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Research Article

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Preliminary phytochemical analysis, antihyperglycemic, antinociceptive activity and toxicity studies on leaves of *Casearia elliptica* Wild.

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ABSTRACT

In oral glucose tolerance tests with methanolic extract of Casearia elliptica leaves (MECE), 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, 6.9, 21.9, 35.8, and 50.0%. In comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, reduced blood glucose level by 47.4%. 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 18.5, 40.7, 48.1, and 51.9% versus the 48.1 and 63.0% 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. Preliminar phytochemical analysis showed the presence of alkaloids, flavonoids and tannins in the extract, which can account for the observed antihyperglycemic and antinociceptive activities.

Key words: *Casearia elliptica*, Flacourtiaceae, OGTT, analgesic, antihyperglycemic

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INTRODUCTION

Casearia elliptica Willd. (Flacourtiaceae) is a tree found in various parts of Bangladesh and India. In Bangladesh, it is known as ‘bon kofi’, while in India, in Hindi it is known as ‘chilla’, in Marathi it is known as ‘modgi’, and in Sanskrit it is known as ‘chilhaka’. The tree is known as ‘katiccai’ in Tamil and ‘chilaka-dududi’ in Telegu. Not much has been reported on the ethnomedicinal uses or pharmacological properties and phytochemical constituents of the plant.

Leaf paste is used to check bleeding of wounds in the Garhwal region of Uttarakhand, India [1]. In The Himalayan regions of India, the root bark is considered an effective remedy for stimulating liver function [2]. Leaves, root and bark are used in traditional medicines against malaria in Tamil Nadu, India [3]. A polyherbal extract containing the plant as one of the components has been found to improve blood sugar levels in alloxan diabetic rats [4].

Diabetes is rapidly becoming endemic in Bangladesh, possibly because of changes in food patterns and lifestyle. Although allopathic drugs as well as insulin injections are available in Bangladesh to reduce elevated levels of blood sugar during diabetes, such drugs are costly and mostly are either in accessible or cannot be afforded by the poorer

segments of the people in Bangladesh. Pain is also a common affliction, particularly among the rural people of the country, because of continuous hard labor either in the agricultural fields or at home. Thus more affordable and readily available drugs need to be found to combat these two afflictions.

We had been systematically screening the plants of Bangladesh for their glucose lowering and antinociceptive potentials [5-12], 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 appropriate scientific validation. Towards that objective, the aim of the present study was to evaluate the antihyperglycemic (through oral glucose tolerance tests or OGTT) and antinociceptive (through acetic acid-induced pain model test) potential of the leaves of *C. elliptica*. At the same time, since not much is known about this plant, preliminary phytochemical screening and toxicity studies were carried out with the methanolic extract of leaves.

EXPERIMENTAL SECTION

Plant material collection

Leaves of *C. elliptica* were collected during November 2013 from Lawachara Forest Reserve in Sylhet Division, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,711).

Preparation of methanolic extract of leaves

Leaves were cut into small pieces, air-dried in the shade, and 150g of dried and powdered leaves were extracted with methanol (w:v ratio of 1:5, final weight of the extract 12.4g).

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 14-18g 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 [13] 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 fruit extract (MECE) 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 [14]. 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 MECE administered mice (Groups 2-6), and control mice (Group 1), respectively.

Antinociceptive activity evaluation through abdominal writhing test

Antinociceptive activity of MECE was examined as previously described [15]. 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 antinociceptive drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MECE 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 MECE, 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 [16],

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 MECE administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute toxicity test

Acute toxicity test was conducted as previously described [17]. 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 MECE 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 [12].

Preliminary phytochemical screening

Preliminary phytochemical analysis of MECE for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before [18].

RESULTS AND DISCUSSION

Toxicity evaluation

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

Antihyperglycemic activity evaluation through OGTT

Dose-dependent reductions in blood glucose levels were observed in glucose-loaded mice following MECE administration. At doses of 50, 100, 200, and 400 mg per kg, MECE, respectively, lowered blood glucose levels by 6.9, 21.9, 35.8, and 50.0%. The results were not statistically significant at the MECE 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 47.4%. Thus at the highest dose of 400 mg per kg, MECE had higher blood glucose lowering effect than glibenclamide. The results are shown in Table 1 and suggest that MECE can be used as a crude drug for lowering glucose.

Table 1: Effect of crude methanol extract of *C. elliptica* leaves (MECE) 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*
(MECE)	50 mg	5.10 ± 0.12	6.9
(MECE)	100 mg	4.28 ± 0.19	21.9*
(MECE)	200 mg	3.52 ± 0.20	35.8*
(MECE)	400 mg	2.74 ± 0.20	50.0*

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

Alkaloids, flavonoids and tannins present in MECE could be responsible for the observed antihyperglycemic 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 [19]. 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 [20]. Ethanolic extract of whole plant of *Tridax procumbens* demonstrating hypoglycemic activity in STZ-diabetic rats revealed the presence of alkaloids, flavonoids, and tannins [21].

Antinociceptive 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 MECE. At doses of 50, 100, 200 and 400 mg per kg body weight, MECE was observed to reduce the number of writhings, respectively, by 18.5, 40.7, 48.1, 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 48.1 and 63.0%, respectively. Thus, a dose of 200 mg/kg MECE was equivalent to that of 200 mg/kg aspirin, and a dose of 400 mg/kg MECE was better than that of 200 mg/kg aspirin regarding antinociceptive potential. The results are shown in Table 2 and suggest that the extract possesses significant antinociceptive properties.

Table 2: Antinociceptive effect of crude methanol extract of *C. elliptica* leaves (MECE) 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*
(MECE)	50 mg	4.4 ± 0.75	18.5
(MECE)	100 mg	3.2 ± 0.73	40.7*
(MECE)	200 mg	2.8 ± 0.37	48.1*
(MECE)	400 mg	2.6 ± 0.40	51.9*

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

Alkaloids, flavonoids and tannins present in MECE can account also for the observed antinociceptive effect. 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 [22]. 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 [23].

The exact mechanism(s) behind the observed antihyperglycemic and antinociceptive effects were not elucidated in this preliminary study; such studies are now being undertaken in the laboratory. To our knowledge, this is the first report on the analgesic and antinociceptive effects of methanolic extract of leaves of *C. elliptica*. However, it is to be noted that stimulation of insulin secretion or inhibition of glucose absorption by the extract can account for the observed antihyperglycemic effects. Such effects have been noted with aqueous extract of *Abutilon indicum* plant and the effects have been attributed to presence of alkaloids, flavonoids, glycosides, saponins and tannins in the extract [24]. The observed antinociceptive effect can be due to inhibition of prostaglandin biosynthesis through inhibition of cyclooxygenase(s) activity, which also can be mediated through presence of alkaloids in MECE. Alkaloids and saponins were present in leaf and stem extracts of *Leucosidea sericea*, which showed inhibition of cyclooxygenases 1 and 2 activities [25].

CONCLUSION

The experimental results suggest that the methanolic extract of leaves of *C. elliptica* possess antihyperglycemic and antinociceptive potential and may be used for lowering blood sugar and alleviating pain.

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