Anti-inflammatory activity of Dalbergia sissoo Roxb. Heart-wood

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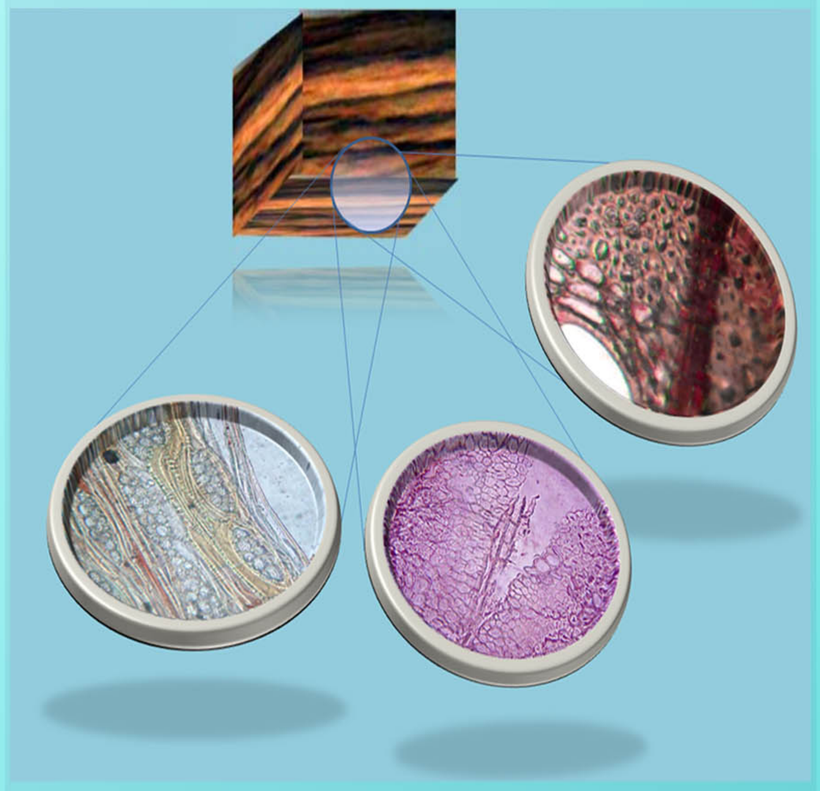
Abstract

Dalbergia sissoo Roxb. is a widely used timber tree growing through out India. The heart wood of the same is reported to be used in several ailments. It is also employed as an anti-inflammatory agent by the traditional healers and the mention of the same can also be traced back to ancient texts. The influence of the drug on carrageenan induced rat paw edema was studied. The drug was found to effective by different angles of examinations. Overall, it can be said that the heart-wood of Dalbergia sissoo Roxb. possesses anti-inflammatory activity.



Introduction

Since time immemorial higher plants used as sources of medicinal compounds continue to play a dominant role in maintenance of human health.

Archaeological discoveries of Neanderthal burial grounds in Iraq, pointed out the use of several plants like Marshmallow, yarrow etc., that still figure in folk medicine (Solecki, 1975).

Traditional medicine is a comprehensive term and has a long history: It is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses.

The World Health Organization has estimated that at least 80 percent of the worlds population relies mainly, if not totally, on traditional medicines (Anonymous, 2000). Over 50% of all modern clinical drugs are of natural product origin (Dietrich, 1971) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker et al, 1995 & Cordell, 1995). In developing countries, people usually turn to traditional healers when in diseased conditions and plants of ethnobotanical origin are often presented for the use.

Dalbergia sissoo Roxb. belonging to the family of Papilionaceae (Fabaceae) is a well known medium-sized deciduous timber tree, generally found to be present in north Indian hills from Punjab to Assam and also commercially cultivated in entire India. The heart wood has concentric bands of annular rings and is hard and dark brown in color. The heart wood is known to have mention in medicinal traditional practices. It is reported to possess or used against astringent, anti-inflammatory, expectorant, anti-emetic, skin diseases, leprosy, leucoderma, ulcers, bronchitis and gout (Chunekar & Pandey, 1999) like medicinal properties. The heart-wood is also known to be abortifacient (Karnick & Hocking) and anthelminthic (Manandhar; 1995).

