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ORIGINAL ARTICLE

An Evaluation of Antinociceptive Effect of Methanol Extracts of *Desmodium Gangeticum* (L.) Dc. Stems and *Benincasa Hispida* (Thunb.) Cogn. Leaves on Acetic Acid-induced Gastric Pain in Mice

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ABSTRACT

Desmodium gangeticum Duchesne (Fabaceae, local name: shalpani) is commonly found in the wild areas of Bangladesh. The leaves of the plant are used by folk medicinal practitioners for treatment of toothache, chest pains, and fungal infections. *Benincasa hispida* (Thunb.) Cogn. (Cucurbitaceae, local name: chal kumra) is cultivated for its edible fruits, which are cooked and consumed as vegetable. The fruits are used by the folk medicinal practitioners for treatment of tumor, gonorrhea, and helminthiasis. The leaves are used by the folk medicinal practitioners for relief from stomach pain. The objective of the present study was to evaluate the antinociceptive potential of stems of *D. gangeticum* and leaves of *B. hispida* in acetic acid-induced gastric pain with consequent abdominal constrictions in Swiss albino mice. Administration of methanol extract of stems of *D. gangeticum* was observed to cause dose-dependent and significant reduction of acetic acid-induced abdominal constrictions in mice. At the highest dose tested of the extract, namely 400 mg/kg body weight, the extract caused 52.6% inhibition of abdominal constrictions, when compared to control mice. In contrast, the standard antinociceptive drug, aspirin, caused 38.6% and 49.2% inhibitions of abdominal constrictions, when administered at doses of 200 and 400 mg/kg body weight, respectively. The results demonstrated that the methanol extract of stems of *D. gangeticum* was highly effective in the reduction of acetic acid-induced gastric pain, and as such validate the use of the plant in folk medicine for treatment of pain. The methanol extract of leaves of *B. hispida* also caused significant reductions in acetic acid-induced abdominal constrictions. However, the number of constrictions was lowest at the lowest dose tested of 50 mg/kg body weight, where it caused 48.1% inhibition in the number of abdominal constrictions (writhings). At higher doses, the percent inhibition of constrictions decreased progressively, and was 31.4% at the highest dose tested (400 mg/kg body weight). The results with *B. hispida*, although validating the use of the plant for pain, also suggest that any treatment with leaves of the plant for pain reduction purposes must be done with low doses. The progressive dose-dependent decrease of inhibitory activities is suggestive of presence of other constituents in the leaves, which may play an antagonistic role to constituents that modulate its antinociceptive effects.

Key words: Antinociceptive, *Desmodium gangeticum*, *Benincasa hispida*, mice

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Introduction

Desmodium gangeticum (L.) DC. is a herbaceous plant found growing in the wild in Bangladesh. The leaves of the plant are used by folk medicinal practitioners (Kavirajes) of Bangladesh for treatment of toothache, chest pains, and fungal infections. *Benincasa hispida* (Thunb.) Cogn. (Cucurbitaceae, local name: chal kumra) is a vinous plant cultivated for its edible fruits, which are cooked and consumed as vegetable. The leaves are also used by the folk medicinal practitioners for treatment of stomach pain.

Diverse pharmacological activities have been reported for various parts of *D. gangeticum*. Antioxidant effects have been demonstrated of ethyl acetate, aqueous and methanol extract of roots of *D. gangeticum* in myocardial ischemia reperfusion injury in rat hearts (Kurian *et al*., 2010; Kurian and Paddikkala, 2009; Kurian *et al*., 2008). Antiinflammatory and antioxidant activities of *D. gangeticum* flavonoid and alkaloid fractions have been reported in carrageenan-induced inflamed rats (Govindarajan *et al*., 2007a). The phenolic fraction obtained from alcoholic extract of the plant also demonstrated antioxidant activity and antiinflammatory activities in arthritic rats (Govindarajan *et al*., 2006). Treatment of diabetic rats with aerial parts of plant extract reportedly led to significant reduction in blood glucose. The extract also caused significant increase in insulin secretion from MIN6 cells (Govindarajan *et al*., 2007b). Antiamnesic effects have also been reported in mice for aqueous extract of the plant (Joshi and Parle, 2006). Crude ethanolic extract of the plant has been shown to be efficacious against experimental visceral leishmaniasis (Singh *et al*., 2005). The plant is also known to contain glycolipids and other constituents with antileishmanial and immunomodulatory activities (Mishra *et al*., 2005). Ethanolic extract of the plant has been shown to demonstrate anti-ulcer activities in various gastric ulcer models in Sprague Dawley rats and histamine-induced duodenal ulcer in guinea pigs (Dharmani *et al*., 2005). The antiinflammatory and anitnociceptive activity of aqueous decoction of roots and aerial parts of the plant has been reported (Rathi *et al*., 2004).

