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Anti-inflammatory, analgesic and antipyretic activity of methanolic *Tecomaria capensis* leaves extract

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ABSTRACT

Objective: To evaluate the analgesic, anti-inflammatory and antipyretic activity of methanolic *Tecomaria capensis* (*T. capensis*) leaves extract using different models in rats. **Methods:** Methanolic *T. capensis* leaves extract (100, 300, 1000 and 2000 mg/kg body weight) was given to rats orally to observe acute toxicity, and observed for 14 days. Analgesic activity was evaluated using tail immersion and formalin induced paw licking models in rats. Anti-inflammatory activity was evaluated using carrageenan induced paw edema model in rats. Antipyretic activity was evaluated using brewer's yeast induced pyrexia model in rats. Methanolic *T. capensis* leaves extract were given at dose of 100, 200 and 500 mg/kg *p.o.* **Results:** Results demonstrated that the no mortality was reported even after 14 days. This indicated that the methanol extract was safe up to a single dose of 2 000 mg/kg body weight. Methanolic *T. capensis* leaves extract (100, 200 and 500 mg/kg *p.o.*) significantly increased the latency period in the tail immersion test, reduced the licking time in both the neurogenic and inflammatory phases in the formalin test. Methanolic *T. capensis* leaves extract (100, 200 and 500 mg/kg *p.o.*) significantly prevented increase in volume of paw edema. Methanolic *T. capensis* leaves extract at the doses of (100, 200 and 500 mg/kg *p.o.*) significantly decreased the rectal temperature of the rats. **Conclusions:** This study exhibites that methanolic *T. capensis* leaves extract possesses analgesic, anti-inflammatory and antipyretic activity which may be mediated by the central and peripheral mechanisms.

1. Introduction

Tecomaria capensis (*T. capensis*) Thunb. (Bignoniaceae), also known as Cape-honeysuckle, a fast growing, scrambling shrub which may grow up to 2-3 m high and spread more than 2.5 m. *T. capensis* is an evergreen plant in warm climate areas but loses its leaves in colder areas. It has pinnately compound leaves that have oval leaflets with blunt teeth. Flowering time for this shrub is very erratic and often it flowers all year round. Flowers are orange in color. Flowers are tubular and bird pollinated, attracting nectar-feeding birds, especially sunbirds. The powdered bark of this plant is used as a traditional medicine to relieve pain and sleeplessness[1]. Bark is indicated as antidiarrheal, antipyretic and anti-inflammatory agent according to Iwalewa *et al*[2]. Analgesic compounds available in the market still present a wide range of undesired effects, leaving an open door for new and better compounds[3]. Thus, a study was made on the analgesic effects of the plant *T. capensis*.

2. Materials and methods

2.1. Plant material

The leaves of *T. capensis* were collected from Jaipur National University, Jaipur, Rajasthan, India on 1 July, 2010. The plant was identified by the Mr. Vinod Sharma, Herbarium Head, Department of Botany, Rajasthan University, Jaipur. A voucher specimen (RUBL 20847) for this plant material was preserved in the herbarium of Department of Botany, Rajasthan University, Jaipur,

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Rajasthan, India. The leaves, dried in shade were powdered and subjected to soxhlet extraction with methanol at 40-60 ℃ for 72 h. The extract collected was evaporated (yield 26.7% w/w), and stored in a vacuum desiccator. The preliminary phytochemical investigations with the methanolic extract revealed the presence of flavonoids, flavones, phenolic compound, tannins, volatile oil, fixed oil, steroids, saponins, glycosides[4-6].

2.2 Drugs and chemical

The following drugs namely, aspirin (Disprin) and paracetamol (Crocin), and chemicals, methanol (Merck) and acetic acid (Fisher Scientific) were used during the experimental study.

2.3 Animals

Albino rats of either sex (150-200 g) were used for the experimental study. The animals were maintained under standard husbandry conditions in polypropylene cages and provided with food and water *ad libitum*. The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

2.4 Acute toxicity studies

The acute toxicity was performed according to OECD guidelines[7]. The selected female albino rats were used for toxicity studies. The animals were divided into four groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded doses like 100, 300, 1000 and 2000 mg/kg body weight. Immediately, after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24 h, and daily for 14 days for any behavioral change or mortality.

2.5 Tail-immersion test

The tail withdrawal response was determined by immersing the lower 3.5 cm of the animals tail into a cup freshly filled with water from a large bath at a constant-temperature of 50 ℃until the typical response was observed. A 25 s cutoff was imposed to avoid tail damage by heat. A control group received vehicle while the aspirin 100 mg/kg *p.o.* administered to group II and methanolic *T. capensis* leaves extract (100, 200 and 500 mg/kg *p.o.*) was given to III, IV and V groups. Analgesic activity was measured at 0, 30, 60, 90, 120 and 180 min after administration of methanolic *T. capensis* leaves extract, aspirin and distilled water[8,9].

