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Evaluation of Anti-Inflammatory Activity of Ethanolic extracts of *Dalbergia sissoo* (ROXB.) Leaves, Pods and Bark

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ABSTRACT

The anti-inflammatory activity of ethanolic extract of *Dalbergia sissoo* leaves, pods and bark was studied in a model of inflammation using a right hind paw edema method in Albino 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 leaves, pods and 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 - edema 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 anti-inflammatory studies, the extract was found to be safe up to 3000 mg/kg. In the Albino Wistar rats. The biological effects increased with increasing doses. The ethanolic extract of *Dalbergia sissoo* bark (DSEB) 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 - edema, Ethanolic extract.

INTRODUCTION

Dalbergia sissoo (Roxb.) belongs to Fabaceae plant family which is native to India and had been long cultivated in Egypt as shade tree on the banks of irrigation canals. It is large deciduous tree; often with crooked trunk and light crown. Under favorable conditions the tree attains a height of about 100 ft and girth up to 8 ft. It grows well on porous soils containing sand, pebbles and boulders. It reaches its finest development in the river and rain tract. *Dalbergia sissoo* includes many members which are broadly used in folk medicine for several diseases [1]. Current literature reveals that the isolation of several compounds of confirmed biological activity such as flavones, isoflavones, quinines and coumarins from *Dalbergia sissoo*. It also contains tectoridin, caviunin-7-*O*-glucosides isocaviunin isoflavones, tectorigenin, dalbergin, biochanin-A, and 7-hydroxy-4-methylcoumarin [2]. The heartwood gave 3,5-dihydroxy-trans-stibene, biochanin A, dalbergichromene, dalbergenone and iso-dalbergin. The concentrated extract of heartwood in milk was prescribed in fevers; extract of leaves in jaundice, bark extract is used as anti-inflammatory agent in piles, sciatica, and as blood purifier. The oil was used externally in the skin diseases and infected ulcers [3]. It has been reported the isolation of several compounds so there is need to confirmed biological activity for *Dalbergia sissoo* leaves, pods, bark- heart wood and sap wood. *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. *Dalbergia sissoo* oil has repellent activity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, and is also resistant to some wood boring insects [4]. *Dalbergia sissoo* leaves have been reported to have anti-inflammatory activity, analgesic and antipyretic activities. 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 [5-6].

MATERIALS AND METHODS

a. Collection of plant material and preparation of extract:

Dalbergia sissoo leaves, pods, and bark were collected from Bhowhari near Bairagarh Bhopal. These were placed in plastic air tight bags and washed by fresh and distilled water. These were powdered and extracted with 95 % ethanol and 5% distilled water by heating under reflux. The ethanolic extract was concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (40°C–50°C). The concentrated ethanolic extract so obtained were suspended in distilled water and fractionated by the light petroleum ether, chloroform and acetic acid [8]. The collected fractions were separately concentrated to yield the reddish brown semi solid residue [9]. The obtained residue was subsequently washed with chloroform and ethyl acetate to get the separated greenish amorphous solid material. [10].

