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Anti-inflammatory activity of aqueous extract of *Cinnamomum tamala* leaves by *in vivo* and *in vitro* methods

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ABSTRACT

Cinnamomum tamala (Family- Lauraceae) was used traditionally in the treatment of inflammation but there is no scientific evidence exists to validate the folkloric use of the plant. Thus the present work was aimed at investigating the anti-inflammatory effect of the aqueous extract of *Cinnamomum tamala* leaves (CTW) by various *in vivo* and *in vitro* screening methods. CTW at dose of 100, 200 and 400mg/kg was evaluated in acute inflammation against carrageenan induced paw edema in rats and acetic acid-induced vascular permeability in mice. *In vitro* anti-inflammatory activity of CTW extract (concentrations 0.2 – 1 mg/ml) was studied by membrane stabilizing activity i.e. red blood cells (RBC's) exposed to hypotonic solution *In vitro* experiment was performed in triplicate to minimize the errors. Results were analyzed by One-way ANOVA followed by Dunnett's test $P < 0.05$ and were considered significant as compared to control. CTW inhibited significantly and dose dependently edema induced by carrageenan in rats also reduced significantly acetic acid-induced vascular permeability in mice. When tested *in vitro*, CTW exhibited significant membrane-stabilizing property in concentration dependent manner up to 1mg/ml. Indomethacin was used as a positive control. The results indicate that *Cinnamomum tamala* possesses significant anti-inflammatory activity and has therapeutic potential for the treatment of inflammatory diseases.

Keywords: Acute, inflammation, membrane stabilization, vascular permeability

INTRODUCTION

Cinnamomum tamala Nees & Eberm (Family- Lauraceae) is one of the economical plant species commonly known as Indian Cinnamon or 'Tejpatta'. It mainly grows in tropical and subtropical Himalayas, Uttar Pradesh, Eastern Bengal, Burma, Khasi and Jaintia Hills of India [1]. Polyphenolic components of *Cinnamomum tamala* leaves have been identified as 3,4',5,7- tetrahydroxyflavone; 3,3',4',5,7-pentahydroxyflavone nonglycosidic compound, kaempferol-3-o-glycopyranoside, quercetin-3-o-sophoroside, kaempferol-3,7-di-orhamnopyraoside and quercetin-3-o- rutinoside glycosidic [2]. Traditionally this plant was useful in the treatment of some of the diseases related to mental alertness, sore throat, immune system, upper air passages, joints and rheumatism [3].

Reported activities of the *Cinnamomum tamala* are CNS activity (hypnotic, anticonvulsant and hypothermic) [4], hypoglycemic [5], anticarcinogenic [6], anti diarrhoeal [7], anti oxidant and radical scavenging [1].

Till now no systematic scientific evidence exists showing anti inflammatory activity of *Cinnamomum tamala*. Thus the present study was planned to investigate the anti-inflammatory activity of aqueous extract of *Cinnamomum tamala* leaves using different experimental animal models and *in vitro* screening method.

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MATERIALS AND METHODS

Chemicals

Carrageenan (Sigma Aldrich, USA), Evan's blue was obtained from Himedia Pvt. Ltd. India, indomethacin [Recon, (Bangalore) India] and all other chemicals used were of analytical grade.

Collection of Plant

Fresh leaves of the plant *Cinnamomum tamala* Nees & Eberm (Family- Lauraceae) were collected from Mumbai region, India in the month of August. The plant material was taxonomically identified by Dr. Ganesh Iyer, Prof. in Botany, Ramnarain ruia college, Mumbai, India. A voucher specimen (No. 9-4/08) has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in an airtight container.

Preparation of Extract

The dried powder material of *Cinnamomum tamala* leaves was defatted with petroleum ether (60⁰-80⁰C) and subsequently extracted with distilled water by hot maceration method. The solvent was completely removed by drying and aqueous extract of *Cinnamomum tamala* leaves (CTW) was obtained (yield 13.7%). The extract was stored at 4⁰C in a sealed container till required. Solution of CTW was prepared freshly in distilled water and used for the present study.

