PRELIMINARY ANTIHYPERGLYCEMIC AND ANALGESIC ACTIVITY STUDIES WITH ANGIOPTERIS EVECTA LEAVES IN SWISS ALBINO MICE

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ABSTRACT

Background. Angiopteris evecta, also known as the Giant Fern is found in Lawachara Forest Reserve of Bangladesh. Not much is known about this species. Since we are screening the plants of Bangladesh for their various pharmacological properties, it was of interest to determine the antihyperglycemic and analgesic properties of the leaves. Methods. Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT). Antinociceptive activity was determined by observed decreases in abdominal constrictions (writhings) in intraperitoneally administered acetic acid-induced pain model in mice. Results. Administration of methanol extract of whole plant led to dose-dependent reductions in blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg body weight, the extract dose-dependently reduced blood glucose levels by 21.3, 23.7, 32.0, and 53.6%, 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 61.2%. In antinociceptive activity tests, the extract at doses of 50, 100, 200 and 400 mg per kg body weight reduced the number of abdominal constrictions by 25.9, 44.4, 55.6, and 59.3%, respectively. A standard pain relieving (antinociceptive) 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 *Angiopteris evecta* leaves. The leaves can be of use in lowering blood sugar and for alleviating pain.

**Key words**: Antihyperglycemic, *Angiopteris evecta*, antinociceptive, Marratiaceae.

**INTRODUCTION**

*Angiopteris evecta* (G. Forst.) Hoffm. is a Marattiaceae (some scientists refer to its family as Angiopteridaceae) family plant and is known in English as the Giant Fern. The plant is not common in Bangladesh, but can be found at Lawachara Forest Reserve in Sylhet Division of the country where it is known locally as ‘Harin khuku’. The huge mature fronds of the plant can grow up to 8 meters long. The plant is also found in India, Australia, and various places of Polynesia and Melanesia.

Ethnomedicinal uses of the plant appear to be scarce. The tribes of Cachar district in Assam, India use the rhizomes of the plant to treat piles. [1] Decoction obtained from leaves of the plant is orally taken with lemon juice to treat intestinal ulcer and stomach ache by indigenous people of Kolli Hills of Eastern Ghats in Tamil Nadu, India. [2]

Angiopteroside, a constituent isolated from the plant, has been reported to inhibit HIV-1 reverse transcriptase and lung cancer cell line (Chaco). [3] *In vitro* antiplasmodial activity against *Plasmodium falciparum* has been shown for tubers of the plant. [4] Ethanolic extract of roots of the plant have been shown to reduce blood glucose in glucose tolerance tests in mice at doses of 300 and 1500 mg/kg. [5] Methanol extract of fronds of the plant have been reported to have antibacterial activity. [6] Dichloromethane and ethyl acetate fractions of leaves and stem bark also have reported antibacterial and antifungal activities. [7]

Diabetes is a disease characterized by high blood sugar levels caused due to insulin deficiency or resistance to insulin. The disease can lead to high risks of cardiovascular disorders, and can cause diabetic retinopathy, neuropathy, and nephropathy. Diabetes is rapidly becoming endemic throughout the world, possibly because of changes in food habits and adoption of a sedentary lifestyle in the world population. The estimated diabetes prevalence in the world for adults between the ages of 20 and 79 has been estimated at 382 million worldwide in 2012 and is expected to reach 592 million people by 2035. The International Diabetes Federation (IDF) estimated that in 2013, five countries of the world, namely, China, USA, Russian Federation, India and Brazil, each had more than 10 million
people with diabetes. A survey has found that in Bangladesh, 9.7% and 22.4%, respectively of the adults had diabetes or pre-diabetes conditions. Diabetes cannot be totally cured with allopathic medicine; moreover, particularly the rural population of Bangladesh lacks access to modern diagnostic centers and clinics or hospitals and in most cases cannot afford the cost of antidiabetic drugs.

