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Research Article

A Study of Analgesic and Antimicrobial Potential of *Mitragyna Parvifolia*

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ABSTRACT
The present study was designed to evaluate the analgesic and antimicrobial activities of ethanolic extract of leaves of *Mitragyna parvifolia* plant (*Rubiaceae*) family. The analgesic activity was carried out on swiss albino male mice by Eddy's hot plate and Acetic acid induced writhing test. The extract showed only moderate analgesic potential in acetic acid induced writhing test at all the test doses while the extract at the dose of 500 mg/kg ($P<0.01$) showed strong analgesic activity comparable to standard drug Diclofenac sodium (50 mg/Kg, i.p.) in hot plate method. The extract in different concentrations was also tested for antibacterial activity using agar well diffusion method. The extract significantly inhibited *S. aureus* and showed some degree of inhibition against *P. aeruginosa* and *E. coli.*

Keywords: *Mitragyna parvifolia*, Analgesic activity, Antimicrobial activity, Acetic acid induced writhing test

INTRODUCTION

The genus Mitragyna (family: *Rubiaceae*) consists of several plants used in local folkare medicine to treat fever, colic & muscular pain. It has been also used for the expulsion of worms. [1] *Mitragyna parvifolia* (Roxb.) Korth is commonly known as Kaim. [2] The plant grows throughout India, in deciduous and evergreen forests. Some of the chemical constituents reported in the plant are pyroligneous acid, methyl acetate, ketones and aldehydes. The plant is credited with innumerable medicinal properties and is widely used by tribal people and other ayurvedic practitioners. The bark and root are used to treat fever, colic, muscular pain, burning sensation, poisoning, gynecological disorders, cough and edema. 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. [1-4] Though the plant has great potential for analgesic and antimicrobial activity, nobody has not been yet documented these activities on the leaves of this plant. In continuation of our earlier reports on the fruit of this plant [5] we hereby reported the analgesic and antimicrobial activities of leaves extract of *M. parvifolia*.

MATERIALS AND METHODS

Plant material

The leaves of *M. parvifolia* Roxb. (Rubiaceae) were collected from local areas during the November month of 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 of Department. The leaves were cleaned and dried in the shade, then powdered to # 40 and stored in an airtight container.

Preparation of Extract

The extract was prepared by simple maceration process. The dried leaves powder (750 g) divided in three parts and was 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 using rotatory evaporator (Heidolph Laborota 4011 digital). The ethanolic extract (yield = 2.06 % w/w) was used for pharmacological studies by suspending a weighed amount of the extract in normal saline (95 ml): tween 80 (5 ml) ratio.

Test animals

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 ± 20 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

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experimentation but allowed free access to water throughout. All studies were carried out by using five groups of animals for 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

A total of four bacterial 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 leaf extract of *M. parvifolia*. All the strains used for these studies were procured from Microbial Type Culture Collection, IMTECH, Chandigarh, India. All the test microorganisms were maintained on Nutrient Agar at 37°C.

Determination of antibacterial activity

Various concentrations (100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml) of ethanolic leaf extract of *M. parvifolia* were evaluated for antibacterial activity by agar well diffusion method. [6] All the bacterial strains were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml. [7] 20 ml of Nutrient agar media was poured into each petri plate and plates were swabbed with 100 µl inocula of each test bacterial strain and kept for 15 min. for adsorption. Wells of 8 mm diameter were punched into seeded agar plates and loaded with a 100 µl volume with different concentrations of leaf extract of *M. parvifolia*, reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 4°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone with zone reader (Hi Antibiotic zone scale). The medium with DMSO as solvent was used as a negative control whereas media with Ciprofloxacin used as positive control. The experiment was carried out in triplicate and mean of the diameter of inhibition zones was calculated.

Acute toxicity test

Acute toxicity tests were performed according to OECD – 423 guidelines (acute toxic class method). [8] 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 ethanolic 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 1500 mg/kg.

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 leave 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. [9] Nociception was induced by an intraperitoneal (i.p.) injection of acetic acid 0.6 %, 0.1 ml/10 g 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 min to 15 min.

Hot-plate test

The hot-plate was used to measure response latencies according to the method described by Eddy and Leimbach, with minor modifications. [10] 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°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.

Statistical analysis

All data were represented as mean ± SEM and as percentage. Results were statistically evaluated using Dunnett's t- test. $P<0.01$ was considered significant.

