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Evaluation of analgesic and anti-pyretic activities of ethanolic extract of *Terminalia pallida* (EETP) stem in experimental animals

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Abstract – The present study was undertaken to evaluate the analgesic and antipyretic activities of ethanolic extract of *Terminalia pallida* Linn. (EETP) stems in experimental animal models. The study was carried out using Albino mice (20-30g) and rats (150-170 g) of either sex. The EETP was prepared by Soxhlet extraction process. The analgesic activity of *Terminalia pallida* Linn was assessed by using hot plate method and acetic acid induced writhing in mice. The antipyretic activity was assessed by Brewer's yeast-induced pyrexia in rats. Doses of ethanolic extract of *Terminalia pallida* used for the present study were 250mg/kg and 500mg/kg. EETP produced a significant decrease in the number of writhes in acetic acid induced writhing model of pain as well as showed a significant increase in the mean reaction time to heat stimuli in hot plate method at both 250mg/kg and 500mg/kg, p.o. doses. Single administration of EETP at doses 250mg/kg and 500mg/kg, p.o. showed significant antipyretic activity throughout the observation period of 3 hours, which was comparable to the standard paracetamol group. The present study suggested that ethanolic extract of *Terminalia pallida* Linn. has significant analgesic and antipyretic activities.

Keywords – *Terminalia pallid* Linn., antipyretic-analgesic, hot plate, acetic acid writhing, yeast induced pyrexia.

INTRODUCTION

Medicinal plants are integral part of human health system from the dawn of civilization. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.

Terminalia pallida Linn. is one of the oldest medicinal herb of India belongs to the family of Combretaceae and it is an ingredient of Indian Ayurvedic drug 'triphala' used for the treatment of digestion and liver disorders [1]. It is a small evergreen endemic tree mainly distributed in the Tirupathi Hills, Andhra Pradesh, India [2]. The fruit of this plant is used in the treatment of hepatic disorders and treatment of diabetes by tribal people. The bark has mild diuretic property, fruits are used in the treatment of ulcers, diarrhea and in venereal diseases (used by the tribal people of Tirupathi Hills)[3] and as anti-diabetic [4].

The present study was designed to evaluate the analgesic and antipyretic activities of the stem extracts of the *Terminalia pallida* Linn.

MATERIALS AND METHODS

Collection and identification of the of plant materials

The plant specimens for the purpose of study were collected from the seshachalam hills of Chittoor district of Andhra Pradesh, India in the month of July, 2011. The specimen was identified and authenticated by Dr.K. Madhava Chetty, SV University, Tirupati.

Preparation of extract of *Terminalia pallida* Linn.

Shade dried stem of *Terminalia pallida* Linn.were grounded to fine powder in an electric grinder. 300 grams of the dried powdered material were extracted with 95% ethanol using Soxhlet apparatus at a temperature of 60°C for about 48 hours which was further evaporated to dryness to obtain the ethanolic extract. The percentage yield of extract was 12.1% w/w with respect to the original air dried powder was obtained. The extract was finally stored in air tight container in a refrigerator at 2-8 °C for further use in the experiment.

Experimental Animals

Albino mice (20-30g) and rats (150-170 g) of either sex were used for experimental study. They were acclimated to laboratory conditions for seven days before the commencement of the experiments, with alternate light-dark cycle of 12 hr each and were allowed free access to standard dry pellet diet and water *ad libitum*. Animals were fasted overnight with free access to water prior to each experiment. The study was performed according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation on Animals) guidelines.

Preliminary phytochemical tests
The ethanolic extract of *Terminalia pallida* stems (EETP) were tested for different phytoconsituents like alkaloids, glycosides, saponinins , tannins, protein, carbohydrates using standard procedures [5-6].

Acute oral toxicity studies
Acute oral toxicity study was carried out for ethanolic extract of *Terminalia pallida* stems (EETP) using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423 in Female Wister rats.

Pharmacological evaluation

Acetic acid induced writhing in mice
The mice were divided into four groups of six animals each. The control group (Group I) mice received vehicle (1% w/v, Tween 80) 2 ml/kg p.o., standard group (Group II) received 40 mg/kg Ibuprofen p.o., Group III and IV received 250 mg/kg and 500mg/kg of EETP p.o. respectively. After 60 minutes, 0.1 ml of 1% acetic acid was injected intraperitonially to each group**.[7-8]** The Number of writhes (abdominal muscle contraction), stretching of the hind limbs and trunk twisting were counted for 10 min after acetic acid injection. Percent inhibition was determined for each experimental group as $(W_c\text{-}W_t / W_c)\times 100$, where W_c is the average number of writhing in control group and W_t is the average number of writhing in test group [9].