Pain is another common affliction that affects people worldwide on a daily basis. Pain can arise from injury or common occurrences like sprain or acidity but also can arise from chronic untreatable diseases like rheumatoid arthritis. Over-the-counter drugs like aspirin or paracetamol have adverse side effects like causing gastric ulceration or hepatotoxicity if taken on a regular basis or from over-dosage. As such this is another area like diabetes where newer and more efficacious drugs are needed, which have less or no side-effects.

Plant kingdom has been and still is the source of many modern drugs. We had been over the last few years, systematically screening various plants of Bangladesh for their antihyperglycemic and analgesic potential. The objective of the present study was to evaluate the antihyperglycemic and analgesic potential of methanolic extract of leaves of *Angiopteris evecta* in Swiss albino mice. Antihyperglycemic potential was determined through oral glucose tolerance tests (OGTT) in glucose-loaded mice, while analgesic potential was determined through intraperitoneally injected acetic acid-induced pain model in mice.

**METHODS**

**Plant material collection**

Leaves of *A. evecta* were collected during November 2013 from Lawachora Forest Reserve, Sylhet Division, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,702).

**Preparation of methanolic extract of leaves**

Leaves were cut into small pieces, air-dried in the shade, and 60g of dried and powdered leaves were extracted with methanol (w:v ratio of 1:5, final weight of the extract 4.54g).

**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) [21] 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 (MEAV) 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. [22] 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) X 100, where W_e and W_c represents the blood glucose concentration in glibenclamide or MEAV administered mice (Groups 2-6), and control mice (Group 1), respectively.

Analgesic activity evaluation through abdominal writhing test
Analgesic activity of MEAV was examined as previously described. [23] 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 MEAV 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 MEAV, 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 [24], 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 MEAV administered mice (Groups 2-7), and control mice (Group 1), respectively.

**Acute toxicity test**

Acute toxicity test was conducted as previously described. [25] 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 MEAV 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. [16]

**Preliminary phytochemical screening**

Preliminary phytochemical analysis of MEAV for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before. [26]

**RESULTS**

**Toxicity evaluation**

The crude extract 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 MEAV indicated the presence of flavonoids and tannins.

**Antihyperglycemic activity evaluation results**

MEAV, when administered at doses of 50, 100, 200 and 400 mg per kg body weight, caused dose-dependent decreases in blood glucose levels in glucose-loaded mice in oral glucose
tolerance tests at the three higher doses of 100, 200 and 400 mg. At the four doses, the percent reductions in blood glucose were, respectively, 21.3, 23.7, 32.0, and 53.6. The decreases in blood glucose levels were significant at the three higher doses but not with the dose of 50 mg MEAV per kg body weight. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 61.2%. The results are shown in Table 1, and indicate that at the highest dose, MEAV has significant antihyperglycemic potential.

**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 MEAV. At doses of 50, 100, 200 and 400 mg per kg body weight, MEAV reduced the number of constrictions, respectively, by 25.9, 44.4, 55.6, 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, even at a dose of 100 mg MEAV, the analgesic activity was nearly equivalent to that of 200 mg aspirin, and at the two highest doses of the extract, MEAV showed analgesic activity better than that of 200 mg per kg aspirin. The results are shown in Table 2 and suggest that the extract possesses significant analgesic properties.

**Table 1: Effect of crude methanol extract of A. evecta leaves (MEAV) 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>5.82 ± 0.25</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.26 ± 0.18</td>
<td>61.2*</td>
</tr>
<tr>
<td>(MEAV)</td>
<td>50 mg</td>
<td>4.58 ± 0.68</td>
<td>21.3</td>
</tr>
<tr>
<td>(MEAV)</td>
<td>100 mg</td>
<td>4.44 ± 0.33</td>
<td>23.7*</td>
</tr>
<tr>
<td>(MEAV)</td>
<td>200 mg</td>
<td>3.96 ± 0.29</td>
<td>32.0*</td>
</tr>
<tr>
<td>(MEAV)</td>
<td>400 mg</td>
<td>2.70 ± 0.14</td>
<td>53.6*</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: Antinociceptive effect of crude methanol extract of *A. evecta* leaves (MEAV) 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>(MEAV)</td>
<td>50 mg</td>
<td>4.0 ± 0.55</td>
<td>25.9*</td>
</tr>
<tr>
<td>(MEAV)</td>
<td>100 mg</td>
<td>3.0 ± 0.45</td>
<td>44.4*</td>
</tr>
<tr>
<td>(MEAV)</td>
<td>200 mg</td>
<td>2.4 ± 0.40</td>
<td>55.6*</td>
</tr>
<tr>
<td>(MEAV)</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.

