Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Evaluation of anti-nociceptive activity of methanolic extract of *Tecomaria capensis* Thunb. (Bignoniaceae) leaves in rats

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ARTICLE INFO

Article history:
Received 24 February 2012
Received in revised form 31 March 2012
Accepted 5 Apirl 2012
Available online 20 October 2012

Keywords:
Pain
Writhing
Tail-flick
Tail-clip

ABSTRACT

Objective: To evaluate the anti-nociceptive activity of *Tecomaria capensis (T. capensis)* leaves extract (TCLE) using different models in rats by acetic acid induced writhing test, (b) tail-flick test (c) tail-clip test. **Methods:** TCLE (100, 300, 1 000 and 2 000 mg/kg body wt.) was given to rats orally to observe acute toxicity for 14 d. Then test drug TCLE were given at dose of 100, 200 and 500 mg/kg *p.o.* and standard drug aspirin were given at a dose of 100 mg/kg *p.o.* **Results:** No mortality was reported even after 14 d. This indicates that the methanol extract is safe up to a single dose of 2 000 mg/kg body weight. TCLE (100, 200 and 500 mg/kg *p.o.*) significantly inhibited abdominal constrictions (writhing) induced by acetic acid and increased the latency period in the tail flick and tail clip test. TCLE at the dose of 500 mg/kg showed significant anti-nociceptive activity compared to standard aspirin. **Discussions:** The results of this study show that methanol extract of *T. capensis* possesses anti-nociceptive activity which may be mediated by the central and peripheral mechanisms.

1. Introduction

The World Health Organization (WHO) has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today[1]. Medicinal herbs are highly highlighted due to their wider use and fewer side effects. An example is *Papaver somniferum* L. (Papaveraceae), from which morphine was isolated. It is regarded as a prototype of opiate anti-nociceptive drugs. For the relief of pain, opiates generally act on the central nervous system, showing their effects through receptors (μ, κ and δ); such drugs are especially important for the treatment of chronic pain. Although morphine has reigned for centuries as the king of pain killers, its rule cannot be considered as totally benign. There are concerns regarding the side effects and addictive properties, which include respiratory depression, drowsiness, decreased gastrointestinal motility, nausea, and several alterations of endocrine and autonomic nervous system[2]. Herbs and medications share a common history, as most of our well-known medications are derived from plants. Herbal remedies are marketed commonly as pills, capsules, tinctures, or dietary supplements and are largely unregulated. According to US federal legislation that was enacted in 1994, herbs and other dietary supplements can be marketed without testing for safety or effectiveness[3].

Tecomaria capensis Thunb. (Bignoniaceae) (*T. capensis*), also known as Cape-honeysuckle, is 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[4]. Dried powdered bark infusions are taken for sleeplessness[5] and are reported to induce sleep[6]. It is included in the list of African plants evaluated for *in vitro* antiplasmodial activity against *Plasmodium*

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falciparum[7].

Pain is produced by the excitation particular receptors, the nociceptors or of their afferent fibers. These remarkable cells respond to a broad spectrum of physical (heat, cold and pressure) or chemical noxious stimuli. In general, perception of noxious stimuli is termed as nociception. Nociception is not exactly same as pain; pain is a subjective experience and includes a strong affective component, whereas nociception lacks affective component. Most of the afferent fibres that are excited by noxious stimuli are nonmyelinated C-fibres with low conduction velocities (<1 m/s), known as C-polymodal nociceptors (PMS), and other fibres are fine myelinated (A δ) fibres with rapid conduction[8]. Anti-nociceptive compounds available in the market still present a wide range of undesired effects, leaving an open door for new and better compounds[9]. Thus, a study was made on the antinociceptive 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, 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[10-12].

2.2. Drugs and chemical

The following drugs namely, aspirin (Disprin) 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[13]. 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 2 000 mg/kg body wt. 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 d for any behavioural change or mortality.