Thermal stimulus-induced pain (hot plate test) in rats
The rats were divided into four groups of six animals each. The test was carried out using Eddy's hot plate apparatus**.** The temperature was set at 55±1 ^{0}C.[9-10] The control group (Group I) mice received vehicle (1% w/v, Tween 80) 2 ml/kg p.o., standard group (Group II) received 5 mg/kg, Pentazocine, s.c., Group III and IV received 250 mg/kg and 500mg/kg of EETP p.o. respectively. Analgesic activity of EETP was assessed by placing the animals on a hot plate and observing the reaction time (paw licking and jumping) in seconds with cut-off time of 15 sec (to prevent injury) The reaction time was noted at 0, 30, 60 and 120 minutes following drug administration.

Brewer's yeast-induced pyrexia
Rats were divided into four groups of six animals each. Fever was induced in rats by subcutaneous injection of 20 mg/kg of 20% suspension of Brewer's yeast in normal saline below the nape of the neck. Initial rectal temperature were recorded. [11]. After 18h, animals that showed an increase of 0.3-0.5 ^{0}C in rectal temperature were selected. The control group (Group I) rats received vehicle (1% w/v, Tween 80) 2 ml/kg p.o., standard group (Group II) received Paracetamol 150 mg/kg p.o., Group III and IV received 250 mg/kg and 500mg/kg of EETP p.o. respectively. Antipyretic activity of EETP was assessed by measuring the rectal temperature with thermometer at 0, 30, 60, 120 and 180 minutes following drug administration.

Statistical analysis
The statistical analysis was carried by one way ANOVA followed by Dunnet's multiple "t" test. P values < 0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism5.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening
The ethanolic extract of *Terminalia pallida* stems (EETP) of stems found to contain tannins , flavonoids and triterpines and Phenolic compounds.

Acute toxicity study
Ethanolic extract of *Terminalia pallida* stems (EETP) was screened for toxicity by oral toxicity studies according to OECD guidelines 423 taking three female Wister rats with starting dose of 2000mg/kg body weight and found to be non-toxic i.e- Category 5 or Unclassified and two test dose level as low 250 mg/kg, and high 500 mg/kg selected for experiment.

Acetic acid induced abdominal writhing:
The standard drug Ibuprofen and ethanolic extract *Terminalia pallida* (EETP) at doses of 250mg/kg and 500mg/kg significantly decreased the number of acetic acid induced writhing in mice, when compared to control ($p<0.05$) [Table 1]. The percentage of inhibition of writhing at 250mg/kg and 500mg/kg *of* EETP were 30.50% and 43.56% respectively, whereas the standard drug Ibuprofen showed a reduction of 73.61%.

Table1: Effect of ethanolic extract of *Terminalia pallida* (EETP) stem on Acetic acid induced writhing in mice

Group	Drugs	Dose (mg/kg)	Mean No. of Writhing±S.E.M (10Mins.)	% Inhibition of writhing
I	Vehicle (1% Tween 80)	2ml/kg	38.40±1.15	---
II	Ibuprofen (p.o.)	40	10.13±0.99***	73.61
III	EETP (p.o.)	250	26.67±1.90*	30.50
IV	EETP (p.o.)	500	21.67±1.20*	43.56

Values are Mean ± S.E.M. (n=6) Significance vs. control group: $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Thermal stimulus-induced pain (hot plate test) in rats:

In the hot plate method, the standard drug Pentazocine and the ethanolic extract *Terminalia pallida* (EETP) at doses of 250mg/kg and 500mg/kg showed significant increase in reaction time i.e. 14.51±0.40 s, 8.32±0.49 s and 9.61±0.30 s, respectively at 120 min. when compared to control (4.17±0.40s) [Table 2]. $p<0.05$ was considered statistically significant. Similarly they showed a significant increase in reaction time at 30 and 60 minutes when compared to the control group. However the analgesic effect of EETP was less when compared to the standard drug Pentazocine.

