



REGULAR ARTICLE

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *TERMINALIA ARJUNA* LEAF

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SUMMARY

Terminalia arjuna Roxb. (Combretaceae), commonly known as Arjuna is traditionally used for several medicinal purposes in India. The present study assessed analgesic and anti-inflammatory activities of its leaf. Dried and crushed leaves of *Terminalia arjuna* were defatted with petroleum ether and then extracted with methanol. The methanol extract (META) at the doses of 100 mg/kg and 200 mg/kg body weight was subjected for evaluation of analgesic and anti-inflammatory activities in experimental animal models. Analgesic activity was evaluated by acetic acid-induced writhing, hot plate and formalin tests in Swiss albino mice; and acute anti-inflammatory activity was evaluated by carrageenan, histamine and dextran-induced paw oedema in Wister albino rats. Aspirin and indomethacin were employed as reference drugs for analgesic and anti-inflammatory studies respectively. In the present study, the methanol extract of the leaves of *Terminalia arjuna* demonstrated significant analgesic and acute anti-inflammatory activities in the tested models.

Key words: *Terminalia arjuna* Roxb., Analgesic, Anti-inflammatory, Pain

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1. Introduction

Terminalia arjuna Roxb. (Combretaceae), commonly known as Arjuna is traditionally used for several medicinal purposes in India. It is a large tree, often with buttressed trunk, smooth grey bark and about 20 - 25 m in height. Its leaves are usually sub-opposite, oblong or elliptic-long, pale dark green above and pale-brown beneath, 10-20 cm long and hard. The flowers are yellowish-white while the fruits are 2.5-5.0 cm ovoid or ovoid-oblong, fibrous-woody, and glabrous. It is common on the banks of rivers, streams and dry watercourses in sub-Himalayan tract, West Bengal as well as in central and South India. The bark of the plant is known to contain a crystalline compound, arjunine, a lactone, arjunetin, essential oil and reducing sugar. Besides these, it also contains 34 % calcium carbonate, 9% of other salts of

calcium, 13% tannin and aluminum, magnesium, organic acids, colouring matter and other substances [1]. In Indian traditional medicine, the fruits of the plant are used as a tonic [2]. Externally, its leaves are used as a cover on sores and ulcer. The bark is anti-dysenteric, antipyretic, astringent, cardiotoxic, lithotriptic and tonic while the powder of the bark acts as a diuretic in cirrhosis of liver and gives relief in symptomatic hypertension [3]. A decoction of the thick bark made with milk is given every morning on an empty stomach or its powder with milk and gurrh as a cardiotoxic [4]. The bark powder is also given with honey in fractures and contusions with echymosis. Furthermore, the extract of the bark, as an astringent, is used for cleaning sores, ulcers and cancers, etc. An ointment

made from the bark by mixing with honey is used to cure acne while the ashes of the bark are prescribed in scorpion stings [5]. The present work was undertaken to evaluate the methanol extract of the leaves of *T. arjuna* for its analgesic and anti-inflammatory activity.

2. Materials and Methods

Plant material

The leaves of *Terminalia arjuna* were collected in January 2008 from Nadia, West Bengal, India. The plant material was taxonomically identified by Dr. Lakhmi Narashimhan, Scientist, Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India. The voucher specimen [CNH/I-I/(216)/2008/Tech.II/216] and the herbarium were preserved in the laboratory of Bengal Institute of Pharmaceutical Sciences, Kalyani 741235, India for future reference.

Extraction

The powdered plant material (400 g) was macerated at room temperature (24-26°C) with methanol (850 ml) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 more days. The macerates were combined, filtered and distilled off in reduced pressure. The resulting concentrate was vacuum dried at 40°C to yield the dry extract (META, yield: 21.45% w/w). The dry extract was kept in a vacuum desiccator until use. Preliminary phytochemical studies of META revealed the presence of alkaloids, triterpenoids, tannins and flavonoids [6].

Animals

Adult male Swiss albino mice of about 2 months of age weighing 20 ± 2 g were obtained from the Laboratory Animal Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India. The mice were grouped and housed in polyacrylic cages (38×23×10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$ with dark/light cycle 12/12 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and

water ad libitum. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All experimental procedures were reviewed and approved by University Animal Ethics Committee, Jadavpur University.

Evaluation of analgesic activity

Acetic acid induced writhing test

Swiss albino mice were divided into four groups (n = 6). Group I received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes. Group II received aspirin (100 mg/kg b.w. p.o.) Group III and IV received META at the doses of 100 mg/kg and 200 mg/kg b.w., p.o. respectively. 30 min after aspirin and META administration, group II and III received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 min [7, 8].

