ANTI-HYPERGLYCEMIC AND ANTINOCICEPTIVE ACTIVITY OF METHANOL LEAF AND STEM EXTRACT OF *NYPA FRUTICANS* WURMB

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ABSTRACT

Nypa fruticans Wurmb. (Arecaceae) is a mangrove palm well-known for its traditional uses by the local practitioners against different ailments in southern regions of Bangladesh. However, the plant is yet to be scientifically studied. The present study was done to evaluate the anti-hyperglycemic and antinociceptive potential of methanolic extract of leaf and stem of *Nypa fruticans* Wurmb. (MENF). The anti-hyperglycemic activity was tested on glucose loaded hyperglycemic mice whereas antinociceptive activity was evaluated using a model of acetic acid-induced writhing in mice. The crude MENF was found to show significant oral anti-hyperglycemic activity on glucose loaded mice at every dose. Maximum anti-hyperglycemic activity was observed at a dose of 500 mg MENF/kg body weight, which was more than what was obtained with a standard drug glibenclamide at a dose of 10 mg glibenclamide/kg body weight). Significant antinociceptive activity was also demonstrated by MENF in acetic acid-induced writhing mice model. The extract caused a maximum of 39.88% ($p<0.001$) inhibition of writhing at the dose of 600 mg/kg body weight, which was better than the result obtained with a standard drug (200 mg aspirin/kg body weight, 49.34% inhibition). These findings indicate that MENF has significant anti-hyperglycemic and antinociceptive activity and thus have great potential as a source of natural products.

Keywords: MENF, anti-hyperglycemic, antinociceptive activity.

INTRODUCTION

The Sundarbans forest, which falls both in Bangladesh and India is extremely rich in biodiversity and has been declared as a World Heritage Site. It is rich in flora, including timber and non-timber species, varieties of orchids, and medicinal plants. *Nypa fruticans* Wurmb. (Arecaceae), the only palm considered a mangrove, called Gol Pata in Bengali, is one of the most important trees that make the botanical wealth of the mangroves. The plant has importance as food additive, as fuel and also as roofing material (http://en.wikipedia.org/wiki/ Nypa_fruticans, accessed on 12 March, 2010; Lawrence and Dennis, 1988). Its leaf extract inhibits the corrosion of zinc (Okorosaye and Oforka, 2004). Chemical constituents reported from the plant include stigmasterol, sitosterol, β-sitostenone, stigmasta-4, 22-dien-3-one, daucosterol, diosgenin, and dioscin (Nan *et al*., 2008). Local people of Khulna, Bagerhat and Satkhira region use this plant for controlling localized pain and blood glucose level. But so far no scientific investigation has been done to screen out its probable anti-hyperglycemic and antinociceptive property. The objective of the present study was to evaluate the glucose tolerance activity of MENF using glucose-loaded hyperglycemic mice and antinociceptive activity by acetic acid-induced gastric pain model mice.

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MATERIALS AND METHODS

Plant material and extraction

The stem and leaf of *Nypa fruticans* Wurmb. were collected from the Sundarbans, Bangladesh in May, 2009. The plant was identified by Mr. Manzur-ul-Kadir Mia, former Principal Scientific Officer and Curator of Bangladesh National Herbarium, Dhaka. The dried leaf and stem (air-dried for 120 hours in shade) of *Nypa fruticans* Wurmb. were pulverized into a fine powder and were mixed with methanol at a ratio of 1:5 (w/v). After 24 hrs, the mixture was filtered; filtrate was collected and the residue was again mixed with methanol at a ratio of 1:3 (w/v) for 24 hrs. After filtration, filtrates were combined and evaporated to dryness using rotary evaporator.

Drugs

Glacial acetic acid was obtained from Sigma Chemicals, USA; glibenclamide and aspirin were from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were analytical grade.

