

ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *DALBERGIA SISSOO* (ROXB.) BARK

MOHAMMAD ASIF* AND ARUN KUMAR

Department of Pharmacy, G.R.D (P.G) I.M.T Rajpur, Dehradun-248901, India

The possible anti-inflammatory activity of a 90% ethanolic extract of *Dalbergia sissoo* bark was studied in a model of inflammation using a right hind paw oedema method in Wistar rats. One percent carrageenan in 0.5% sodium carboxymethyl cellulose (CMC) was administered through the sub-plantar region of the right hind paw of the animals. CMC was used as a suspending agent because it does not produce evident changes in activity response. Phytochemical investigation of bark extract showed that it contained carbohydrates, proteins, amino acids, tannins and flavonoids. After oral administration of ethanolic extract at different doses (300, 500 and 1000 mg/kg), inhibition of right hind paw oedema was observed at 30, 60, and 120 min time intervals. The anti-inflammatory effects of the extract were compared with a standard dose of indomethacin (10 mg/kg). In acute toxicity studies, the extract was found to be safe up to 3000 mg/kg, p.o. in the rats. The biological effects increased with increasing doses. The ethanolic extract of *Dalbergia sissoo* bark at 1000 mg/kg showed the most potent anti-inflammatory activity compared to the other groups (300 and 500 mg/kg) throughout the observation period.

Keywords: *Dalbergia sissoo*, Carrageenan, Indomethacin, Paw oedema, Ethanolic extract

INTRODUCTION

Dalbergia sissoo (Rox.), also called Indian Rosewood, belongs to the legume family (Fabaceae). It is a large deciduous perennial tree found in the lowland region throughout India and is also indigenous to Pakistan, Bangladesh, Afghanistan and Nepal. It is used as timber or fire wood and for the treatment of a variety of ailments by different ethnic groups (Nadkarni 1954; Chopra, Nayer and Chopra 1956; Kritikar and Basu 1975; Singh 1982; Prakash and Hocking 1986; Parrota 1989; Sharma, Yelne and Dennis 2001; Ministry of Health & Family Welfare 2001). *Dalbergia sissoo* has also been used in folk medicine as an aphrodisiac, abortifacient, expectorant, antihelminthic, antipyretic, and in the treatment of various digestive disorders and skin diseases (Kirtikar and Basu 1933; Hajare *et al.* 2000). *Dalbergia sissoo* oil has repellent activity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, and is also resistant to some wood boring insects. *Dalbergia sissoo* leaves have been reported to have

*Corresponding author: Mohammad Asif, email: mohd.mpharm@gmail.com

anti-inflammatory activity, analgesic and antipyretic activities (Shakya 2000; Hajare *et al.* 2001; Ram Krishna *et al.* 2001; Shrestha *et al.* 2008).

The bark is 3-5 cm long, either curved or flat, and fibrous. Its external surface is rough with shallow and broad longitudinal fissures. The leaves and trunk exudates of *Dalbergia sissoo* contain various compounds like dalbergenone, dalbergin and methyl dalbergin, 4-phenyl chromene, dalbergichromene (Mukerjee, Saroja and Seshadri 1971; Farag *et al.* 2001). The plant also contains dalbergichromene, nordalbergin and isodalbergin as minor constituents (Goda *et al.* 1985; Gilman and Watson 1993; Ansari *et al.* 2000; Hajare *et al.* 2000; Hajare *et al.* 2001; Brijesh *et al.* 2006; Shrestha *et al.* 2008).

To the best of our knowledge, studies to evaluate the anti-inflammatory activity of the bark extract of *Dalbergia sissoo* have not been reported. We report herein, the details of isolation, and investigation of the phytochemical and anti-inflammatory activities of its ethanolic extract.

METHODS

Collection & Authentication of Plant Material

Dalbergia sissoo bark was collected from Rajpur Road, Dehradun, (UK), India and was authenticated by Dr. H J Chowdhery, Additional Director, Botanical Survey of India, Dehradun (Specimen No. BSD-112166). Fresh samples were dried at room temperature (25°C–30°C) for 7 to 10 days. The bark was crushed and weighed before extraction.

Preparation of Alcoholic Extraction of Stem Bark

A weighed quantity (500 g) of the crushed bark was defatted with petroleum ether and then extracted with ethanol (90%) in a Soxhlet extractor. The ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rotavapour. The extract was dried in order to produce a dark brown solid extract. The dark brown extract was then subjected to various qualitative phytochemical investigations for the identification of the different phytochemical components.

Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extract of *Dalbergia sissoo* was carried out using standard procedures to assess the different types of phytochemical constituents present in the bark of *D. sissoo* using different chemical tests. Screenings were carried out for carbohydrates, glycosides, proteins, amino acids, phytosterols, saponins, flavonoids, alkaloids and tannins (Trease and Evans 1989; Sofowora 1993; Kokate, Purohit and Gokhle 1990).

Test for Reducing Sugars (Fehling's test)

The ethanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction (a purple ring at the junction of two liquids).

Test for Anthraquinones

0.5 g of the extract was boiled with 10 mL of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken in 5 mL of chloroform. The chloroform layer was pipetted into another test tube, and 1 mL of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Terpenoids (Salkowski's test)

2 mL of chloroform was added to 0.5 g of the extract. Concentrated H_2SO_4 (3 mL) was carefully added to form a layer, and the solution was observed for a reddish brown colouration at the interface, which indicated the presence of terpenoids.

Test for Flavonoids

Three methods were used to test for flavonoids. (i) Dilute ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 mL) was then added. A yellow colouration that disappeared on standing indicated the presence of flavonoids. (ii) A few drops of 1% aluminium solution was added to a portion of the filtrate. A yellow colouration indicated the presence of flavonoids. (iii) A portion of the extract was heated with 10 mL of ethyl

acetate over a steam bath for 3 min. The mixture was filtered, and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.

Test for Saponins

5 mL of distilled water was added to 0.5 g of extract in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The froth was mixed with three drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion.

Test for Phenolic Compounds

50 mg of extract was dissolved in distilled water and to this; 3 mL of 10% lead acetate solution was added. Formation of a bulky white precipitate indicated the presence of phenolic compounds (lead acetate test).

50 mg of extract dissolved in 5 mL of distilled water and to this; 2 mL of a 1% solution of gelatin containing 10% sodium chloride was added. The appearance of white precipitates indicated the presence of phenolic compounds (gelatin test).

Test for Tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added, and the solution was observed for brownish green or a blue-black colouration.

Test for Alkaloids

0.5 g of the extract was diluted to 10 mL with acid alcohol, boiled and filtered. 2 mL of dilute ammonia was added to 5 mL of the filtrate, followed by the addition of 5 mL of chloroform. The mixture was shaken gently to extract the alkaloidal base, and the chloroform layer was extracted with 10 mL of acetic acid. The chloroform layer was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for Cardiac Glycosides (Keller-Killiani test)

0.5 g of extract was diluted to 5 mL in water, and 2 mL of glacial acetic acid containing one drop of ferric chloride solution was added to it. 1 mL of concentrated sulphuric acid was added to form a layer, and the colour at the interphase was recorded. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Experimental Design

The study was approved by the Institutional Animal Ethics Committee (IAEC) of GRD Post Graduate Institute of Management and Technology, Dehradun, India.

Studies of Acute Toxicity

Acute toxicity studies were carried out on Wistar rats according to standard procedures. Alcoholic extracts at doses of 50, 100, 300, 1000, and 3000 mg/kg body weight were administered to separate groups of mice (n = 5) after overnight fasting. Subsequent to administration of drug extract, the animals were observed closely for the first 3 h for any toxic manifestations such as increased locomotor activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 h. The animals were observed for a further week (Ghose 2005).

Experimental Animals

Thirty albino Wistar rats of both sexes weighing (200 to 300 g) were maintained under controlled conditions of light (12 h) and temperature $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in the Animal House of GRD (PG) IMT, Dehradun, for two weeks prior to the experiment for acclimatisation. 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.

Experimental Design

Thirty albino rats of either sex were divided into six groups of five rats each. Drugs were administered to all of the groups (control, standard and tests) through the p.o. route, 30 min prior to administration of 1% carrageenan (0.1 mL i.p.) to the subplantar region of the right hind paw. CMC did not produce evident changes in activity response.

Group-I (control group)

Sodium carboxyl methyl cellulose (0.5% CMC) in distilled water at 10 mL/kg body weight.

Group-II (standard group)

Indomethacin (10mg/kg) suspension in 0.5% sodium CMC to serve as a standard drug at 10 mL/kg body weight.

Groups-III, IV and V (Test groups)

Ethanollic extract (test drug) suspension in 0.5% sodium CMC (300, 500, 1000 mg/kg, respectively) at 10 mL/kg. Doses of *Dalbergia sissoo* leaves were used as an anti-inflammatory up to 1000 mg/kg p.o. (Hajare *et al.* 2001), and 0.1 mL of 1% carrageenan in 0.9% NaCl was administered into the subplantar surface of the right hind paw of the animals (Winter, Risely and Nuss 1962).