Presently, many scientists and organizations are in search o f traditional remedies as alternate medicines. There are challenges ahead for researchers in traditional medicinal practices for validating the claims respective regime in the light of the modern scientific knowledge and understanding therapy, to make the system globally acceptable. This requires a highly integrated approach that combines the best of the traditional wisdom and modern scientific knowledge and expertise. These can be addressed by rigorous application of scientific principles in high quality basic and clinical research, following Reverse Pharmacology path. With the increased demand for scientifically validated (for their efficacy and safety) and standardized herbal product, there is a need for better understanding of molecular mechanisms underlying their biological activity. The influence of the drug on carrageenan induced rat paw edema was studied in detail

Materials and methods

Plant identification

The correct identity and authenticity of the plant was confirmed by studying their morphological characters and comparing them with the characters mentioned in literature. Flowering twigs of both of these were collected, herbarium specimens prepared and deposited at the department museum for further documentation.

Collection and preparation of the heart-wood

The heart-wood was collected from healthy, wild trees just before flowering season from Gandhinagar, Gujarat, India, during the flowering season, in the month of June, 2008. The heart-wood was chopped in small pieces of 10 to 20 cm x 2 to 5 cm. The same was then shade dried and powdered and passed through 60# mesh size.

Extraction of the heart-wood

Powdered heart-wood was weighed (100 gm) and taken in 2 L round-bottom flask and filled with 500 ml methanol (AR grade; SD Fine Chemicals). It was then continuously extracted by refluxing on boiling water-bath 4 h. The mixture was filtered and the mark was re-extracted with methanol by the same procedure. This protocol was repeated till the mark was exhaustively extracted. The resultant extracts were pooled and concentrated under vacuum on a rota-evaporator to leave behind the residue. This process gave reddish-brown free flowing solid residue (methanol extract of Dalbergia sissoo: MED) with a yield of 35% w/w.

Animals

Albino rats of Wistar strain (150-250 g) of either sex were used for the present study. Animals were conditioned in standard polypropelene cages (group of six rats/cage) at 20-25˚C, maintained on standard pellet diet and water ad libitum. They were kept in 12 h light and dark cycle. This experiment complied with the guidelines of our laboratory for animal experimentation.

Drug administration and acute toxcity

MED was dissolved in sterile distilled pyrogen-free water to obtain 100, 300 and 500 mg/kg of body weight. After oral treatment the animals were continuously observed for 1h for overt signs of acute clinical toxicity (Lachrymation, salivation, diarrhea) or stress (exopthalmia, fur erection).

Group No.	Group Specification
1	Control (Vehicle only)
2	MeOH Ext. (100 mg/kg)
3	MeOH Ext. (300 mg/kg)
4	MeOH Ext. (500 mg/kg)

As tabulated below, each group consisting of six rats were starved overnight. All groups were administered respective drugs by oral route. One hour later, the rats were challenged by a subcutaneous injection of 0.5 ml of 0.1% solution of carrageenan (in normal saline), into the plantar side of the left hind paw. The paw volume was measured using a Vernier caliper before the injection and after the injection for 5 hours at 1h interval gap. Each observation was repeated thrice and mean of these observations was considered (Winter et al., 1962). The average foot swelling in test as well as standard groups was compared with that of the control group and the % edema was calculated by using the formula:

$$\% \text{ Reduction of Edema} = \frac{(vt - v0)_{ctrl} - (vt - v0)_{tre}}{(vt - v0)_{ctrl}} \times 100$$

Where, Vt = mean edema at 1 to 5th hour (in cm).

V0= mean edema just before carrageenan injection (in cm).

