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Preliminary phytochemical screening, antihyperglycemic, analgesic and toxicity studies on methanolic extract of aerial parts of *Corchorus olitorius* L.

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ABSTRACT

Corchorus olitorius, also known as Nalta jute or Tossa jute is grown throughout Bangladesh for its fibre and because the aerial parts are edible. It was of interest to phytochemically screen and conduct antihyperglycemic, analgesic and toxicity studies of the aerial parts. Antihyperglycemic and analgesic activities, phytochemical screening and toxicity studies were done through standard methods. Administration of methanol extract of aerial parts (MECO) led to dose-dependent reductions in blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg, the extract reduced blood glucose levels by 18.6, 29.3, 32.9, and 50.7%, respectively compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, reduced blood glucose level by 48.9%. In analgesic activity tests, MECO at doses of 50, 100, 200 and 400 mg per kg reduced the number of writhings by 19.2, 42.3, 53.8, and 57.7%, respectively. A standard analgesic drug, aspirin, reduced the number of writhings by 38.5 and 65.4%, respectively, when administered at doses of 200 and 400 mg per kg. Phytochemical screening of MECO showed presence of alkaloids, flavonoids, saponins and tannins, which may be responsible for the observed effects.

INTRODUCTION

Corchorus olitorius L. (Tiliaceae), known in English as Nalta jute or Tossa jute, is widely cultivated in Bangladesh for its fibre and for its edible aerial parts. In Bangladesh the plant is known as 'mishti paat'. The plant is also considered to have ethnnomedicinal values. Cardenolide glycosides have been reported from seeds of the plant (Nakamura *et al.*, 1998). Antiobesity effect of polyphenolic compounds from leaves of the plant has been reported in LDL receptor deficient mice (Wang *et al.*, 2011). Anti-inflammatory effects of phenolic crude extracts from various parts of the plant have been demonstrated (Yan *et al.*, 2013).

Gastroprotective effects of leaf extract have been observed against ethanol-induced gastric mucosal hemorrhagic lesions in rats (Al Batran *et al.*, 2013).

Diabetes and pain are endemic problems among the population of Bangladesh. Effective allopathic medicines are not available or affordable to the predominantly rural population. Towards finding alternate sources of easily available herbal medicines, we have been conducting extensive investigations of the medicinal plants of Bangladesh as to their antihyperglycemic and analgesic potential (Morshed *et al.*, 2010; Rahmatullah *et al.*, 2010; Ahmed *et al.*, 2011; Shahreen *et al.*, 2012; Haque *et al.*, 2013; Rahmatullah *et al.*, 2013a,b; Ghosh *et al.*, 2014; Hossain *et al.*, 2014; Jahan *et al.*, 2014; Rahman *et al.*, 2014; Tazin *et al.*, 2014).

C. olitorius is one of the common plants of Bangladesh and widely cultivated. The objective of the present study was to evaluate the antihyperglycemic and analgesic potential of methanol extract of aerial parts of the plant along with preliminary phytochemical screening and toxicity studies on the extract.

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MATERIALS AND METHODS

Plant material collection

Aerial parts of *C. olitorius* were collected during September 2013 from Rayer Bazar in Dhaka, Bangladesh and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,715).

Preparation of methanolic extract of aerial parts

Aerial parts were cut into small pieces, air-dried in the shade, and 150g of dried and powdered aerial parts were extracted with methanol (w:v ratio of 1:5, final weight of the extract 5.16g).

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 (OGTT) were carried out as per the procedure previously described by Joy and Kuttan (1999) 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 (MECO) 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 (Venkatesh *et al.,* 2004). 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 MECO administered mice (Groups 2-6), and control mice (Group 1), respectively.

Analgesic activity evaluation through abdominal writhing test

Analgesic activity of MECO was examined as previously described (Shanmugasundaram and Venkataraman, 2005). 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 MECO 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 MECO, 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 (Akter *et al.,* 2014), 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 MECO administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute toxicity test

Acute toxicity test was conducted as previously described (Ganapaty *et al.,* 2002). 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 MECO 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 (Hossain *et al.,* 2014).

Preliminary phytochemical screening

Preliminary phytochemical analysis of MECO for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before (Kumar *et al.,* 2013).