b. Experimental animals

Albino Wistar rats weighing (150-200 g) were used for studying anti-inflammatory activity. Animals were maintained under standard laboratory conditions. Protocol for study was approved by the institutional Animal Ethics Committee (IAEC) [11]. Albino Wistar rats were taken from Laboratory Animal Resource Section. The animals were kept in polypropylene cages and maintained on balanced ration-standard diet with free access to clean water regularly. Animals were further divided into eight groups with four animals in each group. The route of administration of drug was orally [12]. The extracts of *Dalbergia sissoo* used in the doses of 300, 500 and 1000 mg/kg orally in the study test, standard drug used was Indomethacin (20mg/kg), the vehicle drug used was CMC (5ml/kg) were used for comparing Anti-inflammatory effect [13]. Four animals were used in each treatment group. First group was administered with vehicle CMC; second group with standard drug Indomethacin (20mg/kg); third group with ethanolic extract of *D.sissoo* leaves (DSEL-1) 300mg/kg; fourth group ethanolic extract of *D.sissoo* leaves (DSEL-2) 500mg/kg; fifth group ethanolic extract of *D.sissoo* leaves (DSEL-3) 1000mg/kg; sixth group ethanolic extract of *D.sissoo* bark (DSEB-1) 300mg/kg; seventh group ethanolic extract of *D.sissoo* bark (DSEB-2) 500mg/kg; eighth group ethanolic extract of *D.sissoo* bark (DSEB-3) 1000mg/kg; ninth group ethanolic extract of *D.sissoo* pod (DSEP-1) 300mg/kg; tenth group ethanolic extract of *D.sissoo* pod (DSEP-2) 500mg/kg; eleventh group ethanolic extract of *D.sissoo* pod (DSEP-3) 1000mg/kg. The anti-inflammatory activity was marked throughout the observation period up to 5 hrs and the calculated as percentage inhibition of Carrageenan induced paw edema [14-15].

Table-1 Animal Grouping, Treatment and doses

Sno.	Treatment	Dose	No. of animals
1	Vehicle (0.5% CMC)	5ml/kg	4
2	Standard (Indomethacin)	20mg/kg	4
3	DSEL-1	300mg/kg	4
4	DSEL-2	500mg/kg	4
5	DSEL-3	1000mg/kg	4
6	DSEB-1	300mg/kg	4
7	DSEB-2	500mg/kg	4
8	DSEB-3	1000mg/kg	4
9	DSEP-1	300mg/kg	4
10	DSEP-2	500mg/kg	4
11	DSEP-3	1000mg/kg	4

c. Protocol Procedure

Male rats of 100-200 gm. body weight, orally received the test substance 1 hr. prior to Carrageenan injection. A volume of 0.05 ml. of 1% carrageenan in sterile normal saline solution (NSS) was injected intradermally into the plantar side of right hand paw of the rat. The paw diameter was determined using a digital vernier calliper prior to -1, 3, and 5 hrs. after Carrageenan injection was injected. The anti-inflammatory activity was calculated as percentage inhibition of Carrageenan induced paw edema using the following formula (Chu and Kovacs, 1977).

Percent inhibitions- $[1 - \text{paw diameter in treated (dt)/paw diameter in control (dc)}] \times 100$.

d. Experimental Design

Thirty Albino rats of either sex were divided into eleven groups of four rats in each group. Drugs were administered to all of the groups (control, standard and tests) through the orally route, 30 min prior to administration of 1% carrageenan (0.1 ml.) to the region dorsum of the right hind paw. CMC (sodium carboxyl methyl cellulose) did not produce evident changes in activity response.

Group-I (control group)

Sodium carboxyl methyl cellulose (0.5% CMC) in distilled

Water at 5 ml/kg body weight.

Group-II (standard group)

Indomethacin (20mg/kg) suspension in 0.5% sodium CMC to serve as a standard drug at 10 ml/kg body weight.

Groups-III, IV V, VI, VII VIII, IX,X and XI (Test groups)

Ethanol extract (test drug) suspension in 0.5% sodium CMC (300, 500, 1000 mg/kg, respectively) at 10 ml/kg. Doses of *Dalbergia sissoo* extract of leaves (DSEL-1,2,3.), extract of bark (DSEB-1,2,3.) and pods extract of (DSEP-1,2,3.) were used as an anti-inflammatory up to 1000 mg/kg 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 [16].

e. Procedure

The percent inhibition of the edema formed was calculated with comparison to the control group (0.5 % Sodium CMC, 10 mL/kg) after 60 and 120 min.. The anti-inflammatory activity was determined as the percent inhibition of the edema formed after two hours of carrageenan administration [17].

The percent inhibition was calculated using the following formula.

