

ANTINOCICEPTIVE AND ANTI INFLAMMATORY EFFECTS OF *TECTONA GRANDIS* (L.) BARK

Bhangale J.O.¹, Chaudhari S.R.², Shete R.V.², Kale B.N.²

¹Department of Pharmacology, Ideal College of Pharmacy and Research, Kalyan (E), Dist. Thane, 421038 MS, India.

²Department of Pharmacology, Rajgad Dhyanepeeth College of Pharmacy, Bhor, Pune, MS, India.

Summary

The aqueous extract of *Tectona grandis* bark (ATG) was investigated for anti-inflammatory and antinociceptive activity at the doses (p.o.) of 100, 200 and 400 mg/kg body weight. The acetic acid-induced writhing response, tail immersion test and hot plate test were used to assess antinociceptive activity. For evaluation of anti-inflammatory activity, carrageenan induced paw edema served as acute model. At a dose, ATG (100, 200 and 400 mg/kg, p.o.) significantly attenuated the writhing responses induced by an intraperitoneal injection of acetic acid and at ATG (200 and 400 mg/kg, p.o.) significantly increased pain latencies in tail immersion and hot plate test. In addition, the higher doses of ATG (200 and 400 mg/kg, p.o.) were inhibited carrageenan induced paw edema. From acute oral toxicity studies (OECD-423 guidelines), no mortality was observed even at highest dose of ATG (2000 mg/kg, p.o.).

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Address for correspondence:

Jitendra Bhangale

Department of Pharmacology,

Ideal College of Pharmacy and Research,

At.post. Dwarli, Bhal, Kalyan (E),

Dist. Thane, 421 301, Maharashtra, India.

Tel: 02513204294

E mail: jitendra.bhangale2586@gmail.com

Introduction

Inflammation is the response of living tissue to injury. It involves a complex array of enzyme activation mediator release, extravasations of fluid, cell migration, tissue breakdown and repair [1,2]. Inflammation has become the focus of global scientific research area because of its implication in virtually all human and animal diseases. Modern system of anti-inflammatory drugs (NSAID) have various side effects like tolerance and dependence induced by opiates. Thus, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases [3,4]. Therefore, new anti-inflammatory and analgesic drugs lacking these side effect are being researched as alternative to NSAID and opiates [3,5]. Attention is being focused on the investigation of the safety and efficacy of plant based drugs used in traditional medicine because they are economical, have less side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies [3,5,6]. *T. grandis* is used in folkloric medicine in India and its vernacular names are 'sagwan', 'sag', 'Tekku-maram'. The plant *T. grandis* Linn. is widely distributed in Asian countries and is reported to have antimicrobial activity [7], wound healing activity [8] and is used traditionally for treating bronchitis, diabetes, skin disease and cancer. Several studies have been carried out to identify some active constituents, including anti-cancer and lipid peroxidation factors [9].

Material and Methods

Collection of Plant material: Fresh bark of *T. grandis* Linn. (Verbenaceae) was collected from Jalgaon district, Maharashtra. The specimen was authenticated at Agharkar Research Institute, Pune with voucher specimen no. 08-146 and catalogued.

Preparation of extracts: The bark was shed dried and latter powdered. The powder was then extracted by boiling at 100°C in 100 ml of distilled water for 30 min. The mixture was filtered and evaporated to dryness. The dark brownish semisolid mass obtained was stored in a well- closed, airtight and light- resistant container.

Animals: Male Wistar rats of either sex weighing between 180 to 220 g were used for acute inflammatory model. Swiss albino mice of either sex weighing between 20 to 25 g were used for antinociceptive activity. They were maintained at a temperature of 25±4°C under 12-h light:12-h dark cycle. The animals had free access to food pellets and water *ad libitum*.

Drugs and chemicals: Acetic acid (Reagen Co.), Carrageenan (Sigma Aldrich, USA), Diclofenac sodium (Novartis Pharmaceuticals, Thane), Indomethacine (Jacksonpal pharmaceutical Ltd. Faridabad), Aspirin, Pentazocine (Ranbaxy).

