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ORIGINAL ARTICLE

Antinociceptive Activity of Methanol Extract of *Areca catechu* L. (Arecaceae) Stems and Leaves in Mice

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ABSTRACT

The antinociceptive effect of crude methanol extracts of stems and leaves of *Areca catechu* L. (Arecaceae) was evaluated in acetic acid-induced gastric pain writhing model in Swiss albino mice. The methanol extract of *Areca catechu* stems dose-dependently reduced the number of writhings (constrictions) in mice, when tested at doses of 50, 100, 200, and 400 mg extract administered per kg body weight. Significant reductions in the number of writhings were noted with all administered doses. The percent inhibitions of acetic acid-induced writhings with the four different doses were, respectively, 30.8, 36.6, 40.9 and 59.6. The standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight reduced writhings by 42.3 and 55.8%, respectively. A significant dose-dependent inhibition of writhings was also observed with crude methanol extract of *Areca catechu* leaves, where the extract at doses of 50, 100, 200 and 400 mg per kg body weight significantly inhibited writhings by 55.8, 57.7, 86.5 and 88.5%, respectively. Dose for dose, the leaf extract demonstrated higher antinociceptive activity than the stem extract. At even the lowest dose of 50 mg extract per kg body weight, the antinociceptive activity of leaf extract was comparable to that of 400 mg aspirin per kg body weight. The results suggest that both stem and leaf extract possess good antinociceptive activity, which merits further scientific studies as to isolation of responsible phytochemical component(s).

Key words: *Areca catechu*, antinociceptive, mice, gastric pain

Introduction

Areca catechu L. (Arecaceae) (local name: supari) is more commonly known as the areca palm or betel nut palm because its fruit is chewed often with betel leaf. The palm tree can attain heights up to 20 meters tall, and is cultivated throughout the tropical parts of the Indian sub-continent, including Bangladesh (Satyavatia *et al*., 1976). The fruits of the palm (nuts) are known to be in use for more than two thousand years (Bradley, 1979) and are chewed in parts of the world either by themselves for breath freshening effect, digestive aid, expelling intestinal worms (Cawte, 1985), or as in Bangladesh, with betel leaf, lime and other ingredients as a digestive aid and stimulant effect. Chewing of the nut has been reportedly associated with a depressive effect on the heart and a decrease in blood pressure (Kapoor, 1990; Lin *et al*., 2002).

Arecoline, a major alkaloid of the nut, has been shown to inhibit adipogenesis, induce lipolysis and significantly attenuate insulin-induced glucose uptake by fat cells (Hsu *et al*., 2010). However, hypoglycemic activity has also been reported for arecoline (Chempakam, 1993). Extract of the nut has been reported to

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exhibit anti-inflammatory effect on carrageenan-induced edema in mice and rats, and markedly repress prostaglandin E2 and arachidonic acid-induced inflammation. The extract of nut also demonstrated analgesic activity by inhibiting formalin-induced nociception in mice and acetic acid-induced writhing in rats (Khan *et al*., 2011). Analgesic, anti-inflammatory and antioxidant activities have also been reported of hydroalcoholic extract of betel nuts (Bhandare *et al*., 2010). Areca nut extract has been shown to inhibit 12-*O*-tetradecanoylphorbol-13-acetate induced cyclooxygenase-2 protein expression at low doses, thus demonstrating its anti-inflammatory and analgesic potential (Huang *et al*., 2010).

While the nuts of the tree have been studied for their anti-inflammatory and analgesic activities, there is a lack of studies on such properties in leaves and stems. In the folk medicinal system of Bangladesh, the folk medicinal practitioners (Kavirajes) use leaves of the betel nut palm to ease intestinal pain. In our laboratory, we had been studying plants used in the folk medicinal system of Bangladesh, particularly as to their antihyperglycemic and antinociceptive effects (Anwar *et al*., 2010; Jahan *et al*., 2010; Khan *et al*., 2010; Mannan *et al*., 2010; Rahman *et al*., 2010; Rahman *et al*., 2011; Shoha *et al*., 2011; Sutradhar *et al*., 2011). The objective of the present study was to evaluate the antinociceptive potential of methanol extract of *Areca catechu* stems and leaves in acetic acid-induced gastric pain writhing models in mice.

Materials and Methods

The stems and leaves of *Areca catechu* were collected from Gazipur district, Bangladesh during December, 2009. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 35,030). The air-dried stems and leaves of *Areca catechu* were separately grounded into a fine powder, and 100g of the powder was extracted with methanol (1:5, w/v) for 24 hrs each. The extracts were evaporated to dryness. The final weight of the extract was 4.1g for stems and 11.2g for leaves.

