

**ANTIARTHRITIC AND ANTIPYRETIC ACTIVITY OF
MITRAGYNA PARVIFOLIA LEAVES**

A.P. Jain*, M. V. Tote, A. Mittal, N. B. Mahire, V. R. Undale and A. V. Bhosale

PDEA'S, S.G.R.S. College of Pharmacy, Department of Pharmacology Saswad,
Pune-412301. (MS), India.

Summary

The methanolic extract of *Mitragyna parvifolia* (MEMP) leaves were investigated for its antiarthritic and antipyretic potential in animal models. For evaluation of arthritic using Acetic acid-induced vascular permeability in mice and Freund's adjuvant induced arthritis in rats and antipyretic activity was analyzed using Yeast induced pyrexia in rats; MEMP was administered orally at 125,250 and 500 mg/kg and showed significant antiarthritic, antipyretic effect ($p < 0.05-0.01$). The result of acute toxicity test at which maximum toxic dose was above 5 g/kg indicates that the plant extract is relatively safe in mice.

Key words: *Mitragyna parvifolia*, Antiarthritic, Freund's adjuvant.

Corresponding Author:

Ashutosh Pal Jain

PDEA'S, S.G.R.S. College of Pharmacy,

Department of Pharmacology

Saswad, Pune. (MS), India 412301.

(MS), India 412301. Tel. Ph. - +91-9767121926

Fax. No. +91-2115-222213

E-mail- jain.palashutosh@gmail.com

Introduction

The genus *Mitragyna* (family: Rubiaceae) consists of trees growing exclusively in humid conditions. Several of these plants have been used in local folklore medicine for a wide variety of diseases such as fever, colic, muscular pains and for the expulsion of worms and commercially in the timber and paper industry^{1, 2}. The leaves of the plant *Mitragyna parvifolia* have afforded two alkaloids, 16, 17-dihydro-17b-hydroxy isomitraphylline (1) and 16, 17-dihydro-17b-hydroxy mitraphylline (2), together with two known alkaloids, isomitraphylline (3) and mitraphylline (4). The structures of 1 and 2 were elucidated using 1D and 2D NMR spectral methods³. Though various indolic and oxindolic alkaloids have been reported from the species only six alkaloids, all oxindolic, viz. mitraphylline, isomitraphylline, pteropodine, isopteropodine, speciophylline and uncarine F have been reported from the Lucknow region⁴. *Mitragyna parvifolia* (Roxb.) Korth. is an economically important highly endangered tree of Indian Thar Desert belonging to the family Rubiaceae. It has diverse medicinal properties and is widely used by tribal people and ayurvedic practitioners. Over exploitation of the tree together with recurring droughts have reduced its habitat and it is now listed as an endangered species⁵. The temporal and spatial dynamics of arbuscular mycorrhizal fungi (AMF) were investigated in Indian Thar Desert. AMF colonization and spore density were used to compare the responses of AMF to different abiotic parameters. A total of fifteen AMF species were associated with *M. parvifolia*⁶.

Plant material

The leaves of *Mitragyna parvifolia* (Roxb) Korth. were collected in months of September from the Empress botanical garden of Pune, India. The plant was authenticated by Botanical survey of India, Pune (voucher specimen. JAMP1)

Animals

Albino male Swiss mice (25 - 30 g) & Wistar Rat (180-200 g) were housed (5 animals per cage) under the standard laboratory conditions (light period of 12h/day, temperature $25 \pm 2^{\circ}$ C and humidity $55 \pm 5\%$) with free access to food (standard pellets chow, Lipton, India) and water *ad libitum*. Food but not water was deprived overnight and during the experiment. The institutional animal ethics committee (IAEC) approved the experimental protocol.

Drugs and chemicals

Indomethacin, Paracetamol, Diclofenac sodium, Freund's adjuvant yeast and Brewer's yeast All chemicals were obtained from local firms (India) and were of highest pure and analytical grade.