Acute oral toxicity studies: Acute oral toxicity studies were performed [10] according to OECD-423 guidelines (acute toxic class method). Swiss mice ($n = 3$) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The aqueous extract of *T. grandis* was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse out of three animals then the same dose was repeated again to con-

firm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 50, 300 and 2000 mg/kg.

Antinociceptive activity

Writhing test: Male Swiss albino mice (25-30 g) were divided into five groups containing six animals each. ATG (100, 200 & 400 mg/kg, p.o.), Indomethacine (10 mg/kg, p.o.) [11]. All the drug treatments were given 1 hour before i.p. injection of 0.6 % (v/v) acetic acid, at a dose of 10 ml/kg [12]. Writhing is a syndrome characterized by a wave of contraction of the abdominal musculature followed by a wave of contraction of hind limbs. The hind limbs contractions that occurred over a period of 10 min were counted. A reduction in time of writhing initiation & number of writhing as compared to the vehicle treated group was considered as evidence for the analgesia.

Tail immersion test: Mice were divided into groups of five animals each. The lower 5 cm portion of the tail was immersed in a beaker of water maintained at $55 \pm 0.5^\circ\text{C}$ [13]. The time in seconds for tail withdrawal from the water was taken as the reaction time, with a cut-off time of immersion set at 10s. The reaction time was measured 1 h before and 0.5, 1, 2, 3, 4 and 6 h after oral administration of ATG (100, 200 and 400 mg/kg, p.o.), aspirin (100 mg/kg, p.o.) or distilled water (10 ml/kg, p.o.) [14,15].

Hot Plate Method: The hot plate test was used to measure the latencies according to the method described by Eddy and Leimbach (1953) [16]. Animals were placed on a hotplate maintained at a temperature of $55 \pm 1^\circ\text{C}$ for a maximum time of 15s. The time between placement of animal on the hot plate and occurrence of licking of the fore or hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with basal latencies of more than 10 s were eliminated from the study. The testing of response latencies was measured before ATG (100, 200 and 400 mg/kg, p.o.) or distilled water (10 ml/kg, p.o.) or pentazocine (10 mg/kg, i.p.) distraction (basal) and 30, 60 and 90 min. after treatment. The cut off time for hotplate latencies was set at 15s [17].

Anti inflammatory activity

Carrageenan induced rat paw Oedema: Carrageenan induced rat paw edema is a gold model for screening of acute anti-inflammatory activity of test compound. In the present study, the animals were starved overnight. The animals were divided randomly in five groups with 6 rats per group as follows-Group I- vehicle control, Group II- standard i.e. diclofenac sodium (10 mg/kg, p.o.), Group III- ATG (100 mg/kg, p.o.), Group IV- ATG (200 mg/kg, p.o.) and Group V- ATG (400 mg/kg, p.o.). After selection of animals, 0.1 ml of 1% carrageenan solution was injected into the left hind paw. The pretreatment time was 1 h. before carrageenan injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured and the percentage of inhibition was calculated [18-23].

Statistical Analysis: The observations were expressed in mean \pm S.D. The difference in response to test drug was determined by one way analysis of variance followed by Dunnett's multiple comparisons test $P < 0.05$ was considered as significant.

Results

Acute oral toxicity test

T. grandis extract did not produce any mortality even at the highest dose (2000 mg/kg, p.o.) employed. All the doses (5, 50 and 300 mg/kg, p.o.) of ATG were found to be non-toxic. Three doses (100, 200 and 400 mg/kg, p.o.) of ATG were selected for further pharmacological studies.

Writhing test

T. grandis significantly reduced writhings and stretchings induced by 0.6% acetic acid at a dose of 10 ml/kg. The significant protective effect was dose dependent with 39.08% ($P < 0.001$), 54.31% ($p < 0.001$) and 67.51 ($p < 0.001$) reduction observed for 100, 200 and 400 mg/kg respectively. Indomethacine (100 mg/kg) had 73.60% ($P < 0.001$) inhibition.

