

Evaluation of Analgesic, Antipyretic and Anti-Inflammatory Effects of Ethanol Extract from a Fern Species *Macrothelypteris Torresiana* (Gaudich) Aerial Parts

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ABSTRACT

Introduction: *Macrothelypteris torresiana* is a species of fern native to tropical and subtropical region and belonging to family Thelypteridaceae. The present study was conducted to evaluate antipyretic, analgesic and anti-inflammatory activities of ethanol extract from *Macrothelypteris torresiana* aerial parts (EEMTAP) at doses of 200 and 400 mg/kg body weight, *per os*. **Methods:** Analgesic activity was evaluated against both thermal and chemical induced stimuli, which were evidenced from tail immersion, formalin induced paw licking and acetic acid induced writhing test. Antipyretic activity was performed by using the yeast induced hyperpyrexia method. Carrageenan induced rat paw edema and the cotton pellet granuloma model were selected for evaluating anti-inflammatory activities. **Results:** The formalin study showed that both the aphasic and tonic pain was blocked by the extract. Similarly EEMTAP significantly increased the latency period in the tail immersion test and the assessment of peripheral analgesic effect of the test drug exhibited a significant percentage inhibition in the writhings which were induced by acetic acid in mice. EEMTAP also significantly decreased the rectal temperature of the rats. Carrageenan induced rat paw edema showed that the role of EEMTAP was significant in this acute inflammation model at the tested dose level. In the sub-chronic

cotton pellet granuloma model, the tested extract also significantly inhibited granuloma formation and the biochemical parameters alanine transaminase, aspartate transaminase and alkaline phosphatase in serum. **Conclusion:** An ethanolic extract of *Macrothelypteris torresiana* possesses analgesic, anti-inflammatory and antipyretic activity which may be mediated by the central and peripheral mechanisms.

Key words: Aspirin, Ibuprofen, Indomethacin, *Macrothelypteris torresiana*, Morphine sulphate, Paracetamol.

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INTRODUCTION

Macrothelypteris torresiana (Gaudich), syn. *Lastrea torresiana* Moore (family: Thelypteridaceae) is a species of fern which is native to tropical and subtropical region of the world. It is a robust fern with a short creeping rhizome.^{1,2} In traditional medicine *M. torresiana* leaves and roots have a wide range of reputed medicinal application. The aerial parts are used for treatment of fever, pain, granulation, healing and reducing odor in chronic skin ulcer and inflammation by the tribes of Pakistan, India and China.³ It is also used in Chinese folk medicine for the treatment of edema for patient suffering from kidney problems.³ Only few phytochemical and pharmacological properties have been reported on this plant, including the renoprotective potential of *M. torresiana* via ameliorating oxidative stress and proinflammatory activities.³ *In vitro* and *in vivo* antitumor activities were reported by Huang *et al.*, 2010.⁴ A novel flavonoid was isolated from the root and the structure was identified as 5, 7-dihydroxy-2-(1, 2-isopropylidioxy-4-oxocyclohex-5-enyl)-chromen-4-one,⁵ along with four known flavonoids: protoapigenin, apigenin, kaempferol and quercetin.⁶

Literature available from all possible scientific sources revealed very little research work on this selected fern species, whereas tribes claim that *M. torresiana* was used in the treatment of various diseases and ailments, although there is no inbuilt scientific proof in support of the utility of this fern as an analgesic, anti-inflammatory and antipyretics agent. So, the present study explored the analgesic, antipyretics and anti-inflammatory activities of an ethanol extract from *Macrothelypteris torresiana* aerial parts (EEMTAP).

MATERIALS AND METHODS

Plant Material

The aerial parts of the plant *Macrothelypteris torresiana* was collected from in and around East Godavari dist., Andhra Pradesh, India and authenticated by Dr. K. Madhava Chetty, Professor, Dept. of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our research laboratory for further reference. The collected materials were washed with water and shade dried for one week. The dried aerial parts were pulverized using a mechanical grinder to obtain a coarse powder.

Preparation of the extract

The powdered plant material (500 g) was extracted with 1.5 litres of ethanol (90% v/v) for 48 hrs using a Soxhlet extractor. The extract obtained was evaporated under vacuum to remove the solvent completely and concentrated to obtain a dark greenish semisolid residue (10.68 g).