Preparation of *Mitragyna parvifolia* extract

After 10 days of drying under shed, the leaves were powdered using a mixer. The powder was sieved by 40 sieves. Air-dried and powdered leaves of *Mitragyna parvifolia* (100 g) were Soxhlet-extracted with hexane for 4 h to eliminate lipophilic compounds and with MeOH for 24h. The resulting MeOH extract solution was concentrated in vacuum using a rotavapor to obtain a brown powder (20 g). A fresh dilution of dried extract in vehicle (2% acacia) was prepared on the day of the experiments and the employed doses were expressed relative to dried extract.⁷

Isolation of alkaloidal from extract

Air dried powdered leaves were moistend with a dilute solution of ammonia (20%in water) and then extracted with chloroform at 60 C under reflux. The chloroform extract was concentrated under with HCL 2 N. After separation, the acid layer was made alkaline with a strong solution of ammonia and re-extracted with chloroform. The organic layer was dried under reduced pressure to give a purified alkaloidal extract.⁸

Acute oral toxicity studies

The median lethal dose (LD50) of MeOH extract of *Mitragyna parvifolia* was determined in the mice according to a modified method by Lorke⁹. Mice fasted for 24 h were randomly divided into groups of five mice per group either of sexes. Graded doses of MEMP were separately administered orally to the mice in each of the test groups. The mice in test groups were then allowed free access to food and water and observed over a period of 7 days for signs of acute toxicity. The number of deaths (caused by the MEMP) within this period of time was recorded.

Thin layer chromatography

Thin layer chromatography can be performed to monitor the reactions and to access the purity of synthesized compounds. It is performed on microscopic glass slides (2x7.5 cm) coated with silica gel-G and spots are visualized by exposure to iodine vapors. R_f values can be calculated by the following formula:-

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Solvent system: - Methanol

Mobile phase: - Chloroform: Methanol = 9: 1

Preliminary phytochemical screening

Analysis of the methanolic extract was carried out for various constituents like alkaloids, saponins, flavonoides, tannins and glycosides.

Anti arthritis testing in rats Acetic acid-induced vascular permeability in mice

Test drugs or vehicle were orally administered to mice (each weighing 25–30 g). One hour later each mouse was given an intravenous injection of 0.5% Evans Blue solution at 0.1ml/10 g body weight followed by an intraperitoneal injection of 0.6% acetic acid at 0.1ml/10 g body weight. Mice were sacrificed by cervical dislocation 30 min after acetic acid injection and the peritoneal cavity of each animal was washed three times with a total of 10 ml of saline. Saline washes from the same animal were combined and centrifuged for 10min at 581×g in a centrifuge. Supernatants were collected and their absorbance at 590nm was measured with a spectrophotometer (Jasco V-630). The amount of Evans Blue extruded into the peritoneal cavity was estimated from a standard curve.^{10, 11.}

Freund's adjuvant induced arthritis

The method according to Pearsion and Wood et.al; has been adopted for evaluation of anti-arthritic property. Freund,s adjuvant induced Arthritis model was used to assess the anti-arthritic activity in albino rats. Animals were randomly divided into five groups of six animals each. First group received 1 ml of normal saline, Second group received 13.5 mg/kg p.o. diclofenac sodium, remaining group received MEMP at dose 125, 250 and 500 mg/kg. Arthritis was induced by injecting a 0.1 ml suspension of killed mycobacterium tuberculosis homogenized in liquid paraffin into left hand paw. Drug treatment was started from initial day (0 day), 30 min before adjuvant injection and continued till 21 day. Paw volume was measured on 4th, 8th, 14th, and 21 day by using plethismometer. The % inhibition of paw volume of injected paw over vehicle control at day 4,8,14, and 21 was evaluated by using following formula.¹²

