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ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *TOONA CILIATA* M. ROEM (EETC) IN ALBINO RATS

Pharmacology

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ABSTRACT

Background: *Toona ciliata* M. Roem locally known as 'Tairel' in Manipur is an aromatic plant species with many medicinal properties. It is a large forest tree of the mahogany family, native to Australia and Asia, but introduced elsewhere also. The 'Meiteis' of Manipur use *Toona ciliata* leaves for religious ceremony and cultural activities and also therapeutically as antiseptic, anthelmintic, antirheumatic, antidiarrheal, analgesics and counter irritant. Therefore, the present study was undertaken to evaluate the anti-inflammatory properties of ethanolic extract of *Toona ciliata* M. Roem (EETC) in suitable animal models.

Method: A total of 24 rats of either sex were used for each experiment. Acute inflammation was evaluated by carrageenan induced paw edema, subacute inflammation by turpentine oil induced granuloma and chronic inflammation was tested by formalin induced arthritis.

Result: EETC at doses of 200 mg/kg and 400 mg/kg were evaluated. EETC produced significant inhibition of paw edema at the 3rd hour in carrageenan induced paw edema. Decrease in exudates volume was seen in group treated with EETC in granuloma pouch method. EETC also suppressed chronic inflammation in formalin induced arthritis.

Conclusion: The findings in the present study suggested that EETC at the doses 200 mg/kg and 400 mg/kg possesses significant anti-inflammatory activity.

KEYWORDS

Toona ciliata M. Roem, anti-inflammatory, carrageenan, turpentine oil, formalin.

INTRODUCTION

The Indian sub-continent has a rich diversity of plant species in a wide range of ecosystems. There are about 17,000 species of higher plants, of which approximately 8,000 species are considered to have medicinal properties and used by different communities or in traditional medicinal systems, such as the Ayurveda.[1] Medicinal plants are the primary health care system among the people residing in many rural and hard to reach areas in different part of India.

Plants contained functional phytochemicals and their consumption has long been associated with physical wellbeing. Among phytochemicals with health benefits- phenolic acids, flavonoids, and other polyphenols, have been demonstrated to exhibit positive effects on health.[2] They are used as antimicrobial/antifeedant by plants to defend themselves from microbes and insects attack. Many plants has antioxidant, anti-apoptotic, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardio protective, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities.[3]

Manipur being located in the Himalayan region is also blessed with many medicinal herbs and plants. *Toona ciliata* M. Roem locally known as 'Tairel' in Manipur is an aromatic plant species with many medicinal properties. *Toona ciliata* is a large forest tree of the mahogany family, native to Australia and Asia, but introduced elsewhere as a shade tree and for its fast-growing aspect and red timber. In the Indian subcontinent, *T. ciliata* grows from eastern Pakistan to Bangladesh, including the tropical parts of the sub- Himalayan tract and the Western

Ghats in India.[4] These plants are known as medicinal and aromatic plants (MAPs) that provide people with medicines to prevent disease, maintain health or cure ailments.[5]

Manipur has the richest reservoir of plant diversity and supporting about 50% of India's biodiversity.[6] In Manipur, the aromatic plants are associated with religious ceremony and cultural activities and also therapeutically used as diuretic, antiseptic, anthelmintic, antirheumatic, stimulant, anti-diarrheal, analgesics and counter irritant by the local medicinal practitioner called Maiba (male) and Maibi (female) from time immemorial.[7]

The approaches on the use of new herbal products for the treatment of diseases is attracting many scientists and becoming an important area of investigation due to many side effects associated with synthetic drugs. Therefore, the present study was undertaken to evaluate the anti-inflammatory properties of ethanolic extract of *Toona ciliata* M. Roem in suitable animal models.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences (RIMS), Imphal from September 2017 to August 2019 after getting clearance from Institutional Animal Ethics Committee, RIMS, Imphal. The study was non-randomized experimental study conducted on healthy albino rats of either sex were obtained from Central animal house, RIMS, Imphal. Pregnant animals were excluded. A total of 24 albino rats of Wistar strain weighing between 100-150gm of either sex were used for each experiment. They were acclimatized to laboratory condition maintained at 24-28°C temperature and 12 hours day and night cycle and fed a standard pellet diet and water ad libitum before each experiment.

Materials for extraction: Leaves of *Toona ciliata*, Mixer grinder, Soxhlet apparatus, Petroleum ether, Ethanol, Evaporating and storage jar and mesh cotton were utilized for the extraction.