2.6 Formalin induced paw licking

In the formalin-induced paw licking, 100 μL of 3% formalin was injected into the subcutaneous tissue on the planter surface of the left hind paw of rats, 1 h after oral administration of the extracts, normal saline, aspirin and pentazocine. The rats in groups C, D and E were given oral doses of the methanolic *T. capensis* leaves extract as 100, 200 and 500 mg/kg respectively 1 h before formalin injection. The rats in groups B, C and A were given aspirin 100 mg/kg *p.o.*, pentazocine 10 mg/kg *i.p.* and an equivalent amount of normal saline (10 mL/kg), respectively 1 h before the formalin injection. The time spent 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, post injection). The mean of the time spent on licking injected paw in each group was determined[10].

2.7 Carrageenan induced paw edema

Five groups of six animals each were used. Paw swelling was induced by sub-plantar injection of 0.1 mL 1% carrageenan in saline into the right hind paw. Methanolic *T. capensis* leaves extract were administered at 100, 200 and 500 mg/kg orally 60 min before carrageenan administration. Aspirin 100 mg/kg *p.o.* was used as reference drug. Control group received the vehicle only. The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0, 1, 2, 3, 4, and 6 h after carrageenan injection. The percent inhibition of edema was calculated in comparison to the control animals[11].

2.8 Yeast-induced hyperpyrexia in rats

Before experimentation rectal temperature of rats were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Hyperpyrexia was induced in rat by subcutaneous injection of 10 mL/kg b.w. of a 15% aqueous suspension of brewer's yeast in the back below the nape of the rat. Pre-drug control temperatures were taken at 24 h after the yeast injection to determine the pyretic response of yeast. Methanolic *T. capensis* leaves extract (100, 200 and 500 mg/kg body weight) and paracetamol (150 mg/kg body weight) served as the reference drug given orally 24 h after the yeast injection. The temperatures were recorded at 1-4 h after the drug treatment[12,13].

2.9 Statistical analysis

Results are expressed as mean±SEM. Statistical significance was determined by using the one way ANOVA followed by Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Acute toxicity study

In toxicity study four groups of rats were administered with methanolic *T. capensis* leaves extract in graded doses of 100, 300, 1000 and 2000 mg/kg *p.o.*, respectively. The animals were kept under observation for the change in behavior or death up to 14 days following the plant extract administration. The extract administration neither caused any significant change in the behaviors nor the death of animal(s) in all the test groups. This indicates that the methanolic *T. capensis* leaves extract was safe up to a single dose of 2 000 mg/kg body weight. Hence we had selected 200 to 500 mg/kg oral doses of methanolic *T. capensis* leaves extract to evaluate analgesic, anti-inflammatory and antipyretic activity in rats.

3.2 Tail immersion test

Methanolic *T. capensis* leaves extract at doses of 100, 200 and 500 mg/kg *p.o.* significantly increased pain threshold after 30 min. Methanolic *T. capensis* leaves extract exhibited a dose dependent increase in the reaction time at various time intervals of observation exhibited powerful analgesic activity and results were comparable to the reference standard (Table 1).

3.3 Formalin induced paw licking

Administration of methanolic *T. capensis* leaves extract (100, 200, and 500 mg/kg), 1 h before formalin injection, showed significant analgesic effect, reducing the licking time in both the neurogenic and inflammatory phases of the formalin test. Table 2 showed that methanolic *T. capensis* leaves extract cause significant inhibition of early phase 31.46%, 45.19% and 62.74%, and late phase 46.37%, 58.03% and 63.75% at the doses of 100, 200, and 500 mg/kg, respectively. Aspirin and pentazocine inhibited paw licking 22.45% and 61.27% in early phase, and 61.27% and 71.95% in late phase respectively. Inhibition of paw licking, by methanolic *T. capensis* leaves extract was comparable to standard aspirin and pentazocine.

3.4 Carrageenan induced paw edema

Pre-treatment with methanolic *T. capensis* leaves extract at doses 100, 200 and 500 mg/kg significantly prevented increase in volume of paw edema in a dose dependent manner (Table 3). However, a maximal effect is observed at 500 mg/kg *p.o.* dose and was comparable to aspirin (100 mg/kg *p.o.*). The 500 mg/kg of methanolic *T. capensis* leaves extract was able to effectively inhibit increase in paw volume.