Results are presented as mean(s) ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Boneferani t-test against control. Data were considered statistically significant at P value </= 0.05 and highly significant at P? 0.001. The Area Under the Curve (AUC) was calculated from the mean edema.

Result & Discussion

The drug showed no overt signs of signs of acute toxicity during the frame of the study.

The carrageenan-induced paw edema test is widely accepted as a sensitive phlogistic tool for investigating potential anti-inflammatory agents. Particularly the non-steroidal type (Vinegar et al., 1969). Anti-inflammatory activity of methanolic extract against carrageenan induced rat paw edema was found to be inversely related to the dose. The mean paw edema in cm and SEM (Fig.-1) are depicted below (Table-1), for the control group normal saline was used. Percentage reduction of edema across all 5 hours together is given by the percentage reduction in AUC (Table 2). The control animals witnessed rise in edema culminating from 1st hour to 2nd hour, which showed minor decrease up till 4th hour surfacing back increasing to a level equal to the 1st hour. This shows a biphasic response typical to the current phlogistic agent engaged. The phases are separated by roles of amines followed by prostaglandins. Extension of this phase is known to generate chronic inflammation. During the 1st hour MED at 300 mg/kg showed an insignificant increase in edema as compared to control, whereas the rest showed minor decrease in edema. The scenario changed in the 2nd hour, 500 mg/kg (25%; p insignificant) showed lowest activity but 100 mg/kg showed maximum inhibition (48%; $p < 0.001$). At the successive hour the pattern remained same though the difference between the doses was lessened. In the 4th hour due to reduction in edema in control animals the stagnated inhibition of drug at all doses showed up to a lesser extent, except 100 mg/kg, which seemed to lose its effect. The latter regained activity at the last hour drastically (81.45%; $p = 0.001$), in comparison to the control and doses at 500 & 300 mg/kg. The above pattern of dose level and activity relates to possible role of anti-histaminic activity reported in the first phase. The interplay of kinins connecting both the phases is less affected by the drug. Followed by this, the prostaglandin phase is effectively inhibited at lowest dose. At higher dose compounds in lower concentration but with nonspecific potent affinity may be interfering, which are diluted out at lower dose by five times. Due to this those phyto-constituents present in higher concentrations will be more effective at lower doses, and hence may be attributed to anti-inflammatory activity.

TABLE-1 % Inhibition of edema

Time (hour) / Dose (mg/kg)	1	2	3	4	5
100	18.39	48.91	62.06	23.91	81.44
300	-6.51	44.27	55.10	36.08	32.81
500	8.04	25.69	48.91	23.04	41.41

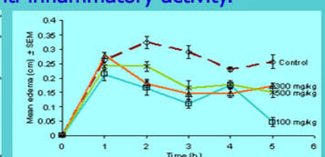
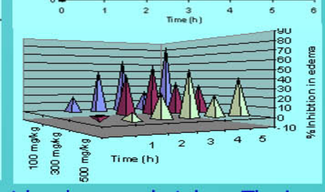


TABLE-2

Dose (mg/kg)	AUC of edema	% Reduction in AUC
Control	1.23	-
100	0.67	44.25
300	0.84	32.14
500	0.89	27.19



In general, less pharmacological work on this plant has been undertaken. The incumbent assay yielded the tested drug to have some anti-inflammatory potential. Attempts to elucidate the mechanism of action of these drugs showed that they inhibit a wide variety of reactions. Important among them were the theories that the anti-inflammatory activity may be produced by modulation of their activity (Zurier and Krakauer, 1979). Currently it is widely accepted that the NSAIDs produce anti-inflammatory activity generally, but not exclusively, through inhibition of prostaglandin synthesis. Vane, (1971) showed that NSAIDs were potent inhibitors of prostaglandin synthesis that cause vasodilation and potentiate the inflammatory effects of other mediators like histamine and bradykinin. NSAIDs do not generally inhibit the formation of leukotrienes, which also contribute to inflammation. These reports focus on the fact that inflammation is a multifactor mediated process and several mechanisms operate during its introduction (Zurier and Krakaue, 1979). Few chemical constituents are been reported to be present in this plant. Some of them are dalbergiphenol (Kulshrestha et al., 1974), sissoidenone, liquiritigenin, isoliquiritigenin, dalbergin, dalbergenone, latifolin (Ramakrishna et al., 2001), stilbene,3-5-dihydroxy-trans-benzenoid (Soni, 1975). Latifolin isolated from the methylene chloride extract of its heartwood was found to exhibit the inhibition of beta-amyloid synthesis with an IC50 of 180 µM (Ramakrishna et al., 2001).

