgesic activity tests conducted with intraperitoneally administered acetic acid-induced pain model in mice, the extract at the aforementioned four doses reduced the number of abdominal constrictions, respectively, by 33.3, 37.0, 44.4, and 59.3%. A standard analgesic drug, aspirin, when administered at doses of 200 and 400 mg per kg reduced the number of abdominal constrictions by 48.1 and 63.0%, respectively. The results suggest that the leaves of the plant possess antihyperglycemic and analgesic potential and may be used for reducing blood sugar and for alleviating pain.

KEY WORDS: Phrynium capitatum, marantaceae, antihyperglycemic, analgesic.

INTRODUCTION
Phrynium capitatum Willed. Is a rhizomatic evergreen plant with broad shiny leaves. The habitat of the plant is the Eastern Himalayas region and the plant is native throughout India.
and eastwards to southern China and the Philippines. It can be observed growing in Lawachara Forest Reserve of Sylhet Division in Bangladesh, where it is known as Lobon pata. The plant belongs to the Marantaceae family. The broad leaves of the plant are used by the Mizos of Mizoram, India for wrapping cooked rice and imparting flavor to the rice.\cite{1} Because of its aromatic nature, the leaves are also used by minority ethnic groups in Laos to wrap rice cakes.\cite{2} Practically nothing is known about the ethnomedicinal uses, phytochemical constituents, and pharmacological properties of the plant or plant parts. We had been systematically screening plants of Bangladesh for their blood glucose lowering and pain relieving potentials.\cite{3-14} Diabetes is rapidly becoming endemic throughout the world because of possible changes in food habits and life style of the people. The disease is characterized by high blood glucose levels in blood and urine, and left untreated can rapidly give rise to other complicating factors like cardiovascular disorders, and diabetic retinopathy, neuropathy and nephropathy. In a study conducted amongst adults aged 35 years or more, the incidences of diabetes and pre-diabetes were 9.7% and 22.4%, respectively.\cite{15} A high incidence of kidney disease has been reported among the diabetic population of Bangladesh.\cite{16} Besides, Types 1 and 2, a high prevalence of gestational diabetes mellitus has also been observed in the women population of the country who are pregnant.\cite{17} Taken together, diabetes is becoming a serious concern for the Bangladeshi population, with rising mortality and associated health-care costs. The problem is more acute for the rural illiterate and below poverty level income people, who do not have access to modern diagnostic and treatment centers, or who simply cannot afford the medical costs of antidiabetic (glucose lowering) drugs or insulin injections. Taking regular injections of insulin is also cumbersome for the people and there is a natural tendency to avoid this form of treatment for rectifying glucose imbalances. Pain is a feeling triggered in the nervous system, which can be acute (like resulting from injury) or chronic (like resulting from incurable diseases like arthritis or cancer). Pain can be countered with drugs like aspirin or paracetamol (over-the-counter or OTC drugs). However, aspirin can cause gastric ulceration and paracetamol can cause hepatic toxicity from over-dosage or continuous use.\cite{18, 19} On top of it, the rural and urban slum people of Bangladesh do not have access to or afford treatment of complications arising from use of pain-relieving drugs. As such it is important to find out alternative means for reducing blood sugar levels in diabetes or impaired glucose metabolism conditions, and for treatment of pain. Plant kingdom has always been a potentially affordable and available means for providing newer and more efficacious drugs. The objective of the present study was therefore to evaluate the antihyperglycemic (through oral glucose tolerance test or
OGTT) and analgesic potential (through intraperitoneally administered acetic acid-induced pain model) of leaves of *Phrynium capitatum*, a plant that is available in Bangladesh.

**METHODS**

**Plant Material Collection**

Leaves of *P. capitatum* were collected during November 2013 from Lawachara Forest Reserve in Sylhet Division, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,701). Leaves were washed thoroughly with distilled water before further processing.

**Preparation of Methanolic Extract of Leaves**

Leaves were cut into small pieces, air-dried in the shade, and 95g of dried and powdered leaves were extracted with methanol (500 ml, final weight of the extract 4.03g).

