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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Anti-hyperglycemic and antinociceptive activity

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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 intraperitoneally 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
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 (PGI2) 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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