

In vitro and *In vivo* anti-inflammatory activity of leaves of *Symplocos cochinchensis* (Lour) Moore ssp *laurina*

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Abstract

The anti-inflammatory activities of different extracts of leaves *Symplocos cochinchensis* Lour ssp *laurina* (Symplococaceae) were investigated for *In-vitro* anti-inflammatory activity by human red blood cell membrane stabilization method. The methanol extract showed effective *in-vitro* anti-inflammatory activity was screened for *in-vivo* anti-inflammatory activity by carrageenan-induced paw edema in rat model. The potency of the extracts was compared with standard diclofenac (50 mg/kg). The methanol extract showed significant membrane stabilizing action on human red blood cell membrane and reduction of edema in carrageenan induced rat paw edema model.

Introduction

Symplocos cochinchensis Lour ssp *laurina* (Symplococaceae) otherwise known as kabli-vetti or Lodh tree is widely distributed in tropical, subtropical areas in Asia, Oceania and America. It is widely used in the treatment of various disorders like leprosy, tumors, diarrhea, dysentery, menorrhagia, inflammation and uterine disorders (Ali et al., 1990). Recently much attention have been paid for *Symplocos* species due to their diverse biological activity in treating various disorders like Anti HIV (Ishida et al., 2001), inhibitory activities against phosphodiesterase (Ahmad et al., 2003) and antitumor (Li et al., 2003) applications.

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane et al., 1995) which are aimed at host defense and usually activated in

most disease condition. Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in amplifying the disease process. In this work the various extracts of *S. cochinchensis* were studied for its *in vitro* and *in vivo* anti-inflammatory activities.

Materials and Methods

The fresh leaves of *S. cochinchensis* were collected from Ooty in the month of August and it was authenticated by Prof. J. Jayaraman, Taxonomist, PARC, Chennai. A voucher specimen is deposited in the department for the future reference. The collected leaves were shade dried and



coarsely powdered. The coarse powder was subjected to continuous extraction in a soxhlet apparatus using n-hexane, chloroform, ethyl acetate and methanol as solvents. The solvents were distilled under reduced pressure using rotary vacuum evaporator. The extracts were dissolved in distilled water using 1% CMC as suspending agent.

Animals: Wister male albino rats weighing 150-200 g were used for the *in-vivo* anti-inflammatory studies. The animals were housed under standard conditions of temperature ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$), 12 hours light/dark cycle and fed with water *ad libitum*. Before performing the experiment the ethical clearance was obtained from institutional animal ethics committee.

In-vitro anti-inflammatory activity (Gandhisani et al., 1991): The human red blood cell membrane stabilization method was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAIDs for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (250, 500 and 1000 mcg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It is incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. the haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (50 mcg/ml) was used as reference standard and a control was prepared omitting the extracts.

Acute toxicity studies (Ecobichon, 1987): Acute toxicity study was carried out for methanol extract using Acute Toxic Class Method as described in OECD [Organization of Economic Cooperation and Development] Guidelines No. 423. The methanol extract was safe up to a dose of 2000 mg/kg body weight so 200 mg/kg and 400 mg/kg were used as moderate dose for the evaluation.

In vivo anti-inflammatory activity (Winter et al, 1962): *In vivo* anti-inflammatory activity was studied for the methanolic extract by carrageenan in-

duced paw edema model in rats. The Wister albino rats were divided in to 4 groups of 6 animals each. Group I served as control, Group II, III received methanol extract at the dose of 200 and 400 mg/kg and Group IV was served as standard (diclofenac 50 mg/kg). Carrageenan was injected in to the sub planter aponeurosis of the right hind paw of rats. An hour before carrageenan injection the animals were given with the extract, standard orally. The paw volumes were measured before and three hours after carrageenan administration by volume displacement method.

Statistical analysis: The data's were expressed as mean \pm SEM, statistical analysis was performed by one way ANOVA followed by Tukey-Kramer multiple comparison test, p values <0.05 were considered as significant. Highest significant difference test performed with Graph pad instat software.

Results and Discussion

The n-hexane, chloroform, ethyl acetate and methanolic extracts of the leaves of *S. cochinchinensis* were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. Among all the extracts methanolic extract showed significant anti-inflammatory activity in a concentration dependent manner. Methanol extract at a concentration of 1000 mcg/ml showed 67% protection of HRBC in hypotonic solution. All the results were compared with standard diclofenac which showed 74% protection.

The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Murugasan, 1981). Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the mem-

Table I: *In vitro* anti inflammatory activity of methanolic extract of *Symplocos cochinchinensis* by HRBC membrane stabilization method

Treatment	Conc (mcg/ml)	Absorbance (540 nm)	% inhibition
Control	--	0.48 ± 0.012	--
n-Hexane	1000	0.35 ± 0.03 ^b	28.6
	500	0.36 ± 0.01 ^b	25.8
	250	0.38 ± 0.005	23.4
Chloroform	1000	0.34 ± 0.003 ^a	31.0
	500	0.33 ± 0.04 ^a	30.0
	250	0.35 ± 0.005 ^a	26.5
Ethyl acetate	1000	0.25 ± 0.001 ^a	47.3
	500	0.28 ± 0.007 ^a	43.1
	25	0.29 ± 0.005 ^a	40.2
Methanol	1000	0.15 ± 0.001 ^a	69.9
	500	0.19 ± 0.002 ^a	60.5
	250	0.20 ± 0.001	58.1
Diclofenac	50	0.13 ± 0.002 ^a	73.9

Values are expressed as mean ± SEM. n=6 animals in each group. ^aP<0.001 ^bP<0.01 compared to control group

Table II: *In vivo* anti inflammatory activity of methanolic extract of *Symplocos cochinchinensis* on Caarageenin-induced paw edema in rats

Treatment	Dose (mg/kg)	Edema volume (ml)	% Inhibition
Control	--	0.72 ± 0.02	--
Methanolic extract	200	0.48 ± 0.029 ^a	33.3
Methanolic extract	400	0.315 ± 0.012 ^a	53.47
Diclofenac	250	0.180 ± 0.001 ^a	75

Values are expressed as mean ± SEM. n=6 animals in each group. ^aP<0.001 compared to control group

brane stabilization by the extract is not known yet, hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimu-

late or enhance the efflux of these intracellular components (Iwueke, 2006).

Since methanol extract showed significant *in-vitro* anti-inflammatory activity it was selected for the evaluation of *in vivo* anti-inflammatory activity by carrageenan induced paw edema model in rats. The extract showed significant anti-inflammatory activity (53%) at the dose of 400 mg/ml while the standard diclofenac showed 75% inhibition of edema. On the basis of the above results it can be concluded that the methanol extract posses significant anti-inflammatory activity studied by *in vitro* and *in vivo* models. The study also provides a strong evidence for the use of the leaves *S. cochinchinensis* in folkloric treatment as anti-inflammatory agent. The activity may be due to the presence of one or more phytochemical constituents present in the extract further study is warranted, for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity.

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