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Anti-inflammatory effect of petroleum ether extract of *Caesalpinia bonduc* (L.) Roxb seed kernel in rats using carrageenan-induced paw edema

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Abstract: Petroleum ether extract of *Caesalpinia bonduc* L. (Roxb) seeds (Family: Caesalpiniaceae) was evaluated for its anti-inflammatory activity in rats. Wistar rats (120-150 g) of either sex were pretreated with petroleum ether extract of seed kernel of *C. bonduc*. Inflammation was induced in rat paw by injecting 0.1 ml of carrageenan (1% w/v) in sub-plantar region of right hind paw. Inflammation induced was significantly (p<0.05) decreased by petroleum ether extract of *Caesalpinia bonduc* (L.) Roxb seeds at dose level of 100 mg/kg is effective till 03 hour. The anti-inflammatory activity of petroleum ether extract of *C. bonduc* may be due to action of phytosterol.
Keywords: *Caesalpinia bonduc* L. (Roxb), oil extract, anti-inflammatory activity.

Introduction

Rheumatoid arthritis is a common disorder with varied clinical signs and symptoms related to multiple anatomical sites, both articular and extra-articular. At present non-steroidal anti-inflammatory drugs are the mainstay therapy in this condition; however, they have serious adverse effect and limitations for long term use [1]. The immunosuppressive drugs are reserved for selected cases, while disease modifying drugs such as gold-salts are costlier and have a low benefit-risk ratio [2].

Hence, there is a need for drugs having good efficacy and a low toxic profile derived from herbal source. *Caesalpinia bonduc* (L.) Roxb. is found throughout the hotter parts of India, Common in West Bengal, South India and Maharashtra. It is often grown as hedge plant [3]. An armed liana, up to 15 m in height, found wild throughout the plains of India and up to an altitude of 1,000 m in the Himalayas; it is also found in deltaic regions of western, eastern and southern India. Branchlets glossy, black armed with recurved prickles at the base of pinnae and elsewhere [4]. Seeds contain bonducin, saponin, a bitter substance - phytosterinin, a thick-yellow fatty oil (20-24%) having a disagreeable odour of the following fatty acid composition: palmitic, 4.5; stearic, 7.5; oleic, 29.0; linoleic, 59.0%, and lignoceric in traces. The defatted kernels contain α-, β-, γ-, δ- and ε-caesalpins [5], furanoditerpenoid-caesalpin F [6], homoisoflavone-bonducillin [7], cassane furanoditerpenoid (Bonducellpin E, F and G) [8] and an amorphous glycoside, bonducin. In addition, seeds also contain starch, sucrose, 2-phytosterols and proteins (25.3%). The amino acid composition of the seed proteins is as follows: arginine, 0.2; cystine, 0.9; histidine, 3.4; leucine and isoleucine, 15.4; lysine, 6.8; methionine, 0.9; phenylalanine, 5.2; threonine, 8.2; tryptophan, 0.4; and valine, 8.5g/16g N. All parts of *C. bonduc* (bark, leaves, roots, seed) are used in traditional system of medicines. The roasted seed powder is used as an anti-leprotic. The seeds are useful as antidiabetic, antiperiodic, antipyretic [9]. Fixed oil expressed from the seeds is a remedy in discharges from the ear; is used as an embrocation in rheumatism, and to remove freckles from the face as a cosmetic [10].

The aim of the present study was to determine the anti-inflammatory activity of *C. bonduc* oil extract in rats using carrageenin-induced paw edema. The effect of

oil extract was also compared with that of the standard drug diclofenac.

Materials and method

Plant material and reagents

Seeds of *Caesalpinia bonduc* were collected in the month of May from the farm of Dindori, District: Nashik, Maharashtra. Authentication of plant was done by Dr. P.G. Diwakar, Joint Director, Botanical Survey of India, Koregaon road, Pune. The herbarium of plant specimen has been deposited at B.S.I., Pune, with the voucher specimen number: **NSC-1** and the reference number: **BSI/WC/Tech./2008/483**.

Preparation of plant extract

The seed coat was broken, the kernel and testa were separated and kernel was size reduced to coarse powder. The kernel powder was loaded in a soxhlet extractor and was extracted with petroleum ether (60-80^0 C). Petroleum ether was distilled off and further air dried to get yellow oil.