2.5. Writhing test

Albino rats of either sex weighing (150-200) g were selected and divided into 5 groups of 6 each. The total number of writhings, following intraperitoneal (*i.p.*) administration of 0.6% (10 mL/kg) acetic acid, was recorded for 20 min after injection of acetic acid. Rats place into the glass jar to correct observation of writhings. The animals were pretreated with test methanolic extract of TCLE 100, 200, 500 mg/kg b.wt and standard aspirin 100 mg/kg b. wt. orally 60 min before injection of acetic acid[14].

2.6. Tail-flick test

Rats of either sex weighing (150-200) g were selected and divided into 5 groups of 6 each. For each dose of the drug, a separate group of animals was used. The tail of the rat was placed on the nichrome wire of an analgesiometer (maintain space 2-4 mm between nichrome wire and tail) and the time taken by the animals to withdraw (flick) its tail from the hot wire was taken as the reaction time. Irresponsive rats were separated by testing all rats with tail-flick test that did not commence efforts to flick the tail within 10 s were rejected. Test TCLE methanol extract were in doses of 100, 200 and 500 mg/kg and standard aspirin 100 mg/kg. Distilled water served as control. Anti-nociceptive activity was measured 0, 30, 60, 90, 120 and 180 min after administration of TCLE methanolic extract, aspirin or distilled water[15].

2.7. Tail clip test

Rats were fasted over night with water given *ad libitum*, maintained at room temperature and irresponsive rats (Rats that did not commence continuous efforts to remove the clip within 10 s) were separated by testing all rats with tail-clip. Responsive rats were tested again before the administration of aqueous suspension of standard drug or drug extract. The rats were divided into 5 groups, each containing six rats. The first group of rats served as control receive vehicle. Second group of rats were administered with standard drug aspirin at a dose of 100 mg/kg body weight, orally. And the remaining groups were treated with the different doses 100, 200, 500 mg/kg body weight, orally of TCLE methanolic extract of test drug. Anti-nociceptive activity was measured 0, 30, 60, 90, 120 and 180 min after administration of TCLE methanolic extract, aspirin and distilled water[16].

2.8. Statistical analysis

Results are expressed as mean ± S.E.M. 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. Result

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 mg/kg, 300 mg/kg, 1000 mg/kg and 2000 mg/kg *p.o.*, respectively. The animals were kept under observation for the change in behavior or death up to 14 d 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 methanol extract is safe up to a single dose of 2 000 mg/kg body weight. Hence we have selected 100 to 500 mg/kg oral doses of methanolic *T. capensis* leaves extract to evaluate anti-nociceptive activity in rats.

3.2. Writhing test

TCLE significantly inhibited writhes in a dose dependent maner. It showed (Table 1) writhing inhibition viz. 56.7%, 75.75% and 87.02% at doses 100, 200 and 500 mg/kg respectively and results were comparable to the reference standard aspirin (74.79%).

3.3. Tail-flick test

There were no significant differences in baseline tail-flick latency in all experimental groups. TCLE at doses of 100, 200 and 500 mg/kg p.o. significantly increased pain threshold after 30 min. TCLE at 500 mg/kg exhibited powerful anti-nociceptive activity and results were comparable to the reference standard at all time intervals (Table 2).

3.4. Tail-clip test

In tail clip method, the anti-nociceptive response (mean time in seconds) increased significantly for the test (TCLE 100, 200 and 500 mg/kg *p.o.*) and standard (Aspirin 100 mg/kg *p.o.*) groups after 30 min drug administration (Table 3). TCLE exhibited a dose dependent increase in the reaction time at various time intervals of observation.

Table 1
Effect of TCLE on acetic acid induced writhes in rats.

Drug	Dose (mg/kg, *p.o.*)	Number of writhings (in 20 min)	Inhibition (%)
Control	–	156.660±1.256	–
Aspirin	100	39.500±1.727	74.79*
TCLE	100	67.830±1.167	56.70*
TCLE	200	38.000±0.856	75.75*
TCLE	500	20.330±0.760	87.02*

Values are expressed as mean±S.E.M. (n=6). *$P<0.01$, as compared to control. One way Anova followed by Dunnett's multiple comparison test.

