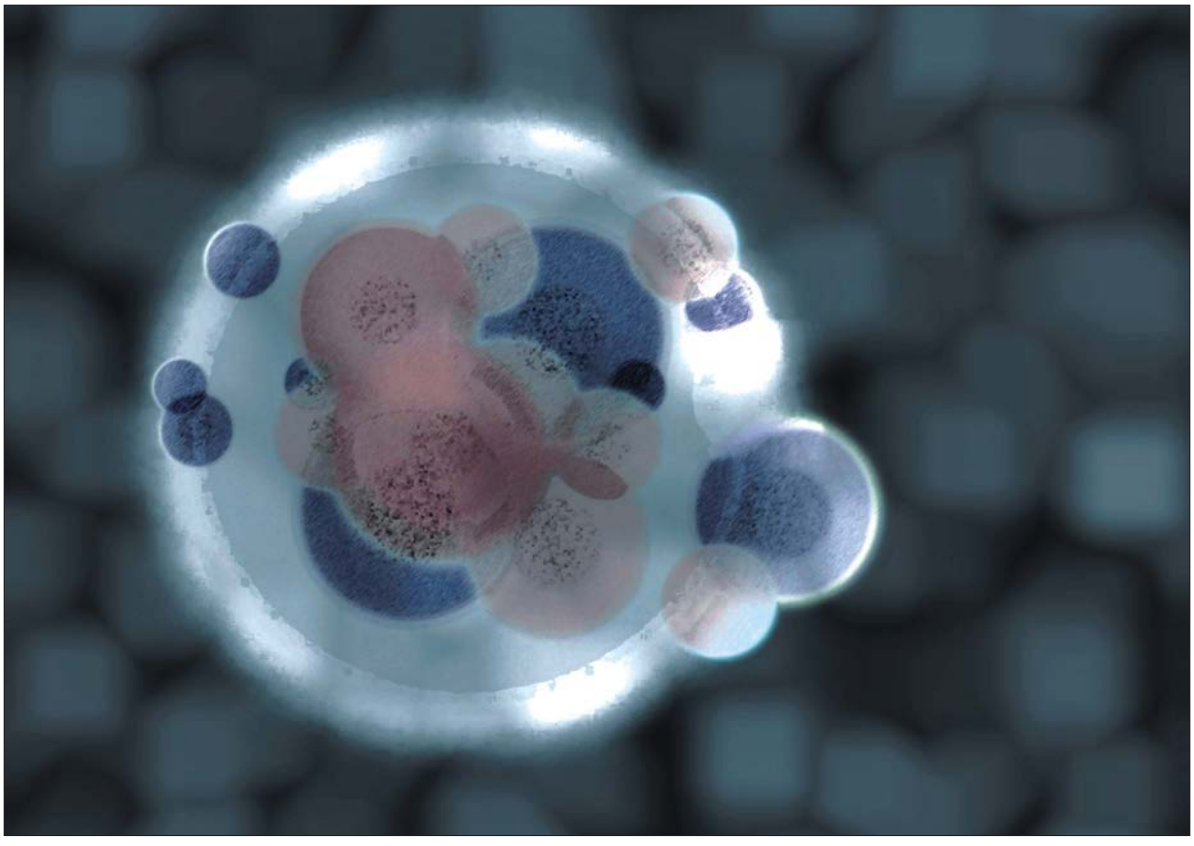

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 12 (3): 202-205, 2012
ISSN 1727-3048 / DOI: 10.3923/jbs.2012.202.205

Antinociceptive Activity of *Dalbergia spinosa* Roxb. Stem Barks

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Abstract: *Dalbergia spinosa* Roxb. (Family: Fabaceae) has many traditional uses in Bangladesh. The crude methanol extract of the stem barks of *D. spinosa* Roxb. was investigated for possible antinociceptive activity using acetic acid induced writhing model in mice. Phytochemical analysis was also performed using standard procedures to detect the presence of phytochemicals in the crude plant extract. The study results showed 37.20% inhibition of writhings in the tested mice when methanol extracts were given intraperitoneally (i.p.) at the dose of 250 mg extract kg^{-1} b.wt. The maximum inhibition (40.01%) of nociception effect was achieved at 500 mg extract kg^{-1} b.wt., i.p. which was also compared with the antinociceptive activity of the standard drug, diclofenac sodium at the dose of 25 mg extract kg^{-1} b.wt. which produced 68.37% inhibition of nociception effect. The inhibition of writhings was calculated in respective to control group and it was found that p-values (<0.0001) calculated by student's t-test were statistically significant. However, the phytochemical screening revealed the presence of alkaloid, steroid, flavonoid, tannin, reducing sugar and gum. Finally, it can be concluded that crude methanol extracts of *D. spinosa* stem barks contain biologically active phytoconstituents exhibiting significant dose-dependent antinociceptive activity in the mice model used. Thus, it is recommended to isolate and characterize the compounds for the development of new analgesics.