Animals

Swiss albino mice (male), weighing 25-30g, were obtained from the animal house of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). The animals were acclimatized for one week prior to the experiments. The study was approved by the Institutional

Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Anti-hyperglycemic activity

Glucose tolerance property of *Nypa fruticans* Wurmb. was performed following the procedure as described previously by Joy and Kuttan (1999). In brief, fasted mice were divided into five groups of seven mice each. Each group received a particular treatment. Group-I served as control and received vehicle (1% Tween 80 in water, 10 ml/kg body weight), group-II received standard drug (glibenclamide, 10 mg/kg body weight) and the three other groups received MENF at three different doses of 100, 200, and 500 mg/kg body weight. Each mouse was weighed and the doses of MENF, glibenclamide, and control vehicle were adjusted according to weights of individual mouse. Test samples, control, and glibenclamide were given orally. After one hour, all mice were orally treated with glucose (2 g/kg body weight). Blood samples were collected after one and two hours following glucose administration. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method (Venkatesh *et al*., 2004).

Acetic acid-induced writhing assay

Antinociceptive activity of MENF was tested using the acetic acid-induced writhing method as described previously by Vasudevan *et al*. (2007) with minor modification. Experimental animals were randomly selected and divided into five groups of seven mice per group. Each group received a particular treatment like group-I received vehicle (1% Tween 80 in water, 10 ml/kg body weight), group-II received standard drug (aspirin, 200 mg/kg body weight) and the three other groups received MENF at three different doses (200, 400 and 600 mg/kg body weight). Each mouse was weighed properly and the doses of MENF, standard drug and control materials were adjusted accordingly. Test samples, control, and aspirin were given orally. An interval of 30 min was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical glacial acetic acid solution (1%) was administered intraperitonealy to each of the animals of all groups (at a dose of 10 ml/kg body weight). A 5 min interval was given for bioavailability of acetic acid, and then the number of writhings was counted for 10 min. The animals always did not accomplish full writhing, because writhing was started but not completed. This partial or incomplete writhing was considered as half writhing.

Acute toxicity study

The study was carried out as previously described (Ganapaty *et al*., 2002). Selected animals were divided into nine groups of six animals each. The control group received 1% Tween 80 in normal saline (2 ml/kg body weight). The other groups received respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg MENF/kg body weight. Animals were monitored closely after dosing for the next 8 hours for any behavioral changes and were kept under observation up to 14 days to find out if there is any mortality.

STATISTICAL ANALYSIS

Student's *t*-test was used to determine a significant difference between the control group and experimental groups. $P < 0.05$ was considered as significant compared to control.

RESULTS

Anti-hyperglycemic effect

The results from the study clearly indicated that MENF has significant glucose lowering capacity at all doses examined in a dose dependent manner. Maximum anti-hyperglycemic activity (59.09% inhibition) of MENF in glucose-induced hyperglycemic mice was observed with a 500 mg/kg body weight dose, while the standard drug, glibenclamide produced 57.28 % activity at 10 mg/kg body weight dose (table 1).

Writhing assay

In the acetic acid-induced writhing model mice, oral administration of MENF also caused writhing inhibition significantly in a dose dependent manner. The test sample showed maximum inhibition of writhing (39.88%) at the dose of 600 mg/kg body weight, whereas aspirin, the standard antinociceptive drug caused 49.34% ($p<0.01$) writhing inhibition at the dose of 200 mg/kg body weight (table 2). The formula for computing percent inhibition was: (average writhes in the control group minus average writhes in the standard- or MENF-treated group) divided by average writhes in the control group, which was then multiplied by 100 (Hossein and Hani, 2002).

Acute toxicity study

There was no mortality in any of the extracts at tested doses till the end of 14 days of observation.

