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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *TILIACORA ACUMINATA* EXTRACT IN RATS

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ABSTRACT: Inflammation is part of the body's immune response. There can be four primary indicators of inflammation: pain, redness, heat or warmth and swelling. Plants have the ability to synthesize a wide variety of phytochemical compounds as secondary metabolites which shows anti-inflammatory activity. The anti-inflammatory activity of ethanol extract of *Tiliacora acuminata* stem and leaf was evaluated using the carrageenan-induced rat paw edema. The ethanol extract of stem and leaf of *Tiliacora acuminata* was injected at different doses such as 200 and 400 µg/kg body weights, and the study was compared with standard drug indomethacin (10 mg/ml). In rat paw edema model induced by carrageenan the extracts were found to reduce similarly ($p < 0.001$). The formation of edema at the 400 µg/ml dose level and showed 77.92% for stem and 84.37% for leaf respectively, inhibition of edema volume at the end of 3 h. These results suggest that *Tiliacora acuminata* has anti-inflammatory property comparable with those of standard drug and may be useful for the treatment of painful inflammatory condition.

INTRODUCTION: Inflammation is a complex biological response of vascular tissue to harmful stimuli caused by injury, infection, environmental agents, malignant, and cellular changes. It is a protective attempt by the body to remove the injurious stimuli as well as initiate the healing process for tissues¹.

The inflammatory response is a complex process that contains activation of white blood cells, the release of immune system chemicals like complements and cytokines, and the production and discharge of inflammatory mediators and prostaglandins².

There are two types of inflammation, and they are acute and chronic. The regime of acute inflammation is warmth, redness, pain, swelling, and loss of function. The sign of chronic inflammation are long-lasting pain, redness, and swelling and are caused by persistence of an irritant which may be biological, physical or chemical in nature³.

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Many synthetic drugs were now available in the market to treat inflammation and pain to side effects. So, the herbal drugs of the utmost importance and there is a need for the production of novel herbal drugs. *Tiliacora acuminata* belongs to the family *Menispermaceae*. This plant has been used as an ingredient in many of the ayurvedic preparations and regards as an anti-tode for snakebite^{4, 5}. The present study was therefore undertaken to evaluate the anti-inflammatory activity of the ethanol extracts of *Tiliacora acuminata* stem and leaf using carrageenan-induced paw edema model.

MATERIAL AND METHODS: The aerial parts (stem and leaf) of *Tiliacora acuminata* (Lam.) Hook. f. & Thomas were collected from Ulakaruvi, Kanyakumari District, Tamil Nadu, India. The plant sample was found out with the help of local flora and confirmed by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of composed plants was deposited in the Ethnopharmacology Unit (VOC 2018), PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu, India.

Solvent Extraction: Ethanol was used as a solvent to prepare the plant extracts. The different plant materials were directly soaked for 12 h in 500 ml ethanol and then subjected to extraction by refluxing for 6 to 8 h below the boiling point of the solvent. The ethanol extracts were concentrated by evaporating at a reduced pressure using rotary evaporator. The concentrated extracts were further dried at 37 °C for 3 to 4 days in order to facilitate complete evaporation of the solvents.

Acute Toxicity Study: For toxicity studies, two different groups of six albino rats of both sexes were administered orally with the test substances, in the range of doses 100 - 2000 mg/kg and the mortality rates were observed after 72 h. The ethanol extracts of stem and leaf of *T. acuminata* showed no mortality at 2000 mg/kg. Therefore, 3000 mg/kg dose was considered as LD₅₀ cut off dose (safe dose) and hence 1/10 (200 mg/kg) 1/5 (400 mg/kg) of LD₅₀ doses were selected as safe doses.

Animals: Wistar albino adult rats of both sexes with 150 - 200 g body weight were used for present

investigation. The animals were kept in individual cages under standard environmental conditions at temperature of 25 ± 2 °C and relative humidity of about 55%. The rats were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* and fasted for 16 h before the start of the experiment (Ethical clearance no. IAEC1012/C/06/CPSEA-Corres-2008-2009).

Drugs (Synthetic Anti-Inflammatory Agents): Commercial name of the reference anti-inflammatory drug used in our study is Indomethacin. It is chemically known as 1-(4-Chlorobenzoyl)-(-methoxy-2-methylindole-3-yl) acetic acid and was obtained from Pharmco Pharmaceuticals Company.

Chemical Used for the Induction of Inflammation: Carrageenan, type IV (Sigma, USA): Carrageenan is a polysaccharide isolated from two species the marine alga, *Gigartina acicularis* and *G. pistillata* which grow together in the sea. Rats were divided into six groups and each group comprising of five rats.

Group I: Control rats received normal saline 0.5 ml/kg.

Group II: Rats received stem extract of *T. acuminata* at the dose of 200 mg/kg body weight.

Group III: Rats received stem extract of *T. acuminata* at the dose of 400 mg/kg body weight.