Key words: *Dalbergia spinosa* Roxb., antinociceptive, methanolic extract, phytochemical, writhings

INTRODUCTION

Dalbergia spinosa Roxb. found in Bangladesh (Senthamarai *et al*., 2003) was selected and tested to justify its medicinal use. This medicinal plant belongs to the family of Fabaceae (Jena *et al.,* 2004). Traditionally, crude drugs have applications for the treatment of a range of pain complications (Eidi *et al*., 2011). *D. spinosa* is a mangrove plant in the Sundarban islands and the medicinal plant is also found in India. It is a shrub having small white flowers. Earlier researchers have identified the presence of glycosides in this plant species (Anjaneyulu *et al*., 2005). Various isoflavones have been isolated and characterized from the roots and stems of *D. spinosa* (Kumar and Muller, 1999). The plant also contains dalspinin, dalspinosin and dalspinin-7-O- β-D-galactopyranoside (Senthamarai *et al*., 2003).

Moreover, the two new isoflavone galactosides such as prunetin 4'-O-β-D-galactoside and 7-methyltectorigenin 4'-O-β-D-galactoside have been isolated from the leaves and stem-bark of this medicinal plant (Narayanan and Nacarajan, 1988). Fever, pain, skin infections and urinary tract infections can be treated with *D. spinosa* (Senthamarai *et al*., 2003). The roots of the plant possess significant anti-inflammatory activity which is comparable to Indomethacin (Jaiganesh and Senthamara, 2010) Different types of diseases are commonly treated by the phytochemical compounds of the traditional medicinal plants (Pareta *et al*., 2011). It is reported that the crude extracts have also significant antimicrobial activities against both gram positive and gram negative bacteria (Senthamarai *et al*., 2003). Due to potential applications of *D. spinosa* in folkloric medicine, the present study was conducted to investigate the antinociceptive activity of the crude methanol extract of stem barks of the plant.

MATERIALS AND METHODS

Collection of plant materials: The stem barks of *D. spinosa* were collected from Sundarban Islands on 2009 and subsequently identified by the taxonomists

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of Bangladesh National Herbarium, Mirpur, Dhaka. The voucher specimen of the plant was deposited at the Faculty of Pharmacy, University of Dhaka.

Preparation of plant extracts: The collected stem barks were separated from undesirable materials and they were air-dried for one week. The dried stem barks were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place. About 350 g of powered material was taken in a clean, flat bottomed glass container and soaked in 900 mL of 80% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean and white cotton material. Then it was filtered through Whatman filter paper. The obtained filtrate was evaporated under ceiling fan and in a water-bath until dried. It rendered a gummy concentrate of reddish black color residue (yield 4.57%) which was designated as crude methanol extract of the stem barks of *D. spinosa*.

Chemicals and standard drug: Acetic acid (0.7%) and methanol were collected from LOBA Chemicals Pvt. Ltd., India. And Diclofenac sodium was obtained from Square Pharmaceuticals Ltd., Bangladesh. All the chemicals used during the study were of analytical grade.

Preliminary phytochemical screening: Preliminary phytochemical analysis of the methanol extracts was carried out using standard procedures (Trease and Evans, 1989).

Animals and experimental model: The experiment of antinociceptive activity was conducted on Swiss albino mice aged 4-5 weeks and weighing 25-30 g of both sexes collected from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Before starting of the experiment, all the mice were subjected to acclimatize for one week under standard experimental conditions (relative humidity 40-65%, room temperature 25±2.0°C and 12 h light/12 h dark cycle). They were fed ICDDR, B formulated rodent food and water *ad libitum*. The antinociceptive activity was determined by acetic acid induced writhing method (Whittle, 1964). The Swiss albino mice were divided into four groups consisting of 5 mice per group.

The first group (Group I) served as control receiving 1% Tween solution in water (10 mg kg^{-1} b.wt.), the second group (Group II: Positive control) received Diclofenac Sodium (25 mg kg^{-1}) orally 30 min before acetic acid injection (0.7%, 1 mL 100 kg^{-1} i.p.) and third and fourth groups received the extract at doses of 250 and 500 mg kg^{-1} i.p., respectively before administration of acetic acid. The mice were then placed in individual cages and the number of abdominal contractions was observed 5 min after stimulation for a period of 10 min.