Conclusion

The MED at 100 mg/kg was found to exhibit anti-inflammatory activity in both phases of inflammation. The second phase was most efficiently inhibited with almost double the activity for this dose. Thus the traditional claims for MED heartwood can be said to true to an extent but is needed to be investigated in detail.

References

1. Anonymous, (2000) General guidelines for methodologies on research and evaluation of traditional medicine, World Health Organization, Geneva.
2. Baker JT, Borris RP, Carte B, Cordell GA, Soejarto DD, Cragg GM, Gupta MP, Iwu MM, Madulid DR and Tyler VE (1995): Natural product drug discovery and development: New perspective on international collaboration. J Nat Prod 58: 1325-1357.
3. Chunekar KC, Pandey GS, Bhāvprakāsh Nighantu (Indian Materia Medica) of Shrī Bhāvamiśra. Varanasi: Chaukhambha Bharati Academy; 1999; p. 105.
4. Cordell GA (1995): Changing strategies in natural products chemistry. Phytohemistry 40: 1585-1612.
5. Dietrich B (1971): Indian Trees. Bishen Singh, Mahendra Pal Singh, Deharadun. pp. 496.
6. Karnick CR, Hocking GM. Ethnobotanical records of drug plants described in valmiki ramayana and their uses in the ayurvedic system of medicine. Q J Crude Drug Res 13 : 143- (1975).
7. Kulshrestha SK, Mukerjee S. K., Seshadri T. R. Dalbergiphenol a new constituent of the heart wood of Dalbergia sissoo Indian Journal of Chemistry. 1974; 12(1): 10-14.
8. Manandhar NP. Medicinal folk-lore about the plants used as anthelmintic agents in Nepal. Fitoterapia 66 2: 149-155 (1995).
9. Ramakrishna, N.V.S.; Vijay Kumar, E.K.S.;Kulkarni, A.S.; Jain, A.K.; Bhat, R.G.; Parikh, S.; Quadros, A.;Deuskar, N.; Kalakoti, B.S. Screening of natural products for new leadsas inhibitors of beta-amyloid production: latifolin from Dalbergia sossoo .Indian Journal of Chemistry v. 40B(6): p. 539-540, 2001.
10. Solecki, (1975) Shanidar 4, a Neanderthal flower burial in northern Iraq, Science, 190, PP. 880-881.
11. Soni Pl. The chemistry of extracts of dalbergia sissoo part i. The occurrence of 3,5-dihydroxy-trans-stilene in the heartwood. J Indian Acad Wood Sci 6: 57-(1975).
12. Vane, J.R. (1972) Prostaglandin In: "inflammation: mechanisms and control." Lepons, I.H., Ward, P.A. (ed.) Academic Press. London, PP. 261-279.
13. Vinegar, R., Schreiber, W., Hugo, R., (1969) Biphasic development of carrageenan edema in rats, J. Pharmacol. Exp. Ther., 166(56):PP. 96-103.
14. Winter CA, Risley EA, Nuss GW (1962): Carrageenan induced edema in hind paw of the rat as an assay of anti-inflammatory drugs. Proc Soc Exp Biol Med 111: 544-547.
15. Zurier RB, Krakaure K (1979) In: Inflammation - Hand Book of Experimental Pharmacology, Ferreira S.H, Vane J.R eds., Springer-Verlag, Berlin, pp. 297.