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Anti-inflammatory and Anti-nociceptive Activity of *Mitragyna parvifolia*

[1]Vikas Gupta, [2]Pawan Kumar, [1]Parveen Bansal and [3]Ranjit Singh
[1]National Institute of Ayurvedic Pharmaceutical Research, Patiala, India
[2]Govt. Polytechnic College for Girls, Patiala, India (Collaborative project with NIAPR)
[3]School of Pharmaceutical Sciences, Shobhit University, Meerut, India

Abstract: The present study was designed to evaluate both anti-inflammatory and antinociceptive activity of the ethanolic extract of dried leaves of *Mitragyna parvifolia* (MPEE), using the Carrageenan-induced paw edema method in rats and Tail-flick method in mice, respectively, at various dose levels. The maximum anti-inflammatory effect of the extract was found to be at 300 mg/kg in carrageenan test and this effect was equivalent to phenylbutazone (PBZ) (80 mg/kg, orally) ($p<0.05$). The extract also demonstrated marked antinociceptive activity at a dose of 300 mg/kg and the effect was comparable to that of standard drug, Ibuprofen (100 mg/kg orally) ($p<0.05$). The results of this study have established the anti-inflammatory activity and antinociceptive activity of leaf extracts of *Mitragyna parvifolia*.

Keyword: *Mitragyna parvifolia*, paw edema method, rubiaceae and tail-flick method

INTRODUCTION

Mitragyna parvifolia commonly called Kadamb (family-Rubiaceae) is a tree of immense cultural, health, and economic importance. It is widely grown commercially in Indian Thar Desert (Shetty *et al*., 1991). The bark and roots of this plant are used to treat fever, colic, muscular pain, burning sensation, poisoning, gynecological disorders, cough, edema and as an aphrodisiac (Panwar and Trafdar, 2006). Wounds and ulcers are dressed with its leaves to alleviate pain, swelling and for better healing. (Pandey *et al*., 2006). *Mitragyna parvifolia* contains scopoletin, thermophyllin, daucosterol, quinovic acid, mitraphyllin, β – sitosterol, indolic and oxindolic alkaloids mainly mitraphylline, isomitraphylline, pteropodine, isopteropodine, speciophylline and uncarine (Shellard *et al*., 1969 a, b). However, there is no experimental evidence for antinociceptive and anti inflammatory activities with respect to the leaves of this plant. Hence, in the present study, an attempt was made to investigate the anti-inflammatory and antinociceptive effects of the ethanolic extract of dried leaves of *Mitragyna parvifolia* in experimental animals.

MATERIALS AND METHODS

Plant Material: The fresh leaves of *Mitragyna parvifolia* were collected during the months of June and July 2008 from Kurukshetra, Haryana, India and authenticated by Dr. B.D.Vashistha Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India.

Drugs: Carrageenan (Sigma-Aldrich, USA), Phenylbutazone and Ibuprofen (MS Pharmaceuticals and Laboratory Ltd., Jalandhar, India) were procured from local market.

Preparation of extract: Dried leaves of *Mitragyna parvifolia* (500 g) were chopped into small pieces, and soaked over night in 1.5 litre of 95% ethanol. This suspension was filtered and the residue was re suspended in an equal volume of 95% ethanol for 48 h and filtered again. The two filtrates were pooled and the solvents were evaporated in a rotary evaporator (Perfit, India) at 40-50°C under reduced pressure. A dark semisolid (greenish black) mass was obtained. It was stored below 4°C until further used. When needed, the extract was suspended/ dissolved in desired solvent and used.

Animals: Male Wistar rats and Swiss mice of either sex weighing 150–200g and 20-25g respectively were used in the present study. The animals were kept at room temperature allowing food and water *ad libitum* and were exposed to normal day and night light cycles. The study was approved by Institutional Animal Ethical Committee.

Anti-inflammatory activity: Experimental animals were divided randomly into five groups with six animals in each group (n=6).

- Group I- carragenan treated control group receiving normal saline.
- Group II- IV - pre-treated with MPEE in doses 100, 200 and 300 mg/kg, orally, respectively.
  Group V - pre-treated with standard drug
- Phenylbutazone 80 mg/kg, orally.

Carrageenan-induced rat paw edema: The ethanolic extract of *Mitragyna parvifolia* (MPEE) was evaluated for the anti-inflammatory activity (Winter *et al*., 1962). Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% carrageenan in normal saline in the right hind paw of the rats, 1h after the administration of the drug/extract. The paw volume was measured by using

Corresponding Author: Vikas Gupta, National Institute of Ayurvedic Pharmaceutical Research, Patiala, India

plethysmometer (Ugo Basile, Italy) at the intervals of 1, 2, 3, 4 and 24 h after the carrageenan injection. Phenylbutazone (80mg/kg, orally.) was used as standard drug. The anti-inflammatory activity was calculated as percentage inhibition of carragenan induced paw edema using the following formula (Chu and Kovacs, 1977).

