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Research Article

EVALUATION OF ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOL EXTRACT OF *MURRAYA PANICULATA* LEAVES IN EXPERIMENTAL RODENTS

M. B. NARKHEDE*, P. V. AJMIRE AND A. E. WAGH

Department of Pharmacology, IBSS College of Pharmacy, Malkapur 443101 Email: maheshnark@gmail.com

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ABTRACT

In present study, the ethanolic extract of *Murraya paniculata* (MPEE) leaves (Family: Ruteceae) was evaluated by hot plate and acetic acid induced writhing methods to assess antinociceptive activity. The MPEE was also evaluated for anti-inflammatory potential of evaluated using carrageenan, histamine, serotonin, dextran induced rat paw edema and chronic cotton pellet method. MPEE at doses of 50mg, 100mg and 200mg / kg exhibited significant anti-inflammatory effect. Maximum inhibition (58.36 %) was noted at the dose of 200 mg/kg after 3 hr of drug treatment in carrageenan induced paw edema. The extract also exhibited significant inhibition on the hind paw edema in rats caused by histamine and serotonin respectively. In cotton pellet induced granuloma model the MPE (200 mg/kg) and standard drug showed decreased formation of granuloma tissue by 51.58 % and 56.44 % ($p < 0.001$) respectively. The extract also found to possess antinociceptive activity against acetic acid-induced writhing and hot plate method. Thus, the present study revealed MPEE that the exhibited significant antinociceptive and anti-inflammatory properties in the tested models.

Keywords: *Murraya paniculata*, Anti-inflammatory, Antinociceptive activity

INTRODUCTION

Nature has provided a complete store-house of remedies to cure all aliments of mankind. From the vast natural resources, the plants are being used for therapeutic purposes from the beginning of the civilization[1]. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs[2]. Because existing synthetic molecules like nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects[3]. So, in order to overcome, there is need to focus on the scientific exploration of herbal drugs having fewer side effects.

Murraya paniculata is an evergreen shrub or occasionally a small tree, usually 2 to 3 m height. The leaves are pinnately compound with three to nine leaflets alternatively on the rachis. It is distributed throughout China, India, Sri Lanka, the Andaman Islands, Myanmar, Thailand, Kampuchea, Viet Nam, Malaysia, northeastern Australia, New Caledonia, and Taiwan[4]. Traditionally most of the plant parts are used therapeutically in treatment of various diseases. Pharmacological studies in plant is comparatively low and earlier studies reported leaves of *Murraya paniculata* possess both stimulant and astringent properties[4], further more it is used to treat venereal disease[5]. The plant is known to have emetic, carminative, antipyretic[6], analgesic[7], free radical scavenging[8], antidiabetic[9] and antiulcer activities. Based on the traditional use of the plant as an anti-inflammatory the present study was carried out in an experimental animal model to substantiate the folklore claim.

MATERIALS AND METHODS

Plant Material

The leaves of the plant *M. paniculata* were collected from the garden of Pharmacy College, Malkapur in month of April. The specimens of the plant were submitted to the Herbarium of Pharmacognosy Department, College of Pharmacy, Malkapur and taxonomically identified and authenticated by the experts.

Preparation of the extract

The freshly collected plant materials were washed with water and dried it in tray drier under the control conditions and powdered. The powdered plant materials (1000g) was macerated with petroleum ether to remove fatty substances, the marc was further exhaustively extracted with of 50% ethanol for 3 days (3 X 3L) and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated under reduced pressure and evaporated at 40^0C (yield 9.5 % w/w). The extract obtained was further subjected to preliminary phytochemical[10, 11] and pharmacological investigations.

Experimental animals

Swiss albino mice of both sexes weighing between 18 to 22 g and Albino Wistar rats of the either sex (180-200 g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The experimental procedures and research protocol used in this study were reviewed and approved by Institutional Animal Ethics Committee (1336/ac/10/CPCSEA) constituted as per the guidelines of Committee for Purpose of Control and Safety on Experiments on Animals, India.

