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Research Article

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Antihyperglycemic, antinociceptive activity, phytochemical analysis and toxicity studies on stems of *Nymphaea nouchali* Burm. f.

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ABSTRACT

In oral glucose tolerance tests with methanolic extract of Nymphaea nouchali stems (MENN), the extract significantly and dose-dependently reduced blood glucose concentrations in glucose-loaded mice. At extract doses of 100, 200 and 400 mg/kg, the reductions in blood glucose levels were, respectively, 20.7, 36.9, 43.0, and 40.1%. In comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, reduced blood glucose level by 45.3%. In antinociceptive activity tests with acetic acid induced pain model mice, the extract at doses of 50, 100, 200 and 400 mg/kg, significantly and dose-dependently reduced acetic acid induced abdominal constrictions in mice by 29.6, 37.0, 44.4, and 51.9% versus the 33.3 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 analysis showed the presence of alkaloids, flavonoids, saponins and tannins in the extract, which can account for the observed antihyperglycemic and antinociceptive activities.

Key words: *Nymphaea nouchali*, Nymphaeaceae, OGTT, antinociceptive, antihyperglycemic

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INTRODUCTION

Nymphaea nouchali Burm. f. (Nymphaeaceae) is a well known aquatic plant widely used in the Ayurveda and Siddha systems of medicines for the treatment of diabetes, inflammation, liver disorders, urinary disorders, menorrhagia, blenorrhagia, menstruation problem, as an aphrodisiac, and as a bitter tonic [1]. The plant bears flowers of different colors depending on which the plant is known as the blue, red or white water lily. In Bengali, the plant again depending on blue, red or white flowers is known as neel shapla, laal shapla or shada shapla. The plant has long slender stems, which is cooked and eaten in Bangladesh as a vegetable.

We had been systematically screening the plants of Bangladesh for their glucose lowering and antinociceptive values [2-14]; both diabetes and pain arising from various causes being common problems suffered by millions of people of the country. As part of this screening process, this study was designed to conduct a preliminary phytochemical analysis of methanolic extract of *N. nouchali* stems, and conduct antihyperglycemic, antinociceptive and toxicity studies on the extract. The overall objective of these screening studies is to identify plants, which can form an affordable and readily available source for alleviation of high blood sugar levels in diabetic patients, and

plants, which can alleviate pain and as such lower dependability on costlier allopathic drugs, which may not be always accessible to the predominantly rural people.

EXPERIMENTAL SECTION

Plant material collection

Stems of *N. nouchali* were collected during August 2014 from a vegetable market in Dhaka city, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 41,587).

Preparation of methanolic extract of stems

Stems were cut into small pieces, air-dried in the shade, and 100g of dried and powdered stems were extracted with methanol (w:v ratio of 1:5, final weight of the extract 6.416g).

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 (OGTT) 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 stem extract (MENN) 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 MENN administered mice (Groups 2-6), and control mice (Group 1), respectively.

Antinociceptive activity evaluation through abdominal writhing test

Antinociceptive activity of MENN 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 antinociceptive drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MENN 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 MENN, 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 [14], 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 MENN administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute toxicity test

Acute toxicity test was conducted as previously described [18]. 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 MENN 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 [9].

Preliminary phytochemical screening

Preliminary phytochemical analysis of MENN for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before [19].

RESULTS AND DISCUSSION

Toxicity evaluation

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

Antihyperglycemic activity evaluation through OGTT

Dose-dependent and statistically significant reductions in blood glucose levels were observed in glucose-loaded mice following MENN administration. At doses of 50, 100, 200, and 400 mg per kg, MENN, respectively, lowered blood glucose levels by 10.7, 20.7, 36.9, and 40.1%. The percent lowering of blood glucose was not statistically significant at a dose of 50 mg/kg but statistically significant at the three higher doses. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, lowered blood glucose level by 45.3%. Thus at the highest dose of 400 mg per kg, MENN had blood glucose lowering effect close to glibenclamide. The results are shown in Table 1 and suggest that MENN can be used as a crude therapeutic substitute for lowering glucose.

Table 1: Effect of crude methanol extract of *N. nouchali* stems (MENN) 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	6.18 ± 0.39	-
Glibenclamide	10 mg	3.38 ± 0.26	45.3*
(MENN)	50 mg	5.52 ± 0.29	10.7
(MENN)	100 mg	4.90 ± 0.26	20.7*
(MENN)	200 mg	3.90 ± 0.31	36.9*
(MENN)	400 mg	3.70 ± 0.26	40.1*

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

Although the exact identification of the bioactive component(s) responsible for the observed antihyperglycemic effect was not done in the present study, preliminary phytochemical analysis of MENN showed the presence of alkaloids, flavonoids, saponins, and tannins, which compounds can be responsible for the observed antihyperglycemic effects. Aqueous extract of seeds of *Persea americana* reportedly 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]. The hypoglycemic effect exhibited by 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 [22].

Antinociceptive activity evaluation results

Dose-dependent and statistically significant reductions ($P < 0.05$) in the number of abdominal constrictions (writhings) induced by intraperitoneal administration of acetic acid were observed with MENN. At doses of 50, 100, 200 and 400 mg per kg body weight, MENN was observed to reduce the number of writhings, respectively, by 29.6, 37.0, 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 33.3 and 51.9%, respectively. Thus, a dose of 400 mg/kg MENN was equivalent to that of 400 mg/kg aspirin regarding antinociceptive potential, while a dose of even 100 mg/kg MENN showed better antinociceptive effects than 200 mg/kg aspirin. The results are shown in Table 2 and suggest that the extract possesses significant antinociceptive properties.

Alkaloids, flavonoids, saponins, and tannins present in MENN can be responsible 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 demonstrated the presence of alkaloids, flavonoids, tannins, saponins, and phenolics [23]. 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 [24].

Table 2: Antinociceptive effect of crude methanol extract of *N. nouchali* stems (MENN) 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	3.6 ± 0.40	33.3*
Aspirin	400 mg	2.6 ± 0.51	51.9*
(MENN)	50 mg	3.8 ± 0.49	29.6*
(MENN)	100 mg	3.4 ± 0.24	37.0*
(MENN)	200 mg	3.0 ± 0.32	44.4*
(MENN)	400 mg	2.6 ± 0.24	51.9*

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

A major finding of the study is that in countries like Bangladesh with poor infrastructure and unhygienic conditions of living among the large rural population and heavy workload leading to various diseases, easily affordable and available plant-based remedies can be a viable alternative to costlier and inaccessible allopathic drugs. If these plant-based remedies prove to be non-toxic and can be validated through proper scientific methods as to the efficacy of their uses, they can prove a boon for the poorer sections of the people who are heavily burdened with health-care costs.

CONCLUSION

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

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