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ANTI-INFLAMMATORY ACTIVITY OF *SAMADERA INDICA* LEAVES BY MEMBRANE STABILIZATION

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ABSTRACT

The present work aims at evaluating the anti-inflammatory activity of *Samadera indica* by HRBC membrane stabilization. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity. The anti-inflammatory activity of the crude ethanol extract, aqueous extract of leaves part of *Samadera indica* were compared to that of the standard drug diclofenac. The extract in concentration of 250-1000 µg/ml showed a dose dependent inhibition of haemolysis of erythrocyte induced by hypotonic solution. The ethanolic extract of *Samadera indica* showed significant anti-inflammatory activity in comparison to aqueous extract and with standard drug diclofenac.

INTRODUCTION: The inflammatory responses involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue brake down and repair which are aimed at host defense and usually activated in most disease condition [1]. Many mediators co ordinate inflammatory and allergic reactions. While some are produced in response to specific stimuli, there is considerable redundancy, and each facet of the response vasodilatation, increased vascular permeability, cell accumulation, etc can be produced by several separate mechanisms [2]. So present study was undertaken to establish scientific evidence for anti-inflammatory activity of leaves extracts of *Samadera indica*

Samadera indica (Simaroubaceae) is distributed throughout India as under growth in forests and along backwaters of South India**.** *Samadera indica* is a small tree up to 11 m in height with stout branches and pale yellow bark and leaves are large, up to 25 cm long and 9 cm broad, elliptic-oblong, shortly acuminate, entire, shining& base rounded.

Flowers are pinkish yellow in few or many flowered umbels, peduncles longer than the leaves, pedicels red. Fruits are large, flat, pear shaped, much compressed, smooth reticulate [3].

The bark and wood is stomachic, emmenagogue, febrifuge and tonic, and are useful in vitiated conditions of vata, dyspepsia, flatulence, colic, dysmenorrhoea and general debility.

The leaves used in puritis, leprosy, scabies, pruritus, skin diseases, constipation and bilious fever. The seed oil is astringent, acrid, thermogenic, depurative, emetic, purgative and febrifuge [4]. Invitro antiplasmodial activity was reported in roots and leaves of *samadera indica* [5]. Samaderin B and C isolated from the seed kernels of *Samdera indica* were shown to exhibit antifeedant activity against *Spodoptera litura* [6].

The present study was aimed to evaluate the *in vitro* anti-inflammatory activity of crude extract of *Samadera indica*.

MATERIALS AND METHODS:

Plant Material: Fresh leaves of *Samadera indica* was collected from TBGRI, Thiruvananthapuram during the month of March 2007. The plant was identified by Mrs. Amina Ali, Associate Professor, Department of Pharmacognosy, Govt. Medical College, Calicut, Kerala, India. Voucher specimen (AA-33/10) is preserved in institute herbarium for future reference.

Preparation of Extract:

1. **Ethyl alcohol extract:** The shade dried powdered leaves (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The extract was concentrated *in vaccuo* to a syrupy consistency. The percentage yield of extract was found to be 3.2 %.

2. **Aqueous extract:** The dried powders (24#) 100gm of the was taken in a 2000ml conical flask with 500ml of distilled water to which 10ml chloroform were added as a preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the marc was pressed and its filtrate dried in hot air oven at 45^{o}C to a semisolid mass. It was stored in airtight container in a refrigerator below 10^{o}C. The percentage yield of extract was found to be 4.1 %.

Membrane stabilization assay: The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity [7] (Gandhisan *et al*., 1991). Blood was collected from healthy volunteer who was not taken any NSAIDS for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%, pH 7.2) and a 10%(v/v) suspension was made with isosaline.

The assay mixture contained the drug (concentration as mentioned in **table 1**), 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclofenac was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control.

All the assay mixture were incubated at 37^{o}C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using Spectro-photometer at 560 nm. The percentage hemolysis was calculated by following equation;

$$\% \text{ inhibition of hemolysis} = 100 \times (OD_1 - OD_2/OD_1)$$

Where OD_1 = Optical density of hypotonic buffered saline solution alone (control) and OD_2 = Optical density of test sample in hypotonic solution.

RESULTS AND DISCUSSION: The ethyl alcohol and aqueous extracts of *Samadera indica* were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method.

Phytochemical investigation reveals that ethyl alcohol extracts contains carbohydrates, steroids, alkaloids, terpenoids, flavonoids, tannins, polyphenols while aqueous extract contains carbohydrates, alkaloids, flavonoids, tannins, poly phenols. The results obtained demonstrate that extracts can significantly and dose dependently inhibits RBC hemolysis. The percentage protection of lysis for standard diclofenac 50 mcg/ml is73%, ethyl alcohol extract at a concentration of 1000 µg/ml is 67% and aqueous extract is 50% (**Table 2**).

The extracts exhibited membrane stabilization effects by inhibiting hypo tonicity induced lysis of erythrocyte membrane [8]. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well as stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which further tissue inflammation and damage up on extra cellular release [9].

The possible anti-inflammatory activity of this extract may due to inhibitory effect on release of inflammation mediators or by membrane stabilizing activity.

The present investigation suggests that the membrane stabilizing activity of *Samadera indica* may be playing a significant role in its anti-inflammatory activity. Further works are in progress to find out its exact mechanism of action.

TABLE 1: *IN VITRO* ANTI INFLAMMATORY ACTIVITY OF ETHYL ALCOHOL AND AQUEOUS EXTRACT OF *SAMADERA INDICA*

Treatment	Conc. (µg/ml)	% inhibition
Control	--------	----------
Ethyl alcohol Extract	1000	68.61
	500	61.03
	250	57.35
Aqueous Extract	1000	50.36
	500	49.90
	250	39.55
Diclofenac Sodium	50	73.08

TABLE 2: PHYTOCHEMICAL SCREENING OF PLANT MATERIAL *SAMADERA INDICA*

Phytochemical constituents	Ethyl Alcohol Extract	Aqueous Extract
Carbohydrates	+	+
Steroids	+	-
Alkaloids	+	+
Saponins	-	-
Terpenoides	+	-
Flavonoids	+	+
Tannins	+	+
Polyphenols	+	+

(+): Present; (-): Absent

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