Evaluation of Anti-inflammatory activity

Carrageenan - induced rat paw oedema

The rats were divided into 5 groups (n = 6). Acute inflammation was produced by the sub plantar administration of 0.1 ml of 1 % carrageenan in normal saline in the right paw of the rats. The different groups were treated with MPEE (50, 100 and 200 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.) and control vehicle were administered orally. The paw volume was measured at 0 h and 3 h after carrageenan injection using plethysmometer[12]. The animals were pretreated with the extract 1 h before the administration of carrageenan. The ratio of the anti-inflammatory effect of MPEE was calculated by the following equation: anti-inflammatory activity (%) = (1-D/C) x 100, where D represents the percentage difference in paw volume after MPEE was administered to the rats, and C represents the percentage difference of volume in the control groups[13].

Dextran induced paw edema

The animals were treated as in case of carrageenan induced paw oedema models, except that in place of carrageenan, dextran (0.1 ml, 1 % w/v in normal saline) was used[12].

Mediator induced inflammation

The anti-inflammatory activity of the extract was measured with phlogistic agents (*viz*. histamine, serotonin) [14, 15]. The paw edema was induced in rats by sub plantar injection of freshly prepared histamine (1 mg/kg) and serotonin (1mg/kg) solutions respectively and the paw edema was measured as mentioned earlier[16].

Cotton pellets-induced granuloma

The rats were divided into four groups (n = 6). After shaving the fur, the rats were anaesthetized and 10 mg of sterile cotton pellets were inserted, one in each axilla. The MPEE (50, 100 and 200 mg/kg, p.o) and indomethacin (10 mg/kg, p.o.) and control vehicle were administered orally for seven consecutive days from the day of cotton pellet implantation. The animals were anaesthetized on the eighth day and cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation[16].

Antinociceptive activity

Peripheral and central analgesic activities of the tested extracts were carried out in albino mice by using acetic acid-induced writhing and hot plate methods respectively.

Acetic acid induced writhing response in mice

For the writhing test[17], mouse was administered intraperitoneally with 0.5 ml of 1% acetic acid dissolved in 0.9% saline. The number of writhes was counted during 30 min period following the injection of acetic acid. A significant reduction in the number of writhes by drug treatments as compared to vehicle control animals was considered as a positive analgesic response and the percentage inhibition of writhing was calculated[18]. Aspirin (100 mg /kg, i.p.) was used as reference standard.

Hot plate reaction time in mice

Mice were screened by placing them on a hot plate maintained at 55±1°C and recording the reaction time in seconds for forepaw licking or jumping[19]. Morphine (5 mg/kg, i.p.) was used as reference standard. The time for forepaw licking or jumping on the heated plate of analgesiometer was taken as the reaction time.

Statistical Analysis

The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of analysis of ANOVA followed by student's *t*-test. P value of < 0.05 was considered as statistically significant.[20, 21]

RESULTS

The phytoconstituents were identified, which showed the presence Alkaloids, Carbohydrate, Proteins, Flavonoids, Phenolic compounds, Phytosterol and amino acids. The ethanolic extract of *Murraya paniculata* was evaluated for anti-inflammatory and analgesic activity in acute and chronic experimental animal models. The MPEE exhibited significant and anti-inflammatory activity at the tested doses of 50, 100 and 200 mg/kg in a dose dependant manner.

Anti-inflammatory

MPEE showed maximum inhibition of 58.36 % at the dose of 200 mg/kg after 3 h of drug treatment in carrageenan induced paw edema, whereas the standard drug showed 59.04 % of inhibition. In dextran induced paw edema, MPEE showed significant inhibition (33.86 %, 45.62 % and 54.37 %) in a dose dependent manner as compared with control (table 1a).