Table1: Effect of aqueous extract of *T. grandis* on acetic acid induced writhing in mice

Treatment	Dose Mg/kg	No of wriths	% inhibition
Vehicle	10	32.83±1.17	-
Indomethacine	10	8.67±0.42	73.60***
ATG	100	20.00±0.86	39.08***
ATG	200	15.00±0.58	54.31***
ATG	400	10.67±0.61	67.51***

Data was expressed as means ± S.E.M and analysed by one way ANOVA followed by Dunnett's test, n=6, ***p<0.001

Tail immersion test

As illustrated in table 2, the reaction time of animal showed a significant increase ($p < 0.001$) with increasing duration (time). Treatment with pentazocine and ATG (200, 400 mg/kg, p.o.) has significantly ($p < 0.001$) increased pain latencies at 1, 2, 3, 4 and 6 h. as compared to vehicle treated group. Treatment with ATG (100 mg/kg, p.o.) did not increase the duration time as compared to vehicle group.

Table2: Effect of aqueous extract of *T. grandis* on latency period (s) in tail immersion method

Treatment	Dose mg/kg	Latency period (h)						
		0	0.5	1	2	3	4	6
Vehicle	10	4.44±0.25	4.23±0.20	3.25±0.08	2.74±0.11	2.38±0.09	2.24±0.05	2.17±0.05
Aspirin	100	4.20±0.15	4.99±0.18 (19.03)**	5.96±0.11 (41.99)***	7.24±0.17 (72.47)***	8.41±0.19 (100.44)***	9.06±0.08 (115.97)***	9.69±0.16 (131.03)***
ATG	100	4.10±0.16	3.55±0.16 (-13.34)	3.14±0.07 (-23.43)	2.68±0.16 (-34.62)	2.50±0.14 (-38.97)	2.40±0.08 (-41.33)	2.27±0.05 (-44.59)
ATG	200	4.03±0.22	4.41±0.25 (9.39)	5.20±0.24 (28.99)***	5.91±0.05 (46.61)***	6.26±0.04 (55.29)***	6.93±0.05 (71.84)***	7.59±0.11 (88.21)***
ATG	400	4.11±0.10	4.82±0.11 (17.23)*	5.75±0.17 (39.76)***	6.46±0.14 (57.07)***	7.32±0.14 (78.07)***	7.92±0.12 (92.62)***	8.55±0.11 (107.90)***

Data was expressed as means \pm S.E.M (% inhibition) and analysed by one way ANOVA followed by Dunnett's test, n=6, *<0.05**p<0.01 ***p<0.001

Hot Plate Method

Pretreatment of animals with ATG extract (200 and 400 mg/kg, p.o.) increased the pain latency in the hot-plate test. The increase in the latency response was found to be statistically significant (p<0.01 and 0.001) at 30, 60 and 90 min post treatment. The known centrally acting analgesic pentazocine also increased the response latencies at 20, 60 and 90 min. The ATG extract (100 mg/kg, p.o.) did not show any significant effect.

Table 3: Effect of aqueous extract of *T. grandis* on hot plate method in mice

Treatment	Dose Mg/kg	Pain latency (min.)			
		0	20	60	90
Vehicle	10	10.72±0.55	8.82±0.22	8.12±0.15	7.00±0.12
Pentazocine	10	9.73±0.35	14.72±0.77***	14.30±0.66***	15.20±0.43***
ATG	100	10.52±0.52	10.10±0.32	8.87±0.14	8.25±0.27
ATG	200	9.72±0.39	11.03±0.57**	13.45±0.32***	15.88±0.40***
ATG	400	9.65±0.57	11.83±0.49***	14.75±0.54***	16.97±0.40***

Data was expressed as means ± S.E.M and analysed by one way ANOVA followed by Dunnett's test, n=6, ***p<0.001

Carrageenan induced rat paw Oedema

Sub-planter injection of carrageenan produced increase in paw volume of all the animals of various groups. At different doses of ATG (200 and 400 mg/kg, p.o.) and diclofenac sodium (10 mg/kg, p.o.) has significantly decreased (p<0.001) carrageenan induced edema at 1, 2, 3, 4 and 6 hr as compared to vehicle treated group. ATG (100 mg/kg, p.o.) did not show significant effect on carrageenan induced rat paw edema.