Preliminary phytochemical tests

Preliminary phytochemical studies of EEMTAP were performed for determination of major phytochemical constituents using standard procedures.^{7,8}

Animals

Swiss albino mice (20-25 g) and Wistar albino rats (150-250 g) of either sex were maintained in the animal house at GITAM institute of pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh under standard environmental conditions of temperature (25°C) and light/dark cycles

(12/12 h). All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of GITAM Institute of Pharmacy, Visakhapatnam, Andhra Pradesh, India (Regd. No. 1287/ac/09/CPCSEA and protocol No: IAEC/GIP-1307/B Pharm/IP/SM-HV/11/2012-13). Experiments were performed according to the guide for the care and use of laboratory animals. All standard drugs and EEMTAP were suspended in normal saline solution using sodium carboxy methyl cellulose (0.5% w/v) for pharmacological studies. All control groups' animals received 0.5% w/v sodium CMC in normal saline as vehicle (3 ml/kg body weight, *per os*) through oral route.

Acute toxicity study

The acute toxicity studies were conducted on Swiss albino mice as per the OECD guidelines 423,⁹ where the test dose limit of 2000 mg/kg, *p.o.*, was used. The test was carried out as suggested by Ganapaty *et al.*,¹⁰ and Shivhare *et al.*¹¹ Immediately after dosing, the animals were closely observed for the initial 4 h after the administration and then once daily during the following days. The behavioral changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep and coma. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/kg, body weight, *p.o.*) of the ethanol extract of *M. torresiana* aerial parts (EEMTAP) were selected for analgesic, antipyretic and anti-inflammatory activities studies.

Evaluation of analgesic activity

Formalin induced paw licking

The method of Dubuisson and Dennis 1977,¹² as modified by Tjolsen *et al.*, 1992,¹³ was used. In formalin induced paw licking, 0.05 ml of formalin (2.5% formaldehyde) was injected into the plantar surface of the rat hind paw, 30 min after treating the rats with EEMTAP (200, and 400 mg/kg, *p.o.*) and standard drug aspirin (100 mg/kg, *p.o.*). The time on licking the injected paw by each rat was observed as soon (early phase 0-5 min, post injection) as the formalin was injected and later (late phase 15-30 min). The mean time spent on licking the injected paw in each group was determined. Pain responses were indicated by elevation or favouring of the paw or excessive licking and biting of the paw. An analgesic response or protection is indicated if both paws are resting on the floor with no obvious favouring of the injected paw.

Tail immersion method

Analgesic activity was also checked in Wistar albino rats by the caudal immersion method.¹⁴ The tail withdrawal response was determined by immersing the tail up to the caudal portion (5 cm from the tip) in hot water at a constant temperature of 55±0.5°C. Group one served as a control and received vehicle (3 ml/kg, *p.o.*), the second group received morphine sulphate (10 mg/kg, *p.o.*) used as reference standard for activity comparison; group three and four received EEMTAP (200, and 400 mg/kg, *p.o.*) respectively. The reaction time for withdrawal of tail was recorded after 60 min from administration of test compounds. Observation was made at an interval of 30, 60 and 90 mins. The maximum time of observation would be about 60 sec throughout to avoid any tissue damage.¹⁵

Acetic acid-induced writhing method

The test was performed according to standard methods.^{16,17} Writhing was induced in mice by a single intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhings was counted over a 20 min period. Group one serve as a control and received only vehicle (3 ml/kg, *p.o.*), the second group received aspirin (100 mg/kg, *p.o.*), used as reference standard for activity comparison; group three, and four received ethanol extract of *M. torresiana* aerial parts (200 and 400 mg/kg, *p.o.*) respectively.

The writhing effect indicated by stretching of abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition was calculated.

Evaluation of antipyretic activity

Yeast induced hyperpyrexia method

The antipyretic activity was screened in Wistar albino rats by using the yeast induced hyperpyrexia method.¹⁶ Fever was induced by subcutaneously injecting 10 ml/kg of 20% aqueous suspension of Brewer's yeast in normal saline below the nape of the neck. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed an increase in temperature of at least 0.7°C,¹⁸ were selected for the study and animals were divided into four groups. Group one which is the control group received vehicle (3 ml/kg, *p.o.*) through oral route, group two received paracetamol (150 mg/kg, *p.o.*) used as a reference standard, group three and four received EEMTAP (200, and 400 mg/kg, *p.o.*) respectively. The rectal temperature of each rat was measured at 1, 2 and 6 hr after treatment.