Table 1a: Effect of MPEE on carrageenan and dextran induced paw edema

Treatment	Dose (mg/kg)	Paw Volume (ml)	Inhibition (%)
Carrageenan control	-	0.735 ± 0.068	-
Indomethacin	10	0.308 ± 0.025	59.04
MPEE	50	0.465 ± 0.042	36.73
MPEE	100	0.384 ± 0.031	47.75
MPEE	200	0.306 ± 0.025	58.36
Dextran control	-	0.629 ± 0.050	-
Indomethacin	10	0.245 ± 0.020	61.04
MPEE	50	0.416 ± 0.040	33.86
MPEE	100	0.342 ± 0.040	45.62
MPEE	200	0.287 ± 0.030	54.37

Values are mean ± SEM (n = 6).

Experimental groups were compared with control $p < 0.001$.

In histamine and serotonin induced paw edema, the methanol extract showed 56.11 % and 51.91 % of inhibition (at 200 mg/kg) whereas indomethacin showed 58.88 % and 60.03 % of inhibition respectively (table 1b).

Table 1b: Effect of MPEE on Histamine and serotonin induced paw edema

Treatment	Dose (mg/kg)	Paw Volume (ml)	Inhibition (%)
Histamine control	-	0.540 ± 0.051	-
Indomethacin	10	0.229 ± 0.018	58.88
MPEE	50	0.348 ± 0.025	35.55
MPEE	100	0.289 ± 0.024	46.48
MPEE	200	0.237 ± 0.019	56.11
Serotonin control	-	0.628 ± 0.042	-
Indomethacin	10	0.251 ± 0.024	60.03
MPEE	50	0.417 ± 0.038	33.59
MPEE	100	0.352 ± 0.021	43.94
MPEE	200	0.302± 0.019	51.91

Values are mean ± SEM (n = 6).

Experimental groups were compared with control p < 0.001.

In cotton pellets-induced granuloma chronic model, the MPEE (200 mg/kg) and standard drug showed decreased formation of granuloma tissue at 50.1 % and 57.3 % (p < 0.001), respectively (table 1c).

Table 1c: Effect of MPEE leaves on cotton-pellets induced granuloma in rat

Treatment	Dose (mg/kg)	Paw Volume (ml)	Inhibition (%)
Control	-	0 47.3 ± 2.7	-
Indomethacin	10	20.6± 0.7	56.44
MPEE	50	32.2 ± 1.1	31.92
MPEE	100	29.4 ± 0.9	37.84
MPEE	200	22.9 ± 0.6	51.58

Values are mean ± SEM (n = 6).

Experimental groups were compared with control p < 0.001.

Antinociceptive (analgesic) activity

The ethanolic extract of *Murraya paniculata* at the doses of 50, 100 and 200 mg/kg exhibited significant (P < 0.01) inhibition of the control acetic acid induced writhes at the rate of 28.84 %, 54.93 % and 67.91 % respectively when compared to the effect of aspirin (100 mg/kg) which was 91.37 % (table 2a).

Table 2a: Effect of MPEE on acetic acid induced writhing in mice

Treatment	Dose (mg/kg)	Number of Writhes (per 30 min)	Inhibition (%)
Control	-	33.04 ± 0.50	-
Aspirin	100	3.21 ± 0.20	91.37
MPEE	50	23.51 ± 0.20	28.84
MPEE	100	14.89 ± 0.40	54.93
MPEE	200	10.6 ± 0.30	67.91

Values are mean ± SEM (n = 6).

Experimental groups were compared with control p < 0.001.

The extract also produces significant (P < 0.01) central analgesic activity at doses of 50 mg/kg to 200 mg/kg when compared with control by using hot plate method (table 2b).

Table 2b: Effect of MPEE on hot plate method

Treatment	Dose (mg/kg)	Basal reaction time (Sec)	Reaction time (Sec)			
			15 min	30 min	60 min	120 min
Control	-	2.78 ± 0.14	2.70 ± 0.19	2.83 ± 0.21	2.71 ± 0.19	2.64 ± 0.12
Morphin	5	2.48 ± 0.12	3.35 ± 0.24	5.26 ± 0.42	7.21 ± 0.51	7.65 ± 0.65
MPEE	50	3.05 ± 0.20	3.2 ± 0.30	5.37 ± 0.43	8.29 ± 0.74	9.82 ± 0.81
MPEE	100	2.83 ± 0.020	3.81 ± 0.17	5.41 ± 0.52	9.37 ± 0.6	13.21 ± 1.2
MPEE	200	3.87 ± 0.020	3.21 ± 0.25	6.21 ± 0.32	10.12 ± 0.48	14.4 ± 1.2

Values are mean ± SEM (n = 6).