DISCUSSION

In the present study, MENF was investigated for possible anti-hyperglycemic and antinociceptive activity. Long term diabetes mellitus is associated with several complications such as retinopathy, atherosclerosis, myocardial infarction, nephropathy etc. (Pushparaj *et al*., 2007). Some of these complications begin within months of the onset of diabetes, although most tend to develop after a few years. Diabetes mellitus is a disorder in which blood sugar (glucose) levels are abnormally high because the body does not produce enough insulin to meet its needs. The present preliminary experimental results indicated that *Nypa fruticans* Wurmb. exhibited a potent blood glucose lowering property in glucose-induced

Table 1: Effect of methanol extract of *Nypa fruticans* (leaf and stem) on serum glucose level in hyperglycemic mice

Treatment	Serum Glucose level (mg/dl)		% of inhibition	
	60 min	120 min	60 min	120 min
Control	162.80±7.92	80.98±3.14	-	-
Glibenclamide (10 mg/kg)	69.55±2.53	59.57±3.12	57.28**	26.44**
MENF (100 mg/kg)	139.89±2.45	71.17±1.5	14.07*	12.11**
MENF (200 mg/kg)	131.06±4.82	55.17±2.95	19.5*	31.87**
MENF (500 mg/kg)	120.93±3.64	33.13±2.48	25.72**	59.09**

All administrations were made orally. Values are represented as mean ± SEM, (n=7); * $P < 0.05$; * * $P < 0.01$ significant compared to hyperglycemic control animals.

Table 2: Antinociceptive effect of crude methanol extract of *Nypa fruticans* (leaf and stem) in the acetic acid-induced pain model

Groups	Dose (mg/kg body weight)	Mean number of writhing	Inhibition (%)
Control (vehicle)	10	21.14±1.2	-
Aspirin	200	10.71±0.86	49.34**
MENF	200	17.29±1.02	18.21*
MENF	400	14.57±0.95	31.08**
MENF	600	12.71±1.17	39.88**

All administrations were made orally. Values are represented as mean ± SEM, (n=7); * $P < 0.05$; * * $P < 0.01$ significant compared to control.

hyperglycemic mice. A plausible mechanism of action is that MENF might have stimulated residual pancreatic β-cell function or produced the anti-hyperglycemic effect through an extra pancreatic mechanism, probably by increasing peripheral utilization of glucose (Farjou *et al*., 1987). It has been noted in a previous study with *Helicteres isora* root extracts that sterols, triterpenoids or glycosides present in the crude extract may be responsible for the observed pharmacological activity (Venkatesh *et al*., 2004). Whether the observed hypoglycemic effect was due to any such sterols present in the plant (Nan *et al*., 2008) is at present under investigation. It is to be noted in this regard that β-sitosterol-3-β-D-glucoside has been reported to be the active anti-diabetic agent of *Centaurea seridis* L. var. *maritima* (Ivorra *et al*., 1990). The anti-hyperglycemic and insulin-releasing effects of β-sitosterol and β-sitosterol-3-β-D-glucoside have also been reported (Ivorra *et al*., 1988).

Acetic acid-induced writhing test is used for detecting both central and peripheral analgesia (Shanmugasundaram and Venkataraman, 2005). Intraperitoneal administration of acetic acid (1%) causes pain and inflammation through production of prostaglandins, mainly prostacyclines (PGI_2) and prostaglandin-E (PG-E) which have been reported to be liable for pain sensation by exciting the Aδ-nerve fibers (Martindale, 1982; Rang *et al*., 2003). In this study, MENF showed significant activity compared to control at all doses investigated. Therefore the result of the acetic acid-induced writhing model mice suggests that the extract may inhibit the writhing via inhibition of prostaglandin synthesis. According to previous study alkaloids, glycosides and tannins may be responsible for antinociceptive activity (Deb *et al*., 2010; Etuk *et al*., 2006; Starec *et al*., 1988). A further exploration of the bioactive molecules in *Nypa fruticans* Wurmb. exactly responsible for these activities is currently under investigation.

In conclusion, the results of this study support the folkloric use of this plant in the management of local pain and controlling diabetes. However, further detailed studies are essential to find out the underlying mechanisms of anti-hyperglycemic and antinociceptive activity and also to isolate the active compound(s) responsible for those pharmacological properties. It is important to note that very few pharmacological activity studies have been conducted with mangrove species. This study is the first of its kind on *Nypa fruticans* Wurmb. and opens up the possibility that other mangrove species of the Sundarbans forest may have important pharmacological effects.

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