Screening for Anti-inflammatory activity

Carrageenan induced rat paw edema

The anti-inflammatory activity of the ethanol extract from *M. torresiana* aerial parts was assessed in selected healthy adult albino rats by the carrageenan induced hind paw edema method¹⁹ using ibuprofen as the reference standard. The selected albino rats were housed in groups of six in each in acrylic cages under laboratory conditions. They were fasted over night and during the experiment but had free access to water. The test samples were suspended in 0.5% w/v sodium carboxy methyl cellulose and administered orally 30 min before injection of carrageenan (0.1 ml of 1%w/v solution) in normal saline into the sub planter region of left hind paw of each rat. The contra lateral paw was injected with an equal volume of saline. All the animal groups (Group I-IV) received the following through oral route: 0.5%w/v sodium CMC (3 ml/kg, *p.o.*), ibuprofen (10 mg/kg, *p.o.*) and EEMTAP (200, and 400 mg/kg, *p.o.*) respectively. The paw volume was measured at 1h, 2h and 4h respectively. The paw swelling was calculated by a plethysmograph as the difference of volume of mercury displaced by the inflamed paw (ml). The anti-inflammatory effect was expressed as percent inhibition of edema.²⁰

Cotton pellet granuloma model

This model was employed to study the sub chronic inflammation;²¹ here two sterilized cotton pellets weighing 10 mg were implanted subcutaneously into axilla in anaesthetized rats. After treatment with test extracts, the standard drug (indomethacin 100 mg/kg, *p.o.*) and vehicle for 7 days, the rats were anaesthetized and blood was collected by the cardiac puncture for biochemical estimation (alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP)). Determination of alanine transaminase (ALT) and aspartate transaminase (AST) was done by the method of Reitman and Frankel (1957).²² Alkaline Phosphatase (ALP) was assayed by the method of Kind and King (1954).²³ After biochemical estimations the cotton pellets were removed, freed from extraneous tissue and dried at 60°C until the weight remained constant. Then the percentage inhibition of the dry weight of the granuloma were calculated and compared.

Statistical Analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's t-test. A p-value <0.05 was considered to be significant. All the values were expressed as mean ± SEM.

RESULTS

Preliminary phytochemical tests

Preliminary phytochemical screening of the ethanol extract from *Macrothelypteris torresiana* aerial parts (EEMTAP) contains sterols, flavonoids, saponins, proteins, reducing sugar, tannins, phlobatannins and phenolic compounds (Table 1).

Acute toxicity study

No mortality or morbidity was observed in animals through the 14 day period following single oral administration. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self mutilation, walking backward etc. were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength were all normal. There was no significant difference in body weights between control and treatment groups. Food and water intake showed daily fluctuations within the range of control animals. This indicates that the ethanol extract from *M. torresiana* aerial parts was safe to a single dose of 2000 mg/kg body weight. Hence, 200 and 400 mg/kg oral doses of EEMTAP were selected to evaluate analgesic, antipyretics and anti-inflammatory activity.

Effect of EEMTAP on analgesic activity

In the formalin test, orally administration of ethanol extract from *M. torresiana* aerial parts at 200 and 400 mg/kg body weight, showed significant analgesic effect, reducing the licking time in both early and late phases (Table 2). The test extract caused significant inhibition of early phase 31.13%, 51.82% and late phase 39.01%, 53.92% at doses of 200 and 400 mg/kg body weight respectively, whereas standard drug aspirin inhibited paw licking 62.04% in early phase and 66.07% in late phase when compared with control group animals. In the tail immersion method, EEMTAP at 200 and 400 mg/kg body weight, p.o., showed significant increase in reaction time up to 60 min after giving thermal stimulus in a dose dependent manner when compared with control group animals (Table 3). Doses of 200 and 400 mg/kg EEMTAP increased the reaction time from 3.7 to 13.0 sec and from 4.0 to 17.2 sec respectively, however the reaction time of standard drug morphine sulphate (10 mg/kg, p.o.) increased from 3.9 to 21.0 sec. Assessment of peripheral analgesic effect through acetic acid induced writhing analysis was evaluated on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of animals (mice) during the writhing test (Table 4). The inhibition in writhing of the test extract were observed for 20 min. Doses of 200 mg/kg and 400 mg/kg produced an inhibition of 21.19% and 52.13% respectively when compared with the control group; whereas the group treated with aspirin (100 mg/kg) possess 57.28% inhibition. When the therapeutic activity of the test extract (EEMTAP) was compared with standard drug aspirin it showed that the observed peripheral analgesic effect for test drug was slightly less than the standard drug aspirin.