Antinociceptive and anti-pyretic activities have been reported for ethanol extract of seeds of *B. hispida* (Qadrie *et al*., 2009). Fruit extract reportedly demonstrated protective effects on oxidative stress in rats with indomethacin-induced gastric ulcers (Shetty *et al*., 2008). Aqueous extract of the plant showed anti-vascular inflammatory activity when studied with high glucose-induced vascular inflammation of human umbilical vein endothelial cells (Moon *et al*., 2009). The anti-angiogenic effect of seed extracts has also been reported (Lee *et al*., 2005). The antioxidative and angiotensin-converting enzyme inhibition capacities of various parts of the fruits from this plant, including pulp, core, seed, and peel has been reported, suggesting that the various parts may provide protective effects against cardiovascular diseases and cancer (Huang *et al*., 2004). The plant has been shown to offer protective effects against various stress conditions-induced ulceration in rats and mice (Grover *et al*., 2001). Fresh juice of the plant reportedly showed significant activity against symptoms of morphine withdrawal in mice (Grover *et al*., 2000).

Considering the reported antinociceptive activities of individual plant parts from both plants, and considering the uses of parts from both plants in folk medicinal treatment of Bangladesh for treatment of pain, the objective of the present study was to conduct a preliminary investigation on methanol extract of stems of *D. gangeticum* and leaves of *B. hispida* on acetic acid-induced gastric pain model in mice. It was felt that such studies on antinociceptive activities have the potential for validating the folk medicinal use of both plants in Bangladesh.

Materials and Methods

Plant material and extraction

The stems of *D. gangeticum* were collected from Dhaka district, Bangladesh in July, 2010. The plant was taxonomically identified by Bangladesh National Herbarium at Dhaka (Accession Number 35,039). The stems of *D. gangeticum* were air-dried in the shade for 120 hours, grounded into a fine powder, and were extracted 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 extracted with methanol at a ratio of 1:3 (w/v) for 24 hrs. Filtrates were combined and evaporated to dryness. The initial weight of dried leaf powder used for extraction was 100g; the final weight of the extract was 4.97g.

The leaves of *B. hispida* were collected from Dhaka district, Bangladesh in April, 2010. The plant was taxonomically identified by Bangladesh National Herbarium at Dhaka (Accession Number 35,031). The leaves of *B. hispida* were air-dried in the shade for 120 hours, grounded into a fine powder, and were extracted with methanol at a ratio of 1:4 (w/v). After 24 hrs, the mixture was filtered; filtrate was collected and the residue was again extracted with methanol at a ratio of 1:3 (w/v) for 24 hrs. Filtrates were combined and evaporated to dryness. The initial weight of dried leaf powder used for extraction was 50g; the final weight of the extract was 1.10g.

Chemicals and Drugs

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

Animals

In the present study, Swiss albino mice (male), which weighed between 20-25g were used. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for one week prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Acetic acid-induced abdominal constriction (writhing) method

Antinociceptive activity of methanol extract of *D. gangeticum* stems and *B. hispida* leaves was examined using previously described procedures of Shanmugasundaram and Venkataraman (2005) with minor modifications. Pain was induced in mice in the abdominal constriction test through intraperitoneal administration of 1% acetic acid at a dose of 10 ml/kg body weight. Mice were separated into seven groups of six mice each. Group-I served as control and was administered vehicle (1% Tween 80 in water, 10 ml/kg body weight). The standard drug, aspirin was administered to Group-II and Group-III mice at a dose of 200 mg and 400 mg per kg body weight, respectively. Groups-IV to VII received extract, respectively, at 50, 100, 200, and 400 mg extract/kg body weight orally 30 min before acetic acid injection. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period the number of abdominal constrictions (writhings) was counted for 10 min.