Hot plate assay

The hot plate test was carried out as described by previous workers [7]. Four groups of mice (n = 6) were treated orally with META (100 and 200 mg/kg b.w. p.o.), morphine sulphate (10 mg/kg b.w. p.o.) and normal saline (5 ml/kg b.w.). Mice were placed on a hot plate (Bibby Sterilin, UK) maintained at $55 \pm 1^\circ\text{C}$ and the reaction latency (in seconds) for licking of hind paw or jumping noted. The mice which reacted within 15 sec and which did not show large variation when tested on four separated occasions were selected for studies. Recordings were taken before treatment with the different drugs and 1, 2, 3, 4, 5 h post treatment. Results were expressed as the difference between the baseline reaction latency and the reaction latency at recorded times.

Formalin assay

The formalin test was carried out as described by previous workers [9]. Four groups of mice (n = 6) were treated orally with META (100 and 200 mg/kg), aspirin (100 mg/kg) and normal saline (5 ml/kg b.w.). Formalin solution (1% v/v) was injected into the sub-plantar region of the right hind paw of the animals 30 min post-

treatment. The number of times paw was licked/bitten within the time frames of 0-5 min (neurogenic phase) and 15-30 min (inflammatory phase) after formalin administration was counted.

Evaluation of anti-inflammatory activity Carrageenan induced rat paw oedema

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% carrageenan in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The META was administered to the rats 1 h before carrageenan injection. Different groups were treated as follows:

Group I: Carrageenan (0.1 ml of 1.0% carrageenan/rat to the sub plantar region).

Group II: Carrageenan + Indomethacin (10 mg/kg b. w., p. o.)

Group III and IV: Carrageenan + META (100 mg/kg and 200 mg/kg b. w., p. o. respectively). The paw volume was measured initially and at 1, 2, 3 and 4 h after carrageenan injection, using Plethysmograph, inflammation was calculated for comparison [10, 11].

Dextran induced rat paw oedema

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% dextran in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The META was administered to the rats 1 h before dextran injection. Different groups were treated as follows:

Group I: Dextran (0.1 ml of 1.0% dextran/rat to the sub plantar region).

Group II: Dextran + Indomethacin (10 mg/kg b. w., p. o.)

Group III and IV: Dextran + META (100 mg/kg and 200 mg/kg b.w., p.o. respectively). The paw volume was measured initially and at 1, 2, 3 and 4 h after dextran injection, using Plethysmograph, inflammation was calculated for comparison [10, 11].

Histamine induced rat paw oedema

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% histamine sulphate in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The META was administered to the rats 1 h before histamine injection. Different groups were treated as follows:

Group I: Histamine (0.1 ml of 1.0% histamine/rat to the sub plantar region).

Group II: Histamine + Indomethacin (10 mg/kg b. w., p. o.)

Group III and IV: Histamine + META (100 mg/kg and 200 mg/kg b.w., p.o. respectively). The paw volume was measured initially and at 1, 2, 3 and 4 h after histamine injection, using Plethysmograph, inflammation was calculated for comparison [10, 11].

Statistical analysis

The values are represented as mean \pm standard error of mean (SEM). Statistical significance was analyzed by One way ANOVA with Tukey-Kramer multiple comparison post test. *P* values of < 0.001 were considered as statistically significant.

3. Results and Discussion

The present study evaluated the analgesic and anti-inflammatory activities of methanol extract from *T. arjuna* leaf in experimental rodent models. The results are presented in Tables 1-5. The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs and chemicals. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway [14, 15]. Therefore, it may be inferred that the inhibitory effect of the compound could be due to the inhibition of prostaglandin pathway. Effectiveness in the hot plate test, on the other hand, indicated central analgesic action of META.