$$i = \frac{[(V) \text{ control} - (V) \text{ treated}]}{(V)} \times 100$$

Where i = % inhibition of paw oedema

V_{treated} = Paw volume of treated rat

V_{untreated} = Paw volume untreated rat

Yeast induced pyrexia in rats

The subcutaneous injection of Brewer's yeast suspension is known to produce fever in rats. A decrease in temperature can be achieved by administration of compounds with antipyretic activity. A 15% suspension of Brewer's yeast in 0.9% saline is prepared. By insertion of a thermometer to a depth of 2 cm into the rectum the initial rectal temperatures are recorded. The animals were fevered by injection of 10 ml/kg of Brewer's yeast suspension subcutaneously in the back below the nape of the neck. The site of injection is massaged in order to spread the suspension beneath the skin. Immediately after yeast administration, food is withdrawn 19 h post challenge; the rise in rectal temperature is recorded. Rectal temperatures are recorded again 30, 60, 120 and 180 min post dosing^{13,14}.

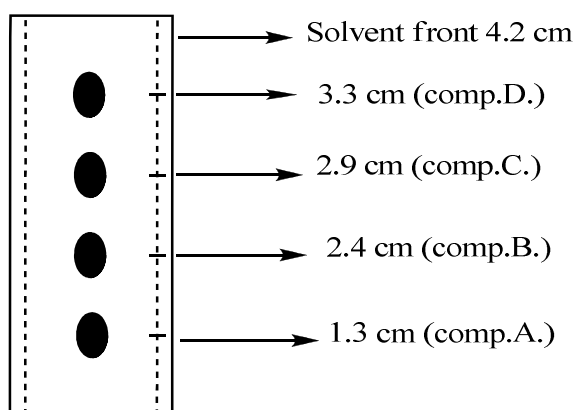
Statistical analysis

The results were expressed as means \pm S.E.M. The data obtained in experimental groups were evaluated by ANOVA followed Dunnett test. Values of *P* less than 0.05 were considered significant.

Results

In a group of six mice, no death occurred within 7 days after a dose of MEMP at 5.0 g/kg, orally. Symptoms associated with toxicity such as convulsion, locomotor ataxia and diarrhea were not observed.

From the TLC four spots observed in an acid base treated chloroform fraction of *M. parvifolia* leaves extract and orange brown spots are visualized by exposure to Dragendroff, s reagent. R_f Values: - Compound A.: 0.30, Compound B.: 0.57, Compound C.: 0.69, Compound D.: 0.78.



The phytochemical screening revealed the presence of alkaloids, saponins, flavonoides, and glycosides.

Acetic acid-induced vascular permeability in mice

As shown in Fig. 3, indomethacin at 10 mg/kg inhibited acetic acid-induced dye extrusion into the peritoneal cavity by 53.4%. MEMP produced a dose-dependent inhibitory effect on dye extrusions at 125, 250 and 500mg/kg, the inhibition rates of MEMP were 22.1, 35.9 and 51.3%, respectively. (Table-1)

Table-1- Effect of MEMP on acetic acid induce vascular permeability

Groups	Dose mg/kg	Evan's blue (ppm)	% inhibition
Control	vehicle	12.15±0.2742	0
Indomethacin	10	5.66±0.1994**	53.4
MEMP	125	9.46±0.2333*	22.1
MEMP	250	7.78±0.2272**	35.9
MEMP	500	5.91±0.2023**	51.3

Values are expressed as mean ± S.E.M ($n=6$). * $p<0.05$ ** $p<0.01$ compared with vehicle control (ANOVA followed by Dunnet's t-test).