Animals

Wistar albino rats of either sex weighing 180–200 g and Swiss albino mice of either sex weighing 18–22 g were used for animal studies. The animals were grouped in polyacrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) and relative hu-

midity (50±5%) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet ('Amrut' brand, M/s. Nav Maharashtra Chakan oil mills Ltd., Pune, India) and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee of Institute and care of the animal was taken according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Acute Toxicity Study

Acute toxicity study was performed according to OECD-423 guidelines [8]. *Swiss albino* mice of either sex were used for study. The animals were fasted for 4 h, but were allowed free access to water *ad libitum* throughout. The animals were divided into six groups containing six animals each. CTW was dissolved in distilled water and administered orally as a single dose to mice at different dose levels viz. 500, 750, 1000, 1250, 1500 and 2000 mg/kg of body weight (b.w.). Mice were observed periodically for the symptoms of toxicity and death within 24 hours and then daily for next 14 days.

Evaluation of Anti-inflammatory Activity

Carrageenan-induced rat paw edema

This test was followed by the method described by Winter *et al* [9]. Rats were divided into five different groups (n = 6). Group I served as control and received vehicle orally. Group II, III and IV received CTW extract orally at the dose levels of 100, 200 and 400 mg/kg. Group V received indomethacin orally at a dose of 10 mg/kg. One hour after the respective treatment, 100µl of 1% freshly prepared carrageenan in normal saline was injected in sub-plantar region of right hind paw of rats. The paw volume was measured at 0 h i.e. immediately after carrageenan injection and then at 1, 2, 3 and 4 h using plethysmometer. The anti-inflammatory effect of CTW was calculated by the following equation:

Anti-inflammatory activity (%) Inhibition = $(1-D/C) \times 100$,

Where D represents the percentage difference in increased paw volume after the administration of test drugs to the rats and C represents the percentage difference of increased paw volume in the control group [10].

Acetic acid-induced vascular permeability in mice

This test was carried out according to the method described by Whittle [11] with some modifications. Five groups of six mice each were used. Group I served as control and received vehicle orally, groups II, III and IV were treated with 100, 200 and 400 mg CTW extract/kg orally respectively, while group V received indomethacin 10 mg /kg orally. One hour after the treatment, 0.2% Evan's blue in normal saline was injected intravenously through tail vein at a dose of 0.1 ml/10 g. Thirty minutes later, each mouse was injected intraperitoneally with 0.2 ml of 0.6% acetic acid in normal saline. After 1 h, the mice were sacrificed and the abdominal wall was cut to expose the entrails. The abdominal cavity was washed using 5ml of normal saline to collect pigments in a test tube. After centrifuging the contents of the tube to eliminate contaminants, the solution was subjected to colorimetric analysis using a spectrophotometer at a wavelength of 590 nm. The vascular permeability effects were expressed as the absorbance (A), which represented the total amount of dye leaked into the intraperitoneal cavity. Percentage inhibition of vascular permeability was calculated by comparing with control group.

Membrane stabilizing activity

This test was followed by the method described by Shinde *et al* [12] with some modifications. Whole human blood was obtained from healthy human volunteer and transferred to heparinized centrifuge tube. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) for 10 minutes at 3000g. The test sample consisted of stock erythrocyte (RBC's) suspension (0.5 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the CTW extract (0.2- 1.0 mg/ml) or indomethacin (0.1 mg/ml). The control sample consisted of 0.5 ml of RBC's mixed with hypotonic buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000g and the absorbance of the supernatant was measured at 540 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of hemolysis or membrane stabilization was calculated by the following equation.

% Inhibition of hemolysis = $100 \times (A_1 - A_2 / A_1)$

Where:

A₁ = Absorption of hypotonic-buffered saline solution alone

A₂ = Absorption of test sample in hypotonic solution

Statistical analysis

The experimental data was expressed as mean ± SEM, the significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's multiple comparison tests using Graphat Instat Software (San Diego, CA, USA). The level of significance was set at $P < 0.05$.

RESULTS

Acute Toxicity Test

In the acute toxicity study no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis. The median lethal dose (LD₅₀) was determined to be higher than the dose tested i.e. 2.0 g/ kg.

