ISSN 0974 – 5211

Journal of Natural Products

Volume 2 (2009)

www.JournalofNaturalProducts.com

Research Paper

Evaluation of activities of *Mitragyna parvifolia* fruit extract

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(Received 11 February 2009; Revised 14 February 2009; Accepted 03 March 2009)

ABSTRACT

Ethanolic extract of the *Mitragyna parvifolia* was evaluated for anti-inflammatory, analgesic and antimicrobial activities. The extract showed very significant analgesic and anti-inflammatory potential. The analgesic activity was significant at the dose of 500 mg/kg ($P< 0.01$) while the doses of 250 and 100 mg/kg showed only promising results. The extract at the dose of 500 mg/Kg showed very high % inhibition in edema volume comparable to standard drug Diclofenac sodium (50 mg/Kg, i.p.). The plant extract did not exhibit any anti-bacterial potential against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Keywords: *Mitragyna parvifolia*, Anti-inflammatory, Analgesic, Antimicrobial, Activity, Fruit extract.

INTRODUCTION

Mitragyna parvifolia (Roxb.) Korth belongs to family Rubiaceae is commonly known as Kaim (Panwar and Tarafdar, 2006). The plant grows throughout India, in deciduous and evergreen forests. The chemical constituents of the plant are pyroligneous acid, methyl acetate, ketones and aldehydes. It is credited with innumerable medicinal properties and is widely used by tribal people and other ayurvedic practitioners. The bark and roots are used to treat fever, colic, muscular pain, burning sensation, poisoning, gynecological disorders, cough, edema and as aphrodisiac. The fruit juice augments the quantities of breast milk in lactating mothers and also work as lactodepurant. Wounds and ulcers are dressed with its leaves to alleviate pain, swelling and for better healing (Panwar and Tarafdar, 2006; Prajapati, et al., 2003; Pandey, et al., 2006; Shellard and Houghton, 1971). Though the plant has great potential for anti-inflammatory, analgesic and antimicrobial activity, nobody has not been yet documented these activities neither on this plant nor on any of its parts. So, in this study we have attempted to investigate the analgesic, anti-inflammatory and antimicrobial activities of *Mitragyna parvifolia* fruit extract.

MATERIALS AND METHODS

Plant material: The fruits of *Mitragyna parvifolia* Roxb. (Rubiaceae) were collected from local areas during the month of November 2008. The plant got identified and authenticated by Department of Botany, Kurukshetra University, Kurukshetra, Haryana, (India) and a voucher specimen of the sample (Sr. No. KUK/IPS/2008/MP-105) has deposited in the Herbarium collection at Department. The fruits were cleaned and dried in the shade, then powdered to 40 mesh and stored in an airtight container.

Preparation of Extract: Dried fruits powder (900 gm) was divided in three parts, treated each three times with fresh ethanol (1000 ml) separately for 48 h. The ethanolic extracts thus obtained were combined, filtered and distilled on a water bath. The last traces of the solvent were evaporated under reduced pressure in rotatory evaporator. The yield of the ethanolic extract was 1.83 % w/w. Pharmacological studies were carried out by suspending a weighed amount of the extract in normal saline (95 ml): tween 80 (5 ml) ratio.

Test animals: Wistar rats weighing 180-200 gram and Swiss albino mice weighing 25-30 gm were obtained from Haryana Agriculture University, Hisar, Haryana, (India). The animals were housed in Animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra (Haryana) in polycarbonate cages, in a room maintained under controlled room temperature 22 ± 2^0 C, relative humidity 60 - 70% and provided with food and water ad libitum. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Register Number: 562/02/a/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India. The animals were deprived of food for 24 h before experimentation but allowed free access to water throughout. All studies were carried out by using five groups of animals for both anti-inflammatory and analgesic activity.

Drugs: All the standard drugs (Ciprofloxacin and Diclofenac sodium) were obtained from various chemical units – E. Merck India Ltd. and S. D. Fine Chem. Ltd. (India).

Test microorganisms: Four microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram positive bacteria - *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) and two Gram negative - *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) were chosen for evaluation of antibacterial activity of the extract of the fruits of *M. parvifolia.* All the strains used for these studies were procured from MTCC, IMTECH, Chandigarh, India.

