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ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *DALBERGIA SISSOO* ROXB. HEARTWOOD

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ABSTRACT: *Dalbergia sissoo* Roxb. is a extensively used as timber growing throughout India. Its heartwood is found to be used in several ailments. The same is employed as an anti-inflammatory agent by various traditional healers and its use is also mentioned in the number of ancient texts. The influence of the drug on carrageenan induced rat paw edema was studied. The drug was found effective by different perspectives of examinations proving its 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 [1]. 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 terms complementary or alternative or non-conventional medicine are used interchangeably with traditional medicine in some countries [2].

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More than 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs of the pharmaceutical industry [3-5]. In developing countries, especially in rural contexts, people usually turn to traditional healers when in diseased conditions and plants of ethnobotanical origin are often presented for use. *Dalbergia sissoo* Roxb. belonging to the family of Papillionaceae (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 heartwood has concentric bands of annular rings and is hard and dark brown in color. The heartwood is used as an alternative, in leprosy, boils, eruptions, cutaneous affections and to allay vomiting [6, 7]. It is reported to possess astringent, anti-inflammatory, expectorant, abortifacient and anthelminthic activities [7-9].

But, there are challenges ahead for researchers in traditional medicinal practices for validating the claims in the light of the modern scientific knowledge and understanding of modern therapeutics to make the traditional-practice globally acceptable. In correlation to this, the influence of the drug on carrageenan induced rat paw edema was studied in detail, in order to verify the claims of the traditional practices.

MATERIALS AND METHODS:

Plant Material: The heartwood was collected from healthy, wild trees just before flowering season from Gandhinagar, Gujarat, India, during the flowering season and authenticated by the taxonomist of Gujarat University. Its herbarium specimen was prepared and deposited at the author's department for further documentation.

Extraction of the Heartwood: The heartwood was chopped in small pieces of 10 to 20cm x 2 to 5cm. The same was then shade dried and powdered and passed through 60# mesh size. Powdered heartwood (100g) was exhaustively extracted with 500ml methanol (AR grade; SD Fine Chemicals) by refluxing on boiling water bath. The resultant extracts were pooled and concentrated under vacuum using 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 (150-250g) 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 12h light and dark cycle. This experiment complied with the guidelines of our laboratory for the animal ethical committee.

Drug Administration and Acute Toxicity: MED was dissolved in sterile distilled pyrogen free water. Animals were divided into four groups. The animals administered the dose of 100, 300 and 500mg/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). Each group consisting of six rats were starved overnight. Animals were administered respective drugs by oral route. One hour later, the rats were challenged by a subcutaneous injection of 0.5ml 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 [10]. 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:

$$\% \text{ Reduction of Edema} = [(V_t - V_0)_{ctrl} - (V_t - V_0)_{tre}] \times 100 / (V_t - V_0)_{ctrl}$$

Where, V_t is mean edema at 1 to 5th hour (in cm); V_0 is 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.

RESULTS AND DISCUSSION: The drug showed no overt 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 [11]. 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 are depicted below **Fig. 1**, for the control group normal saline was used.

Percentage inhibition **Table 1** and reduction of edema across all 5 hours altogether is given by the percentage reduction in AUC **Table 2.** The control animals witnessed rise in edema from 1st hour culminating 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 300mg/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, 500mg/kg (25%; p insignificant) showed lowest activity but 100mg/kg showed maximum inhibition (48%; $p < 0.001$). At successive hours the pattern remained the 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 100mg/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 and 300mg/kg. The above pattern of dose level and activity relates to the 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. The standard drug, valdecoxib at 10mg/kg showed significant decrease in edema visible in all quarters of time prolific in the 5th hour. The area under curve of standard was 33.525, exhibiting a 33.24% reduction in AUC.

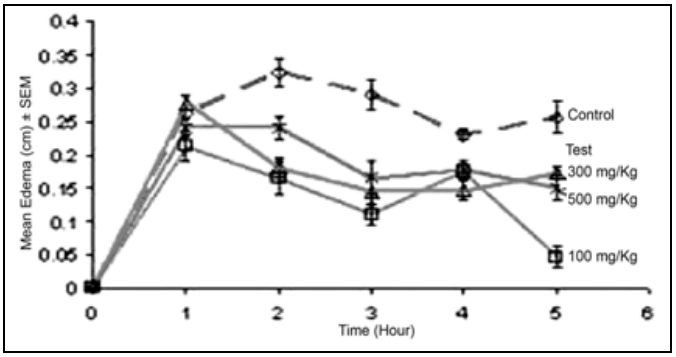

FIG. 1: MEAN RAT PAW EDEMA (± SEM) AT 100, 300 AND 500MG/KG PO MED AFTER CARRAGEENAN CHALLENGE BETWEEN 1ST AND 5TH HOUR

TABLE 1: % INHIBITION OF EDEMA

Drug	Time (h)	1	2	3	4	5
MED	100mg/kg	18.39	48.91	62.06	23.91	81.44
	300mg/kg	-6.51	44.27	55.10	36.08	32.81
	500mg/kg	8.04	25.69	48.91	23.04	41.41
Valdecoxib	10mg/kg	8.82	52.8	77.67	89.72	96.3

TABLE 2: % REDUCTION IN AUC USING METHANOLIC EXTRACT OF *D. SISSOO*

Drug	Dose (mg/kg)	AUC of edema	% Reduction in AUC
Control		1.23	-
MED	100	0.67	45.5
	300	0.84	31.7
	500	0.89	27.6
Valdecoxib	10mg/kg	0.845	31.3

In general, less pharmacological work on this plant has been undertaken. The 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 including the anti-inflammatory activity which may be produced by modulation of their activity [12]. Currently it is widely accepted that NSAIDs produce anti-inflammatory activity generally, but not exclusively, through inhibition of prostaglandin synthesis. Vane showed that NSAIDs were potent inhibitors of prostaglandin synthesis that cause vasodilation and potentiate the inflammatory effects of other mediators like histamine and bradykinins [13]. NSAIDs do not generally inhibit the formation of leukotrienes, which also contributes to inflammation. These reports focus on the fact that inflammation is a multifactor mediated process, and several mechanisms operate during its introduction [12]. Few chemical constituents like dalbergiphenol, sissoidenone, liquiritigenin, isoliquiritigenin,

dalbergin, dalbergenone, latifolin, dalbergi-chromene 3-5-dihydroxy-trans- stilbene, biochanin A are reported to be present in this plant[14-17]. Latifolin isolated from the methylene chloride extract of its heartwood was found to exhibit the inhibition of beta-amyloid synthesis with an IC_{50} of 180µM [15].

The MED at 100mg/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 a better understanding of the underlying mode of action would require further investigation.

CONCLUSION: The MED when tested at three different doses (100mg/kg, 300mg/kg and 500mg/kg) was found to exhibit anti-inflammatory activity at dose of 100mg/kg 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 a better understanding of the underlying mode of action would require further investigation.

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