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

The animals were fasted for 24 h prior to the experiment. A mark was made on the right hind paw just beyond the tibia-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 [18].

Statistical evaluation

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

RESULTS AND DISCUSSION

In this present study demonstrates that DSE extracts was used in the doses of 200, 300 and 1000 mg/kg orally in the study test. Indomethacin (20mg/kg, p.o.) and CMC (5mg/kg) were used as standard drugs. The Anti-inflammatory effects by standard drugs CMC and Indomethacin which offer relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins was suggested to play an important. Therefore, it is likely that DSE might suppress the formation of these substances or antagonize the action of these substances and thus exerts its Anti-inflammatory activity in Carrageenan induced paw edema in Albino Wistar Rats. It has been seen in the present study, DSE (1000 mg/kg) shows significantly Anti-inflammatory activities.. The ethanolic bark extract of *Dalbergia sissoo* showed marked anti-inflammatory activity compared to ethanolic extracts of leaves and pods. *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 with standard drugs. Presence of flavonoids was reported in *Dalbergia* species and flavonoids are known to inhibit prostaglandin synthetase. Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it could be suggested that reduced avail. The Anti-Inflammatory activity as studied by Carrageenan induced paw edema increase in diameter of paw at DSE of leaves, pods and bark (1000mg/kg) significantly, inhibition % are shown in Table of different for Anti-Inflammatory activity

4- Table- Result table for Anti inflammatory activity-Carrageenan Induced paw edema (Paw diameter in mm)

S.N	Treatment	Dose	1 hr.	3 hr.	5 hrs.	% inhibition
1.	Vehicle (0.5% CMC)	5 ml/kg	1.66± 0.123	1.88±0.216	2.49±0.149	-
2.	Standard (Indomethacin)	10mg/kg	0.69±0.067	0.60±0.076	0.37 ± 0.075	84.96
3.	DSEL-1 leaves	300mg/kg	1.21±0.070	1.36±0.131	1.45 ± 0.126	41.88
4.	DSEL-2 leaves	500mg/kg	1.16±0.115	1.01±0.116	0.82 ± 0.089	66.83
5.	DSEL-3 leaves	1 1000mg/kg	0. 89±0.122	0. 72±0.080	0. 61 ± 0.054	7 67.55
6.	DSEB-1 Bark	300mg/kg	1.36±0.163	1.54±0.165	1.74± 0.132	30.16
7.	DSEB-2 Bark	500mg/kg	1.09±0.136	1.13±0.131	1.00 ± 0.120	59.81
8.	DSEB-3 Bark	1000mg/kg	1.12±0.124	0.97±0.114	0.76± 0.091	69.23
9.	DSEP-1 Pod	300mg/kg	1.78±0.134	1.97±0.213	2.123±0.871	49.21
10.	DSEP-2 Pod	500mg/kg	1.31±0.070	1.46±0.151	1.25 ± 0.127	52.13
11.	DSEP-3 Pod	1000mg/kg	0.59±0.080	0.45±0.056	0.39±0.136	59.09

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. It has been seen in the present study, DSE (1000 mg/kg) shows significantly Anti-inflammatory activities. The ethanolic bark extract of *Dalbergia sissoo* showed marked anti-inflammatory activity compared to ethanolic extracts of leaves and pods. *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 with standard drugs. Presence of flavonoids was reported in *Dalbergia* species and flavonoids are known to inhibit prostaglandin syntheses. Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it could be suggested that reduced avail. The Anti-Inflammatory activity as studied by Carrageenan induced paw edema increase in diameter of paw at DSE of leaves, pods and bark (1000mg/kg) significantly, inhibition % are shown in Tables of different for Anti-Inflammatory activity.

SN	Extract contents	Unit (SI)	Time	Unit (SI)
1	leaves	mg/kg	15,30 &60	Mins.
2	pod	mg/kg	15,30 &60	Mins.
3	bark	mg/kg	15,30 &60	Mins.

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