***In-vitro antibacterial activity*:** The antimicrobial activity (bacteria) of the compounds was evaluated by agar well diffusion method (Ahmad and Beg, 2001)**.** All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml (Andrews, 2001). 20ml of Mueller Hinton agar media was poured into each petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 1.0 mg/ml of ethanolic extract reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 hrs. Antimicrobial activity of plant extract was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). The medium with DMSO as solvent was

used as a negative control whereas media with Ciprofloxacin were used as positive control. The experiments were performed in triplicates.

Acute toxicity test: Acute toxicity tests were performed according (Ecobichon, 1977) to OECD – 423 guidelines (acute toxic class method). Swiss mice (n = 6) 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 alcoholic extract of *M. parvifolia* suspended in normal saline:tween 80 (95:5) was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 5/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in less than four mice out of six animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 100, 300 and 2000 mg/kg.

Carrageenan-induced rat paw edema method: The anti-inflammatory activity of ethanolic extract of *M. parvifolia* using carrageenan-induced paw edema was studied according to Winter *et al* (Winter *et al.*, 1962)**.** The animals were divided into five groups each consisting of ten rats. The control group received normal saline : tween 80 (95:5), the standard group received diclofenac sodium (50 mg/kg i.p.) and the test groups received the fruit extracts at the dose of 100, 250 and 500 mg/kg p.o. Thirty minutes after administration of test and standard drugs, 0.1 ml of 1% w/v of carrageenan suspension in normal saline was injected to all animals in the left hind paw (plantar region). The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer (model 7140, Ugo Basile, Italy). The measures were determined at 0 h (before carrageenan injection) and 30, 60, 90 and 120 minutes after drug treatment.

Analgesic activity: The analgesic activity was measured against chemical and thermal stimulus. For analgesic activity the animals were divided into five groups consisting of ten mice. The control group received normal saline:tween 80 (95:5) p.o., the standard group received diclofenac sodium (50 mg/kg i.p.) and the test groups received the fruit extract at the doses of 100, 250 and 500 mg/kg p.o.

Acetic acid-induced abdominal writhing test: The test was performed as described by Collier et al (Collier et al., 1968)**.** Nociception was induced by an intraperitoneal (i.p.) injection of acetic acid 1.0%, 0.1 ml/10g body weight. Five groups of ten mice each pretreated with normal saline: tween 80, diclofenac sodium and test drugs received acetic acid (i.p.) 30 minutes later. The number of streching or writhing was recorded from 5 minutes to 15 minutes.

Hot-plate test: The hot-plate was used to measure response latencies according to the method described by Eddy and Leimbach (Eddy and Leimback, 1953)**,** with minor modifications. The paws of mice are very sensitive to heat at temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. The animals were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5^0 C. A cut off period of 15 sec, was observed to avoid damage of the paw. Reaction time and the type of response were noted using a stopwatch. The latency was recorded before and after 15, 30, 60 and 120 min of both test and standard. Average reaction times were then calculated and the percentage variation calculated using following relation:-

% inhibition = [(After treatment /before treatment) - 1] x 100

Statistical analysis: All data were represented as mean ± S.E.M. and as percentage. Results were statistically evaluated using Dunnett's *t*- test. $P<0.01$ was considered significant.

RESULTS

Acute toxicity test: *M. parvifolia* fruit extract did not produce any mortality even at the dose of 1500 mg/kg, p.o. All the doses (5, 50 and 300 mg/kg, p.o.) of *M. parvifolia* were thus found to be non-toxic. On the basis of above results, three doses (100, 250, 500 mg/kg, p.o.) of *M. parvifolia* were selected for further pharmacological studies.

Acetic acid-induced writhing test: The analgesic effect of the ethanolic extract of *M. parvifolia* is shown in Table 1. The ethanolic extract at the doses of 100, 250 and 500mg/kg p.o. caused an inhibition on the writhing response induced by acetic acid. The maximal inhibition of the nociceptive response was achieved at a dose of 500 mg/kg ($P<0.01$).

Hot plate test: The oral doses of fruit extract 100, 250 and 500 mg/kg elicited a significant analgesic activity as evidenced by increase in latency time on comparison with negative control at the end of 15, 30, 60, 120 min. The increase in latency time was found in a dose dependent manner and was found to be very significant at the dose of 500 mg/kg.

