

Phytochemical investigation and evaluation of antinociceptive activity of ethanolic extract of *Dalbergia sissoo* (Roxb.) bark

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Abstract

The antinociceptive activity of ethanolic extract of the plant bark of *Dalbergia sissoo* (Roxb.) was investigated using tail flick method on Wistar rats. Three different dose levels (300, 500, and 1000 mg/kg) in 0.5% carboxyl methyl cellulose (CMC) were administered by p.o. route. The antinociceptive activities of the all doses were compared with that of the standard drug aspirin (300 mg/kg) administered by p.o. route and the results were found to be significant ($P < 0.01$). At the above doses, the extract exhibited significant and dose-dependent antinociceptive activity. Phytochemical investigation of the ethanolic extract indicated the presence of carbohydrates, proteins, amino acids, phenolic compounds, and flavanoids. The antinociceptive activity of the bark extract of *D. sissoo* may be due to the presence of phytochemical constituents such as flavanoids. The acute toxicity study revealed that ethanolic extract was not toxic up to 3000 mg/kg body weight.

Key words: Antinociceptive activity, *Dalbergia sissoo*, phytochemicals, tail flick method

INTRODUCTION

Dalbergia sissoo (Roxb.), Indian rosewood, belonging to legume family (Fabaceae), is a perennial tree found in the low land region (300 m to about 1000 m) of India. Its distribution extends across the sub-Himalayan region in Nepal, Pakistan, Bangladesh and Afghanistan. In addition to its use as a timber or firewood, it is also used by different ethnic groups to treat a variety of ailments.^[1-4]

D. sissoo has also been reported to possess various biological activities such as effective against blood diseases, syphilis, stomach problems, dysentery, nausea, eye and nose disorders, ulcers, skin diseases; has been used as

an aphrodisiac and expectorant; also for its nitric oxide production inhibition activity, anti-inflammatory, analgesic, antipyretic, larvicidal activities and the mosquito repellent action of *D. sissoo* oil against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, as well as resistance to some wood boring insects.^[5-10]

The bark of *D. sissoo* is 3–5 cm long, curved or flat and fibrous. Its external surface is rough with shallow and broad longitudinal fissures exfoliating in irregular, woody strips and scales. It is pale yellow to dark reddish-brown in color. It has fibrous fracture.^[1,2]

D. sissoo contains different compounds like dalbergenone, dalbergin, methyl dalbergin, 4-phenyl chromene, and dalbergichromene, and also contains dalbergichromene, nordalbergin and isodalbergin as minor constituents.^[11-13] To the best of our knowledge, since no information is available on the antinociceptive activity of *D. sissoo* bark, the present study was undertaken to investigate the extract yield, phytochemicals present in it and its antinociceptive activity.

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MATERIALS AND METHODS

Collection and authentication of plant material

The bark of *D. sissoo* was collected from Dehradun, Uttarakhand, India. The plant material collected was authenticated by Dr. H. J. Chowdhery, (Additional Director, Botanical Survey of India, Dehradun). The collected bark of *D. sissoo* was washed thoroughly with water to remove any unwanted matter. Then, it was dried in shade, ground to a coarse powder with a mechanical grinder and passed through sieve no. 40 and stored in an air-tight container.

Alcoholic extraction of bark

A weighed quantity (500 g) of the air-dried powdered stem bark of *D. sissoo* was taken and extracted with ethanol (90%) in a Soxhlet extractor. The ethanolic extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C to get a solid residue.

Phytochemical investigation

The crude extract of the plant was subjected to preliminary phytochemical screening and thin layer chromatography (TLC) to determine the presence of carbohydrates, glycosides, amino acids, phytosterol, saponins, flavanoids, alkaloids, and tannins.^[14]

Experimental animals

Wistar rats weighing 200–300 g of either sex were maintained under controlled conditions of light (12 hours) and temperature $25 \pm 1^\circ\text{C}$ in the animal house of GRD (PG) IMT, Dehradun, 2 weeks prior to the experiment for acclimatization. Animals had access to food and water *ad libitum*. All pharmacological activities were carried out as per the norms of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) after obtaining the approval from the institutional animal ethical committee.

Acute toxicity studies

Acute toxicity studies were carried out on Wistar rats according to the method proposed by Ghosh. Alcoholic extracts at dose of 50, 100, 300, 1000, and 3000 mg/kg body weight were administered to separate groups of mice ($n = 6$) after overnight fasting. Subsequent to administration of drug (bark) extract, the animals were observed closely for the first 3 hours for any toxic manifestations like increased motor activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 hours. The animals were observed for a further 1 week.^[15]

Experimental design

Thirty Wistar rats of either sex were grouped into five groups of six animals each. Group 1 received 0.5% CMC in distilled water 10 ml/kg body weight, which served as a

control group. Group 2 received aspirin (300 mg/kg) and served as the standard group. Groups 3, 4 and 5 received ethanolic extracts with 0.5% CMC at doses of 300, 500 and 1000 mg/kg, respectively, and served as test groups. All the doses were administered. by p.o. route.

