PHYTOCHEMICAL SCREENING, ANTIHYPERGLYCEMIC AND ANALGESIC ACTIVITY STUDIES WITH METHANOL EXTRACT OF *TREVESIA PALMATA* LEAVES

K.M. Hasanur Rahman, Joyanto Kumar Nandi, Samira Sultana, Shahnaz Rahman, Shahadat Hossan, Mohammed Rahmatullah*

Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh.

ABSTRACT

**Background.** *Trevesia palmata*, also known as the Snowflake Aralia is found in Lawachara Forest Reserve of Bangladesh. It was of interest to phytochemically screen and determine the antihyperglycemic and analgesic properties of the leaves. **Methods.** Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT). Analgesic activity was determined by observed decreases in abdominal constrictions (writhings) in intraperitoneally administered acetic acid-induced pain model in mice. Phytochemical screening was done through standard methods. **Results.** Administration of methanol extract of leaves led to dose-dependent reductions in blood glucose levels in glucose-loaded mice. At doses of 100, 200 and 400 mg per kg body weight, the extract dose-dependently reduced blood glucose levels by 17.9, 28.1, and 47.4%, respectively compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 47.4%. In analgesic activity tests, the extract at doses of 50, 100, 200 and 400 mg per kg body weight reduced the number of abdominal constrictions by 33.3, 40.7, 48.1, and 55.6%, respectively. A standard pain relieving (analgesic) drug, aspirin, reduced the number of writhings by 48.1 and 63.0%, respectively, when administered at doses of 200 and 400 mg per kg body weight. **Conclusion.** Antihyperglycemic and antinociceptive activities have not previously been reported for *Trevesia palmata* leaves. The leaves can be beneficial in lowering blood sugar and for alleviating pain.
**Key words**: Antihyperglycemic, *Trevesia palmata*, analgesic, Araliaceae.

**INTRODUCTION**

*Trevesia palmata* (Roxburgh ex Lindley) Visiani belongs to the Araliaceae family and is known in English as the Snowflake Aralia and in Bengali as Bon papay. The tree can be found in Bangladesh, Cambodia, India, Laos, Nepal, Thailand and Vietnam. In Bangladesh, the plant is known to exist in Lawachara Forest Reserve in Sylhet Division in the northeast part of the country. *T. palmata* is an evergreen tree, which grows to 15-20 feet tall with few or no side branches and with leaves that are 1-2 feet wide. As far as is known, the tree has not been reported outside Lawachara Forest Reserve.

Very few scientific reports exist on the plant. The presence of lactone glycosides has been reported in the plant. Antiproliferative triterpenoid saponins have also been reported from the plant. In Arunachal Pradesh of India, the leaves are eaten as vegetable. The Kry ethnic group in Laos use decoction of roasted stems and roots for postpartum recovery, perineal healing, retraction of the uterus, abdominal pain, and as a lactagogue. The Adi tribe of Arunachal Pradesh, India use fruits of the plant as fish poison to stupefy the fish resulting in an easy catch.

The whole world, including Bangladesh, is witnessing a rapid increase in the number of people who are suffering from diabetes or impaired glucose tolerance. Diabetes is characterized by high blood sugar levels and can cause not only other serious conditions like increased risk of cardiovascular disorders but also cause disruptions in the social life and financial status of the patient and the patient’s family. Almost one in ten adults in Bangladesh have diabetes, and 56% of diabetics are not even aware that they have diabetes, thus highly increasing the risk factor of mortality and health-care costs. It has been estimated that in 2012, among adults of the world with ages ranging between 20 and 79, 382 million people had diabetes, and which number is estimated to rise to 592 million by the year 2035. Thus the world is currently undergoing a serious burden of diabetes and which is projected to rise dramatically in the future. The burden will fall more on the poorer and rural segments of the population in countries like Bangladesh, who do not have access to or cannot afford modern health-care and clinics.
Pain is another problem which disrupts the life of millions of people throughout the world on a daily basis. People can suffer from chronic pain during incurable diseases like rheumatoid arthritis or cancer or from acute pain, which can occur through sprains or even minor injuries and accidents. Pain is usually treated with over-the-counter drugs like aspirin or paracetamol, but these drugs can lead to gastric ulceration or hepatotoxicity from prolonged use or over-dosage. [8, 9] As such, it is a necessity to find out newer pain-relieving drugs without adverse side-effects.

Towards finding out newer drugs to combat diabetes (which cannot be cured by existing allopathic drugs) and to relieve pain, we had been screening the plants of Bangladesh for their antihyperglycemic and analgesic potentials. This screening has involved plants on which both ethnomedicinal reports exist of their uses against diabetes and/or pain, but also plants with hitherto unreported pharmacological studies. [10-21] As such, the objective of the present study was to evaluate the antihyperglycemic and analgesic potential of a not so well-studied plant, namely, Trevesia palmata.

METHODS
Plant material collection
Leaves of T. palmata were collected during November 2013 from Lawachora Forest Reserve, Sylhet Division, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,700).

Preparation of methanolic extract of leaves
Leaves were cut into small pieces, air-dried in the shade, and 82g of dried and powdered leaves were extracted with methanol (w:v ratio of 1:5, final weight of the extract 5.46g).

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 15-20g 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)\(^{[22]}\) 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 (METP) 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.\(^{[23]}\) The percent lowering of blood glucose levels were calculated according to the formula described below.