Pharmacological Evaluation

Anti-inflammatory activity

The animals were divided into six groups and were housed individually in polypropylene cages. The anti-inflammatory potential of the ethanolic extract of *Dalbergia sissoo* bark was assessed by the carrageenan-induced right hind paw oedema method. The ethanolic bark extract was given at three different doses, 300, 500 and 1000 mg/kg, to different groups 30 min prior to administration of carrageenan (0.1 mL of 1%, i.p.) in the subplantar region of right hind paw. For evaluation of an anti-inflammatory effect, the paw volume was measured 15, 30, 60 and 120 min after administration of drugs. The percent inhibition of the oedema formed was calculated with comparison to the control group (0.5 % Sodium CMC, 10 mL/kg) after 60 and 120 min. (Leticia *et al.* 2004;

Tijani, Uguru and Salawu 2008; Rajendran and Lakshmi 2008). The anti-inflammatory activity was determined as the percent inhibition of the oedema formed after two hours of carrageenan administration. The percent inhibition was calculated using the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Mean paw inflammation of control} - \text{Mean paw inflammation of test}}{\text{Mean paw inflammation of control}}$$

Procedure

The animals were fasted for 24 h prior to the experiment. A mark was made on the right hind paw just beyond the tibio-tarsal junction to ensure that the paw volume, as measured with a plethysmograph, was measured consistently every time it was dipped in the mercury (Hg) column up to the fixed mark. The initial volume was noted for each rat by the mercury (Hg) displacement method. After 15, 30, 60 and 120 min of carrageenan administration, the paw volumes of all groups were measured using a plethysmograph.

Statistical evaluation

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. All values are expressed as the mean \pm SEM.

RESULTS AND DISCUSSION

After the extraction of stem bark, 7.14% w/w yield of dry extract was obtained. Acute toxicity studies did not reveal any toxic symptoms or death in any of the animals up to the dose of 3000 mg/kg body weight of the ethanolic bark extract. Preliminary phytochemical screening showed that the alcoholic extract of *Dalbergia sissoo* contains carbohydrates, proteins, amino acids, tannins and flavonoids.

The ethanolic bark extract of *Dalbergia sissoo* showed anti-inflammatory activity compared to indomethacin (Table 1). Alcoholic bark extract of *Dalbergia sissoo* possessed anti-inflammatory activity after 15, 30 and 60 min at the 1000 mg/kg dose. A dose of 1000 mg/kg elicited a greater percent inhibition of inflammation after 60 and 120 min than

Table 1: Anti-inflammatory activity of the ethanolic extract of *Dalbergia sissoo* bark.

Groups	Dose	Change In Mean Paw Volume (mL)			
		15 min	30 min	60 min	120 min
Control (Group I)	10 ml/Kg	0.40 ± 0.029	0.41 ± 0.029	0.41 ± 0.029	0.43 ± 0.070
Standard (Group II)	10 mg/kg	0.25 ± 0.009	0.292 ± 0.047	0.314 ± 0.024	0.344 ± 0.076
Test I (Group III)	300 mg/kg	0.242 ± 0.007***	0.32 ± 0.004	0.394 ± 0.003	0.40 ± 0.035
Test II (Group IV)	500 mg/kg	0.222 ± 0.008***	0.302 ± 0.018	0.368 ± 0.004	0.368 ± 0.004
Test III (Group V)	1000 mg/kg	0.22 ± 0.009***	0.294 ± 0.008*	0.32 ± 0.008*	0.33 ± 0.008

SEM - Standard Error Mean, n: five animals in each group;

*** p < 0.001 and * p < 0.05 compared to control.

other groups (Table 2). These results showed that test drug at the dose level of 1000 mg/kg have the most potent anti-inflammatory activity. *Dalbergia sissoo* leaves have been used as an anti-inflammatory, analgesic and antipyretic in doses up to 1000 mg/kg p.o. At this dose level, the leaves produced a significant inhibition of carrageenan-, kaolin- and nystatin-induced paw oedema. It was also devoid of ulcerogenic effects on the gastric mucosa of rats in acute and chronic tests (Hajare *et al.* 2000; Hajare *et al.* 2001).