Materials for experiments: Albino mice and rats, plant extract (EETC), tab. aspirin, analgesiometer, rat restrainer, gum acacia, distilled water, bell jar, weighing machine, towel, feeding tube, tuberculin syringe, markers, carrageenan powder, turpentine oil, formaldehyde, Plethysmometer, mercury, 50 ml and 2 ml syringes, diethyl ether, cotton roll.

The fresh leaves of *Toona ciliata* M. Roem were collected during the month of August-September, 2017 from Tarung village, Imphal. The plant was identified and authenticated by Botany Department, D.M College, Imphal, having the Acc. No. DM 201/2010. The plant materials were cleansed and dried under shade, powdered by mixer grinder and stored in airtight container.

Preparation of ethanolic extract[8]: 100gm of the powder leaves of Toona ciliata M.Roem were

packed into a Soxhlet apparatus and defatted with petroleum ether. The dried defatted powder was extracted sequentially with 90% ethanol. After the completion of extraction, it was filtered and the solvent was removed by distillation under pressure. The yield was 9%. The dried ethanolic extract obtained was subjected to phytochemicals and qualitative analysis.

Acute toxicity testing was done in healthy albino mice according to OECD guidelines 423[9]. Limit test with 2000 mg/kg was carried out with six animals (three animals per step). There was no mortality observed at the dose of 2000 mg/kg of EETC. So, maximum safe dose 200 mg/kg (1/10th of maximum test dose) and 400 mg/kg of EETC were selected for the study.

Animals were divided into 4 groups of 6 animals each and fasted overnight before the experiment. Aspirin and test drugs were suspended in 2% gum acacia and administered orally to standard and test groups respectively. The control animals were given only 2% gum acacia in distilled water(DW). The volume of the medications was kept constant at 10ml/kg body weight of the animals. Both the control and the test groups in the particular series received the same phlogistic agents.

The rats were divided as follows:

Groups	Drugs administered
I (Control)	2% gum acacia in DW 10ml/kg
II (Standard)	Aspirin 100mg/kg in 2% gum acacia
III (Test 1)	EETC 200mg/kg in 2% gum acacia
IV (Test 2)	EETC 400mg/kg in 2% gum acacia

1. Carrageenan induced paw edema[10]: The animalas were treated with drugs one hour before carrageenan injection. Freshly prepared 1% carrageenan in 0.9% sodium chloride solution was injected in a volume of 0.1ml into the sub-planter region of right hind paw of albino rats. The paw volume was measured by a plethysmometer immediately after and 3 hours after carrageenan injection. Percentage inhibition of paw oedema was calculated as shown below:

% inhibition= Vc – Vt x 100 / Vc
where Vc and Vt are the mean increase in paw volume after 3 hours of carrageenan injection in control and test respectively.

2. Turpentine oil induce granuloma[11]: Subcutaneous dorsal granuloma pouch was made in ether anaesthetized rats by injecting 20 ml of air, followed by injection of 0.5 ml of turpentine oil into it. Test groups were given EETC in 2% gum acacia, standard group were given aspirin in 2% gum acacia per orally one hour prior to turpentine oil injection and continued for seven consecutive days. On day 7, the pouch was opened under ether anaesthesia, the amount of exudate was taken out with a syringe, the volume was measured and compared with those of the control and treated groups.

The percentage inhibition of granuloma formation was calculated by the following formula:
% Inhibition = (VC –VT) × 100/VC

Where, VC = Volume of exudates in control group, VT = Volume of exudates in test group

3. Formalin induced arthritis[12]: Arthritis was induced by sub plantar injection of 0.1 ml of 2% (v/v) formaldehyde solution. The paw will be marked with ink at the level of the lateral malleolus and arthritis was assessed with the help of plethysmometer by measuring the volume of mercury displaced by the paw before the induction of arthritis and once every day for 10 days, starting from day one, after induction of arthritis. The treatment was started one day before the injection of formaldehyde and continued once daily for the next ten days. Arthritis was assessed by measuring the mean increase in paw thickness and edema volume over a period of 10-day.

Results and observation
The phytochemical testing revealed the presence of tannins, flavonoids, starch, carbohydrates, protein, amino acids, steroids, triterpenoids and terpenoids in EETC.

Table 1: Acute anti-inflammatory activity of ethanolic extract of *Toona ciliata* M.Roem on carrageenan induced rat paw edema.