3.5 Brewer's yeast induced hyperpyrexia

Subcutaneous injection of yeast suspension markedly

Table 1
Analgesic effect of TCLE with tail-immersion test.

Drug	Dose (mg/kg, *p.o.*)	Reaction time after administration					
		0 min	30 min	60 min	90 min	120 min	180 min
Control	-	3.64±0.16	3.79±0.24	4.04±0.23	4.07±0.20	4.08±0.19	3.64±0.11
Aspirin	100	4.60±0.14	8.10±0.13***	12.12±0.28***	15.41±0.19***	21.51±0.54***	14.43±0.85
TCLE	100	5.08±0.22	7.42±0.19***	9.06±0.30***	12.10±0.24***	18.10±0.48***	9.74±0.56
TCLE	200	4.15±0.18	7.97±0.17***	10.07±0.16***	15.22±0.28***	20.60±0.20***	12.25±0.69
TCLE	500	4.40±0.15	9.94±0.12***	13.35±0.19***	17.28±0.58***	24.06±0.39***	17.11±0.37

Values are expressed as mean±SEM (n=6). TCLE: *T. capensis* leaves extract. *P<0.05, **P< 0.01, ***P<0.001 as compared to control. One way Anova followed by Dunnett's multiple comparison test.

Table 2
Analgesic effect of TCLE on formalin induced paw licking.

GROUP/ Dose	Response(time spent in licking)	
	Early phase within 0-5 min (% inhibition)	Late phase within 15 -30 min (% inhibition)
CONTROL -	91.14±1.72	110.83±2.65
Aspirin 100 mg/kg *p.o.*	70.68±1.95*** (22.45%)	42.92±1.44*** (61.27%)
Pentazocine 10 mg/kg *i.p.*.	25.17±1.46*** (72.38%)	31.09±1.22*** (71.95%)
TCLE 100 mg/kg *p.o.*	62.47±1.55*** (31.46%)	59.44±1.72*** (46.37%)
TCLE 200 mg/kg *p.o.*	49.95±1.76*** (45.19%)	46.51±1.78*** (58.03%)
TCLE 500 mg/kg *p.o.*	33.96±2.30*** (62.74%)	40.18±2.26*** (63.75%)

Values are expressed as mean±SEM (n=6). TCLE: *T. capensis* leaves extract. *P<0.05, **P<0.01, ***P<0.001 as compared to control. One way Anova followed by Dunnett's multiple comparison test.

Table 3

Effect of TCLE in carrageenan induced paw edema

Group/ Dose	Paw volume before carragenan admin.	Paw volume after Carrageenan administration (% increase)					
		0 min	1 h	2 h	3 h	4 h	6 h
Control	0.45±0.01	0.67±0.01 (48.88%)	0.87±0.03 (93.33%)	1.06±0.02 (135.55%)	1.15±0.02 (155.55%)	1.28±0.02 (184.44%)	1.28±0.02 (184.44%)
Aspirin 100 mg/kg *p.o.*	0.53±0.02	0.71±0.02 (33.96%)	0.80±0.01*** (50.94%)	0.86±0.02*** (62.26%)	0.88±0.02*** (66.04%)	0.89±0.02*** (67.92%)	0.89±0.02*** (67.92%)
TCLE 100 mg/kg *p.o.*	0.45±0.01	0.68±0.01 (51.11%)	0.76±0.01*** (68.89%)	0.86±0.01*** (91.11%)	0.93±0.01*** (106.07%)	0.95±0.01*** (111.11%)	0.96±0.01*** (113.33%)
TCLE 200 mg/kg *p.o.*	0.50±0.01	0.68±0.01 (36.00%)	0.74±0.01*** (48%)	0.76±0.01** * (52%)	0.82±0.01*** (64%)	0.84±0.01*** (68%)	0.86±0.01*** (72%)
TCLE 500 mg/kg *p.o.*	0.58±0.01	0.69±0.01 (18.97%)	0.76±0.01*** (31.03%)	0.79±0.01*** (36.21%)	0.81±0.01*** (39.66%)	0.82±0.01*** (41.38%)	0.82±0.01*** (41.38%)

Values are expressed as mean±SEM (n=6). TCLE: *T. capensis* leaves extract.* P<0.05, ** P< 0.01, *** P<0.001 as compared to control. One way Anova followed by Dunnett's multiple comparison test.

Table 4

Effect of TCLE on Brewer's yeast induced hyperpyrexia.