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

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

**Analgesic activity evaluation through abdominal writhing test**

Analgesic activity of METP was examined as previously described.\(^{[24]}\) 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 METP 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 METP, 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\(^{[25]}\), 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 = \((1 - W_e/W_c) \times 100\)
where \( W_e \) and \( W_c \) represents the number of writhings in aspirin or METP administered mice (Groups 2-7), and control mice (Group 1), respectively.

**Acute toxicity test**

Acute toxicity test was conducted as previously described. \(^{[26]}\) 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 METP 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. \(^{[17]}\)

**Preliminary phytochemical screening**

Preliminary phytochemical analysis of METP for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before. \(^{[27]}\)

**RESULTS**

**Toxicity evaluation**

The crude extract (METP) did not show any toxicity in mice even at the highest dose tested. There were no changes in behavioral pattern and mortality was not observed.

**Preliminary screening of phytochemicals**

Various tests conducted for presence of phytochemicals in METP indicated the presence of alkaloids, flavonoids, saponins, and tannins.

**Antihyperglycemic activity evaluation results**

In oral glucose tolerance tests (OGTT), METP at doses of 100, 200 and 400 mg/kg caused, respectively, 17.9, 28.1, and 47.4% reductions in blood glucose levels. At a dose of 50 mg/kg, any reduction in blood glucose level was not observed. Glibenclamide (a standard antihyperglycemic drug), when administered at a dose of 10 mg/kg lowered blood glucose by 47.4%, which was the same as observed with the highest dose of the extract. The results suggest that the extract at the highest dose tested possess strong antihyperglycemic activity.
The results are shown in Table 1. To our knowledge, any antihyperglycemic activity of leaves of the plant have not been reported before, and as such, the leaves merit further scientific attention towards discovery of possibly newer antihyperglycemic drugs.

**Analgesic activity evaluation results**

Dose-dependent and significant reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with METP. At doses of 50, 100, 200 and 400 mg per kg body weight, METP was observed to reduce the number of constrictions, respectively, by 33.3, 40.7, 48.1, and 55.6%. 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 METP was equivalent to that of 200 mg/kg aspirin. The results are shown in Table 2 and suggest that the extract possesses significant analgesic properties. Since analgesic activity has previously not been reported from the whole plant or any part of the plant to our knowledge, the results suggest possible presence of potentially new analgesic phytoconstituents in leaves.

**Table 1: Effect of crude methanol extract of *T. palmata* leaves (METP) 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 (METP)</td>
<td>10 mg</td>
<td>2.88 ± 0.19</td>
<td>47.4*</td>
</tr>
<tr>
<td>(METP)</td>
<td>50 mg</td>
<td>5.58 ± 0.32</td>
<td>-</td>
</tr>
<tr>
<td>(METP)</td>
<td>100 mg</td>
<td>4.50 ± 0.28</td>
<td>17.9*</td>
</tr>
<tr>
<td>(METP)</td>
<td>200 mg</td>
<td>3.94 ± 0.26</td>
<td>28.1*</td>
</tr>
<tr>
<td>(METP)</td>
<td>400 mg</td>
<td>2.88 ± 0.32</td>
<td>47.4*</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.

**Table 2: Analgesic effect of crude methanol extract of *T. palmata* leaves (METP) 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>
</tbody>
</table>
All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *\(P < 0.05\); significant compared to control.

DISCUSSION

It is interesting that alkaloids, flavonoids, saponins and tannins were present in the methanolic extract of leaves of the plant. These groups of compounds, either singly or in combination can account for the observed antihyperglycemic and analgesic properties. For instance, stem bark extract of *Tamarindus indica* reportedly demonstrated antihyperglycemic activity in alloxan diabetic rats. Phytochemical screening of the extract showed the presence of glycosides, saponins, flavonoids, cardiac glycosides, tannins, alkaloids and triterpenes.\(^{[28]}\) The analgesic activity of *Aconitum baikalnensis* has been attributed to diterpene alkaloids.\(^{[29]}\)

The antihyperglycemic effect of polyphenol flavonoids like quercetin has been reported in alloxan induced type 2 diabetic mice.\(^{[30]}\) Pharmacognostic and phytochemical investigation of leaves of *Malvastrum coromandelianum* indicated presence of alkaloids, tannins and flavonoids along with analgesic and anti-inflammatory activities.\(^{[31]}\) Aqueous extract of *Vernonia condensata* leaves has been reported to exhibit antinociceptive activity in writhing tests; the extract was found to contain alkaloids, flavonoids, and saponins.\(^{[32]}\) Phytochemical analysis of the ethanolic extract of *Sida cordifolia* roots exhibiting analgesic activity indicated the presence of reducing sugar, alkaloids, steroids and saponins.\(^{[33]}\) Ethanolic extract of whole plant of *Tridax procumbens* reportedly showed hypoglycemic effect in STZ-diabetic rats. Alkaloids, flavonoids and saponins were present in the extract.\(^{[34]}\)

Irrespective of the nature of the phytochemical constituent(s) responsible for the observed antihyperglycemic and analgesic effects, the results suggest that the plant possesses constituents, which may be beneficial in reducing blood glucose and relieving pain, and so can be beneficial to the people suffering from pain and/or diabetes or impaired glucose metabolism.

CONCLUSION

The results suggest that methanolic extract of *T. palmata* leaves can be used for lowering of blood glucose and for alleviating pain.
CONFLICTS OF INTEREST
The author(s) declare that they have no competing interests.

REFERENCES