Carrageenan-induced rat paw edema: The anti-inflammatory effect of the ethanolic extract of M. parvifolia is shown in Table 1. The extract at the doses of 250 and 500 mg/kg p.o. showed very good results and caused an significant inhibition in the carrageenan - induced rat paw edema. The maximal inhibition in edema volume was achieved at a dose of 500 mg/kg ($P<0.01$), comparable to standard drug diclofenac sodium.

In-vitro antimicrobial activity: In the present study, ethanolic extract was evaluated against both Gram- positive and Gram-negative bacteria. Ciprofloxacin was used as standard for comparison. The extract at concentration of 1mg/ml failed to show any zone of inhibition while standard drug showed its zone of inhibition. The results have been presented in Table 1.

DISCUSSION

The genus *Mitragyna* (family: Rubiaceae) consists of several plants used in local folkare medicine to treat a variety of disease such as fever, colic, muscular pain and for the expulsion of worms (Shellard and Phillipson, 1964).

M. parvifolia (Roxb.) is reported to have some alkaloid of significant biological importance (Shellard et al., 1969b; Pandey et al., 2006).

In these studies, the *M. parvifolia* fruit extract was found to be very non-toxic even at the high doses of 1500 mg/kg. The extract was found to have both analgesic and anti-inflammatory potential. The analgesic potential of the extract was very significant at the dose of 500 mg/kg ($P<0.01$) only. But the anti-inflammatory potential shown by the extract was significant at both the doses i.e. 250 and 500 mg/kg. The analgesic and anti-inflammatory potential of the extract was comparable to diclofenac sodium. The extract failed to show any activity against Gram positive and Gram negative bacterial strains. So, the plant needs further investigation of chemical constituents responsible for the above activity.

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Table-1: Analgesic, anti-inflammatory and antimicrobial activity of ethanolic extract of fruits of *M. parvifolia*

S. No.	Drug treatment	Dose mg/ kg (p.o.)	No. of animals	Acetic acid induced writhing method		Hot plate method			Anti-inflammatory activity			Antibacterial activity (Zone of inhibition in mm)			
				Change in No. of wriths (Mean ± SEM)	't' value	Reaction time in minutes (Mean ± SEM)	% inhibition	't' value	Change in paw volume (ml) (Mean ± SEM)	% inhibition	't' value	*S. aureus*	*B. subtilis*	*E. coli*	*P. aerugino*
1.	N.Saline: Tween 80	10 ml/ kg	10	13.30±0.37	--	1.30 ± 0.15	--	--	0.43±0.02	--	--	NS	NS	NS	NS
2.	Diclofenac sodium	50 i.p.	10	4.90 ± 0.35	16.62	4.80 ± 0.36	72.91	8.97	0.23±0.01	38.3	12.51	NS	NS	NS	NS
	Ciprofloxacin	20 µg/ml	---	NS	NS	NS	NS	NS	NS	NS	NS	26.3	25.6	25.0	23.3
3.	*M. parvifolia*	100	10	9.40 ± 0.50	6.30	1.60 ± 0.16	18.75	1.34	0.44 ± 0.01	22.6	1.04	NS	NS	NS	NS
4.	*M. parvifolia*	250	10	8.00 ± 0.70	6.71	1.90 ± 0.28	31.57	1.90	0.33 ± 0.02	34.7	4.25	NS	NS	NS	NS
5.	*M. parvifolia*	500	10	5.90 ± 0.48	12.22	3.10 ± 0.28	58.06	5.69	0.25 ± 0.01	37.8	10.94	NS	NS	NS	NS
6.	*M. parvifolia*	1 mg/ ml	--	NS	NS	NS	NS	NS	NS	NS	NS	--	--	--	--

NS- Not studied; -- NIL

For Acetic acid induced withing: F = 44.5; df = 4, 25; P < 0.01; n = 10, values are mean ± SEM. The data were analyzed by Dunnett's *t*-test. P < 0.01 compared to control group.

For Hot plate: F = 31.0; df = 4, 45; P< 0.01; n = 10. The data were analyzed by one way ANOVA followed by Dunnett's *t* -test. P < 0.01 compared to control group.

For Anti-inflammatory activity: F = 67.6; df = 4, 45; P < 0.01; n= 10. The data were analyzed by one-way. ANOVA followed by Dunnett's *t*- test. P< 0.01 compared to the control group.