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.

Results and Discussion

Dose-dependent and significant antinociceptive activity was observed with methanol extract of *D. gangeticum* stems, when administered prior to intraperitoneal injection of acetic acid in mice. The standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, respectively, caused 38.6% and 49.2% reductions in the number of abdominal constrictions (writhings) induced by intraperitoneal injection of acetic acid in mice. In comparison, the methanol extract of *D. gangeticum* stems, even at the lowest dose tested of 50 mg per kg body weight caused 31.6% inhibition in the number of abdominal constrictions in mice. Highest inhibition of abdominal constrictions was observed with a dose of 400 mg/kg body weight. The results are summarized in Table 1. It is evident that the methanol extract of stems of *D. gangeticum* is a potent inhibitor of nociception or pain. As such, the results validate the use of plant in folk medicine of Bangladesh for treatment of pain like toothache or chest pains. It is to be noted that the leaves of the plant are used in folk medicinal preparations of Bangladesh for treatment of pain. Our results suggest that the stems may also be utilized for the same purpose. It may further be noted in this connection that the antiinflammatory and anitnociceptive activity of aqueous decoction of roots and aerial parts of the plant has been reported (Rathi *et al*., 2004). In the present study we confirm antinociceptive activity of methanol extract of an aerial part, namely stem of the plant.

The leaves of *B. hispida* are used by folk medicinal practitioners of Bangladesh for treatment of stomach pain. The results obtained in the present study, which has been summarized in Table 2, also show significant antinociceptive activity in the methanol extract of leaves, when evaluated in acetic acid-induced gastric pain model in mice. However, the results also pointed to an interesting feature. The percent inhibition of abdominal constrictions induced through intraperitoneal injection of acetic acid with consequent induction of gastric pain diminished progressively with increasing doses of the extract administered. Thus, for instance, while a dose of 50 mg extract per kg body weight caused a 48.1% reduction in the number of abdominal constrictions, there was a 37% reduction in the number of abdominal constrictions with an extract dose of 200 mg per kg body weight, and a reduction of 31.4% in the number of abdominal constrictions with the highest dose tested of 400 mg per kg body weight. The obvious cause for antinociceptive activity demonstrated by any crude extract is

the presence of phytochemical(s) in the extract. In this case, since the antinociceptive activity diminished with increasing doses of the extract, the only rational hypothesis would be that there are other phytochemica(s) present in the extract, which antagonizes the antinociceptive activity modulated by one or a group of phytochemical(s). Whether this hypothesis is true needs further investigations, which are currently being conducted in our laboratory.

Intraperitoneal administration of acetic acid induces gastric pain in mice, which can be observed from the number of abdominal constrictions or writhings. Both central and peripheral analgesia can be detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005). Pain is caused by the synthesis of prostaglandins (mainly prostaglandin E2) or prostacyclines (mainly PGI_2) in response to any internal or external stimuli (Reynolds, 1982; Rang and Dale, 1993). Any substance that inhibits prostaglandin biosynthesis can then act as a factor for alleviating the sensation of pain. In acetic acid-induced gastric pain model in mice, it would be apparent through reduction in the number of abdominal constrictions. It can then be postulated that methanol extracts of stems of both *D. gangeticum* as well as leaves of *B. hispida* possess constituent(s) that are acting in the cessation or reduction of the pain through inhibition of synthesis of prostaglandins. However, the exact mechanism needs further studies, but it may involve possible inhibition of cyclooxygenase and/or lipooxygenase enzymes. In fact, a similar mechanism has been proposed for antinociceptive activity of *Ficus deltoidea* aqueous extract in acetic acid-induced gastric pain model (Sulaiman *et al*., 2008).

Previous pharmacological activity studies conducted in our laboratory have validated the folk medicinal uses of different plants of Bangladesh (Morshed *et al*., 2010; Ahmed *et al*., 2010; Moushumi *et al*., 2010). The present study is no exception. The further conclusion that can be derived from this study is that other medicinal plants used in folk medicine of Bangladesh need to be studied as soon as possible for their potential for cheaper and more efficacious drugs.

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