Drugs from plant sources have been used for the treatment of various diseases since ancient times. Nowadays, the use of herbal drugs to cure inflammation and pain is gaining popularity due to their effectiveness, fewer side effects, low cost and availability. Non-steroidal anti-inflammatory drugs act by inhibiting cyclooxygenase and the production of prostaglandins. Indomethacin offers relief from inflammation by suppressing the production of prostaglandins and bradykinin. The presence of flavonoids has been reported in *Dalbergia* species, and flavonoids are known to inhibit prostaglandin synthetase (Hirose *et al.* 1984; Goda *et al.* 1985). Previous studies have shown similar relationships between flavonoids and anti-inflammatory effects (Martini, Katerere and Eloff 2004; Toker *et al.* 2004). Therefore, it is possible that the anti-inflammatory action of *Dalbergia sissoo* bark extract may be related to

Table 2: Percent inhibition of inflammation in various groups.

S. no.	Groups	Inhibition after 15 min (%)	Inhibition after 30 min (%)	Inhibition after 60 min (%)	Inhibition after 120 min (%)
1	Group II (Standard 10 mg/kg)	37.5 %	29.26 %	24.39 %	20.93 %
2	Group III (Extract 300 mg/kg)	40.0 %	21.95 %	4.87 %	6.97 %
3	Group IV (Extract 500 mg/kg)	45.0 %	26.82 %	12.19 %	16.27 %
4	Group V (Extract 1000 mg/kg)	45.0 %	29.26 %	21.95 %	23.25 %

the inhibition of prostaglandin synthesis. The phytochemical profile of *Dalbergia sissoo* may be explored further to identify the active constituents responsible for its anti-inflammatory activity.

CONCLUSION

The active extract of *Dalbergia sissoo* bark contained carbohydrates, phenolic compounds, flavonoids and tannins. Its ethanolic extract at a dose of 1000 mg/kg had the most potent anti-inflammatory activity throughout the observation period. Our results showed that *Dalbergia sissoo* has the potential to be developed as an anti-inflammatory agent.

REFERENCES

- ANSARI, M. A., RAZDAN, R. K., MAMTA, T. & VASUDEVAN, P. (2000) Larvicidal and repellent actions of *Dalbergia sissoo* Roxb. (F. Leguminosae) oil against mosquitoes. *Bioresource Technology*, 73: 207-211.
- BRIJESH, S., DASWANI, P. G., TETALI, N. P, ANTIA, H & BIRDI, T. J.(2006). Studies on *Dalbergia sissoo* (Roxb.) leaves: possible mechanism(s) of action in infectious diarrhea, *Indian Journal Pharmacology*, 38: 120-124.

- CHOPRA, R. N., NAYER, S. L., & CHOPRA, I. C. (1956). *Glossary of Indian Medicinal Plants*, pp 90-91 (New Delhi: CSIR).
- FARAG, S. F., AHMED, A. S., TERASHIMA, K., TAKAYA, Y. & NIWA, M. (2001) Isoflavonoid glycosides from *Dalbergia sissoo*, *Phytochemistry*, 57: 1263-8
- GHOSE, M. N. (2005). *Fundamental of Experimental Pharmacology*, 3rd edition (Kolkata: Hilton & Company).
- GILMAN, E. F. & WATSON, D. G. (1993) *Dalbergia sissoo: Indian Rosewood*. Fact Sheet ST-227. November. Environmental Horticulture Department, Florida Cooperative Extension Service, (University of Florida: Institute of Food and Agricultural Sciences).
- GODA, Y., KATAYAMA, M., ICHIKAWA, K., SHIBUYA, M. & KIUCHI, F. (1985). Inhibition of prostaglandin biosynthesis from *Dalbergia odorifera*, *Chemical & Pharmaceutical Bulletin*, 33: 5606-5609.
- HAJARE, S. W., CHANDRA, S., TANDAN, S. K., SHARMA, J., LAL, J. & TELANG, A. G. (2000). Analgesic and antipyretic activities of *Dalbergia sissoo* leaves, *Indian Journal of Pharmacology*, 32: 357-60.
- HAJARE, S. W., CHANDRA, S., SHARMA, J., TANDON, S. K. & TELONG, A.G. (2001). Anti-inflammatory activity of *Dalbergia sissoo* leaves, *Fitoterapia*, 72: 131-139.
- HIROSE, K., JYOYAMA, H., KOJIMA, Y., EIGYO, M., HATAKEYAMA, H., ASANUMA, F., UMEHARA, H. & YAMGUCHI, T (1984). Pharmacological properties of 2-[4,4-(2triazolyloxy)-phenyl propionic acid (480156-5)], a new non-steroidal anti-inflammatory agent, *Arzneimittelforschung - Drug Research*, 34: 280-386.
- KIRTIKAR, K. R., & BASU, B. D. (1933). *Indian Medicinal Plants*, 2nd edition, Vol 1, pp. 818-819 (Allahabadi: Lahit Mohon Basu).
- KOKATE, C.K., PUROHIT, A.P. & GOKHLE, S.B. (1990) (Eds.). *Pharmacognosy*, 1st edition, pp 178-181 (Pune: Nirali Prakashan).
- KRITIKAR, K. R. & BASU, B. D. (1975). *Indian Medicinal Plants*, 2nd edition., Vol 4. (New Delhi: Jayyed Press).
- LETÍCIA, V. C., DAVID, F. L., MANOEL, A., JOSÉ, N. S. B., LUZIA, K. A. M. L., FRANCISCA, C. L. F. S., & GLAUCE, S. B. V. (2004). Analgesic, antiinflammatory and central depressor effects of the hydroalcoholic extract and fractions from *Aeolanthus suaveolens*, *Biological & Pharmaceutical Bulletin*, 27: 821-824.
- MARTINI, N. D., KATERERE, D. R. P. & ELOFF, J. N. (2004). Biological activity of five antibacterial flavanoids from *Combretum erythrophyllum* (Combretaceae), *Jornal of Ethnopharmacology*, 93: 207-212.