**DISCUSSION**

Ethanolic extract of roots of the plant reportedly reduced blood glucose in glucose tolerance tests in mice at doses of 300 and 1500 mg/kg. [5] However, to our knowledge, this is the first reported instance of antihyperglycemic and analgesic activity demonstration of leaves of the plant. The observed blood glucose lowering effect can be through any of the possible mechanisms like increased secretion of insulin, increased peripheral utilization of glucose, or through decreased absorption of glucose from gut. Whatever mechanism was responsible was not determined in this preliminary study and further experiments are underway in the laboratory to determine the exact mechanism(s).

Acetic acid-induced pain can arise from increased synthesis of prostaglandins like PGE2 and PGF2α or through other cyclooxygenase and lipooxygenase mediated pathway products. [27] Thus the decrease in abdominal constrictions as observed with MEAV can be through inhibition of the cyclooxygenase and lipooxygenase pathways. The exact mechanism and the component(s) responsible for the antihyperglycemic and analgesic effects of MEAV merit further investigation.

It is to be noted that preliminary phytochemical screening of MEAV indicated the presence of flavonoids and tannins. Ethanolic leaf extract of *Ficus glumosa* has been shown to demonstrate hypoglycemic and antilipidemic properties in diabetic rats; the extract contained flavonoids and tannins among other groups of phytoconstituents. [28] Inhibition of α-amylase and α-glucosidase activities by aqueous extract of *Morinda lucida* leaf has also been
attributed to presence of flavonoids, saponins and tannins in the extract.\textsuperscript{[29]} Antidiabetic and antihyperlipidemic effect in streptozotocin diabetic rats has been observed with ethanolic extract of whole plant of \textit{Tridax procumbens}; the extract reportedly contained alkaloids, flavonoids, saponins, phenolic compounds, and tannins.\textsuperscript{[30]} Antihyperglycemic activity has also been observed with stem bark extract of \textit{Tamarindus indica} in alloxan diabetic rats; the extract reportedly contained alkaloids, flavonoids, saponins and tannins.\textsuperscript{[31]} Thus flavonoids and tannins present in MEAV can account for the antihyperglycemic effect as noted in the present study.

These two groups of phytochemicals (flavonoids and tannins) can also play a role in the observed analgesic effects of MEAV. Analgesic effect observed through inhibition of acetic acid-induced writhings by ethanolic leaf extract of \textit{Ixora coccinea} has been attributed to presence of flavonoids, tannins, and triterpenes.\textsuperscript{[32]} Analgesic activity of fertile fronds of \textit{Drynaria quercifolia} has been reported; the extract contained coumarins, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and terpenoids.\textsuperscript{[33]} Antinociceptive property of methanolic extract of \textit{Teucrium stockstianum} has been demonstrated; the extract contained flavonoids, saponins and tannins among other constituents.\textsuperscript{[34]} Flavonoids, terpenes, and tannins were also found in methanol extract of leaves of \textit{Phlogacanthus thyrsiflorus}, which extract showed analgesic, anti-inflammatory, and anti-oxidant activities.\textsuperscript{[35]}

\textit{Angiopteris evecta} has been a relatively unexplored plant thus far regarding its pharmacological potential. The present study can spur scientific interest towards isolation of phytochemical constituents from the plant and examine their pharmacological properties. Also, the plant can prove to be a cheap source for antidiabetic and analgesic lead compounds from the plant, which can be beneficial to people suffering from diabetes and pain.

**CONCLUSION**

The results suggest that methanolic extract of \textit{A. evecta} 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.
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