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Analgesic Activity of *Dalbergia lanceolaria* Bark Extract in Swiss Albino Mice

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Abstract

The ethanol extract of *Dalbergia lanceolaria* Linn. bark was subjected to pharmacological screening using various animal models. The extract showed analgesic activity when tested in acetic acid–induced writhing, tail-flick response, and formalin-induced licking tests in Swiss albino mice. The plant extract, at doses of 100, 200, and 400 mg/kg body weight, showed significant central as well as peripheral analgesic activity by oral route. Therefore, the current study indicates that the ethanol extract of *Dalbergia lanceolaria* bark has significant central and peripheral analgesic activity.

Keywords: Analgesic activity, *Dalbergia lanceolaria* Linn., Swiss albino mice.

Introduction

Herbal medicines derived from plant extracts are being increasingly used to treat a wide variety of clinical diseases, although relatively little is known about their mode of action. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. In our earlier screening program, we evaluated the antidiarrheal activity of the ethanol extract of the bark of *Dalbergia lanceolaria* Linn. in albino mice based on the ethnobotanical lead (Mujumdar et al., 2005).

Dalbergia lanceolaria Linn. (Fabaceae), commonly known as *dandusi*, is a shade tree grown in rural areas of India. The wood of the plant is used for tool handles and agricultural implements and is suitable for carving, boarding, rafters, packing cases, and general construction purposes. Decoction of the bark is used for dyspepsia, and the seed oil is used for rheumatism (Kashyapa & Chand, 2000). Few preliminary findings report the activity of the plant against amavata, which is equated with rheumatoid arthritis. The clinical evidence of this plant for the treatment of amavata was mentioned in the Indian Medicinal System–Ayurveda (Sushruta Samhita, 5000 BC). Amavata is equated with arthritis-like condition, in which pain is one of the main components. In view of this, in the current investigation the ethanol extract of the bark of this plant was evaluated for analgesic activity using an animal model.

Materials and Methods

Plant material and preparation of extract

Dalbergia lanceolaria Linn. bark was collected from Khanapur, an area of Western Ghats of India, in the winter season of 2003. An authentic specimen of material was deposited in the Agharkar Herbarium of Maharashtra Association at Agharkar Research Institute, Pune, India (voucher specimen no. AHMA: S/B 049).

The bark of *Dalbergia lanceolaria* was washed with distilled water to remove dirt. The bark was further shade-dried and then coarsely powdered. This was first defatted with petroleum ether (60–80°C) and then successively extracted with ethanol. These extracts were concentrated under reduced temperature and pressure on a rotary evaporator. The yields of the pet-ether and ethanol extracts were 0.4% and 6.25%, respectively.

Experimental animals

Swiss albino mice (20–25 g) of either sex were originally obtained from the National Institute of Virology, Pune,

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India. The animals have been inbred in the animal house facilities at Agharkar Research Institute (ARI) for several generations for the past 20 years. They were housed in polypropylene cages in an air-conditioned area at 25 ± 2°C with 10:14 h light:dark cycle. They were given Amrut brand balanced animal feed and water *ad libitum*. The animal experiments were carried out after approval from the Institutional Animal Ethics Committee of ARI.

Acute toxicity study

The resulting extracts were subjected to acute oral toxicity studies as per revised OECD Organization of Economic Co-operation and Development guidelines (OECD No. 423). These extracts were devoid of any toxicity up to 2000 mg/kg body weight in albino mice for a single oral dose monitored for 14 days. The optimum conditions for experiments were decided on the basis of pilot experiments carried out using three animals per group. For further experiments, a group of at least six animals was used for individual treatment. Based on exploratory studies, analgesic activity was investigated using the ethanol extract only.

Drugs used

The *Dalbergia lanceolaria* bark ethanol extract in distilled water was used for oral dosing. Formalin and acetic acid were purchased from Merck India Ltd., and the standard drugs aspirin from Reckitt & Colman and pentazocine from Ranbaxy Pharmaceuticals were used.

Acetic acid–induced writhing test

Mice were injected with 10 ml/kg of 3% aqueous acetic acid by the intraperitoneal (i.p.) route 30 min after treatment of extract 100–400 mg/kg or aspirin 30 mg/kg kg orally to various groups of mice. The number of writhing episodes of an individual mouse was recorded for 20 min after acetic acid treatment (Koster et al., 1959).

Tail-flick method

Mice were treated with 100, 200, and 400 mg/kg doses of extract orally or pentazocine 25 mg/kg i.p. (Kumar, 2001; Vedhanayaki, 2003). After 30 min, the animal was held firmly to immerse its tail in a water bath that was maintained at constant temperature of 54°C. The time required for the typical reaction, a violent jerk of the tail, was recorded to assess response to the noxious stimulus (Turner, 1965).