Table 1. Analgesic effect of META on acetic acid induced writhing in mice

Treatment	Dose	Mean no of writhing \pm SEM	% Inhibition
Acetic acid (1% v/v)	10 ml/kg	52.83 \pm 1.400	-
Acetic acid + Aspirin	100 mg/kg	17.66 \pm 1.606*	66.57
Acetic acid + META	100 mg/kg	26.00 \pm 1.261*	50.79
Acetic acid + META	200 mg/kg	21.05 \pm 1.371*	60.16

Values are mean \pm SEM (n = 6). * $p < 0.001$ when compared to control

Table 2. Analgesic effect of META on hot plate test in mice

Treatment	Dose	% Inhibition
Normal saline	5 ml/kg	-
Morphine sulphate	10 mg/kg	67.56*
META	100 mg/kg	51.79*
META	200 mg/kg	66.16*

Values are mean \pm SEM (n=6). * $p < 0.001$ when compared to control

Table 3. Analgesic effect of META on formalin induced pain in mice

Treatment	Dose	% Inhibition
Normal saline	5 ml/kg	----
Aspirin	100 mg/kg	70.55*
META	100 mg/kg	53.74*
META	200 mg/kg	69.18*

Values are mean \pm SEM (n = 6). * $p < 0.001$ when compared to control

Table 4. Anti-inflammatory effect of META on carrageenan induced rat paw oedema

Treatment		1 h	2 h	3 h	4 h	% Inhibition
Carrageenan (1% w/v)		0.73 \pm 0.08	1.40 \pm 0.57	1.80 \pm 0.57	1.66 \pm 0.08	-
Carrageenan + Indomethacin (10 mg/kg)	5*	0.20 \pm 0.05*	0.50 \pm 0.05*	0.36 \pm 0.03*	0.23 \pm 0.03*	86.12
Carrageenan + META (100 mg/kg)	5*	0.33 \pm 0.05*	0.58 \pm 0.03*	0.45 \pm 0.05*	0.36 \pm 0.03*	78.34
Carrageenan + META (100 mg/kg)	6*	0.31 \pm 0.05*	0.51 \pm 0.07*	0.41 \pm 0.04*	0.32 \pm 0.06*	80.70

Values are mean \pm SEM (n = 6). * $p < 0.001$ when compared to control

Table 5. Anti-inflammatory effect of META on dextran induced rat paw oedema

Treatment	1 h	2 h	3 h	4 h	% Inhibition
Dextran (1% w/v)	1.13 \pm 0.08	1.90 \pm 0.57	1.76 \pm 0.57	1.67 \pm 0.08	-
Dextran + Indomethacin (10 mg/kg)	0.29 \pm 0.05*	0.58 \pm 0.05*	0.46 \pm 0.03*	0.38 \pm 0.03*	77.25
Dextran + META (100 mg/kg)	0.33 \pm 0.05*	0.69 \pm 0.03*	0.58 \pm 0.05*	0.46 \pm 0.03*	72.50
Dextran + META (200 mg/kg)	0.31 \pm 0.06*	0.61 \pm 0.07*	0.52 \pm 0.04*	0.43 \pm 0.06*	74.25

Values are mean \pm SEM (n = 6). * $p < 0.001$ when compared to control

Table 6. Anti-inflammatory effect of META on Histamine induced rat paw edema

Treatment	1 h	2 h	3 h	4 h	% Inhibition
Histamine (1% w/v)	1.33±0.08	1.96±0.57	1.86±0.57	1.78±0.08	-
Histamine + Indomethacin (10 mg/kg)	0.32±0.05*	0.59±0.05*	0.51±0.03*	0.47±0.03*	73.60
Histamine + META (100 mg/kg)	0.41±0.05*	0.79±0.03*	0.68±0.05*	0.58±0.03*	67.42
Histamine + META (200 mg/kg)	0.38±0.06*	0.69±0.07*	0.62±0.04*	0.49±0.06*	72.47

Values are mean ± SEM (n = 6). * $p < 0.001$ when compared to control

The carrageenan-induced hind paw oedema model in rats is known to be the acute inflammatory model sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents (NSAID), which primarily inhibit the cyclooxygenase involved in prostaglandin (PG) synthesis. In case of the time course of oedema development in carrageenan induced paw edema model in rats is generally two phases are found. The first phase, which occurs between 0 to 2.5 h of injection of the phlogistic agent, has been attributed to the release of histamine or serotonin. The edema volume reaches to its maximum approximately 3 h post treatment and then begin to decline. The second phase of inflammatory reaction which is measured at 3h is caused by the release of bradykinin, protease, prostaglandin and lysosome [14, 15]. Therefore, it can be inferred that the inhibitory effect of the extract on the carrageenan induced inflammation could be due to the inhibition of enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

Thus, the results of the present study demonstrates that the methanol extract obtained from leaves of *Terminalia arjuna* exhibited analgesic activity and acute anti-inflammatory activity in the tested models which was found to be the most effective at higher concentrations employed. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extracts and its active compound(s).

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