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Phytochemical investigation and evaluation of analgesic activity of ethonolic extract of *Dalbergia sissoo* (Roxb.) bark

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ABSTRACT

The alcoholic extract of the stem bark of Dalbergia sissoo was evaluated for analgesic activity using tail flick method on Wistar Rats. The ethanolic extract was prepared by using soxhlet apparatus. Three different doses (300 mg/ kg, 500 mg/kg and 1000mg/kg) in 0.5 % CMC (carboxyl methyl cellulose) were administered by p.o. route, 300 mg/kg, 500 mg/kg failed to alter pain threshold capacity but increased significantly at the dose of 1000 mg/kg at 30 min. But the results were significant ($p < 0.01$) for analgesic activity at the all doses at 1 hr. Asprin (300mg/kg) by p.o. route used as standard. Phytochemical investigation showed that ethanolic extract contained carbohydrates, proteins, amino acids, phenolic compounds, and flavanoids. The ethanolic extract showed significant dose dependent analgesic activity. These findings support the use of this drug Dalbergia sissoo in the treatment of pain. The analgesic activity of the bark extract of Dalbergia 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: *Dalbergia sissoo*, analgesic Activity, 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 to about 1000 meter) of India. Its distribution range extended across the sub Himalayan region in Nepal, Pakistan, Bangladesh and Afghanistan. In addition to its use as a timber or fire wood, It also is used by different ethnic groups to treat a variety of ailments (1- 4).

Dalbergia sissoo has also been reported to posses various biological activities like blood diseases, syphilis, stomach problems, dysentery, nausea(5), eye and nose disorders, aphrodisiac, expectorant, ulcers, digestive disorders, skin diseases, nitric oxide Production inhibitory , anti-inflammatory, analgesic and antipyretic activities, larvicidal, growth inhibitor and mosquito repellent actions 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 *Dalbergia sissoo* 3-5 cm long, curved or flat, fibrous, cut pieces; external surface rough with shallow, broad longitudinal fissures, exfoliating in irregular, woody strips and scales; pale yellow to dark reddish-brown; fracture, fibrous(1, 2).

Dalbergia sissoo contained different compounds like dalbergenone, dalbergin, methyl dalbergin, 4-phenyl chromene, dalbergichromene and also contained dalbergichromene, nordalbergin and isodalbergin as minor constituents (11-13). To the best of our knowledge, since no information is available on analgesic activity of *Dalbergia sissoo* bark, the present study was under taken to investigate the extraction, phytochemical investigations and analgesic activity of *Dalbergia sissoo* bark**.**

MATERIALS AND METHODS

Collection & authentication of plant material

The bark of *Dalbergia sissoo* was collected from Dehradun, Uttrakand, India. The bark of *Dalbergia sissoo* was collected washed thoroughly with water to remove any unwanted matter. Then dry in the shade, grinded to a course powder with a mechanical grinder and passed through sieve no. 40 and stored in air tight container.

Alcoholic Extraction of bark

A weighed quantity (500 g) of the air dried powdered stem bark of *dalbergia sissoo* was taken and extracted with ethanol (90 %) in a Soxhlet extractor. The ethanolic extract was concentrated in a rotary flash evaporator at a temprature not exceeding 50 ^{0}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, flvanoids, alkaloids, and tannins (14).

Experimental animals

Wistar Rats weighing 200 – 300 g of either sex were maintained under controlled conditions of light (12 hr) and temperature 25 ± 1 ^{0}C in the animals had access to food and water *ad libitum.* All pharmacological activities were carried out as per CPCSEA (Committee for the purpose of control and supervision of experiments on animals) norms 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. Alcohalic extracts at dose of 50, 100, 300, 1000, and 3000 mg/kg body weight were administered to the separate groups of mice (n=5) after over night fasting. Subsequent to administration of drug (bark) extract, the animals were observed closely for the first three hours, for any toxic manifestations, like increased motor activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 hour. The animals were observed for further one weak (15).

Experimental design

30 Wistar rats of either sex were grouped into 6 groups of six animals each. Group-1 received distilled water, which served as a control group. Group- 2 received Aspirin (300 mg/kg) and served as standard group. Group- 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 group. All the drugs were administered orally.

