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Research Article

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Oral glucose tolerance, antinociceptive and acute toxicity studies with *Trichosanthes dioica* fruits

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ABSTRACT

In oral glucose tolerance tests with methanolic extract of Trichosanthes dioica fruits (METD), 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, 2.9, 21.5, 39.8, and 46.4%. 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, 33.3, 37.0, and 40.7% 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.

Key words: *Trichosanthes dioica*, Cucurbitaceae, OGTT, analgesic, antihyperglycemic

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INTRODUCTION

Trichosanthes dioica Roxb. belongs to the Cucurbitaceae family and is known as pointed gourd in English, parwal in Hindi and patal in Bengali. The plant is cultivated mainly for its edible fruits, which are cooked along with the seeds inside and consumed as vegetable in Bangladesh. Juice of leaves of *T. dioica* is used as tonic, febrifuge, in edema, alopecia, and in sub-acute cases of enlargement of liver [1].

In rats with streptozotocin induced severe diabetes mellitus, aqueous extract of *T. dioica* fruits at a dose of 1000mg/kg body weight daily once for 28 days has been shown to reduce the levels of fasting blood glucose, postprandial glucose, aspartate amino transferase, alanine amino transferase, alkaline phosphatase, creatinine, urine sugar and urine protein where as total protein and body weight was increased [2]. In glucose loaded rats, normal rats and hyperglycemic rats the aqueous extract at doses (800 mg/kg/p.o and 1600 mg/kg/p.o) reduced blood glucose significantly when compared to control [3]. Antinociceptive and anti-inflammatory effects of root extract of the plant at doses of 50 and 100 mg per kg body weight have been reported in rodent models [4].

Diabetes and pain are common afflictions in Bangladesh and indeed throughout the world. The rural people of Bangladesh are generally poor, and moreover lack access to qualified physicians and modern medicines. As a result, they rely on traditional medicinal practitioners, who in turn administer various plants or plant parts for alleviation of

many diseases. Scientific validation of such uses is important not only from the aspect of treatment but also such validations can lead to scientific research on the plants used, which in turn may lead to discovery of novel and more efficacious drugs. It may be noted in this regard that plants have always formed a natural resource for discovery of many important allopathic medicines.

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 fruits (containing seeds) of *T. dioica*, which plant is readily available in the rural parts of the country and affordable by all segments of the population because it is cultivated on a wide basis.

EXPERIMENTAL SECTION

Plant material collection

Fruits of *T. dioica* were collected during May 2013 from a local market in Dhaka city, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,575).

Preparation of methanolic extract of fruits

Fruits 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 1:5, final weight of the extract 6.32g).

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 (METD) 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 METD administered mice (Groups 2-6), and control mice (Group 1), respectively.

Antinociceptive activity evaluation through abdominal writhing test

Antinociceptive activity of METD 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 analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered METD 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 METD, 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 METD 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 METD 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].

RESULTS AND DISCUSSION

Toxicity evaluation

The crude extract (METD) 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.

Antihyperglycemic activity evaluation through OGTT

At doses of 50, 100, 200, and 400 mg per kg, METD, respectively, lowered blood glucose levels dose-dependently by 2.9, 21.5, 39.8, and 46.4%. The results were significant ($P < 0.05$) at the higher three doses administered. A standard antihyperglycemic drug, glibenclamide, lowered blood glucose level by 47.4%. The results are shown in Table 1 and suggest that METD can be used as a crude drug for lowering glucose.

Table 1: Effect of crude methanol extract of *T. dioica* fruits (METD) 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*
(METD)	50 mg	5.32 ± 0.27	2.9
(METD)	100 mg	4.30 ± 0.22	21.5*
(METD)	200 mg	3.30 ± 0.46	39.8*
(METD)	400 mg	2.94 ± 0.25	46.4*

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

Table 2: Antinociceptive effect of crude methanol extract of *T. dioica* fruits (METD) 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*
(METD)	50 mg	4.4 ± 0.24	18.5*
(METD)	100 mg	3.6 ± 0.40	33.3*
(METD)	200 mg	3.4 ± 0.24	37.0*
(METD)	400 mg	3.2 ± 0.20	40.7*

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

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 METD. At doses of 50, 100, 200 and 400 mg per kg body weight, METD was observed to reduce the number of writhings, respectively, by 18.5, 33.3, 37.0, and 40.7%. 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 48.1 and 63.0%, respectively. The results are shown in Table 2 and suggest that the extract possesses significant antinociceptive properties.

Various cucurbitacin compounds (cucurbitacins B, D, E) have been reported to be present in different parts of the plant. Cucurbitacin glycosides are reported to have glucose lowering effect [18]. A cucurbitacin isolated from *Lagenaria breviflora* fruit has been reported to exhibit analgesic and anti-inflammatory activities [19]. Thus *T. dioica* may possess cucurbitacin compounds, which can account for the observed antihyperglycemic and antinociceptive effects. As such, it is essential to isolate and identify these compounds along with other pharmacologically active components, if any, towards possible development of new drugs.

CONCLUSION

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

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