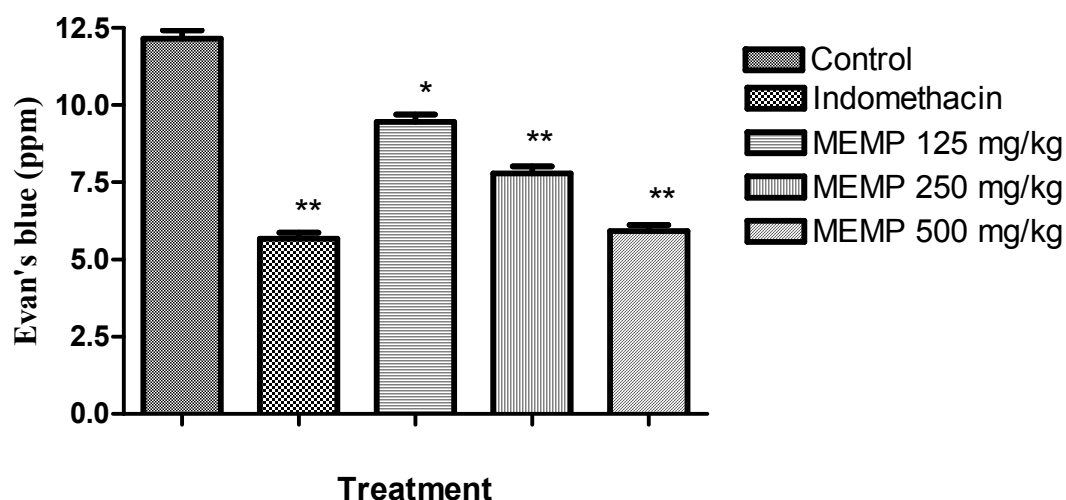


Figure 1. Effect of MEMP on acetic induced vascular permeability in mice

Freund,s adjuvant induced arthritis

MEMP produced a dose-dependent inhibitory effect on dye extrusions at 125, 250 and 500mg/kg, the inhibition rates of MEMP were 36.74, 39.94 and 59.54%, respectively. (Table-2)

Table-2- Effect of MEMP on adjuvant induced arthritis in rats

Groups	Dose mg/kg	Paw volume (ml \pm SEM) in rats				% inhibition of paw oedema On 21 st day
		4 th day	8 th day	14 th day	21 st day	
Control	vehicle	3.851 \pm 0.0510	3.651 \pm 0.0404	3.686 \pm 0.0311	3.715 \pm 0.02790	0
Diclofenac sodium	13.5	2.293 \pm 0.0903	1.543 \pm 0.1410	1.475 \pm 0.1369	1.396 \pm 0.1323	62.42
MEMP	125	2.960 \pm 0.0620	2.526 \pm 0.1053	2.460 \pm 0.0993	2.35 \pm 0.09924	36.74
MEMP	250	2.693 \pm 0.1566	2.426 \pm 0.1154	2.355 \pm 0.1408	2.231 \pm 0.1386	39.94
MEMP	500	2.135 \pm 0.1259	1.691 \pm 0.0823	1.606 \pm 0.0717	1.503 \pm 0.0654	59.54

Values are expressed as mean \pm S.E.M ($n=6$). * * $p<0.01$ compared with vehicle control (ANOVA followed by Dunnet's t-test).

Antipyretic activity

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 19 hr of administration to rats. Treatments with MEMP at dose of 125,250 and 500 mg/kg decreased the rectal temperature dose-dependent manner. The antipyretic effect started from the half hour and was maintained for 3.0 hr, after administration of MEMP .The standard drug paracetamol at the 100 mg/kg body wt. dose significantly reduced the yeast provoked elevation of body temperature .The result obtained from MEMP and Paracetamol treated rats were compared with control group and significant reduction in yeast induced elevated rectal temperature was observed. ($p< 0.05$, $p<0.01$). (Table-3)