Statistical analysis: The percentage inhibition of writhing was obtained using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhing (control)-Mean No. of writhing (test)}}{\text{Mean No. of writhing (control)}}$$

The results of the experiment were expressed as means±Standard Error of Mean (SEM). Student's t-test (GraphPad Software) was used to determine a significant difference between the control and experimental groups where p values of less than 5% ($p<0.05$) was chosen as the level of significance.

RESULTS

Preliminary phytochemical screening: The results of phytochemical analysis of the methanol extract of *D. spinosa* stem barks are summarized in Table 1. Phytochemical study revealed the presence of alkaloid, steroid, flavonoid, tannin, reducing sugar and gum.

Antinociceptive activity: In acetic acid-induced writhing model, the crude methanol extract showed a significant dose-dependent decrease in the number of writhings. The extracts at the dose of 250 and 500 mg crude extract kg^{-1} body weight showed 37.20% and 40.01% inhibition of writhings in the experimental animals in a dose dependent manner. The obtained results were also comparable to the inhibition observed with a standard antinociceptive drug, Diclofenac sodium at 25 mg kg^{-1} b.wt. (77.21% inhibition). The results of antinociceptive activities are shown in Table 2.

DISCUSSION

Preliminary phytochemical analysis showed the presence of alkaloid, steroid, flavonoid, tannin, reducing sugar and gum in the crude methanol extract of the plant. It was revealed that the pain perception was inhibited by flavonoids, alkaloids and tannins (Ramaswamy *et al*., 1985; Zakaria *et al*., 2006; Rahman *et al*., 2011) which were also confirmed by this study. Moreover, flavonoids could show the anti-inflammatory, antioxidant and free radical scavenging activities (Hossinzadeh *et al*., 2002; Okwu and Orji, 2007). Thus, the plant might also possess anti-inflammatory and antioxidant activities which have been investigating in our laboratory.

Table 1: Results of preliminary phytochemical screening of methanol extract of *D. spinosa* (MEDS) stem bark

Extract	Alkaloid	Steroid	Flavonoid	Tannin	Reducing sugar	Saponin	Gum
MEDS	+	+	+	+	+	-	+

+: Present, -: Absent

Table 2: Effect of MEDS stem bark on acetic acid-induced writhing in mice

Animal group	Treatment	Writhing (Mean±SEM)	Inhibition of writhing (%)	95% CI	p-value
Group I: Control (n = 5)	1% Tween solution in water (10 mg kg^{-1} b.wt.)	36.24±0.17	-	-	-
Group II: Positive control (n = 5)	Diclofenac sodium (25 mg kg^{-1} b.wt.)	8.26±0.32	77.21	27.15 to 28.81	<0.0001
Group III: Test group (n = 5)	Methanolic extract (250 mg kg^{-1} b.wt.)	22.76±0.26	37.20	12.76 to 14.20	<0.0001
Group IV: Test group (n = 5)	Methanolic extract (500 mg kg^{-1} b.wt.)	21.74±0.30	40.01	13.70 to 15.30	<0.0001

Values are expressed as Mean±SEM, n: No. of mice, CI: Confidence interval

Clinical tests have confirmed the efficacy and safety of traditional medicinal plants to control pain and inflammation (Musa *et al*., 2007; Narendhirakannan *et al*., 2007; Woode *et al*., 2009). Acetic acid induced abdominal constriction is a sensitive procedure to establish peripherally acting analgesics. The response is thought to be mediated by the prostaglandin pathways (Le Bars *et al*., 2001). The promising antinociceptive activity of the crude methanol extract of *D. spinosa* stem barks might be due to the presence of analgesic principles which interfere in the biosynthesis of prostaglandins and some other autacoids. It has also been found that certain flavonoids play an important role in the inhibition of production of prostaglandins which also inhibit the key enzymes such as lipoxygenase, phospholipase and cyclooxygenase responsible for prostaglandin biosynthesis. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory processes (Manthey, 2000).

It is therefore possible that the inhibitory effects of nociceptive activities observed in the extract may be attributed in part to its flavonoid content. From the study, it may be concluded that the crude methanol extract of *D. spinosa* stem barks possesses significant antinociceptive activities. Thus, the results tend to corroborate the traditional use of this plant in the treatment of pain. However, further investigations are required to identify the active constituent(s) and to verify the therapeutic merits of the active constituent(s).

CONCLUSION

In conclusion, it can be claimed that *D. spinosa* possesses significant analgesic action which gives a scientific support to the traditional use of the plant in the management of pain. However, a lot of research work is required to identify the exact mode of action for antinociceptive activity and to isolate the active phytoconstituent(s) responsible for such potential.

ACKNOWLEDGMENTS

All authors are thankful to the authority of Square Pharmaceuticals Limited and International Centre for Diarrhoeal Disease and Research, Bangladesh for providing support to complete this research work successfully.

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