Analgesic Activity

The analgesic potential of the ethanolic bark extract of *Dalbergia 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 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 with in 3-5 sec), the value of the cut off time was considered as latency period for that animal. The time until this reaction occurs is measured. A cut off period 10-12 sec is observed to prevent damage of the tail. Any animal falling to withdrawal its tail 3-5 sec is rejected from the study (17).

Table 1 - Analgesic activity of *Dalbergia sissoo* barks extract:

		Mean Response Time (in seconds)				
Group	Dose	Initial	15 minutes	30 minutes	60 minutes	120 minutes
Normal (Group I)	5 ml/Kg	15.002±0.737	15.126±0.776	15.28± 0.737	15.048± 0.789	15.48± 0.585
Standard (Group2)	300m g/kg	15.068±2.713	26.98±3.663	28.916±4.406	30.856± 4.430	27.98± 3.663
Test – I (Group3)	300 mg/k	16.75 ± 0.867	23.462± 4.666	24.56 ± 4.564	26.274*±3.29	19.378± 2.919
Test-II (Group4)	500 mg/kg	14.02 ± 0.786	24.184± 3.860	26.56 ± 4.964	27.88* ± 5.039	22.142± 3.418
Test-III (Group5)	1000mg/kg	15.982±3.717	25.256±6.335	27.54*±7.55	29.42* ± 9.125	24.520± 5.404

SEM- Standard Error Mean, n = five animals in each group; values are mean ±SEM;
** p < 0.01, when compared to control.*

Statistical Analysis- Statistical Analysis was performed; one way analysis of varience (ANOVA) fallowed by Tukey- Kramer Multiple Comparisons Test. All the values were expressed as mean ± SEM.

RESULTS AND DISCUSSION

The dry weight of the ethanolic bark extract of *Dalbergia sissoo* was found to be (7.14 % w/w). Qualitative phytochemical analysis of the ethanolic bark extract of *Dalbergia sissoo* contained carbohydrates, proteins, amino acids, phenolic compounds and flavanoids. Acute toxicity studies did not reveal any toxic symptoms or death in any of the animal up to the dose level 3000 mg/kg boy weight.

The bark extract of *Dalbergia sissoo* showed significant analgesic activity as evidenced by the increase in reaction time to the pain stimulus. The extract 300 mg/ kg and 500 mg/kg failed to alter pain threshold capacity but increased significantly at the dose of 1000 mg/kg at 30 min. But the results were significant ($p < 0.01$) for analgesic activity at the all doses at 1 h. The analgesic activity of different groups presented in table 1. Asprin significantly increased the pain threshold throughout the observation period of 30 min to 2 h. Test group 5 showed the maximum significant effect amongst all the test groups.

The analgesic activity of Dalbergia sissoo bark extract was study for the peripheral activity (non norcotic). Analgesic activity of bark extract in acute inflammatory pain moderate as compared to the potent inhibitory action of Asprin. Asprin offer relief from pain by suppressing the formation of pain mediator/ substance in the peripheral tissue, where the prostaglandin and bradykynin were suggest to play an important role in the pain process(18-20). Therefore, it is likely to that bark extract of *Dalbergia sissoo might* suppress the preparation of these substances or inhibit the action of these substances and thus exert the analgesic activity. In the present study showed significantly increased the latency period in tail flick method.

Presences of flavanoids were reported in *Dalbergia* species and flavanoids were reported as prostaglandin synthatase inhibitor (21). Since prostaglandins are involve in the pain perception and are inhibit by flavanoids, it could be suggest that reduced availability of the prostaglandin by flavanoids that might be responsible for analgesic activity.

Therefore, the present study demonstrates that *Dalbergia sissoo* bark extract has marked analgesic activity and establishes the effectiveness and pharmacological rationale. The drug may be further explored for its phytochemical profile to identify the exact active constituents for the analgesic 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 showed phytochemical investigation and analgesic activity of the ethanolic bark extract of the Dalbergia sissoo (Roxb.).This activity was related to the dose and this result corroborates the tradition use of the plant in peripheral pain condition.

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