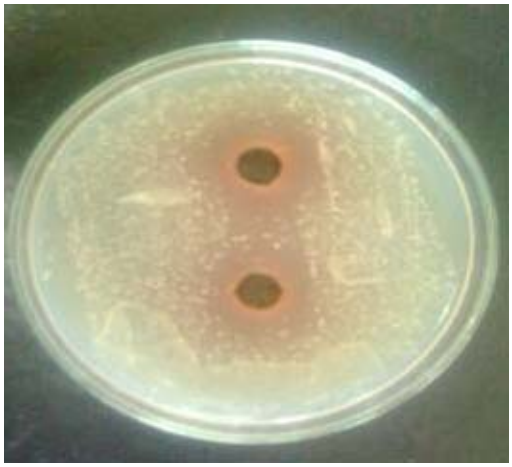

Fig. 1: Zone of Inhibition Against *S. aureus* at Concentration of 25 and 50 mg/ml

Fig. 2: Zone of Inhibition Against *S. aureus* at Concentration of 75 and 100 mg/ml

RESULTS

Acute toxicity test

M. parvifolia leaves extract did not produce any mortality even at the dose of 1500 mg/kg, p.o. All the doses (5, 50 and

Table 1: Analgesic effect of ethanolic extract of leaves of *M. parvifolia*

S. No.	Drug treatment	Dose mg/kg *(p.o.)*	No. of animals	Acetic acid induced writhing method			Hot plate method		
				Change in no. of wriths (Mean ± SEM)	't' value	'P' value	Reaction time in minutes (Mean ± SEM)	't' value	'P' value
1.	Normal Saline: Tween 80 (95:5)	10	10	27.16±0.60	--	--	1.60 ± 0.16	--	
2.	Diclofenac sodium	50 i.p.	10	6.50 ± 0.70	13.70	< 0.01	6.00 ± 0.21	8.97	< 0.01
3.	*M. parvifolia*	100	10	23.50 ± 0.50	2.431	> 0.05	2.50 ± 0.16	1.34	< 0.01
4.	*M. parvifolia*	250	10	20.50 ± 1.40	4.419	< 0.01	3.20 ± 0.20	1.90	< 0.01
5.	*M. parvifolia*	500	10	17.60 ± 1.50	6.29	< 0.01	4.40 ± 0.16	5.69	< 0.01

For Acetic acid induced withing: F = 34.316; df = 4, 25; n = 10, values are mean ± SEM. The data were analyzed by Dunnett's *t*-test. - - NIL
For Hot plate: F = 16.630; df = 4, 45; n = 10. The data were analyzed by one way ANOVA followed by Dunnett's *t* -test.

Table 2: Antibacterial activity of ethanolic leaf extract of *M. parvifolia*

Organisms	Diameter of zone of inhibition (mm)[a]					
	Extract concentration (mg/ml)				Control	
	100	75	50	25	Ciprofloxacin (20 µg/ml)	DMSO
S. aureus	27.6	20.6	21.3	17.3	26.3	-
B. subtilis	-	-	-	-	25.6	-
E. coli	12.6	11.6	-	-	25	-
P. aeruginosa	13.3	12.6	12	11.6	23.3	-

- No activity
[a] Values, including diameter of the well (8mm), are means of three replicates

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 extract of *M. parvifolia* at different dose is shown in Table 1. The extract at all the doses of 100, 250 and 500 mg/kg p.o. caused an inhibition on the writhing response induced by acetic acid. The maximum analgesic effect were at the doses of 250 and 500 mg/kg (*P<0.01*) as it showed good results while the dose of 100 mg/kg (*P>0.05*) showed only negligible effect.

Hot plate test

The oral doses of leave 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 significant at the dose of 500 mg/kg (*P<0.01*). The doses of 250 and 100 mg/kg showed good analgesic activity (*P>0.01*) as shown in Table 1.

***In-vitro* antimicrobial activity**

The crude ethanolic leaf extract of *M. parvifolia* (Shown in Table 2) inhibited all the tested bacterial strains except *B. subtilis*. The inhibitory zone of the extract against *S. aureus* was comparable to that of ciprofloxacin as shown in Fig. 1 and 2. However, the crude ethanolic leaf extract was not able to inhibit *E. coli* at concentrations of 25 and 50 mg/ml.

DISCUSSION

In these studies, the *M. parvifolia* leave extract was found to be non-toxic even at the high doses of 1500 mg/kg. The extract was found to have both analgesic and antimicrobial potential. In acetic acid induced writhing method, the plant extract failed to show any promising results at dose of 100 mg/kg but at 250 mg/kg and 500 mg/kg it showed some good results. The analgesic potential of the plant extract was very good at all the doses (100, 250 and 500 mg/kg) in Hot plate method. The results of these finding are slightly different from previous studies on analgesic effects of fruit extract of *M. parvifolia*. [5] In the previous studies the fruit extract of the plant was found to have good activity in both Hot plate and acetic acid induced writhing methods at all the doses (100, 250 and 500 mg/kg). In the present study on leaf extract, the extract was found to have good results at all the doses in Hot plate method while in acetic acid induced writhes good results were at the doses of 250 and 500 mg/kg. The ethanolic leaf extract of *M. parvifolia* inhibited all the tested strains except *B. subtilis*. The zone of inhibition of the extract at all concentrations (25, 50, 75, 100 mg/kg) against *S. aureus* was comparable to that of Ciprofloxacin (20µg/ml). The extract showed only moderate zone of inhibition against *E. coli* and *P. aeruginosa.* Hence, antibacterial action against *S. aureus*, which is very pathogenic to human being such as skin infection (folliculitis, surunple & carbuncle), septicaemia, bacteremia & otitis esterna. The antimicrobial results these studies are contradictory to studies on fruit extract because fruit extract was not found to have any zone of inhibition against above bacterial strains. So, the further investigation of chemical constituents responsible for the above activity is needed.

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