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Research Article

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Preliminary phytochemical screening, oral glucose tolerance, analgesic and acute toxicity studies with *Dendrocalamus giganteus* aerial parts

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ABSTRACT

In oral glucose tolerance tests with methanolic extract of Dendrocalamus giganteus aerial parts (MEDG), the extract dose-dependently reduced blood glucose concentrations in glucose-loaded mice. At extract doses of 50, 100, 200 and 400 mg/kg, the reductions in blood glucose levels were, respectively, 9.9, 31.7, 43.5, and 53.4%. In comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, reduced blood glucose level by 50.8%. In analgesic activity tests with acetic acid induced pain model mice, the extract at the afore-mentioned four doses, dose-dependently reduced acetic acid induced abdominal constrictions in mice by 11.1, 22.2, 44.4, and 51.9% versus the 40.7 and 51.9% reductions obtained with a standard analgesic drug, aspirin, administered respectively, at doses of 200 and 400 mg per kg. The extract when administered to mice did not cause any acute toxicity when administered at doses up to 3000 mg per kg. Preliminary phytochemical screening of the extract showed the presence of alkaloids, flavonoids, saponins and tannins, which compounds may be responsible for the observed antihyperglycemic and analgesic effects.

Key words: *Dendrocalamus giganteus*, Poaceae, OGTT, analgesic, antihyperglycemic

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INTRODUCTION

Dendrocalamus giganteus Wall. ex Munro (Poaceae), also known as Giant Bamboo in English, is a tropical genus of bamboo and can be found in southeast Asian countries like Bangladesh, India, Myanmar and Thailand. In Bangladesh, the plant is known as 'Bombai'. The plant can reach heights exceeding 40 meters.

Not much has been reported about the ethnobotanical uses, phytochemical constituents or pharmacological properties of the plant. The Mizos of Mizoram, India eat the tender shoots of the plant along with fish or meat [1]. Twelve coumarin compounds, namely, skimin, scopolin, scopoletin, umbelliferone, 6,7-dimethoxycoumarin, coumarin, psoralen, xanthotoxin, 5,7-dimethoxycoumarin, pimpinellin, imperatorin, and osthole have been reported from leaves [2].

Among the diseases that are commonly present in the Bangladesh population are diabetes and pain. Diabetes, characterized by high blood glucose levels, is rapidly reaching endemic proportions in Bangladesh. About 9.7% had diabetes and 22.4% had pre-diabetes as has been found in a survey conducted among the Bangladesh adult population aged 35 years or more [3]. This is also accompanied by a rise in juvenile diabetes within the country [4]. In fact, diabetes is on the rise world-wide, and the World Health Organization (WHO) estimates that more than 220 million people worldwide had diabetes in 2004, and diabetes deaths will double between 2005 and 2030.

Pain is a common affliction suffered by people on occasions. Pain can be acute, arising from injury or sprains, or can be chronic, arising from other diseases like cancer or arthritis. Pain can arise in almost any part of the body, and people who do heavy labor suffer (like the poorer segments of the Bangladesh population) more acutely from pain, necessitating in the daily use of over-the-counter (OTC) drugs like aspirin or paracetamol, resulting in gastric ulceration or hepatotoxicity from prolonged use or over-dosage [5, 6].

The majority of the people of Bangladesh are poor and more than a third of the total population has poverty level incomes, which has been defined as less than US$ 1 per day. Moreover, the rural and the urban slum dwellers cannot afford or lack access to modern doctors and clinics. As such, the diabetic patients not only lack access to modern blood sugar lowering medications, but also a substantial number relies on traditional medications, which may or may not be effective in treatment of diabetes or impaired glucose metabolism. On the other hand, Bangladesh has over 5,000 floral species, which can serve as a base for blood glucose lowering or pain relieving drugs, even in the form of crude extract. We had been systematically screening the plants of Bangladesh for their antihyperglycemic and analgesic potentials [7-14], for these plant resources can form a cheap and effective basis for blood sugar lowering and pain relieving drugs, which would be more affordable and accessible to the general population and can be safely taken following proper scientific validation. Towards that objective, the aim of the present study was to evaluate the antihyperglycemic (through oral glucose tolerance tests or OGTT) and analgesic (through acetic acid-induced pain model test) potential of the aerial parts of *D. giganteus*, which plants are readily available in the rural parts of the country and affordable by all segments of the population.

EXPERIMENTAL SECTION

Plant material collection

Aerial parts of *D. giganteus* were collected during June 2014 from the National Botanical Garden, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 39,563).

Preparation of methanolic extract of aerial parts

Aerial parts were cut into small pieces, air-dried in the shade, and 100g of dried and powdered fruits were extracted with methanol (w:v ratio of 1:6, final weight of the extract 2.17g).

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 [15] 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 aerial part extract (MEDG) 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 [16]. 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 MEDG administered mice (Groups 2-6), and control mice (Group 1), respectively.

Analgesic activity evaluation through abdominal writhing test

Analgesic activity of MEDG was examined as previously described [17]. 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 MEDG 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 MEDG, 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 [18], 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 = $(1 – W_e/W_c)$ X 100,

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

Acute toxicity test

cute toxicity test was conducted as previously described [19]. 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 MEDG per kg body weight. All animals were closely observed for the next 8 hours to notice any behavioural 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 [14].

