Evaluation of antinociceptive and antihyperglycemic activities in methanol extracts of whole plants of *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae) in mice

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Abstract: The present study evaluated the antinociceptive and antihyperglycemic effects of crude methanol extract of whole plants of *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae) in Swiss albino mice. Antinociceptive activity was evaluated by attenuation of the number of constrictions in acetic acid-induced gastric pain, while antihyperglycemic activity was evaluated through oral glucose tolerance tests in glucose-loaded mice. Dose-dependent and significant inhibitions in the number of constrictions were seen in mice administered with extract at doses of 50, 100, 200 and 400 mg per kg body weight. At these concentrations, the numbers of constrictions were reduced, respectively, by 31.0, 32.7, 37.9 and 44.8%. In comparison, a standard antinociceptive drug, aspirin reduced the number of constrictions by 37.9 and 67.2%, when administered at doses, respectively, of 200 and 400 mg per kg body weight. The extract also exhibited dose-dependent and significant antihyperglycemic activity when administered to mice at the afore-mentioned four doses. Serum glucose concentrations were reduced, respectively, by 36.3, 58.6, 65.0 and 65.6% at the four doses administered. The results compare favorably with a standard antihyperglycemic drug, glibenclamide, which when administered at a dose of 10 mg per kg body weight reduced serum glucose level by 42.7%. Taken together, the results obtained indicate that the extract merit further scientific studies towards discovery of components, which may prove beneficial in ameliorating pain, as well as high sugar levels of diabetic patients.

Keywords: *Alternanthera philoxeroides*, antinociceptive, antihyperglycemic, Amaranthaceae.

INTRODUCTION

Alternanthera philoxeroides (Mart.) Griseb. (Amaranthaceae) is an aquatic plant, which although originating in South America can now be found in many parts of the world including Bangladesh. The plant is known in English as the alligator weed and in the local Bengali language as haicha shak. Only a few studies can be found in the scientific literature concerning phytochemical components or pharmacological activities about this plant.

The preventive and therapeutic effects of the plant against influenza have been reported (Niu, 1986). Aqueous extract of the plant reportedly demonstrated inhibitory activities against the human immunodeficiency virus (Zhang *et al*., 1988). Petroleum ether, ether, and ethyl acetate extracts of plant parts have shown promising inhibitory effects against epidemic hemorrhagic fever virus (Yang *et al*., 1989). The plant has also been reported to give protection against fetal epidemic hemorrhagic fever virus infection in suckling mice (Qu *et al*., 1993). Extract of the plant has also been found to possess antiviral effects against dengue virus *in vitro* (Jiang *et al*., 2005). Oral preparation of the plant has been shown to be effective against respiratory syncytical virus in mice (Jiang *et al*., 2007).

Reported components of the plant include phaeophytin a, phaeophytin a´, oleanoic acid, β-sitosterol, 3β-hydroxystigmast-5-en-7-one, α-spinasterol, 24-methyl-enecycloartanol, cycloeucalenol, and phytol (Fang *et al*., 2006). From the aerial parts of the plant, the antitumor compounds, alternanthin B and N-*trans*-feruloyl-3,5-dimethoxytyramine has been isolated (Fang *et al*., 2007). Pentacyclic triterpene saponins (philoxeroidesides A-D) isolated from the plant exhibited cytotoxic activities against SK-N-SH and HL60 cell lines (Fang *et al*., 2009a). Oleanolic acid 3-*O*-β-D-glucuronopyranoside, isolated from n-butyl extract of the plant showed significant inhibitory effect against Hela and L929 cell lines (Fang *et al*., 2009b).

Dhaka City, the capital of Bangladesh is surrounded by marshy lands and rivers and is protected from yearly monsoon floods by embankments (locally called Bheri Bundh). Huge numbers of *A. philoxeroides* (Mart.) Griseb. can be found growing in the marshy lands as well as riversides. The embankments are usually occupied by slum dwellers, who have built up their slums on the high land of the embankments. These slum dwellers, more often than not, are serviced by folk medicinal practitioners, because they either due to habit or because of non-affordability cannot visit modern allopathic

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doctors. During an ethnomedicinal survey conducted amongst these slum dwellers and the folk medicinal practitioners, it was observed that they occasionally consume juice obtained from macerated whole plants of *A. philoxeroides* (Mart.) Griseb. When queried, they mentioned that the juice obtained from this plant has apparent beneficial effects during fever, pain, and diabetes. Since the antinociceptive and antihyperglycemic properties of this plant have not been reported, it was the objective of the present study to conduct such evaluation with crude methanol extract of the plant.

