

PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL STUDIES WITH *ANGIOPTERIS EVECTA* ROOTS

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ABSTRACT

Objective: The objective of the present study was to phytochemically screen and evaluate the antihyperglycemic and analgesic potential of methanolic extract of *Angiopteris evecta* roots. **Methods:** Extract was administered orally at doses of 50-400 mg/kg. Oral glucose tolerance test (OGTT) was used to evaluate antihyperglycemic activity in glucose-loaded mice. Analgesic activity was assessed in intraperitoneally injected acetic acid-induced pain model. **Results:** The extract, at doses of 50, 100, 200, and 400 mg/kg, reduced blood glucose levels by 11.3, 23.0, 32.8, and 46.7%, respectively. By comparison, a standard antihyperglycemic drug, glibenclamide (10 mg/kg) reduced blood glucose levels by 47.4%. In acetic acid-induced pain model, the extract reduced the number of abdominal constrictions

by 25.9, 40.7, 48.1, and 59.3%, respectively, at doses of 50, 100, 200 and 400 mg/kg. A standard analgesic drug, aspirin (200 and 400 mg/kg) reduced abdominal constrictions by 48.1 and 63.0%, respectively. **Conclusion:** The extract possesses significant antihyperglycemic and analgesic potential.

KEYWORDS: *Angiopteris evecta*, Marratiaceae, antihyperglycemic, analgesic, oral glucose tolerance, glibenclamide.

1. INTRODUCTION

Angiopteris evecta (G. Forst) Hoffm. Is a Marattiaceae family plant and is known in English as the Giant Fern and in Bangladesh as 'horin khuku'. The plant is not a very common plant of Bangladesh and can mainly be found in Sylhet Division in the northeast part of the country in and around Lawachara Forest Reserve. Besides Bangladesh, the plant can also be found in India, Australia, and various places of Polynesia and Melanesia.

There are a few reports on ethnomedicinal uses of the plant. Leaf decoction is taken orally with lemon juice to treat intestinal ulcer and stomach ache by indigenous people of Kolli Hills of Eastern Ghats in Tamil Nadu, India. ^[1] In Assam, India, the tribes of Cachar District use rhizomes of the plant to treat hemorrhoids. ^[2] In bio-activity studies, tubers of the plant have been shown to have anti-plasmodial activity. ^[3] Methanol extract of fronds of the plant have been reported to have antibacterial activity. ^[4] Ethanolic extract of roots of the plant have been shown to reduce blood glucose in glucose tolerance tests. ^[5]

Diabetes is rapidly becoming endemic throughout the world, possibly because of changes in lifestyle and food habits of people. Bangladesh is no exception to this spread of diabetes, which is characterized by high blood glucose levels, and which disease can, if left untreated lead rapidly to other disorders of the heart, kidney and eyes. A survey has found that in Bangladesh, 9.7% and 22.4%, respectively of the adults have diabetes or pre-diabetes conditions, including impaired glucose metabolism. ^[6] The problem is compounded by the fact that at least a third of the people of Bangladesh has below poverty level incomes and so cannot afford or access the symptomatic cures of anti-diabetic drugs.

Pain is another problem suffered by people throughout the world in acute or chronic form. Pain can result from simple matters like cuts, wounds, sprains and injuries but also can arise from complicated diseases like cancer or rheumatoid arthritis. Over-the-counter (OTC) drugs like aspirin or paracetamol are present to treat pain, but they suffer from adverse effects like gastric ulceration or causing hepatotoxicity from over-dosage or prolonged use. The rural people of Bangladesh have agriculture as their main occupation, which necessitates hard labor in the fields particularly during cultivation and harvesting times. This results in pain. A majority of the urban slum dwellers are occupied in hard labor like being engaged as construction workers or as rickshaw or van pullers; these occupations also lead to pain on a frequent and almost daily basis. Thus overuse and misuse of OTC drugs are common.

Towards finding out suitable anti-diabetic and pain relieving drugs, we had been systematically conducting efforts to identify plants of Bangladesh with anti-hyperglycemic and analgesic potential. [7-17] Plant kingdom has always been an excellent source for modern allopathic drugs; examples include quinine, artemisinin, reserpine, atrophine, to name only a few. In a previous report, we have analyzed the anti-hyperglycemic and analgesic potential of leaves of *A. evecta*. [18] The objective of the present study was to evaluate the roots of *A. evecta* for their anti-hyperglycemic and analgesic potential.

2. MATERIALS AND METHODS

Plant Material Collection

Roots 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 Roots

Roots were thoroughly cleaned with distilled water and the excess water was soaked off with tissue paper. They were then cut into small pieces, air-dried in the shade, and 100g of dried and powdered roots were extracted with methanol (w:v ratio of 1:5, final weight of the extract 4.78g).

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 14-19g 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 Anti-Hyperglycemic Activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999). [19] 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 root 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.^[20] 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 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.^[21] 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^[22], 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 MEAV administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute Toxicity Test

Acute toxicity test was conducted as previously described.^[23] 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 \pm 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. ^[13]

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of MEAV for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before. ^[24]

3. 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 alkaloids, flavonoids and tannins.