Table 2
Effect of TCLE in tail flick response of rats.

Drug	Dose(mg/kg, *p.o.*)	Reaction time after administration of control/standard/test					
		0 min	30 min	60 min	90 min	120 min	180 min
Control	–	5.560±0.135	5.720±0.225	6.940±0.214	6.970±0.296	7.290±0.108	6.590±0.154
Aspirin	100	5.420±0.135	8.180±0.111*	13.180±0.202*	15.140±0.236*	18.820±0.150*	9.850±0.215
TCLE	100	5.130±0.099	7.970±0.182*	11.080±0.131*	12.910±0.325*	16.880±0.256*	8.200±0.234
TCLE	200	6.260±0.145	8.980±0.155*	12.790±0.432*	16.110±0.161*	20.120±0.308*	13.520±0.376
TCLE	500	5.220±0.087	9.180±0.193*	12.520±0.226*	19.950±1.459*	25.310±0.250*	13.820±0.245

Values are expressed as mean±S.E.M. (n=6). *$P<0.01$ as compared to control. One way Anova followed by Dunnett's multiple comparison test.

Table 3
Effect of TCLE with tail clip test.

Drug	Dose(mg/kg, *p.o.*)	Reaction time after administration of control/standard/test					
		0 min	30 min	60 min	90 min	120 min	180 min
Control	–	3.760±0.070	4.090±0.098	4.710±0.079	5.020±0.089	4.960±0.189	4.750±0.230
Aspirin	100	3.510±0.116	8.600±0.382*	12.580±0.444*	15.100±0.294*	17.250±0.427*	11.110±0.249
TCLE	100	4.120±0.123	6.490±0.143*	9.210±0.116*	12.090±0.248*	13.980±0.220*	7.920±0.255
TCLE	200	4.040±0.068	8.060±0.261*	12.760±1.493*	15.130±0.234*	18.270±0.180*	10.990±0.151
TCLE	500	4.200±0.154	10.960±0.225*	15.320±0.274*	18.050±0.182*	21.060±0.302*	15.830±0.240

Values are expressed as mean±S.E.M. (n=6). *$P< 0.01$ as compared to control. One way Anova followed by Dunnett's multiple comparison test.

4. Discussion

The writhing test, tail flick, tail clip test is generally used for screening of anti-nociceptive activity[17]. In writhing test, acetic acid used as an inducer for writhing syndrome, causing pain by releasing endogenous substances including histamine, serotonin, bradykinin, substance P, and prostaglandins, which then stimulate the pain nerve endings leading to the abdominal writhing[18]. Peripheral anti-nociceptive effects were observed by reduction in writhing and central anti-nociceptive effects were observed by central delay in reaction time to thermal pain[19-20].

On preliminary phytochemical screening the methanol extract of TCLE was found to contain flavonoids, flavones, phenolic compound, tannins, volatile oil, fixed oil, steroids, saponins, glycosides. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception[20]. Hence, the presence of flavonoids may be contributory to the anti-nociceptive activities of TCLE. TCLE showed significant inhibition on acetic acid induced writhing response. The reference drug aspirin (100 mg/kg) also produced significant protective effects towards the acetic acid-induced pain in rat. In comparison, the TCLE was more potent than aspirin at 500 mg/kg. It is observed that the TCLE exhibited potent anti-nociceptive activity in acetic acid-induced writhing, tail flick, tail clip anti-nociceptive models in rats. The results indicate that the TCLE possessed both peripheral (reduction in writhing) and central (delay in reaction time to thermal pain) anti-nociceptive effects.

The results of this study exhibited that methanol extract of Tecomaria capensis possesses antinociceptive activity which may be mediated by the central and peripheral mechanisms. Further research is important for both drug development and establishment of the ethno medicinal use of this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This study was supported by the management and faculty of Jaipur National University, Jaipur, Rajasthan, India.

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