Group/ Dose	Normal temperature before yeast administration	Pre-drug control, 1 h before drug admin.	Rectal temperature after drug administration (% decrease)			
			1 h	2 h	3 h	4 h
Control	95.56±0.88	101.27±0.34	101.10±0.42 (0.17%)	101.07±0.38 (0.20%)	101.17±0.38 (0.10%)	101.07±0.44 (0.20%)
Paracetamol 150 mg/kg *p.o.*	95.43±0.48	100.17±0.30	98.20±0.49** (1.97%)	97.20±0.56*** (2.96%)	96.50±0.40*** (3.66%)	95.47±0.42*** (4.69%)
TCLE 100 mg/kg *p.o.*	96.13±0.62	100.10±0.85	98.57±0.57 (1.53%)	96.80±0.35** (3.30%)	96.33±0.52*** (3.77%)	95.97±0.58*** (4.13%)
TCLE 200 mg/kg *p.o.*	95.57±1.35	100.07±0.29	99.03±0.29* (1.04%)	97.57±0.29** (2.50%)	96.80±0.15*** (3.27%)	96.40±0.20*** (3.67%)
TCLE 500 mg/kg *p.o.*	95.23±0.49	100.50±0.12	99.40±0.29** (1.09%)	97.47±0.67*** (3.01%)	96.17±0.44*** (4.31%)	95.47±0.50*** (5%)

Values are expressed as mean±SEM (n=6). TCLE: *T. capensis* leaves extract. *P<0.05, ** P< 0.01, *** P<0.001 as compared to control. One way Anova followed by Dunnett's multiple comparison test.

elevated the rectal temperature after 24 h of administration. Treatment with the methanolic *T. capensis* leaves extract at the doses of 100, 200 and 500 mg/kg significantly decreased the rectal temperature of the rats in a dose-dependent manner. The antipyretic effect started as from the first hour and the effect was maintained for 4 h, after administration of the extract. The result obtained from both the standard (paracetamol) and methanolic *T. capensis* leaves extract treated rats were compared with that of control and a significant reduction in the yeast induced elevated rectal temperature was observed (Table 4).

4. Discussion

It is well known that pharmaceutical companies around the world are interested in developing safer and more effective drugs to treat pain, inflammation and fever. The present study evaluated the analgesic, anti-inflammatory and antipyretic effect of methanolic *T. capensis* leaves extract in several animal models.

Methanolic *T. capensis* leaves extract prolonged the tail-immersion latency, indicating an increase in the nociceptive threshold. The tail-immersion response is believed to be a spinally mediated reflex[14]. Moreover, Grumbach *et al* has shown that the effectiveness of analgesic agents in the tail flick pain model is highly correlated with human pain relief[15].

The action of analgesic drugs differs in the two phases of the formalin test. Opiates, which act centrally for the most part, inhibit both phases similarly. However, nonopiate analgesics, including dipyrone, with both central and peripheral site of actions, produce an analgesic effect in both phases of the formalin test, especially in the second phase, in which pain is inhibited at lower doses than those necessary to be inhibited in the first phase. In the present investigation, the activity of methanolic *T. capensis* leaves extract was observed in the first phase of the formalin test, a phase in which, as demonstrated by morphine, the action occurs at the level of the central nervous system[16]. Additionally, methanolic *T. capensis* leaves extract produced a significant effect on the second phase.

Inflammation induced by carrageenan was observed to have two phases *i.e.* early phase (up to 2 h) and late phase

(1-6 h). The early phase was associated with significantly severe inflammation, whereas late phase was observed to have slow increase in volume of paw edema. The initial phase has been attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability[17]. The late phase edema has been shown to be a result of over production of prostaglandins[18]. The result of pre-treatment of methanolic *T. capensis* leaves extract (at all the doses) is effective in the early phase of inflammation which has been reported because of release of histamine and serotonin primarily. Based on this an assumption can be made that the extract may be showing its effect through inhibition of histamine release.

Methanolic *T. capensis* leaves extract significantly reduced the pyrexia induced by yeast in rats. The reference drug aspirin also suppressed the yeast-induced fever in rats by inhibiting the synthesis of prostaglandin E2[19,20-26]. These results support the use of *T. capensis* as an antipyretic for the treatment of fever.

The results of this study exhibited that methanolic *T. capensis* leaves extract possesses analgesic, anti-inflammatory and antipyretic activities which may be mediated by the central and peripheral mechanisms. An activity-guided fractionation of this extract is presently being carried out. The isolation of new and effective analgesic, anti-inflammatory and antipyretic compounds is important for both drug development and establishment of the ethno medicinal use of this plant.

Conflict of interest statement

We report no conflict of interest

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