Group N=6	Mean increase in paw volume (cm^3) after 3h (Mean±SEM)	% inhibition of paw edema
I (Control)	0.53±0.04	0
II (Aspirin 100mg/kg)	0.18±0.03*	66.03%
III (EETC 200mg/kg)	0.35±0.04**ŧ	33.96%
IV (EETC 400mg/kg)	0.33±0.04**	37.73%0

Values are expressed as Mean±SEM, n=6, One way ANOVA (SPSS21), *P<0.001 when compared to control. **P<0.05 when compared to control, ţP<0.05 when compared to standard.

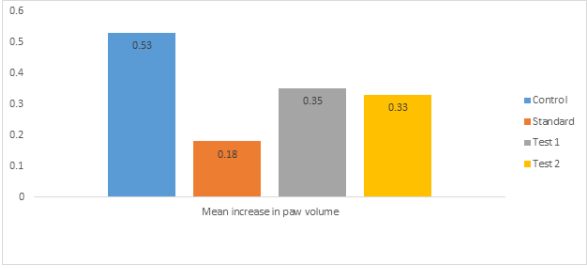

Figure III. Mean increase in paw volume in Carrageenan induced rat paw edema after 3 hours.

Table 6: Effect of ethanolic extract of *Toona ciliata* M.Roem on turpentine oil induced granuloma in rats.

Group	Drug dose	Mean volume(ml) of granulomatous exudates (Mean±SEM)	% inhibition of granuloma formation
I (Control)	10ml/kg of 2% gum acacia in DW	2.28±0.09	0
II (Standard)	Aspirin 100mg/kg	0.50±0.05*	78.07%
III (Test 1)	EETC 200mg/kg	1.56±0.06*ŧ	31.57%
IV (Test 2)	EETC 400mg/kg	1.06±0.06*ŧŧ	53.50%

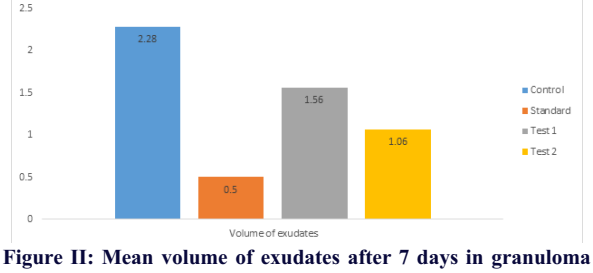

Figure II: Mean volume of exudates after 7 days in granuloma pouch test

Table 3: Effect of ethanolic extract of *Toona ciliata* M.Roem on formaldehyde induced arthritis.

Group N=6	Mean increase in paw volume (cm^3) Mean±SEM			
	3rd day	5th day	7th day	10th day
I(Control)	0.50±0.03	0.51±0.03	0.46±0.02	0.36±0.02
II(Aspirin 100mg/kg)	0.11±0.03*	0.10±0.03*	0.05±0.02*	0.03±0.02*
III(EETC 200mg/kg)	0.26±0.21*ŧ	0.21±0.03*	0.18±0.04*	0.11±0.03*
IV(EETC 400mg/kg)	0.23±0.33*	0.18±0.03*	0.13±0.04*	0.08±0.04*

Values are expressed as Mean±SEM, n=6, One way ANOVA (SPSS21), *P<0.001 when compared to control, [1]P<0.05 when compared to standard.

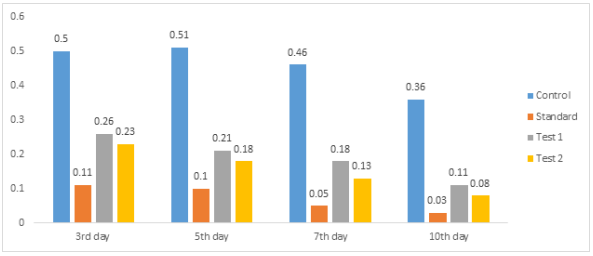

Figure III. Mean increase in paw volume in Formalin induced arthritis

DISCUSSION

Phytochemical study:

The preliminary phytoconstituent screening revealed the presence of various phytochemicals such as Tannins, Flavonoids, Starch, Proteins, Amino acids, Carbohydrates, Steroids, Terpenoids and Triterpenoids. Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidal compounds. They have been also reported to have anti-viral, antibacterial, and antiparasitic effects.[13] Flavonoids exhibit several biological effects such as antihepatotoxic, anti-inflammatory and anti-ulcer activity. They also inhibit enzymes such as aldose reductase, cyclooxygenase, Ca ATPase, xanthine oxidase, phosphodiesterase and lipoxygenase.[14] Plant steroid plays a crucial role in the development of the human body. These steroids can be used medicinally as cardiotonic, growth promoter, anti-tumor, antifungal, hepatoprotective, anti-microbial, anti-tussive etc.[15]