Effect of EEMTAP on antipyretic activity

Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 17 hrs of administration (Table 5). The result obtained from both the standard paracetamol (150 mg/kg, p.o.) and EEMTAP (200 and 400 mg/kg) treated rats showed significant ($P < 0.05$; $P < 0.01$) activity against induced pyrexia when compared with control group.

Effect of EEMTAP on anti-inflammatory activity

The circumference on carrageenan-induced rat hind paw edema signifies the acute anti-inflammatory effect of EEMTAP. Pretreatment with EEMTAP at 200 and 400 mg/kg prevented significant increase in volume of paw edema when compared with control group animals (Table 6). The extract showed maximum inhibition of 25.96% and 33.65% at the dose

of 200 and 400 mg/kg, p.o., after 4 h of drug treatment, whereas the standard drug ibuprofen (10 mg/kg, p.o.) showed 37.01% inhibition. The percentage inhibition of the test extract in acute inflammation in the paw of rats was found to be comparable but little lower when compared to the standard drug ibuprofen. In the cotton pellet granuloma test, the ethanol extracts from *M. torresiana* aerial parts inhibited both exudatory as well as granulatory phases of inflammation (Table 7). In granuloma induced sub chronic inflammation the test extract in dose of 200 and 400 mg/kg, p.o., had significant anti-inflammatory activity. The percentage of inhibition of granuloma after drug administration shows that 200 and 400 mg/kg of EEMTAP had 41.49% and 54.21% inhibition respectively. The standard drug indomethacin (100 mg/kg, p.o.) had 68.97% of inhibition when compared with the control group. Simultaneously biochemical parameters like ALT, AST and ALP in serum were significantly prevented by concomitant treatment of EEMTAP as well as standard drug Indomethacin (Figure 1). The results shows that after administration of EEMTAP at doses of 200 and 400 mg/kg the value of ALT is 35 ± 4.03 IU/ml and 28 ± 3.01 IU/ml respectively. Standard drug indomethacin (100 mg/kg, p.o.) has ALT value 23 ± 2.66 IU/ml when compared with control group (53 ± 3.33 IU/ml). AST value for EEMTAP at doses of 200 and 400 mg/kg is 92 ± 3.02 IU/ml and 83 ± 2.01 IU/ml respectively, whereas that of standard drug indomethacin (100 mg/kg, p.o.) is 71 ± 1.03 IU/ml when compared with control group (113 ± 4.01 IU/ml). ALP value for EEMTAP at doses of 200 and 400 mg/kg is 64 ± 2.78 IU/ml and 58 ± 2.03 IU/ml respectively and that of that of standard drug indomethacin (100 mg/kg, p.o.) is 51 ± 1.61 IU/ml when compared with control group (76 ± 3.06 IU/ml).

DISCUSSION

There has been a tremendous development in the field of synthetic drugs during recent era. But they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects.^{24,25} Therefore various pharmaceutical companies around the world are interested in developing safer and more effective drugs to treat pain, fever and inflammation.

Ethanol extract of *Macrothelypteris torresiana* aerial parts (EEMTAP) protected against both thermal and chemical induced stimuli, which were evidence from tail immersion, formalin induced paw licking and acetic acid induced writhing test. The formalin test is sensitive to NSAIDs. This test has two different phases; the early phase which may be due to direct effects on nociceptors and the late phase which is due to an inflammatory response partly mediated by prostaglandins and can be inhibited by NSAIDs.²⁶ In the present investigation the activity of ethanol extract of *M. torresiana* aerial parts was observed in both the phase at the doses

Table 1: Preliminary phytochemical tests to identify presence of various phytoconstituents in ethanol extract of *Macrothelypteris torresiana* aerial parts

Sl. No.	Test groups	Inference
1	Alkaloids	-
2	Carbohydrates	+
3	Cardiac glycosides	-
4	Proteins and amino acids	+
5	Tannins and phenolic compounds	+
6	Steroids and sterols	+
7	Triterpenoids	-
8	Saponins	+
9	Flavonoids	+

(-) Absent, (+) Present.