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin was obtained from Square Pharmaceuticals Ltd., Bangladesh.

In the present study, Swiss albino male mice, which weighed between 20-25g were used. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Prior to experiments, all the animals were acclimatized for one week. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternatives, Dhaka, Bangladesh.

Antinociceptive activity of methanol extract of *Areca catechu* stems and leaves was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into eleven groups of six 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 methanolic stem extract of *Areca catechu* at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Groups 8-11 were administered methanolic leaf extract of *Areca catechu* 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 extract, 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 bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

Student's *t*-test was used to analyze any significant differences between control and experimental groups. $P < 0.05$, was considered significant as compared to control.

Results and Discussion

In acetic acid-induced gastric pain writhing tests, both methanolic extract of stems and leaves demonstrated significant and dose-dependent reductions in the number of writhings when compared to non-extract treated control animals. The results are shown in Table 1. The standard drug, aspirin, at doses of 200 and 400 mg per kg body weight, reduced the number of constrictions, respectively, by 42.3 and 55.8%. Compared to aspirin, the methanolic stem extract at doses of 50, 100, 200 and 400 mg per kg body weight reduced the number of constrictions by 30.8, 36.6, 40.9, and 59.6%, respectively. Thus at the highest dose of the stem extract tested, the percent reduction in writhings were comparable to that of aspirin, administered at a dose of 400 mg per kg body weight.

The methanolic extract of leaves of *Areca catechu* showed an even greater inhibition in the number of constrictions when evaluated at doses of 50, 100, 200 and 400 mg per kg body weight. The percent inhibitions were significant and dose-dependent. At the above four afore-mentioned doses, the leaf extract inhibited constrictions or writhings by 55.8, 57.7, 86.5 and 88.5%, respectively. Dose for dose, the leaf extract had higher antinociceptive activity than the stem extract. In fact, even at the lowest dose of leaf extract, the percent

Table 1: Antinociceptive effect of crude methanol extract of *Areca catechu* stems and leaves in the acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	8.67 ± 0.76	-
Aspirin (Group 2)	200 mg	5.00 ± 1.21	42.3*
Aspirin (Group 3)	400 mg	3.83 ± 0.95	55.8*
Areca catechu stem (Group 4)	50 mg	6.00 ± 1.24	30.8*
Areca catechu stem (Group 5)	100 mg	5.50 ± 0.76	36.6*
Areca catechu stem (Group 6)	200 mg	5.17 ± 0.79	40.9*
Areca catechu stem (Group 7)	400 mg	3.50 ± 0.76	59.6*
Areca catechu leaf (Group 8)	50 mg	3.83 ± 0.87	55.8*
Areca catechu leaf (Group 9)	100 mg	3.67 ± 0.61	57.7*
Areca catechu leaf (Group 10)	200 mg	1.17 ± 0.79	86.5*
Areca catechu leaf (Group 11)	400 mg	1.00 ± 0.45	88.5*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=6); *$P < 0.05$; significant compared to control.

inhibitions in writhings was directly comparable to that of aspirin, when administered at 400 mg per kg body weight. When compared to aspirin, administered at a dose of 200 mg per kg body weight, which inhibited the number of constrictions by 42.3%, the leaf extract showed greater potency even at the lowest dose of 50 mg per kg body weight tested.

Both central and peripheral analgesia can be suitably detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005). Intraperitoneal administration of acetic acid (1%) leads to pain and inflammation mediated through production of prostaglandins [mainly prostacyclines (PGI_2) and prostaglandin-E (PG-E)], which are reported to be responsible for excitation of the Ad-nerve fibers, leading to sensation of pain (Reynolds 1982). Therefore any agent that lowers the number of abdominal constrictions will demonstrate analgesia by inhibition of prostaglandin synthesis. It is to be noted that extract of nuts of *Areca catechu* has already been implicated in anti-inflammatory and analgesic activities (Bhandare *et al*., 2010), and repress prostaglandin E2 and arachidonic acid-induced inflammation (Khan *et al*., 2011). Areca nut extract has further been shown to inhibit cyclooxygenase-2 protein expression (Huang *et al*., 2010), a protein directly involved in the synthesis of prostaglandins. It is, therefore very plausible, that the stems and leaves of the plant might contain similar phytochemicals like the nuts, and which may be the reason for the antinociceptive activity obtained in the present study.

The present study results validate the use of leaves of *Areca catechu* by folk medicinal practitioners of Bangladesh for treatment of intestinal pain. The strong antinociceptive activities demonstrated by both methanolic stem and leaf extract of the plant merits further scientific studies towards discovery of the responsible phytochemical component(s)

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