MATERIALS AND METHODS

Plant material and extraction

Whole plants of *A. philoxeroides* (Mart.) Griseb. were collected from the Bheri Bundh area of Dhaka city, Bangladesh in April 2010. The plant was taxonomically identified by the Bangladesh National Herbarium at Dhaka (Accession Number: 35,056). Whole plants were cut in to small pieces and air-dried in the shade for 120 hours, following which they were grounded in to a fine powder. 100g of the powder was extracted with 500 ml methanol for 24 hours at room temperature (30-32^{o}C). After 24 hours, the mixture was filtered and the filtrate evaporated to dryness. The final weight of the extract was 9.5g.

Chemicals and drugs

Aspirin, glibenclamide and glucose were purchased from Square Pharmaceuticals Ltd., Bangladesh. Glacial acetic acid was obtained from Sigma Chemicals, USA. All other chemicals used were of analytical grade.

Animals

For purposes of the present study, Swiss albino mice weighing between 20-25g were purchased from the International Centre for Diarrhoeal Disease Research, Bangladesh. The animals were acclimatized for one week prior to commencement of any experiment. The study was approved by the Institutional Animal Ethical Committee of University of Development Alternative.

Acetic acid-induced constriction method

Previously described procedures (Shanmugasundaram and Venkataraman, 2005) with minor modifications were used for evaluating antinociceptive activity of methanol extract. Abdominal pain as demonstrated by constrictions was induced in mice through intraperitoneal injection of acetic acid (1% acetic acid, 10 ml per kg body weight). Mice were divided into seven groups, each group consisting of eight mice. Group 1 mice served as control and were administered vehicle (1% Tween 80 in water, 10 mg per kg body weight). Groups 2 and 3 were administered a standard antinociceptive drug, aspirin, at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered extract, respectively, at 50, 100, 200 and 400 mg per kg body weight orally 30 min prior to acetic acid administration. Five minutes was given to each mouse to ensure bio-availability of acetic acid. The number of constrictions induced by gastric pain in mice was then measured for the next 10 minutes.

Antihyperglycemic activity

Antihyperglycemic activity of extract was evaluated by the oral glucose tolerance test in glucose-loaded mice as previously described by Joy and Kuttan (1999) with some minor changes. Briefly, fasted mice were divided into six groups of six mice each. Group 1 received vehicle in the form of 1% Tween 80 in water (10 ml per kg body weight). Group 2 received the standard drug, glibenclamide at a dose of 10 mg per kg body weight. Groups 3-6 were administered the crude methanol extract at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Dose per mouse was individually adjusted following weighing of individual mouse. Vehicle, glibenclamide and extract were orally administered to each mouse. Mice were given a 60 min period following administration of vehicle, glibenclamide or extract and then each mouse was orally administered 2g glucose per kg body weight. Blood samples were collected 120 min following glucose administration through puncturing heart. The procedure of Venkatesh *et al*. (2004), which uses the glucose oxidase method, was used for measuring concentrations of glucose in serum.

Acute toxicity test

Acute toxicity test was as previously described (Ganapaty *et al*., 2002). Mice were divided into nine groups of six animals each. 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 crude methanol extract per kg body weight. Animals were closely observed for the next 8 hours to notice any behavioral changes or mortality and were kept under observation for the next two weeks.

STATISTICAL ANALYSIS

Student's *t*-test was used to compare any significant differences between control and experimental animals. $P < 0.05$, was considered significant as compared to control.

RESULTS

Antinociceptive activity

The crude methanol extract of *A. philoxeroides* (Mart.) Griseb. demonstrated dose-dependent and significantly reduced number of constrictions in mice intraperitoneally administered with acetic acid. At doses of 50, 100, 200 and 400 mg per kg body weight, the extract reduced

constrictions in mice, respectively, by 31.0, 32.7, 37.9 and 44.8%. In comparison, the antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of constrictions by 37.9 and 67.2%, respectively. The two highest doses of the extract, namely 200 and 400 mg per kg body weight, thus demonstrated equivalent or better results than aspirin, the latter when administered at a dose of 200 mg per kg body weight. The results are shown in table 1.

Antihyperglycemic effect

The crude methanol extract also caused dose-dependent and statistically significant reductions in serum glucose levels, when administered at doses of 50, 100, 200 and 400 mg per kg body weight to glucose-loaded mice in oral glucose tolerance tests. At the afore-mentioned four doses, the respective reductions in serum glucose concentrations were 36.3, 58.6, 65.0 and 65.6%. Glibenclamide by comparison caused reduction of serum glucose level by 42.7%. The results indicate that the extract possess considerable antihyperglycemic properties, for even at a dose of 100 mg per kg body weight, the extract was more potent than glibenclamide (the latter being administered at 10 mg per kg body weight) in reducing serum glucose levels. The results are shown in table 2.

Acute toxicity test

Any mortality or behavioral changes were not observed with the extract in mice even when administered at a level of 3000 mg per kg body weight.