Preliminary phytochemical screening

Preliminary phytochemical analysis of MEDG for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before [20].

RESULTS AND DISCUSSION

Toxicity evaluation

The crude extract (MEDG) 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 MEDG indicated the presence of alkaloids, flavonoids, saponins, and tannins.

Antihyperglycemic activity evaluation through OGTT

Dose-dependent reductions in blood glucose levels were observed in glucose-loaded mice following MEDG administration. At doses of 50, 100, 200, and 400 mg per kg, MEDG, respectively, lowered blood glucose levels by 9.9, 31.7, 43.5, and 53.4%. The results were not statistically significant at the MEDG dose of 50 mg per kg, but significant ($P < 0.05$) at the higher doses administered. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, lowered blood glucose level by 50.8%. Thus at the highest dose of 400 mg

per kg, MEDG had higher blood glucose lowering effect than glibenclamide. The results are shown in Table 1 and suggest that MEDG can be used as a crude drug for lowering glucose.

Table 1: Effect of crude methanol extract of *D. giganteus* aerial parts (MEDG) 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	5.24 ± 0.22	-
Glibenclamide	10 mg	2.58 ± 0.21	50.8*
(MEDG)	50 mg	4.72 ± 0.37	9.9
(MEDG)	100 mg	3.58 ± 0.39	31.7*
(MEDG)	200 mg	2.96 ± 0.29	43.5*
(MEDG)	400 mg	2.44 ± 0.20	53.4*

*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 reductions ($P < 0.05$) in the number of abdominal constrictions (writhings) induced by intraperitoneal administration of acetic acid were observed with MEDG. At doses of 50, 100, 200 and 400 mg per kg body weight, MEDG was observed to reduce the number of writhings, respectively, by 11.1, 22.2, 44.4, and 51.9%. 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 40.7 and 51.9%, respectively. Thus, a dose of 200 mg/kg MEDG was better than that of 200 mg/kg aspirin, and a dose of 400 mg/kg MEDG was equivalent to that of 400 mg/kg aspirin. The results are shown in Table 2 and suggest that the extract possesses significant analgesic properties.

Table 2: Analgesic effect of crude methanol extract of *D. giganteus* aerial parts (MEDG) 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	3.2 ± 0.58	40.7*
Aspirin	400 mg	2.6 ± 0.40	51.9*
(MEDG)	50 mg	4.8 ± 0.20	11.1*
(MEDG)	100 mg	4.2 ± 0.37	22.2*
(MEDG)	200 mg	3.0 ± 0.45	44.4*
(MEDG)	400 mg	2.6 ± 0.51	51.9*

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

Preliminary phytochemical analysis of MEDG showed the presence of alkaloids, flavonoids, saponins, and tannins, which compounds could be responsible for the observed antihyperglycemic and analgesic effects. The hypoglycemic effect of stem bark extract of *Tamarindus indica* in alloxan-diabetic rats has been attributed to presence of alkaloids, flavonoids, and tannins among other groups of compounds [21]. Aqueous extract of seeds of *Persea americana* showed hypoglycemic activity in alloxan-diabetic rats; phytochemical screening of the extract indicated the presence of alkaloids, flavonoids, and tannins [22]. Ethanolic extract of whole plant of *Tridax procumbens* demonstrating hypoglycemic activity in STZ-diabetic rats revealed the presence of alkaloids, flavonoids, and tannins [23]. Methanolic root bark extract of *Afzelia africana* showed hypoglycemic activity in alloxan diabetic mice. Investigation on the phytochemical constituents of the extract revealed the presence of flavonoids, tannins, alkaloids, steroids and saponins [24].

Methanol extract of the stem bark of *Prosopis africana* containing flavonoids, saponins, carbohydrates, cardiac glycosides, tannins, and alkaloids reportedly exhibited analgesic and anti-inflammatory activities in mice and rats [25]. Aqueous extract of *Felicia muricata* leaves has been shown to possess anti-inflammatory, antinociceptive and antipyretic activities; phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolics [26]. Analgesic activity has been seen with aqueous leaf extract of *Lagenaria breviflora*; phytochemical analysis revealed the presence of alkaloids, flavonoids, and tannins in the extract [27].

To our knowledge, this is the first study on antihyperglycemic and analgesic effects of methanol extract of aerial parts of *D. giganteus*, which is a Poaceae family plant. However, other reports exist on other Poaceae family plant parts exhibiting antidiabetic activity. The antidiabetic effect of germinated wheat and barley has been shown [28];

notably, both are Poaceae family plants. Tricin has been reported from various species of bamboo leaves [29, 30]. Increased synthesis of prostaglandins (particularly PGE2) and other cyclooxygenase and lipooxygenase enzyme products are responsible behind the sensation of pain. Tricin has been reported to inhibit cyclooxygenase enzymes [31]. Thus tricin can be a compound present in MEDG mediating the analgesic effect of the extract. However, the exact identification of phytoconstituent(s) present in MEDG and exhibiting the antihyperglycemic and analgesic effects needs to be done and such work is currently undergoing in our laboratory.

CONCLUSION

The experimental results suggest that the methanolic extract of aerial parts of *D. giganteus* possess antihyperglycemic and analgesic potential and may be used for lowering blood sugar and alleviating pain.

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