Percent inhibition=100-[(edema volume in treated/edema volume in control) x 100].

Antinociceptive activity: Experimental animals were divided randomly into five groups with six animals in each group (n=6).

- Group I- control group receiving normal saline.
- Group II- IV - pre-treated with MPEE in doses 100, 200 and 300 mg/kg, orally, respectively.
- Group V - pre-treated with standard drug Ibuprofen 100 mg/kg, orally.

Tail flick method: The analgesic activity was determined by radiant heat Tail-flick method in mice (D'Amour and Smith, 1941). Ibuprofen (100 mg/kg orally) was used as standard drug. Tail-flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nichrome wire was kept constant at 5A. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was within 2 cm, measured from the root of the tail. Cut-off reaction time was 10s to avoid any tissue injury during the process. Tail-flick latency was measured after 1h of the administration of the drug/extract.

Tail flick latencies were converted to maximum possible effect (MPE), according to the following formula:

MPE (%) = 100× (post-extract latency− pre-extract latency)/ (cut-off time− pre-extract latency).

Statistical analysis: Results are expressed as mean ± SEM. The statistical significance of the observed data was determined by One Way Analysis of Variance (ANOVA) followed by Dunnet's test and results were reported to be statistically significant at $p < 0.05$.

RESULTS

Carrageenan-induced rat paw edema: The anti inflammatory effect of the ethanolic extract of leaves of *Mitragyna parvifolia* is shown in Table 1. The extract at the oral dose of 300mg/kg showed good results and caused an significant inhibition in the carrageenan induced rat paw edema. The maximum inhibition in edema volume was noted to be 37.99% as comparable to the standard drug, phenylbutazone, which caused maximum inhibition of 42.02% ($p<0.05$). The extract at lower doses of 100 and 200 mg/kg orally showed lesser anti-inflammatory effect.

Tail flick method: The antinociceptive effect of the ethanolic extract of the leaves of *Mitragyna parvifolia* is shown in Table 2. The extract at the dose of 300mg/kg orally showed 39.59 % of Maximal Possible Effect (%MPE) which was comparable to standard drug, ibuprofen which showed 43.63% of Maximal Possible Effect at dose of 100 mg/kg, orally.($p<0.05$)

DISCUSSION

Mitragyna parvifolia is an important medicinal plant which is used in traditional medicine to treat many diseases (Panwar and Tarafdar, 2006). The fruit extract of *Mitragyna parvifolia* is reported to have both analgesic and anti inflammatory activities (Saneja et.al. 2009). The carrageenan-induced paw edema in rats is believed to be bi-phasic (Vinegar, *et al.*, 1969). The first phase of inflammation is due to the release of histamine and serotonin and the second phase is due to the release of bradykinin, protease, prostaglandins and lysosomes (Crunkhorn and Meacock, 1971). Therefore, it can be concluded that inhibitory effect of MPEE on carrageenan-induced paw edema could be due to the inhibition of cyclooxygenase leading to the inhibition of prostaglandin synthesis. In Tail-flick method for antinociceptive activity

Table 1: Anti-inflammatory activity of MPEE on carrageenan induced rat paw edema

Groups	Dose (mg/kg)	% inhibition of paw volume				
		1h	2h	3h	4h	24h
Control	0.0	0.00	0.00	0.00	0.00	0.00
MPEE	100	6.61±0.29	11.71±0.45	16.21±0.19*	21.11±0.16*	9.12±0.32
MPEE	200	12.57±0.64	21.59±0.29*	32.51±0.34*	25.90±0.29*	10.32±0.19
MPEE	300	19.49±0.21*	28.89±0.38*	37.99±0.63*	32.25±0.87*	17.71±0.18
PBZ	80	29.17±0.29*	35.90±0.17*	42.02±0.44*	37.02±0.90*	21.96±0.11*

Values are Mean±SEM (n=6); one way ANOVA. *$p<0.05$ compared to control, MPEE= *Mitragyna parvifolia* ethanolic extract, PBZ= Phenylbutazone.

Table 2: Antinociceptive activity of MPEE on tail-flick assay of mice

Groups	Dose (mg/kg)	% Maximum Possible Effect		
		1h	2h	3h
Control	0.0	0.00	0.00	0.00
MPEE	100	8.19±0.42	12.22±0.22	14.38±0.54
MPEE	200	13.49±0.26	22.19±0.47*	31.74±0.15*
MPEE	300	19.99±0.29*	34.19±0.39*	39.59±0.28*
Ibuprofen	100	20.05±0.21*	37.39±0.69*	43.63±0.47*

Values are Mean±SEM (n=6); one way ANOVA. *$p<0.05$ compared to control, MPEE= *Mitragyna parvifolia* ethanolic extract.

indicated that MPEE increased the stress tolerance capacity of the animal possibly due to involvement of a higher centre (Whittle, 1964). However, further work for the isolation of active constituents and elucidating the exact mechanism underlying the observed pharmacological effects is recommended.

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