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. [*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 pyrroligneous 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 fruits 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

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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 16522) 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 x 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 leaf extract at the doses of 100, 250 and 500 mg/kg p.o.

**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...
The analgesic effect of the extract of *M. parvifolia* was comparable to that of ciprofloxacin as shown in Fig. 1 of 250 and 100 mg/kg showed good analgesic activity from previous studies on analgesic effects of fruit extract of *M. parvifolia*. The results of these finding are slightly different above activity is needed. In these studies, the method. The results of these finding are slightly different from previous studies on analgesic effects of fruit extract of *M. parvifolia*. In the previous studies the study 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, surulence & carbuncle), septicaemia, bacteremia & otitis externa. 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.

| Organisms | Diameter of zone of inhibition (mm) | Extract concentration (mg/ml) | Ciprofloxacin (20 µg/ml) | DMSO |
|-----------|-----------------------------------|------------------------------|--------------------------|------|
|           |                                   | 100                          | 75                       | 50   | 25  |
| *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

*Values, including diameter of the well (8mm), are means of three replicates*

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

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**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 (Mean ± SEM) | Hot plate method (Mean ± SEM) |
|--------|---------------------------------------|---------------------|----------------|-----------------------------------------------|-------------------------------|
| 1.     | Normal Saline:                        | 10                  | 10             | 27.16±0.60                                   | 1.60±0.16                     |
| 2.     | Diclofenac sodium:                    | 50 i.p.             | 10             | 6.50±0.70                                    | 6.00±0.21                     |
| 3.     | *M. parvifolia*:                      | 100                 | 10             | 23.50±0.50                                   | 2.50±0.16                     |
| 4.     | *M. parvifolia*:                      | 250                 | 10             | 20.50±1.40                                   | 3.00±0.20                     |
| 5.     | *M. parvifolia*:                      | 500                 | 10             | 17.60±1.50                                   | 4.40±0.16                     |

For Acetic acid induced writhing: *F = 34.316; df = 4, 45; n = 10.* The data were analyzed by one way ANOVA followed by Dunnett’s *t*-test. 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.