RESULTS AND DISCUSSION

Toxicity evaluation

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

Antihyperglycemic activity evaluation results

In oral glucose tolerance tests, MECO when administered at doses of 50, 100, 200 and 400 mg per kg body weight, dose-dependently and significantly reduced the amount of blood glucose in experimental animals. At these four doses, MECO, respectively, decreased blood glucose levels by 18.6, 29.3, 32.9, and 50.7%. A standard antihyperglycemic drug, glibenclamide when administered at a dose of 10 mg per kg body weight, reduced blood glucose levels by 48.9%. Thus MECO at the highest concentration tested showed better antihyperglycemic activity than glibenclamide. The results are shown in Table 1 and suggest that MECO can be used to lower blood glucose levels in hyperglycemic subjects.

Table 1: Effect of crude methanol extract of *C. olitorius* aerial parts (MECO) 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	5.60 ± 0.27	-
Glibenclamide	10 mg	2.86 ± 0.26	48.9*
(MECO)	50 mg	4.56 ± 0.12	18.6*
(MECO)	100 mg	3.96 ± 0.33	29.3*
(MECO)	200 mg	3.76 ± 0.20	32.9*
(MECO)	400 mg	2.76 ± 0.28	50.7*

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

The bioactive component(s) responsible for the antihyperglycemic activity or the mechanism through which MECO lowered blood glucose levels were not ascertained in this preliminary study and further work is being undertaken by us to determine them. However, the extract showed the presence of alkaloids, flavonoids, saponins and tannins. These groups of compounds have variously been reported by other authors to possess antihyperglycemic effects. For instance, the analgesic activity of *Aconitum baikalnensis* has been attributed to diterpene alkaloids (Nesterova *et al.*, 2014).

Stem bark extract of *Tamarindus indica* reportedly demonstrated antihyperglycemic activity in alloxan diabetic rats. Phytochemical screening of the extract showed the presence of glycosides, saponins, flavonoids, cardiac glycosides, tannins, alkaloids and triterpenes (Yerima *et al.*, 2014). The preliminary phytochemical analysis of an ethanolic extract of the whole plant of *Tridax procumbens* indicated the presence of alkaloids, tannins, flavonoids, saponins, and phenolic compounds. The ethanolic extract of the whole plant at 250 and 500 mg/kg demonstrated significant antidiabetic and antihyperlipidemic activities (Petchi *et al.*, 2013).

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 MECO. At doses of 50, 100, 200 and 400 mg per kg body weight, MECO was observed to reduce the number of constrictions (writhings), respectively, by 19.2, 42.3, 53.8, and 57.7%. 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 38.5 and 65.4%, respectively.

Thus, a dose of 100 mg/kg MECO showed better analgesic activity than that of 200 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 *C. olitorium* aerial parts (MECO) in acetic acid-induced pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	5.2 ± 0.20	-
Aspirin	200 mg	3.2 ± 0.58	38.5*
Aspirin	400 mg	1.8 ± 0.58	65.4*
(MECO)	50 mg	4.2 ± 0.49	19.2*
(MECO)	100 mg	3.0 ± 0.55	42.3*
(MECO)	200 mg	2.4 ± 0.24	53.8*
(MECO)	400 mg	2.2 ± 0.37	57.7*

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

It is interesting to note that alkaloids, flavonoids, saponins and tannins (which are present in MECO) have also been implicated in showing analgesic effects. Pharmacognostic and phytochemical investigation of leaves of *Malvastrum coromandelianum* indicated presence of alkaloids, tannins and flavonoids along with analgesic and anti-inflammatory activities (Sanghai *et al.*, 2013). Phytochemical analysis of the ethanolic extract of *Sida cordifolia* roots exhibiting analgesic activity indicated the presence of reducing sugar, alkaloids, steroids and saponins, which components were deemed responsible for the observed analgesic effects (Momin *et al.*, 2014).

Aqueous extract of *Vernonia condensata* leaves has been reported to exhibit antinociceptive activity in writhing tests; the extract was found to contain alkaloids, flavonoids, and saponins (Risso *et al.*, 2010). Thus these four groups of compounds, namely alkaloids, flavonoids, saponins and tannins can be responsible for the observed antihyperglycemic and analgesic effects of methanolic extract of aerial parts of *C. olitorius*. Since the plant is easily available, it opens up possibilities of obtaining new blood sugar lowering and pain alleviating drugs from the plant. The crude extract may itself serve the same purpose since it did not show any toxic effects.

CONCLUSION

The results suggest that methanolic extract of *C. olitorius* aerial parts can be used for lowering of blood glucose and for alleviating pain.

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