Experimental groups were compared with control p < 0.001.

DISCUSSION

The extract was evaluated for its anti-inflammatory activity in acute and chronic models. Significant anti-inflammatory activity was observed for MPEE in carrageenan, dextran and mediators induced edema models. Carrageenan induced edema is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-HT followed by kinin release and then prostaglandin in the later phase[22, 23]. Dextran induced paw edema is known to be mediated both by histamine and serotonin. Dextran induces fluid accumulation, which contains little protein and few neutrophils, whereas carrageenan induces protein rich exudation containing large number of neutrophils[24]. The extract effectively suppressed the inflammation produced by both carrageenan and dextran. The extract also effectively suppressed the inflammation produced by mediators *viz*. histamine and serotonin. The MPEE exhibited a significant inhibition against histamine and 5-HT induced hind paw edema, which indicates that the extracts exhibits its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators *viz*. histamine, serotonin and prostaglandins might be involved in inflammation and it can be suggested that the anti-inflammatory activity is possibly backed by its antihistaminic activity.

Chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The MPEE showed significant anti-inflammatory activity in cotton-pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation[25].

The potential analgesic effects of the ethanolic extract of *Murraya paniculata* were investigated. The analgesic test used in the present study was chosen in order to test different nociceptive stimuli, namely cutaneous thermic (hot plate) and chemical visceral (writhing) stimuli. The results indicate that the extract exerted a significant and dose dependent effect on chemical (acetic acid

induced) and thermic painful stimuli from the respective doses of 100 and 200 mg/kg.

In acetic acid induced abdominal writhing, which is the viceral pain model, the processor the release of arachidonic acid via cyclooxygenase and prostaglandin via synthesis plays a role in the neoceptive mechanism. Various peripherally acting analgesic drugs have inhibited acid induced writhing in mice [26, 27]. The abdominal constriction is thought to involve local peritoneal receptors[28] and mediated by peritoneal mast cells[29, 30], acid sensing ion channels and the prostaglandin pathways[30]. Intra-peritoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE2 and PGF2 and their levels will be increased in the peritoneal fluid of the acetic acid induced mice [31] increased levels of protanoids as well as lipoxygenase products have also been found in the peritoneal fluid after the injection of the acetic acid [32]. The study revealed that all the doses of the MPEE produce exhibit antineoceptive effect and significantly reduced the number of acetic acid induced writhes in a dose dependent manner with an increasing percentage inhibition with increasing extract dose. This effect may be due to inhibition of the synthesis of the arachidonic acid metabolite. The Hot plate method test has been found to be suitable for evaluated of centrally but not of peripherally acting analgesic[33]. The hot plate test as been found to be suitable for evaluation of centrally acting analgesic. The validity of this test has been shown even in the presence of substantial impairment of motor performance[34]. The present study findings indicate the MPEE may be centrally acting. The analgesic activity of the tested plants could be explained partly by their radical scavenging property. In similar way to that of morphine, the extracts were found to have radical scavenging potentials [8]. Moreover, morphine was found to possess effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities at concentration 25, 50 and 72 lg/ml. This may explain some of its central analgesic activity [35] and in turn, the central analgesic effects of the extracts.

CONCLUSION

In conclusion, the present study indicates that the test plants may be useful in the protection against inflammatory diseases, especially if free radicals are a part of its pathophysiology. These results demonstrate that ethanolic extract of *Murraya paniculata* leaves exhibited significant anti-inflammatory and analgesic activity in both acute and chronic inflammatory models. However, more detailed phytochemical studies are necessary to identify the active principles and exact mechanisms of action.

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