- MINISTRY OF HEALTH & FAMILY WELFARE (2001). *The Ayurvedic Pharmacopoeia of India*. Part - I, Volume - III. Department of Indian Systems of Medicine & Homeopathy. (New Delhi: Govt. of India).
- MUKERJEE, S. K. SAROJA, T. & SESHADRI, T. R. (1971). Dalbergichromene a new neoflavonoid from stem-bark and heartwood of *Dalbergia sissoo*, *Tetrahedron*, 27: 799 - 803.
- NADKARNI, K. M. (1954). *Indian Materia Medica*, 3rd edition, Vol. 1. pp. 432 (Bombay: Popular Book Depot).
- PARROTA, J. A. (1989). *Sissoo, Indian Rosewood*. (New Delhi: Oxford and IBH Publishing Company).
- PRAKASH, R. & HOCKING, D. (1986). *Some favourite trees for fuel and fodder*, (New Delhi, India: Society for Promotion of Wastelands Development).
- RAJENDRAN, V. & LAKSHMI, K. S. (2008). In vitro and In vivo anti-inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp *laurina*, *Bangladesh Journal of Pharmacology*, 3: 121-124.
- RAM KRISHNA, N. V. S., KUMAR, E. K. S., KULKARNI, A. S., JAIN, A. K., BHAT, R. G., PRIK, S, QUADROS, A., DEUSKAR, N. & KALOKOI, B. S. (2001). *Indian Journal of Chemistry*, 40B: 539-540.
- SHAKYA, P. R. (2000). IN: *Intellectual Heritage on Folk Medicine in Nepal: Proceedings of Nepal- Japan Joint Symposium*, Kathmandu, Nepal, November 6-11, pp 43-49.
- SHARMA P. C, YELNE, M. B. & DENNIS, T. J. (2001). *Database on Medicinal Plants Used in Ayurveda*, pp. 481-489, (New Delhi: Central Council for Research in Ayurveda and Siddha).
- SINGH, R. V. (1982) *Fodder trees of India*. (New Delhi: Oxford and IBH Publishing Company).
- SOFOWORA, A. (1993). *Medicinal plants and Traditional Medicine in Africa*, pp. 150 (Ibadan: Spectrum Books).
- SHRESTHA, S. P., AMANO, Y., NARUKAWA, Y., & TAKEDA, T. (2008) Nitric oxide production inhibitory activity of flavonoids contained in trunk exudates of *Dalbergia sissoo*, *Journal of Natural Products*, 71: 98-10.
- TIJANI, A. Y., UGURU, M. O. & SALAWU, O. A. (2008). Anti-pyretic, anti-inflammatory and anti-diarrhoeal properties of *Faidherbia albida* in rats, *African Journal of Biotechnology*, 7: 696-700.
- TOKER, G., KUPELI, E., MEMISOOLU, M. & YESILADA, E. (2004). Flavanoids with antinociceptive and antiinflammatory activities from the leaves of *Tilia argentea* (Silver linden), *Journal of Ethnopharmacology*, 95: 393-397.

TREASE, G. E. & EVANS, W. C. (1989). *Pharmacognosy*, 13th edition, pp. 176–180 (London: Bailliere Tindall).

WINTER, C. A., RISELY, E. A. & NUSS, G. W. (1962). Carrageenan-induced oedema in hind paw of rat as an assay of anti-inflammatory drugs, *Experimental Biology and Medicine*, 111:19–26.