Table 2: Analgesic effect of EEMTAP on formalin induced paw licking

Treatment	Dose	Response (time spent in licking) (sec)	
		Early phase within 0-5 min	Late phase within 15-30 min
Control (vehicle treated)	3 ml/kg, p.o.	85.1±3.6	112.0±2.0
Aspirin	100 mg/kg, p.o.	32.3±2.0**	38.0±1.3**
EEMTAP	200 mg/kg, p.o.	58.6±3.0**	68.3±2.0**
EEMTAP	400 mg/kg, p.o.	41.0±2.1**	51.6±1.3**

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA;

*P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

Table 3: Analgesic effect of EEMTAP on tail immersion test

Treatment	Dose	Reaction time after administration (sec)			
		0 min	30 min	60 min	90 min
Control (vehicle treated)	3 ml/kg, p.o.	3.8±0.1	3.6±0.2	4.0±0.2	4.0±0.1
Morphine Sulphate	10 mg/kg, p.o.	3.9±0.1	9.3±0.1**	13.0±0.1**	21.0±0.2**
EEMTAP	200 mg/kg, p.o.	3.7±0.1	6.3±0.1*	10.1±0.2**	13.0±0.3**
EEMTAP	400 mg/kg, p.o.	4.0±0.2	7.9±0.2**	12.0±0.2**	17.2±0.4**

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA;

*P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

Table 4: Analgesic activity of EEMTAP on acetic acid induced writhing in mice

Treatment	Dose	Avg. no. of writhing	Percentage Inhibition (%)
Control (vehicle treated)	3 ml/kg, p.o.	33.03±3.02	-
Aspirin	100 mg/kg, p.o.	14.11±1.33**	57.28
EEMTAP	200 mg/kg, p.o.	26.03±2.01*	21.19
EEMTAP	400 mg/kg, p.o.	15.81±1.09**	52.13

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA;

*P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

Table 5: Effect of EEMTAP on yeast induced pyrexia in rats

Treatment	Dose	Initial rectal temperature (°C)	Rectal temperature (°C) after drug administration			
			0 h	1 h	2 h	6 h
Control (vehicle treated)	3 ml/kg, p.o.	37.41±0.02	38.87±0.04	39.22±0.13	39.7±0.04	38.84±0.05
Paracetamol	150 mg/kg, p.o.	37.23±0.01	38.85±0.03	38.18±0.01**	38.06±0.03**	37.21±0.02**
EEMTAP	200 mg/kg, p.o.	37.41±0.07	38.92±0.02	38.73±0.02	38.42±0.01**	38.05±0.02*
EEMTAP	400 mg/kg, p.o.	37.53±0.05	38.63±0.05	38.33±0.01*	38.13±0.01**	37.77±0.07**

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA;

*P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

Table 6: Effect of EEMTAP on carrageenan induced paw edema in rats

Treatment	Dose	Paw volume (ml) at different hrs			
		0 h	1 h	2 h	4 h
Control (vehicle treated)	3 ml/kg, p.o.	0.122±0.007	0.187±0.007	0.214±0.012	0.208±0.009
Ibuprofen	10 mg/kg, p.o.	0.124±0.006	0.142±0.007**	0.136±0.005**	0.131±0.007**
EEMTAP	200 mg/kg, p.o.	0.101±0.007	0.148±0.005**	0.158±0.004**	0.154±0.007**
EEMTAP	400 mg/kg, p.o.	0.102±0.007	0.132±0.005**	0.142±0.004**	0.138±0.007**

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA;