DISCUSSION

Intraperitoneal administration of acetic acid induces gastric pain in mice, which effect can be visualized through abdominal constrictions in mice as a result of the gastric pain. According to Shanmugasundaram and Venkataraman (2005), both central and peripheral analgesia may be detected with the acetic acid-induced constriction model. Peripheral analgesia involves the peripheral nociceptive afferent neuron, while central analgesia involves an afferent input thus generating a sensation of pain. Under normal circumstances, pain is associated with electrical activity in small diameter primary afferent fibers of peripheral nerves. These fibers can be non-myelinated C-fibers or fine myelinated Aδ fibers. Pain and inflammation occurs primarily through the production of prostaglandins, among which are prostacyclines (PGI_2) and prostaglandin E, which in turn are responsible for excitation of the Aδ-nerve fibers, leading to sensation of pain, which may be sharp and localized or else cause a dull, burning pain (Reynolds, 1982; Rang *et al.*, 2003). The crude methanolic extract of *A. philoxeroides* (Mart.) Griseb. whole plant reduced the number of abdominal constrictions in mice, suggesting that components in the extract may be acting through inhibition of prostaglandin synthesis, which may further involve inhibition of cyclooxygenases and/or lipo-oxygenases activities or expression.

Analgesics, which are centrally acting, act through opioid μ and κ receptors, which activity can be more suitably detected with hot plate and tail flick tests (Abbott and Young, 1988; Furst *et al.*, 1988). These tests were not conducted in the present study for two reasons; first, this is a preliminary screening of antinociceptive activity in the extract, and second, as per previous report, the acetic acid-induced gastric pain model includes both central and peripheral analgesic effects (Shanmugasundaram and Venkataraman, 2005). The major conclusion of the present study was that the extract demonstrated dose-dependent and significant antinociceptive activity, which was comparable to aspirin, and as such can be considered for further scientific researches leading to isolation of efficacious pain-killing chemical components from the extract. Pain can be caused through many causes and is suffered by countless people on a daily basis throughout the world. The usual pain killing drugs like aspirin or paracetamol causes side-effects like gastric ulceration and internal bleeding or hepatotoxicity from long-term usage, which can happen in e.g. patients suffering from rheumatic pain. From this point, plant species like *A. philoxeroides* (Mart.) Griseb. can prove to be a valuable

Table 1: Antinociceptive effect of methanol extract of *Alternanthera philoxeroides* whole plants in the acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control (Group 1)	10 ml	7.25 ± 0.84	-
Aspirin (Group 2)	200 mg	4.50 ± 0.53	37.9*
Aspirin (Group 3)	400 mg	2.38 ± 0.73	67.2*
Alternanthera philoxeroides (Group 4)	50 mg	5.00 ± 0.63	31.0*
Alternanthera philoxeroides (Group 5)	100 mg	4.88 ± 0.97	32.7*
Alternanthera philoxeroides (Group 6)	200 mg	4.50 ± 0.42	37.9*
Alternanthera philoxeroides (Group 7)	400 mg	4.00 ± 0.89	44.8*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=8); *$P < 0.05$; significant compared to control.

Table 2: Effect of methanol extract of *Alternanthera philoxeroides* whole plants on serum glucose level in hyperglycemic mice.

Treatment	Dose (mg/kg body weight)	Serum Glucose level (mg/dl)	% of inhibition
Control (Group 1)	10 ml	65.42 ± 1.87	-
Glibenclamide (Group 2)	10 mg	37.50 ± 6.80	42.7*
A. philoxeroides (Group 3)	50 mg	41.67 ± 5.97	36.3*
A. philoxeroides (Group 4)	100 mg	27.08 ± 4.25	58.6*
A. philoxeroides (Group 5)	200 mg	22.92 ± 2.77	65.0*
A. philoxeroides (Group 6)	400 mg	22.50 ± 4.13	65.6*

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

source for efficacious pain killers with less serious or no side-effects.

The methanolic extract further exhibited potent antihyperglycemic properties by lowering serum glucose concentrations in glucose-loaded mice. Since the experiment was conducted with normal and not diabetic mice, the results preclude any antihyperglycemic effect occurring through a mechanism like regeneration of β-cells of the pancreas. The extract, therefore, may be acting through mechanisms like inhibiting glucose absorption from gut (Bnouham *et al*., 2003; Bhowmik *et al*., 2009) or potentiating the pancreatic secretion of insulin or increasing the glucose uptake from blood (Nyunai *et al*., 2009; Farjou *et al*., 1987). The exact mechanism underlying the antihyperglycemic activity of the extract is currently under investigation in our laboratory. Since diabetes is a debilitating disease affecting millions of people worldwide and for which allopathic medicine has no known cure, the extract merits further consideration for scientific research, which may lead to discovery of novel antidiabetic drugs.

The present study also validates the folk medicinal use of the plant in Bangladesh for pain and diabetes. Many modern drugs have been discovered through observing the use of plant species by indigenous people or their use in traditional medicinal systems of the world. It is expected that the validation of the folk medicinal use of *A. philoxeroides* (Mart.) Griseb. can also serve a similar purpose.

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