Anti-inflammatory Activity

Carrageenan-induced rat paw edema

The anti-inflammatory activity of CTW at the doses of 100, 200 and 400 mg/kg against paw edema induced by carrageenan is shown in Figure 1. CTW extract at the doses of 100 and 200 mg/kg moderately inhibited paw edema (25.65 and 31.57 % respectively) where as at the dose of 400 mg/kg and indomethacin at dose of 10 mg/kg significantly ($P < 0.05$) inhibited paw edema (54.4 and 62.5 % respectively) at the end of 4 h after carrageenan injection.

Acetic acid-induced vascular permeability in mice

The CTW at the doses of 100, 200, 400 mg/kg and indomethacin (10 mg/kg) were significantly ($P < 0.05$) inhibited vascular permeability (13.59%, 24.81%, 54.78% and 68.45% respectively) induced by acetic acid in mice when the results compared with vehicle control.

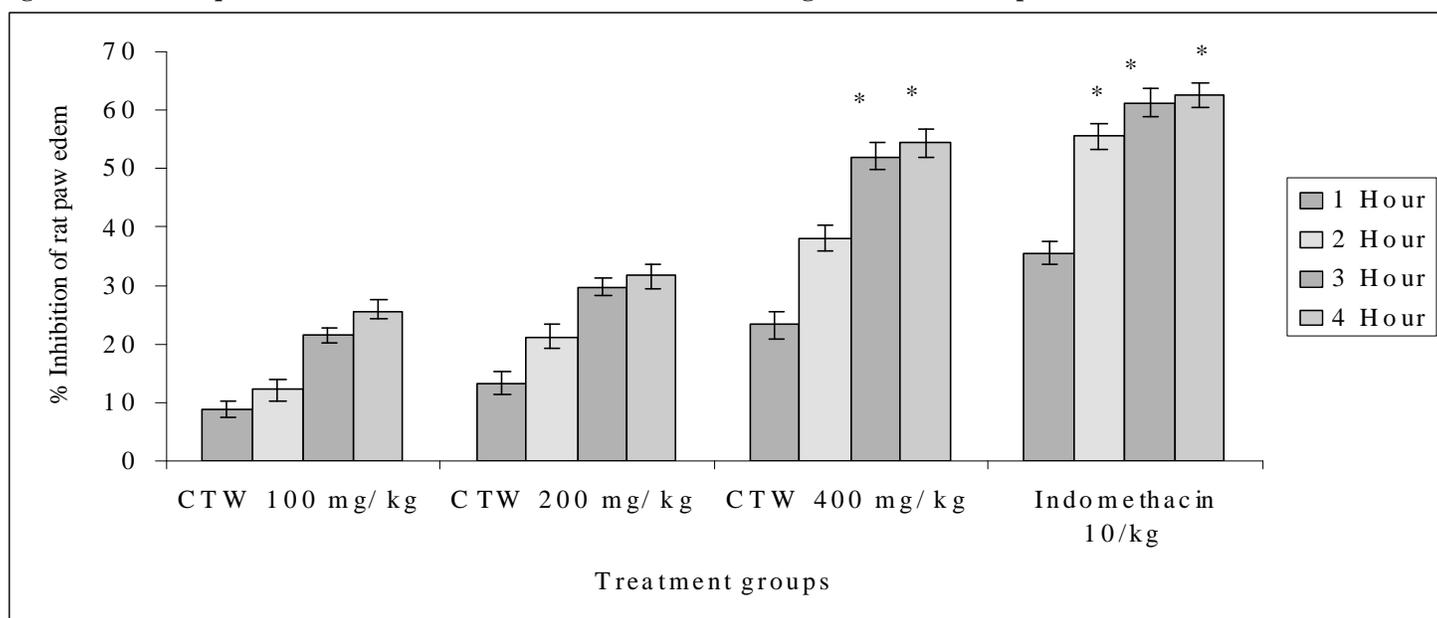
Membrane stabilizing activity

The CTW extract at concentrations range of 0.6-1.0 mg/ml and indomethacin (0.1 mg/ml) protected significantly the erythrocyte membrane against lysis induced by hypotonic solution. The CTW (1 mg/ml) and indomethacin (0.1 mg/ml) showed 43.83% and 54.79% respectively inhibition of RBC haemolysis (Table 1).