*P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

Table 7: Effect of EEMTAP on cotton pellet induced granuloma in rats

Treatment	Dose	Weight of cotton pellet granuloma (mg)	Protection percentage (%)
Control (vehicle treated)	3 ml/kg, p.o.	61.33±2.03	-
Positive control (Indomethacin)	100 mg/kg, p.o.	19.03±3.01**	68.97
EEMTAP	200 mg/kg, p.o.	35.88±4.06**	41.49
EEMTAP	400 mg/kg, p.o.	28.08±3.22**	54.21

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA;

*P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

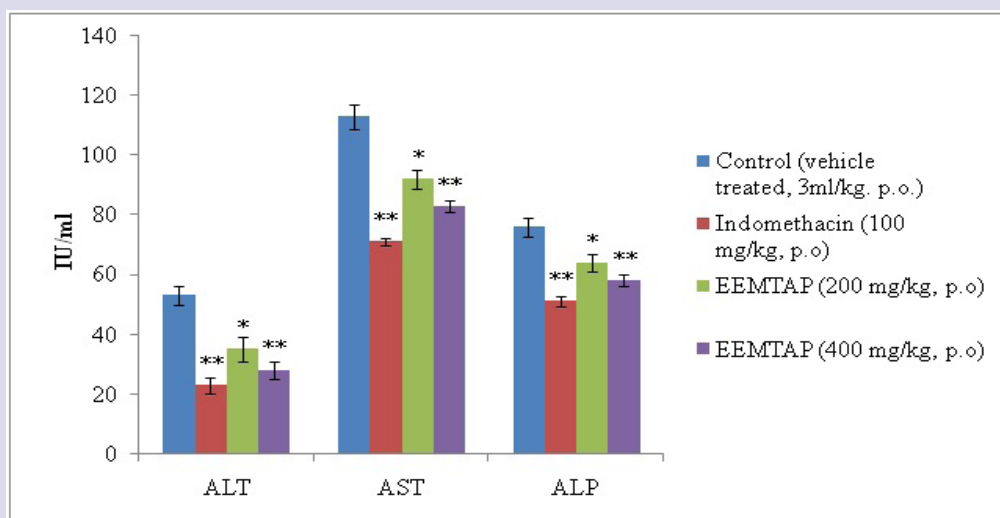


Figure 1: Effect of ethanol extract from *M. torresiana* aerial parts on various biochemical parameters in cotton pellet granuloma in rats.

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA;

*P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase

of 200 and 400 mg/kg body weight, p.o., and the results of the formalin study, showed that both the aphasical (early phase) and tonic pain (late phase) were blocked by the extract. The formalin test was conducted to distinguish analgesic from anti-inflammatory properties.²⁷ It was found that EEMTAP, as well as aspirin, exerted a marked analgesic activity in the late phase of the formalin test, suggesting an effect on acute inflammation. Similarly, EEMTAP possesses prolonged tail immersion latency, which indicates an increase in the non-nociceptive threshold and it is believed that tail immersion response is spinally mediated reflex and it is highly correlated with human pain relief.²⁸ The assessment of peripheral analgesic effect of the test drug exhibited significant percentage inhibition in the writhings which were induced by acetic acid in mice at both the tested doses of EEMTAP when compared with the control group. The percentage inhibition of writhings indicated the pronounced peripheral analgesic effect in the context of visceral pain which was comparable to the standard drug aspirin (100 mg/kg, p.o.) within 20 min of test. The overall analgesic effect of EEMTAP (200 and 400 mg/kg body weight, p.o.) was little lower than the standard drugs (aspirin and morphine sulphate). The presence of flavonoid compounds in ethanol extract of *M. torresiana* aerial parts may be responsible for the analgesic effect by inhibiting the synthesis, release or receptor responses in prostaglandin mediated effects.^{5,6,15}

During antipyretics test, the extract was demonstrated in yeast induced hyperthermia in rats. Yeast induced pyrexia is called pathogenic fever. Subcutaneous injection of Brewer's induces pyrexia by increasing the synthesis of prostaglandin.²⁹ The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretics action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity. Here our test extract significantly reduced the pyrexia induced by yeast in rats. The reference drug paracetamol (150 mg/kg, p.o.) also suppressed the fever in rats by inhibiting the synthesis of prostaglandin E₂.^{14,30} Therefore, it is reasonable to assume that the inhibition of prostaglandin biosynthesis may take part in the antipyretic activity study by the EEMTAP.