Experimental animals

Wistar rats (120-150 g) of either sex were purchased from National Institute of Virology, Pune. They were housed in polypropylene cages in a controlled room temperature 22±1^{o}C and relative humidity of 60-70%. They were kept under standard conditions of 12/12 h light and dark cycle. The animals were maintained with standard pellet diet (Chakan Mill, Pune) and water *ad libitum*. The animals were acclimatized to laboratory conditions for seven days before commencement of experiment. All studies were carried out using 6 rats in each group. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC).

Chemicals and instrument

Diclofenac 5 mg/kg (Biochem) was purchased from local market, Carrageenan (1% w/v in saline solution). All the chemicals used for the study were of analytical grade. Digital Plethysmometer–LE7500 (Panlab-USA) was used for measurement of paw volume.

Phytochemical evaluation

Seeds were subjected to successive solvent extraction followed by qualitative chemical examination [11].

Acute oral toxicity study

Acute oral toxicity study of petroleum ether extract *C. bonduc* was carried out according to OECD guidelines. Extract at different doses up to 2000 mg/kg was administered and animals were observed for toxicity and mortality up to 48 h.

Anti-inflammatory activity [12, 13]

Rats were divided into four groups each containing six. Wherein group I served as control and received vehicle (10 ml/kg) whereas group II served as standard and received diclofenac 5 mg/kg. Group III and group IV served as test drug dose 1 (100 mg/kg, p.o.), and 2 (300 mg/kg, p.o) respectively. Aforementioned drugs were administered to the respective animals on the day of evaluation.1 hour after this; the sub plantar injection of 0.1 ml of carrageenan (1% w/v) in right hind paw was given. The paw volume was measured as a displacement of triton mixed saline solution at 0, 0.5, 1, 3, 5 and 24 h using Digital Plethysmometer.

Statistical analysis

The results are expressed as the mean ± SEM for each group. Statistical differences were evaluated using one way ANOVA followed by Dunnett's t test. Results were considered to be statistically significant at $p<0.05$.

Results

The phytochemical evaluation of seeds of *C. bonduc* revealed presence of carbohydrates, phytosterol, fixed oil, saponins, gums and mucilage, proteins, amino acids, phenol and tannins while alkaloids were found to be absent. Animals treated with petroleum ether extract of *C. bonduc* didn't show any toxic reaction or mortality. The extract was found to be safe at the dose of 2000 mg/kg.

The test drug is effective at a dose of 100 mg/kg while at higher dose effectiveness abolishes. Moreover this dose (100 mg/kg) is effective till 03 hour (Table 1).

Discussion

The carrageenan induced paw edema is a collective role of release of histamine and 5-HT during first 1hour,after which increase vascular permeability is maintained by the release of kinins up to 2.30h and from 2.30 to 6h, the mediators appear to be prostaglandins, the release of which is closely associated with migration of leukocytes into the inflamed cells [14]. The present results indicate the specificity of test drug towards selective inflammatory mediators. However the role of phytochemicals shall not be ignored. The exact mechanism may be revealed after detailed phytochemical analysis. The acute oral toxicity study carried out as per OECD guidelines showed safety till 2000mg/kg and hence above two doses were selected as per the standard criteria applied to acute oral toxicity results.

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Table: 1 Rats paw volume (Displacement value in ml)

Drug Treatment	0 hrs	0.5 hrs	1 hrs	3 hrs	5 hrs
Control (Saline sol.)	0.66 ± 0.019	1.05 ± 0.014	1.00 ± 0.019	0.935 ± 0.016	0.81 ± 0.018
100mg/kg	0.63 ^{ns} ±0.015	0.83 * ± 0.079	0.93 * ± 0.014	0.866 * ± 0.013	0.815 ^{ns} ± 0.012
300mg/kg	0.66 ^{ns} ± 0.022	1.05 ^{ns} ± 0.017	1.02 ^{ns} ± 0.015	0.908 ^{ns} ± 0.016	0.846 ^{ns} ± 0.024
Diclofenac 5mg/kg	0.678 ^{ns} ± 0.019	0.94 ^{ns} ± 0.019	0.86 ** ± 0.017	0.746 ** ± 0.019	0.705 ** ± 0.018

n=6, Value are mean ± for six rats.* p< 0.05, **<0.01 compared to respective group, ns: non significant.

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