Group IV: Rats received leaf extract of *T. acuminata* at the dose of 200 mg/kg body weight.

Group V: Rats received leaf extract of *T. acuminata* at the dose of 400 mg/kg body weight.

Group VI: Rats received standard Indomethacin 10 mg/kg body weight.

Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan in physiological saline into the sub-plantar tissues of the left hind paw of each rat⁶. The ethanol extracts of stem and leaf of *T. acuminata* were administered orally 30 min before carrageenan administration. The paw volume was determined at intervals of 60, 120, 180 and 240 min by the mercury displacement technique using a plethysmograph. The inhibition percentage of paw

volume in drug-treated groups was compared with the carrageenan control group. Percentage inhibition was calculated using the formula:

$$\text{Percentage inhibition} = [(V_c - V_t) / V_c] \times 100$$

Where, V_t represents the percentage difference in increased paw volume after the administration of test drugs to the rats and V_c represents the difference in increased volume in the control groups.

RESULTS AND DISCUSSION: The ethanol extract of *T. accuminata* did not show any sign of toxicity up to 2000 mg/kg body weight, and hence

it was considered to be safe. The anti-inflammatory activity of the extract was measured at a dose of 200 and 400 $\mu\text{g}/\text{kg}$ body weights against acute paw edema induced by carrageenan. Strong inhibition of the paw edema was observed with the different dose of the ethanol extract of *T. accuminata* stem and leaf and with indomethacin. The two doses treated (200 and 400 $\mu\text{g}/\text{kg}$) produced significantly ($p < 0.001$) anti-inflammatory activity and reduced paw edema by 71.17%, 77.92%, 81.61% and 84.37% for stem and leaf extract of *T. accuminata* respectively whereas indomethacin caused 83.74% reduction when used as a standard drug **Table 1**.

TABLE 1: EFFECT OF STEM AND LEAF OF ETHANOL EXTRACTS OF *TILIACORA ACUMINATA* (TAS AND TAL) ON THE PERCENTAGE OF INHIBITION OF PAW EDEMA IN RATS

Groups	Dose (mg/kg body weight)	Edema volume (ml)			% inhibition after 180 min	
I	Control (saline 1%)	36.40 \pm 1.84	80.50 \pm 2.40	110.50 \pm 3.16	136.55 \pm 2.15	
II	TAS (200)	34.16 \pm 1.56	68.33 \pm 1.54ns	51.15 \pm 1.65**	39.36 \pm 1.96 **	71.17
III	TAS (400)	30.10 \pm 1.27	60.56 \pm 1.31ns	42.66 \pm 1.39**	30.15 \pm 1.26**	77.92
IV	TAL (200)	35.15 \pm 1.30	63.91 \pm 1.84	38.31 \pm 1.65***	25.11 \pm 1.08 ***	81.61
V	TAL (400)	31.11 \pm 1.65	55.16 \pm 1.27*	31.27 \pm 1.16***	21.33 \pm 1.16***	84.37
VI	Indomethacin	30.65 \pm 1.31	52.16 \pm 1.93*	32.16 \pm 1.27***	22.19 \pm 1.31***	83.74

Values are expressed as mean \pm SEM, n = 5 in each group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ - Comparison made between normal control to paw edema induced control and drug-treated rats; ns - not significant.

The inhibition of carrageenan-induced inflammation in rats is an established model for evaluating anti-inflammatory drugs, which has been used frequently to assess anti-edematous effect of natural products. The development of carrageenan-induced edema is biphasic⁷; the first phase occurs within one hour of carrageenan inflammation and is attributed to the release of cytoplasmic enzymes, histamine, and serotonin, from the mast cells. The second phase (>1 h) is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two phases is provided by kinins.

Since, the extract note worthy inhibited paw edema induced by carrageenan in the second phase, this result suggests a possible inhibition of cyclooxygenase combination by the extract, for the reason that the carrageenan inflammatory model basically reflects the action of prostaglandins^{8, 9}. This effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme, which catalyzes the synthesis of cyclic endoperoxides important in the formation of prostaglandins.

The presence of the reported phytochemical constituents in the stem and leaf of *T. accuminata* extracts may contribute to its observed anti-inflammatory activity. Many flavonoids and alkaloids have been found to exhibit anti-inflammatory effects¹⁰. 9, 12-Octadecadienoic acid, methyl ester 9, 12, 15-Octadecatrienoic acid (Z, Z) - 9, 12, 15-Octadecadienoic acid (Z, Z, Z)-, Vitamin E and 9, 12-Octadecadienoic acid, methyl ester (E, E) - were reported in the ethanol extract of *T. accuminata* by GC-MS. These compounds may have the function in anti-inflammatory effects.

CONCLUSION: This finding have proved that ethanol extract of *T. accuminata* possess potent anti-inflammatory activity possibly due to presence of flavonoids and phenolic compounds.

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CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest to disclose.

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