Finally, anti-inflammatory effects of the ethanol extract of *M. torresiana* aerial parts were demonstrated in carrageenan induced and cotton pellet granuloma model. Carrageenan induced on rat paw is a suitable test for evaluating acute inflammatory model.³¹ Carrageenan induced paw edema is a biphasic event. In the first phase histamine, kinins and serotonin are released, whereas in second phase edema is imposed by release of prostaglandins lysosome and protease, this phase is very sensitive to most clinically effective anti-inflammatory drugs.³² The result of carrageenan induced rat paw edema showed that the role of EEMTAP was significant in this acute inflammation model. The extract at dose

of 200 and 400 mg/kg body weight, p.o., showed significant dose dependent reduction in paw size and elicited anti-inflammatory response comparable with standard drug ibuprofen (10 mg/kg, p.o). This might be due to the inhibition of biphasic response induced by the carrageenan. Similarly to assess the efficacy of EEMTAP against proliferative phase of inflammation we have selected cotton pellet granuloma animal model in which fibrosis and tissue degeneration occurs. During the repair process of inflammation, there is proliferation of macrophages, fibroblasts, neutrophils and multiplication of small blood vessels which are the basic sources of forming a highly vascularised reddish mass, termed as granulation tissue.³³ Our tested extract significantly inhibited granuloma formation and the efficacy of the tested extract was found to be comparable but little lower when compared to the standard drug indomethacin (100 mg/kg, p.o). Therefore, from the above discussion it is possible that acute and sub chronic anti-inflammatory effect of ethanol extract from *M. torresiana* aerial parts may be involve multiple mechanism like inhibition of either cyclooxygenase and/or lipoxygenase enzyme or inhibition of synthesis, release and action of above inflammatory mediators. Anti-inflammatory effect of ethanol extract from *M. torresiana* aerial parts may be attributed due to the presence of flavonoid because several authors reported that various types of flavonoids are present in the aerial parts *M. torresiana*^{5,6} and as we know that flavonoids have various biochemical effects, which inhibit a number of enzymes like aldose reductase, xanthine oxidase, phosphodiesterase, lipoxygenase, cyclooxygenase etc.³⁴⁻³⁶

The detailed scientific evaluations of the ethanol extract from *M. torresiana* aerial parts in terms of its analgesic, antipyretics and anti-inflammatory actions indicates therapeutic efficacy, which was found to be comparable with the standard drugs. The EEMTAP was found to be significantly effective against pain, fever and inflammation and there was no noticeable acute toxicity observed during the preclinical study. Analgesic, antipyretic and anti-inflammatory properties have been known to be inter related and inter dependent and could be attributed due to the presence of flavonoids in the ethanolic extract of *M. torresiana* aerial parts.

CONCLUSION

The ethanol extract from *M. torresiana* aerial parts possesses analgesic, antipyretic and anti-inflammatory activities. Thus, it may presage further studies to better understand the mechanism of such action scientifically.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATION USED

EEMTAP: Ethanol extract from *Macrothelypteris torresiana* aerial parts; **p.o.:** per os; **OECD:** Organization for Economic Community Development; **ALT:** alanine transaminase; **AST:** aspartate transaminase; **ALP:** alkaline phosphatase; **NSAIDs:** Nonsteroidal anti-inflammatory drugs.

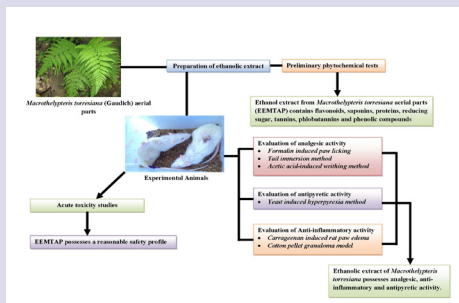
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PICTORIAL ABSTRACT



- *Macrothelypteris torresiana* is used in inflammation, pain and fever by tribal people in different parts of Asia but there is no scientific proof.
- Only a few phytochemical and pharmacological properties have been reported on this plant.
- This plant has a reasonable safety profile.
- This study confirms that the ethanol extract from *Macrothelypteris torresiana* aerial parts possesses analgesic, antipyretic and anti-inflammatory properties which confirm its folkloric use.



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