DISCUSSION

The present study establishes the anti-inflammatory activity of the

Figure 1. Effect of aqueous extract of *Cinnamomum Tamala* leaves on carrageenan-induced rat paw edema.



Each value represents the mean \pm S.E.M., n=6. *P<0.05; **P<0.01 compared with control, Dunnett's multiple comparison test after analysis of variance.

Table 1. Effect of aqueous extract of *Cinnamomum Tamala* leaves on erythrocyte hemolysis

Sample (s)	Concentrations	Optical density	% Inhibition of hemolysis
Hypotonic medium	50 mM	0.730 \pm 0.08	—
Aqueous extract of <i>Cinnamomum Tamala</i> leaves	0.2 mg/ml	0.679 \pm 0.1*	6.98
	0.4 mg/ml	0.652 \pm 0.07*	10.68
	0.6 mg/ml	0.561 \pm 0.06**	23.15
	0.8 mg/ml	0.440 \pm 0.09**	39.72
	1 mg/ml	0.410 \pm 0.11**	43.83
Indomethacin	0.1 mg/ml	0.330 \pm 0.04**	54.79

Each value represents the mean \pm S.E.M., n=3. *P<0.05; **P<0.01 compared with control, Dunnett's multiple comparison test after analysis of variance.

aqueous extract of *Cinnamomum tamala* leaves (CTW) in different *in vivo* and *in vitro* screening methods, representing different phases of inflammation.

Carrageenan-induced paw edema has been commonly used as an experimental animal model for acute inflammation study and is believed to be biphasic. The early phase (1– 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The later phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [13]. The results of the present study indicated that the CTW 400 mg/kg acts more significantly in later phases probably by inhibiting prostaglandin release involving arachidonic acid metabolites [14]. Where as indomethacin (10 mg/kg) was found to be effective through out the study.

The inflammatory response is a physiological characteristic of vascularized tissues [15]. Increased vascular permeability occurs as results of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasma proteins and fluid [16] that leads to exudation of fluid rich in plasma proteins including immunoglobulins (antibodies), coagulation factors [17] and cells [18] into the injured tissues. Exudation, which is a consequence of increased vascular permeability, is considered a ma-

ajor feature of acute inflammation [19]. Chemical-induced vascular permeability (acetic acid) causes an immediate sustained reaction that is prolonged over 24 h [20] and its inhibition suggests that the CTW extract may effectively suppress the exudative phase of acute inflammation in dose dependent manner showing maximum inhibition (54.78%) at 400mg/kg.

The vitality of cells depends on the integrity of their membrane, exposure of red blood cells to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin [21, 22]. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Compounds with membrane-stabilizing properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators [23]. CTW has shown significant membrane stabilizing property, which suggests its anti-phospholipases activity. The anti-inflammatory effect of CTW observed in above methods tested may be due to the presence of flavonoids [1, 2] in the plant. Therapeutic applications of flavonoids on inflammation have been reported [24]. Devi *et al* [1] reported anti oxidant and radical scavenging activity of *Cinnamomum tamala*. Free radicals involved in the process of lipid peroxidation are considered to play a cardinal role in numerous chronic pathologies, such as cancer, inflammation, cardio-

vascular diseases. Inhibitions of free radicals suggest utility of *Cinnamomum tamala* in anti-inflammatory activity. Antidiarrhoeal activity of *Cinnamomum tamala* was reported [7]. The gut wall contains prostaglandins E and F with prostaglandin synthetase activity mainly in the mucosa. In human, prostaglandins cause intestinal cramps and diarrhoea which is due to effect on intestinal smooth muscle and secretion [25]. Therefore antidiarrhoeal activity of *Cinnamomum tamala* also supports its anti-inflammatory activity.

Thus, from the present study it is concluded that the aqueous extract of *Cinnamomum tamala* leaves produced significant anti-inflammatory activities in dose dependent manner and also possesses significant membrane stabilization property in concentration dependent manner.

Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a better therapeutic index.

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