**Chemicals and Drugs**

Glibenclamide, aspirin, and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

**Animals**

Swiss albino mice, which weighed between 15-19g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). 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.

**Oral Glucose Tolerance Tests for Evaluation of Antihyperglycemic Activity**

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999) \[^{20}\] with minor modifications. Briefly, fasted mice were grouped into six groups of five 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 methanolic leaf extract of *P. capitatum* (MEPC) at doses of 50, 100, 200 and 400 mg per kg body weight. All substances were orally administered. Following a period of one hour, all mice were orally administered 2g 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. The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = \(1 - \frac{W_e}{W_c}\) \times 100,

Where \(W_e\) and \(W_c\) represents the blood glucose concentration in glibenclamide or MEPC administered mice (Groups 2-6), and control mice (Group 1), respectively.

**Analgesic Activity Evaluation through Abdominal Writhing Test**

Analgesic activity of MEPC was examined as previously described. Mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MEPC 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 MEPC, 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 bioavailability and onset of chemically induced irritation of acetic acid, following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

Percent inhibition = \(1 - \frac{W_e}{W_c}\) \times 100

where \(W_e\) and \(W_c\) represents the number of writhings in aspirin or MEPC administered mice (Groups 2-7), and control mice (Group 1), respectively.

**Acute Toxicity Test**

It was of interest to determine the toxicity of the extract (MEPC), since no previous such reports exist for the plant. Acute toxicity test was conducted as previously described. Mice were divided into nine groups, each group consisting of six animals. Group 1 was given 1% Tween 80 in normal saline (2 ml per kg body weight). The other eight groups (Groups 2-9) were administered, respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg of MEPC per kg body weight. All animals were closely observed for the next 8 hours to notice any behavioral changes or mortality and were kept under close observation for the next two weeks.
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. [10]

Preliminary Phytochemical Screening
Preliminary phytochemical analysis of MEPC for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before. [25]

RESULTS AND DISCUSSION
Toxicity Evaluation
The crude extract (MEPC) did not show any mortality in mice even at the highest dose tested.

Preliminary Screening of Phytochemicals
Various tests conducted for presence of phytochemicals in MEPC indicated the presence of alkaloids, flavonoids, saponins, and tannins.

Antihyperglycemic Activity Evaluation Results
MEPC, when administered to mice at doses of 50, 100, 200 and 400 mg/kg showed dose-dependent improvements in glucose tolerance. The antihyperglycemic potential of MEPC was demonstrated through dose-dependent reductions of blood glucose concentrations in glucose-loaded mice by 16.8, 26.3, 42.7, and 48.9%, respectively, at the afore-mentioned four doses. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg/kg, reduced blood glucose concentration by 47.4%, compared to control mice. Although the 50 mg MEPC/kg results were statistically insignificant, at the highest dose tested (400 mg/kg), MEPC showed better antihyperglycemic results than that of glibenclamide. The results are shown in Table 1.

Table 1: Effect of Crude Methanol Extract of P. capitatum Leaves (MEPC) 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</td>
<td>10 ml</td>
<td>5.48 ± 0.34</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide (MEPC)</td>
<td>10 mg</td>
<td>2.88 ± 0.19</td>
<td>47.4*</td>
</tr>
<tr>
<td></td>
<td>50 mg</td>
<td>4.56 ± 0.47</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>100 mg</td>
<td>4.04 ± 0.38</td>
<td>26.3*</td>
</tr>
<tr>
<td></td>
<td>200 mg</td>
<td>3.14 ± 0.30</td>
<td>42.7*</td>
</tr>
<tr>
<td></td>
<td>400 mg</td>
<td>2.80 ± 0.33</td>
<td>48.9*</td>
</tr>
</tbody>
</table>

All administrations were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to hyperglycemic control animals.
Analgesic Activity Evaluation Results

Intraperitoneal administration of acetic acid to mice results in pain of peripheral origin and is manifested by abdominal constrictions, otherwise known as writhings or squirming.\[26\] Dose-dependent and significant reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with MEPC. At doses of 50, 100, 200 and 400 mg per kg body weight, MEPC was observed to reduce the number of constrictions, respectively, by 33.3, 37.0, 44.4, and 59.3%. A standard analgesic drug, aspirin, when administered to experimental animals at doses of 200 and 400 mg per kg body weight, reduced the number of constrictions by 48.1 and 63.0%, respectively. Thus, a dose of 200 mg/kg MEPC was nearly equivalent to that of 200 mg/kg aspirin, and a dose of 400 mg MEPC/kg was better than that obtained with 200 mg/kg aspirin in reducing pain. The results are shown in Table 2.