Table 4: Effect of aqueous extract of *T. grandis* on carrageenan induced rat paw oedema

Treatment	Dose Mg/kg	Change in paw vol (h)				
		1	2	3	4	6
Vehicle	10	0.28±0.02	0.39±0.01	0.48±0.02	0.57±0.02	0.64±0.01
Diclofenac sodium	10	0.11±0.01 (60.24)***	0.13±0.01 (65.94)***	0.13±0.01 (72.28)***	0.13±0.01 (76.69)***	0.15±0.01 (76.36)***
ATG	100	0.22±0.01 (20.48)	0.30±0.02 (22.41)	0.36±0.02 (24.21)	0.40±0.02 (29.20)	0.44±0.02 (31.68)
ATG	200	0.19±0.01 (31.32)***	0.25±0.01 (35.34)***	0.27±0.01 (43.15)***	0.32±0.01 (43.65)***	0.33±0.01 (48.57)***
ATG	400	0.15±0.01 (45.78)***	0.16±0.01 (58.62)***	0.16±0.00 (66.31)***	0.19±0.00 (66.37)***	0.20±0.00 (68.83)***

Data was expressed as means ± S.E.M (% inhibition) and analysed by one way ANOVA followed by Dunnett's test, n=6, ***p<0.001

Discussion

The study indicated that aqueous extract of *T. grandis* has both peripheral and central analgesic properties. Its peripheral analgesic activity was deduced from its inhibitory effects on chemical (acetic acid, inflammatory phase) induced nociceptive stimuli. At 100mg/kg (39.08%), 200 mg/kg (54.31%), 400 mg/kg (67.51%) of ATG, and 100 mg/kg (73.60%) of indomethacine, the peripheral analgesic action of the extract on acetic acid induced pain was found to be significant as comparable to vehicle treated animals. The centrally acting protective effects of the extract were corroborated by the first phase of tail immersion and hotplate test results. The tail immersion test indicated that the pharmacological actions were mediated by mu (μ) opioid receptors rather than kappa (κ) and delta receptors [24,15]. At 200 and 400 mg/kg of ATG, the central analgesic action of the extracts on hot plate test were found to be significant ($p < 0.001$) comparable to vehicle treated animals. With respect to the writhing test, the research group of Deraedt et al. (1980) [25] described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators [26-28]. Thus, the results obtained for the writhing test using acetic acid were similar to those obtained for the edematogenic test using carrageenan, since ATG (100, 200 and 400 mg/kg, p.o.) was effective in inhibiting the acetic acid induced writhing in mice. Therefore, an anti-inflammatory substance may also be involved in the peripheral analgesic activity.

Carrageenan induced oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic the first phase (60 min) involves the release of serotonin and histamine while the second phase (over 60 min) is mediated by prostaglandins, the cyclooxygenase products, and the continuing between the two phase is provided by kinins [29]. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [30]. This study has shown that the aqueous extract of the bark of the *T. grandis* (200 and 400 mg/kg, p.o.) possess a significant ($p < 0.001$) anti-oedematogenic effect on paw oedema induced by carrageenan compared to vehicle treated animals. Since carrageenan induced inflammation model is a significant test for anti-inflammatory agent acting by the mediators of acute inflammation [31]. The results of this study showed that *T. grandis* can be effective in acute inflammatory disorder. These data validated the traditional uses of this plant to assuage pain resulting from headache, dysmenorrhoea, and toothache as well as inflammatory diseases like gout, rheumatism, cystitis and nephritis.

Conclusion

It can be concluded that *Tectona grandis* is endowed with peripheral and centrally acting analgesic properties as well as antiinflammatory activity on acute inflammatory processes.

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