Table-3 Effect of MEMP on Yeast-induced pyrexia in rats

Groups	Dose mg/kg	Rectal temperature ($K^0 \pm SEM$) in rats					
		-19 hr ^a	0 hr ^a	0.5 hr ^b	1.0 hr ^b	2.0 hr ^b	3.0 hr ^b
Control	vehicle	98.83 \pm 0.1382	101.55 \pm 0.4282	101.75 \pm 0.0846	101.90 \pm 0.1483	101.96 \pm 0.1726	101.98 \pm 0.1579**
Paracetamol	100	98.88 \pm 0.0792	101.55 \pm 0.1668	100.41 \pm 0.1956**	100.11 \pm 0.2242**	99.38 \pm 0.1014**	99.16 \pm 0.0760**
MEMP	125	98.63 \pm 0.1282	101.76 \pm 0.1116	101.28 \pm 0.1195*	100.11 \pm 0.1579**	99.45 \pm 0.0428**	99.31 \pm 0.0254**
MEMP	250	98.98 \pm 0.1108	101.43 \pm 0.2246	100.05 \pm 0.0885**	99.51 \pm 0.0945**	99.26 \pm 0.0666**	99.13 \pm 0.0802**
MEMP	500	98.75 \pm 0.1310	101.46 \pm 0.1430	100.65 \pm 0.1147**	99.50 \pm 0.1155**	99.11 \pm 0.0945**	98.98 \pm 0.1276**

Values are expressed as mean \pm S.E.M (n = 6). * P<0.05 ** P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

^a Before drug administration (K^0)

^b After drug administration (K^0)

Discussion

Rheumatoid arthritis is a chronic inflammatory disease affecting about 1% of the population in developed countries¹⁵. Rheumatoid arthritis is a chronic, systemic disorder with symmetrical, inflammatory polyarthritis that may produce progressive joint damage. Inflammation of the joint tissues is associated with the release of toxic substances in the synovium that lead to cartilage destruction.¹⁶ The vascular permeability was induced by acetic acid, which causes an increase in peritoneal fluids of prostaglandin E2 (PGE2), prostaglandin F2 α (PGF2 α), serotonin, and histamine. This leads to a dilation of the capillary vessels and the increase in vascular permeability. As a consequence, fluid and plasma proteins are extravagated, and edema forms.^{17, 18}. MEMP markedly inhibited the acetic acid-induced increase in vascular permeability in mice. This result suggests that MEMP may produce anti-inflammatory action through inhibiting the inflammatory mediators of the acute phase of inflammation.

Arthritis induced paw oedema was taken as prototype of exudation phase of inflammation where development of oedema being described as biphasic. The initial phase is attributed to release of histamine, serotonin and kinin after injection of MEMP. A more second phase is related to release of prostaglandins like substance. The knowledge of these mediator involved in different phases is each important for interpreting mode of drug action¹⁹.

The determination of paw swelling is an apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic efficacy of the drugs. In this study, administration of *M. parvifolia* leaf extract showed significant inhibition of paw oedema volume. Reduction of paw swelling from third week onwards may be due to immunological protection rendered by the plant extract.

This postulation is supported by the antipyretic effect of the extract, evidenced by its impact on pathogenic fever induced by the administration of a yeast injection. Its etiology includes production of prostaglandins in the central nervous system, which is the final common pathway responsible for fever induction²⁰. Inhibition of prostaglandin synthesis could then be the possible mechanism of antipyretic action as that of ASA.²¹ Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states. Antipyretics are drugs which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between the population and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point is elevated, and drugs like paracetamol do not influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature.^{22,23}

Fever is thought to be produced by certain endogenous substances which include tumour necrosis factor- α (TNF- α) and prostaglandins. Paracetamol has been shown to suppress fever by inhibiting prostaglandin synthetase resulting in the blockade of synthesis of prostaglandin in the brain. The present results show that the methanol extract of *Mitragyna parvifolia* leaves at 125, 250 and 500 mg kg⁻¹ doses possesses a significant anti-pyretic effect, in yeast-provoked elevation of body temperature (101.76 °K) in rats as the temperature was reduced to 99.31 °K, 99.13 °K and 99.11 °K respectively, and this effect is comparable to that of the standard anti-pyretic drug paracetamol which at 100 mg kg⁻¹ reduced the temperature to 98.98 °K.

Acknowledgement

The authors are thankful to Dr. S.C. Majumdar, Scientist-D, Botanical survey of India (BSI), Pune for authenticating the collected plant material; Poona District Education Association for providing us laboratory facilities to carry out this research work.

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