Table 2: Effect of ethanolic extract of *Terminalia pallida* (EETP) stem on Thermal stimulus induced pain (Hot Plate Test) in Rats

			Reaction Time in seconds			
Groups	Drugs	Dose mg/kg	0 min	30 min	60 min	120 min
I	Vehicle (1% Tween 80)	2 ml/kg	4.17±0.47	4.10±0.36	4.34±0.30	4.17±0.40
II	Pentazocine (s.c.)	5	4.83±0.30	10.83±0.54***	12.17±0.30***	14.51±0.34***
III	EETP (p.o)	250	4.43±0.30	6.33±0.21^{ns}	6.73±0.33^{ns}	8.32±0.49***
IV	EETP (p.o.)	500	4.10±0.25	5.83±0.40^{ns}	7.17±0.30**	9.61±0.30***

Values are Mean ± S.E.M. (n=6) Significance vs. control group: $^{*}p<0.05$, *p<0.01, ***p<0.001.

Antipyretic activity:

The results of the antipyretic effect of the control, standard drug (Paracetamol) and the ethanolic extract *Terminalia pallida* (EETP) at doses of 250mg/kg and 500mg/kg are depicted in Table 3. The Paracetamol as well as EETP at doses of 250 mg/kg and 500mg/kg started showing significant antipyretic activity after 1h (60 min.) of post dosing when compared with the control group. Antipyretic activity was observed up to 3 h (180 min.) after paracetamol and test extracts administration.

Table 3: Effect of Ethanolic extract of *Terminalia pallida* (EETP) stem on Brewer's yeast-induced pyrexia in Rats.

			Rectal temperature in °C at time (min.)				
Groups	Drugs	Dose (mg/kg)	0	30	60	120	180
I	Vehicle (1% Tween 80), p.o	2 ml/kg	38.48 ± 0.15	38.65 ± 0.13	38.86 ± 0.09	38.96 ± 0.08	39.08 ± 0.09
II	Paracetamol (p.o)	150	38.56 ± 0.11	38.88 ± 0.07	**37.61 ± 0.14	**37.26 ± 0.16	**36.97 ± 0.16
III	EETP (p.o)	250	38.58 ± 0.17	38.86 ± 0.11	**38.18 ± 0.17	**37.94 ± 0.18	**37.80 ± 0.18
IV	EETP (p.o)	500	38.56 ± 0.14	38.81 ± 0.11	**37.89 ± 0.11	**37.61 ± 0.12	**37.38 ± 0.13

Values are Mean ± S.E.M. (n=6) Significance vs. control group: $^{*}p<0.05$, *p<0.01, ***p<0.001.

Acetic acid-induced writhing and Eddy's hot plate induced thermal stimulation are models of pain that mainly involve peripheral and central mechanisms, respectively. Analgesic effect observed in these two models with 250 mg/kg and 500mg/kg ethanolic extracts of *Terminalia pallida* (EETP) indicates the involvement of both peripheral and central mechanisms. The acetic acid-induced writhing has been associated

with an increased level of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products [13]. The present results revealed a significant reduction in acetic acid-induced writhing, and increase reaction time to heat stimuli, strongly suggests that the mechanism of the extract may be linked partly to cyclooxygenase and/or lipoxygenase inhibition. In addition, the flavonoids are known to inhibit prostaglandin synthetase [14]. Apart from flavonoids, tannins are also known to possess analgesic activity. Since prostaglandins involved in pain perception are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavonoids and tannins present in *Terminalia pallida* might be responsible for its analgesic effect.

It is well known that most of the anti-inflammatory and analgesic drugs possess antipyretic activity. The extract markedly decreased the rectal temperature of pyretic rats. This postulation is supported by the antipyretic effect of the extract, evidenced by its impact on the pathogenic fever induced by the administration of a yeast injection. Its etiology includes the production of prostaglandins in central nervous system which is the final common pathway responsible for fever induction. In general, NSAIDS produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus [15]. Therefore, it appears that the flavonoids content of *Terminalia pallida* may also be responsible for its antipyretic activity by inhibiting prostaglandin synthesis in hypothalamus.

CONCLUSION

The present study concludes that the ethanolic extract of *Terminalia pallida* (EETP) has analgesic and antipyretic activities in mice and rats at the doses of 250mg/kg and 500 mg/kg. However, this is a preliminary study and further study needs to be carried out for knowing the possible mechanism of actions and isolation of active principle(s) responsible for such activities.

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