Table 2: Analgesic Effect of Crude Methanol Extract of *P. capitatum* Leaves (MEPC) In Acetic Acid-Induced Pain Model Mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of abdominal constrictions</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml</td>
<td>5.4 ± 0.24</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>2.8 ± 0.37</td>
<td>48.1*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.0 ± 0.32</td>
<td>63.0*</td>
</tr>
<tr>
<td>(MEPC)</td>
<td>50 mg</td>
<td>3.6 ± 0.40</td>
<td>33.3*</td>
</tr>
<tr>
<td>(MEPC)</td>
<td>100 mg</td>
<td>3.4 ± 0.68</td>
<td>37.0*</td>
</tr>
<tr>
<td>(MEPC)</td>
<td>200 mg</td>
<td>3.0 ± 0.32</td>
<td>44.4*</td>
</tr>
<tr>
<td>(MEPC)</td>
<td>400 mg</td>
<td>2.2 ± 0.37</td>
<td>59.3*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to control.

The exact mechanisms behind the observed antihyperglycemic and analgesic effects as observed with MEPC or the responsible phytochemicals in MEPC were not elucidated in this preliminary study and is currently being undertaken in our laboratory. However, preliminary phytochemical screening of MEPC showed presence of alkaloids, flavonoids, saponins and tannins. These phytochemicals, individually or in combination, have been described in other reports to exert antihyperglycemic as well as analgesic effects.

Lowering of blood glucose has been reported in alloxan induced diabetic mice with methanolic root bark extract of *Afzelia africana*. The extract was found to contain flavonoids,
tannins, alkaloids, steroids and saponins. [27] Aqueous extract of *Persea americana* seeds also demonstrated a hypoglycemic effect in alloxan diabetic rats; the extract contained glycosides, tannins, saponins, carbohydrates, flavonoids, and alkaloids. [28] Significant antidiabetic activity exhibited by ethanolic extract of whole plant of *Tridax procumbens* may be due to presence in the extract of alkaloids, tannins, flavonoids, saponins, and phenolic compounds. [29] Phytochemical screening revealed the presence of carbohydrates, glycosides, saponins, flavonoids, cardiac glycosides, tannins, alkaloids and triterpenes in stem bark extract of *Tamarindus indica* exhibiting hypoglycemic effects in alloxan-induced hyperglycemic rats. [30] Aqueous extract of *Felicia muricata* leaves reportedly showed anti-inflammatory and antinociceptive activities, the latter in acetic acid-induced writhing tests; phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins, and phenolics in the extract. [31] Different solvent ((acetone, dichloromethane, ethanol and ethyl acetate) extracts of *Vernonia condensata* leaves demonstrated antinociceptive activity; phytochemical screening showed that all the extracts contained alkaloids, phenolic compounds, flavonoids, tannins and saponins. [32] In acetic acid-induced writhing test, analgesic activity was observed with aqueous leaf extract of *Lagenaria breviflora*; phytochemical screening showed the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides and flavonoids. [33]

This is the first reported instance (to our knowledge) of antihyperglycemic and analgesic activity in methanol extract of *P. capitatum* leaves. Diabetes cannot be cured with allopathic medicine, although some traditional medicinal systems claim to have cures (still unproven). From that view point, leaves of this plant may provide a novel means for treatment of diabetes, pre-diabetes and impaired glucose metabolism. As per search in PubMed, there are no scientific reports on Marantaceae family plants for antihyperglycemic or analgesic effects. The present report can pave the way for scientific studies on plants belonging to this family.

REFERENCES