Anti-inflammatory activity:

Carrageenan induced paw edema is a standard experimental model of acute inflammation. Acute inflammation conveniently described as vascular and cellular events, alteration in the microvasculature is the earliest response to tissue injury. These alterations include hemodynamic changes such as transient vasoconstriction, persistent progressive vasodilatation, followed by local hydrostatic pressure, stasis, leukocyte migration, and vascular changes in which accumulation of oedema fluid. In cellular events, phagocytosis, that is

engulfment solid particulate materials by cells, causes the inflammation[16].

Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic affects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandins and slow reacting substances which peak at 3 hours. The mean increase in paw volume of the control group was 0.53±0.03. The test drug at doses 200mg/kg and 400mg/kg produced 33.96% and 37.73% inhibition of paw edema ($p<0.05$) respectively when compared to 66.03% inhibition produced by standard drug aspirin 100mg/kg. Thus, the EETC produced a significant inhibition of carrageenan induced paw edema. The inhibition was however less than that of standard drug aspirin which correspond to the findings of Manoj Kumar Sagar and Kumud Upadhyaya[17]. The ability of the ethanolic extract of the leaves of *Toona ciliata* M.Roem to inhibit carrageenan induced paw edema suggested that it possesses a significant effect against acute inflammation which might be due to the presence of flavonoids and steroids.

The granuloma pouch technique using turpentine oil as irritant as described by Selye H[11] was employed with slight modification. An aseptic inflammation resulting in large volume of haemorrhagic exudate was elicited which resembles the subacute type of inflammation. Turpentine oil induced granuloma pouch offer a model for exudative type of inflammation. Though, the chemical mediators of this type of response are unknown, protein synthesis is necessary for the formation of granuloma[18]. The mean volume of exudates in control group was 2.28±0.09.

The test drug at the dose of 200mg/kg and 400mg/kg and standard drug aspirin 100mg/kg produced 31.57%, 53.50% and 78.07% inhibition respectively of granuloma formation which was similar to the findings of Shastry VM et al[19]. The standard drug aspirin drug aspirin produced an inhibition of 78.07%. The findings of this study suggested that the ethanolic extract of *Toona ciliata* M. Roem possesses significant effect against sub-acute inflammation.

Formaldehyde-induced arthritis is the most commonly used model for determining anti-arthritic activity. Formaldehyde causes release of chemical mediators like histamine, serotonin and prostaglandin[20]. Inflammation caused by formaldehyde is biphasic in nature, i.e., it shows neurogenic elements which effect brain activity and then lead to tissue-mediated response[21]. Neurogenic phase directly stimulates paw swelling along with centrally mediated effects of pain through the release of pain mediators in the later phase. It has been proclaimed that drugs that works on CNS inhibit both phases uniformly, whilst peripherally acting drugs inhibit late phase[22]. The mean increase in paw volume in the control group was found to be 0.50±0.03 (mean±SEM) on the 3rd day and 0.51±0.03 on the 5th day, thereafter it decreases to 0.36±0.02 on the 10th day. With the test drug in doses 200mg/kg and 400mg/kg there was an increase in paw volume to 0.26±0.21 and 0.23±0.33 respectively on 3rd day and decreasing gradually. On the 10th day the volume was decreased to 0.11±0.03 and 0.08±0.04 respectively. The paw volume in the standard group treated by aspirin 100mg/kg increased to 0.11±0.03 on 3rd which decreased to 0.03±0.02 on the 10th day.

From the present study it is evident that the test drug at doses 200mg/kg and 400mg/kg significantly inhibited rat paw edema induced by formaldehyde. Inhibition of paw oedema observed in the formaldehyde model might be due to the ability of EETC to inhibit histamine, serotonin and prostaglandin, which are responsible for inflammation.

Limitations:

The biochemical and histopathological analysis were not done in the present study.

CONCLUSION

The findings of the present study showed that the ethanolic extract of *Toona ciliata* M. Roem leaves (EETC) has significant anti-inflammatory activity. Further studies on the active constituents present in *Toona ciliata* is necessary to understand the mechanism of action.

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