Formalin-induced licking behavior

This test was carried out according to the method of Dubuisson (1977). Thirty minutes after oral administration of extract, 0.05 ml of 1% formalin in distilled water was injected subcutaneous to the right hind paw of the mouse. Each animal was then returned to the chamber, and pain response was recorded for 30 min. The summation of time (in seconds) spent in the licking and biting response of the injected paw during first 5 min block and in the 20–30 min time period was measured as an indicator of central and peripheral pain response, respectively. Aspirin was used as the standard control.

Statistical analysis

A statistical analysis of the results was carried out to calculate mean ± SEM. Further analysis was carried out using Student's *t*-test to calculate significance of the results.

Table 1. Analgesic activity of *Dalbergia lanceolaria* using various animal models.

	Animal model (n = 6)			
	Tail flick	Acetic acid induced–writhing	Formalin-induced licking	
Dose	Time required for flicking (s) (Mean ± SE)	No. of writhings (Mean ± SE)	Time spent in licking and biting (s) in 0–5 min (Mean ± SE)	Time spent in licking and biting (s) in 20–30 min (Mean ± SE)
Control	3.67 ± 0.21	49.17 ± 4.74	65.5 ± 4.1	31.33 ± 3.13
Aspirin (30 mg/kg)	—	19 ± 3.2***	48.67 ± 3.83*	4.83 ± 1.56***
Pentazocin (25 mg/kg)	10.5 ± 0.43***	—	—	—
Extract 100 mg/kg	4.33 ± 0.33	25.5 ± 2.12**	45.33 ± 4.81**	27.17 ± 3.06
Extract 200 mg/kg	5.83 ± 0.17***	13.83 ± 3.78***	43.33 ± 3.32**	19.33 ± 2.93*
Extract 400 mg/kg	8.5 ± 1.21**	11.5 ± 3.17***	40.5 ± 3.83**	0.0 ± 0.0***

*Significant as compared with control, $p < 0.05$.
**Significant as compared with control, $p < 0.01$.
***Significant as compared with control, $p < 0.001$.

Results

The results of the analgesic studies, summarized in Table 1, indicate that there is a significant reduction in the acetic acid–induced writhing in mice treated with the extract of *Dalbergia lanceolaria* (or aspirin) in comparison with the control group.

The time required for the flicking tail response was also shown to be delayed in the drug-treated animals as compared with controls. This suggests the presence of a significant analgesic activity correlating to doses of 200 and 400 mg/kg; however, this activity was not found to be dose dependent.

The analgesic activity was also observed in the formalin-induced pain model. As shown in Table 1, treatment with extract reduced the duration of licking and biting activity in the later phase with lower doses, whereas in the case of higher doses, it significantly inhibited licking and biting activity both in early and later phases of formalin-induced nociceptive action.

Discussion

The results of the acetic acid–induced writhing response treated with extract exhibited potential antinociceptive action. Antinociceptive drugs are generally classified into centrally or peripherally acting with respect to the site of action. The result of the writhing test alone did not provide proof of whether the antinociceptive effects were central or peripheral. To understand the mode of action, the ethanol extract was examined using the tail-flick response and formalin-induced licking behavior. The extract showed analgesic activity in the tail-flick response, which is generally considered a central action. The formalin test was initiated by injecting formaldehyde under the skin of the mouse's paw. This resulted in two phases of pain behavior observed as frequent biting and licking of the injured paw. The first phase occured within 5 min after the injection. These rapid rises and falls of nociception were mediated through C and A [Delta] fibers (Puig et al., 1996), which reflected the sensation of acute tissue damage. After a quiescent period, which indicated the immediate nociception stimuli had subsided, pain behavior in the second phase could be observed at 20–30 min after injection. The second phase is more closely associated with the chronic pain model. Stimuli of the first phase were transmitted through the lateral spinothalamic tract, whereas stimuli in the second phase were transmitted through the medial spino-thalamic tract (Melzack, 1990). According to Dubuission and Dennis (1977), centrally acting drugs inhibit the pain response in both phases equally, whereas peripherally acting drugs inhibit an effect only in the second phase. The extract only inhibits the second-phase effects at lower doses, whereas at higher doses the inhibition of both phases suggests central mechanism of action. Thus, net analgesic activity of the extract at lower doses was peripheral, confirming the results of acetic acid–induced writhings, and at higher doses the effect was a central one, confirming the results of the tail-flick response. Hence, it is through these mechanisms that the analgesic effects of the *Dalbergia* bark against amavata might be explained.

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