Antihyperglycemic Activity Evaluation Results

Extract of roots of *A. evecta*, when administered to glucose-loaded mice, led to dose-dependent reductions in the level of blood glucose. At extract doses of 50, 100, 200 and 400 mg/kg, MEAV administration led to reductions in blood glucose levels, respectively, by 11.3, 23.0, 32.8, and 46.7%. The reductions were statistically significant ($P < 0.05$) at the three higher doses of the extract, but not with the dose of 50 mg MEAV per kg. A standard anti-hyperglycemic drug, glibenclamide, when administered to mice at a dose of 10 mg per kg led to 47.4% reduction in blood glucose level in mice. Thus the highest dose of the extract (400 mg per kg) demonstrated significant anti-hyperglycemic activity as manifested through improved glucose tolerance and was comparable to that of the standard drug, glibenclamide. The results are shown in Table 1.

Table 1: Effect of crude methanol extract of *A. evecta* roots (MEAV) on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.48 ± 0.34	-
Glibenclamide	10 mg	2.88 ± 0.19	47.4*
(MEAV)	50 mg	4.86 ± 0.25	11.3
(MEAV)	100 mg	4.22 ± 0.27	23.0*
(MEAV)	200 mg	3.68 ± 0.27	32.8*
(MEAV)	400 mg	2.92 ± 0.21	46.7*

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

Dose-dependent and significant (*P* < 0.05) reductions in the number of abdominal constrictions or writhings 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, 40.7, 48.1, 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, at the two highest doses of the extract, MEAV showed analgesic activity equivalent to or 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 2: Antinociceptive effect of crude methanol extract of *A. evecta* roots (MEAV) in acetic acid-induced pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	5.4 ± 0.24	-
Aspirin	200 mg	2.8 ± 0.37	48.1*
Aspirin	400 mg	2.0 ± 0.32	63.0*
(MEAV)	50 mg	4.0 ± 0.32	25.9*
(MEAV)	100 mg	3.2 ± 0.49	40.7*
(MEAV)	200 mg	2.8 ± 0.37	48.1*
(MEAV)	400 mg	2.2 ± 0.49	59.3*

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

4. DISCUSSION

The observed decreases in blood glucose levels following MEAV administration can be due to a number of mechanisms like decreased absorption of glucose from gut, potentiating insulin secretion, or increase in peripheral utilization of glucose. The exact mechanism responsible for the observed anti-hyperglycemic action was not elucidated in the present study and further work needs to be done in this regard. Intraperitoneal administration of acetic acid can lead to sensation of pain mediated through increased synthesis of prostaglandins like PGF₂ α and PGF₂ β or through other cyclooxygenase and lipoxygenase mediated pathway products.^[25] Thus MEAV could be decreasing the pain sensation (as manifested by reductions in abdominal writhings) through inhibition of cyclooxygenase and/or lipoxygenase pathways. However, the exact mechanism needs to be elucidated and currently work is being focused in our laboratory to elucidate the mechanism(s) behind the observed anti-hyperglycemic and analgesic effects of MEAV.

Preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, and tannins in MEAV. Hypoglycemic and tissue protective effects have been shown with aqueous extract of *Persea americana* seeds in alloxan diabetic rats; phytochemical analysis revealed the presence of glycosides, tannins, saponins, carbohydrates, flavonoids, and alkaloids in the extract.^[26] Similarly, ethanolic extract of whole plant of *Tridax procumbens* reportedly showed anti-diabetic and anti-hyperlipidemic activity in streptozotocin (STZ) diabetic rats. Phytochemical analysis of the extract showed the presence of alkaloids, tannins, flavonoids, saponins, and phenolic compounds.^[27] The anti-hyperglycemic activity of stem bark extract of *Tamarindus indica* has also been attributed to presence of carbohydrates, glycosides, saponins, flavonoids, cardiac glycosides, tannins, alkaloids and triterpenes in the extract.^[28]

Alkaloids, flavonoids, and tannins have also been implicated in analgesic and anti-inflammatory activity studies with other plant extracts. Analgesic and anti-inflammatory activities of the aqueous stem and leaf extract of *Asystasia gangetica* has been reported; preliminary phytochemical screening showed the presence of alkaloids, tannins, cardiac glycosides and flavonoids.^[29] Antinociceptive and anti-inflammatory activities have been seen with aqueous and ethanolic extracts of *Myrtus communis*; the extracts contained alkaloids, flavonoids, and tannins.^[30] Aqueous leaf extract of *Lagenaria breviflora* exhibiting analgesic and anti-inflammatory activities also had alkaloids, flavonoids, and tannins present in the extract.^[31]

The present study and the previous study demonstrating anti-hyperglycemic and analgesic potential, respectively, of roots and leaves of *A. evecta* suggest that the plant may prove useful in isolation of lead compounds, which could prove effective in lowering blood sugar and for alleviating pain. The plant thus merits further scientific studies, since this is the first documented instance of such studies with roots of the plant.

5. CONCLUSION

The present study demonstrates that the methanolic extract of roots of *A. evecta* has anti-hyperglycemic and analgesic potential.

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