Antinociceptive activity

The antinociceptive potential of the ethanolic bark extract of *D. sissoo* bark was measured by the Radiant Heat method (tail flick method).^[16] For each animal, the tail flick latency was obtained thrice before drug administration, and the mean was used as pre-drug latency. The flick latencies were measured at 0, 15, 30, 60 and 160 min after oral administration of vehicle or extracts (drugs), placing the tip (last 1–2 cm) of the tail on the radiant heat source. The withdrawal from the heat (flicking response) is taken as the end point or cut-off time (normally within 3–5 sec); the value of the cut-off time was considered as the latency period for that animal. The time until this reaction occurred was measured. A cut-off period 10–12 sec was observed to prevent damage of the tail. Any animal failing to withdraw its tail in 3–5 sec was rejected from the study.^[17]

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparisons Test. All the values were expressed as mean \pm SEM.

RESULTS AND DISCUSSION

Exactly 7.14 % w/w dry weight (% yield) of the ethanolic bark extract of *D. sissoo* was obtained. Preliminary phytochemical investigation of the ethanolic bark extract of *D. sissoo* showed that it contained carbohydrates, proteins, amino acids, phenolic compounds and flavanoids. Acute toxicity studies did not reveal any toxic symptoms or death in any of the animals up to the dose level of 3000 mg/kg body weight.

The bark extract of *D. sissoo* showed significant antinociceptive activity as evidenced by an increase in reaction time to the pain stimulus. The extract doses, 300 and 500 mg/kg, failed to alter pain threshold capacity which increased significantly at the dose of 1000 mg/kg at 30 min. But the results were significant ($P < 0.01$) for antinociceptive activity at all doses at 1 hour. The antinociceptive activity of different groups is presented in Table 1. Aspirin significantly increased the pain threshold throughout the observation period of 30 min to 2 hours. Test group 5 showed the maximum significant effect amongst all the test groups.

The antinociceptive activity of *D. sissoo* bark extract was studied for the peripheral activity (non-narcotic).

Table 1: Antinociceptive activity of *Dalbergia sissoo* bark extract

	Group Dose	Initial	Mean response time (in seconds)			
			After 15 minutes	After 30 minutes	After 60 minutes	After 120 minutes
Normal (Group 1)	5 ml/kg	15.00±0.737	15.12±0.776	15.28±0.737	15.04±0.789	15.48±0.585
Standard (Group 2)	300 mg/kg	15.06±2.713	26.98±3.663	28.91±4.406	30.85±4.430	27.98±3.663
Test-I (Group 3)	300 mg/kg	16.75±0.867	23.46±4.666	24.56±4.564	26.27*±3.29	19.37±2.919
Test-II (Group 4)	500 mg/kg	14.02±0.786	24.18±3.860	26.56±4.964	27.88*±5.039	22.14±3.418
Test-III (Group 5)	1000 mg/kg	15.98±3.717	25.25±6.335	27.54*±7.55	29.42*±9.125	24.52±5.404

Values are mean ± SEM (SEM, Standard Error Mean); n = five animals in each group, *P < 0.01, when compared to control

Antinociceptive activity of the bark extract in acute inflammatory pain was moderate as compared to the potent inhibitory action of aspirin. Aspirin offers relief from pain by suppressing the formation of pain mediator/substance in the peripheral tissue, where the prostaglandin and bradykinin are suggested to play an important role in the pain process.^[18-20] Therefore, it is likely that the bark extract of *D. sissoo* might suppress the preparation of these substances or inhibit the action of these substances and thus exert the antinociceptive activity. The present study showed significant increase in the latency period in tail flick method.

Presence of flavanoids was reported in *Dalbergia* species and flavanoids are reported to be prostaglandin synthetase inhibitors.^[21] Since prostaglandins are involved in the pain perception and are inhibited by flavanoids, it could be suggested that reduced availability of the prostaglandin by flavanoids might be responsible for the extract's antinociceptive activity.

Therefore, the present study demonstrates that *D. sissoo* bark extract has marked antinociceptive activity and establishes the effectiveness and pharmacological rationale. The drug may be further explored for its phytochemical profile to identify the exact active constituents responsible for the antinociceptive activity. Also, the medical application of the drug for use in the treatment of pain in traditional system of medicine is substantiated.

CONCLUSION

The results have shown the phytochemical properties and antinociceptive activity of the ethanolic bark extract of the *D. sissoo* (Roxb.). These findings support the use of the drug *D. sissoo* in the treatment of pain. This activity was related to the dose and this result corroborates the traditional use of the plant in peripheral pain condition.

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