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Original Research

Pharmacological evaluation of *Mallotus philippinensis* (Lam.) Muell.-Arg. fruit hair extract for anti-inflammatory, analgesic and hypnotic activity

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ABSTRACT

Objective: Recently, we observed wound healing activity of 50% ethanol extract of *Mallotus philippinensis* Muell. Arg (MP) fruit hairs extract (MPE). In several intestinal infections, localized inflammation is of common occurrence and hence we evaluated the anti-inflammatory, analgesic and hypnotic activity of MPE in different rat experimental models. **Materials and Methods:** Anti-inflammatory activity was evaluated by carrageenan (acute) and turpentine oil induced formalin (subacute) induced paw edema and while granuloma pouch (subacute) in rats. Analgesic and hypnotic activity of MPE was undertaken by tail-flick, hot-plate, and acetic acid-induced writhing tests while pentobarbitone-induced hypnotic potentiation in rats. **Results:** MPE at a dose of 200 mg/kg at 3 h after their administration showed inhibition of formalin-induced paw edema by 41.60% ($P < 0.001$) and carrageenan-induced paw edema by 55.30% ($P < 0.001$). After 7 days of treatments, MPE showed 38.0% ($P < 0.001$) inhibition against formalin-induced paw edema and reduced weight of turpentine-induced granuloma pouch by 29.6% ($P < 0.01$) and volume of exudates by 26.1% ($P < 0.01$), respectively. MPE (200 mg/kg) showed dose-dependent elevation in pain threshold and peak analgesic effect at 120 min as evidenced by increased latency period in tail flick method and increased reaction time in the hot-plate test while the reduction in the number of acetic acid-induced writhes by 45.7% ($P < 0.001$). The pentobarbitone-induced hypnosis model showed potentiation, as defined by increased duration of sleep in treated group rats as compared to control. **Conclusion:** Thus, the study revealed MPE is effective in reducing acute and subacute inflammation and showed effective and similar analgesic activity. This seemed to be safe in the treatment of pain and inflammation.

KEY WORDS: Carrageenan, Eddy's hot plate, formalin, granuloma pouch, *Mallotus philippinensis*, tail-flick method

INTRODUCTION

Inflammation is the symptom in various infective diseases such as inflammatory bowel disease bronchitis, cardiovascular diseases, asthma, inflammatory and autoimmune disorders, neurodegenerative conditions, and cancer [1]. Anti-inflammatory activity refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs, steroids, mainly glucocorticoids, mainly decreases inflammation by combining with the cortisol receptors. Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by counteracting the cyclooxygenase (COX) enzyme [2]. The inflammatory response are mediated *via.* immune system components at infectious region and will manifested by increased blood supply and vascular permeability which results in immigration of peptides, mononuclear cells, and neutrophils. Literature suggests various standard anti-inflammatory animal models *viz.* air pouch granuloma, paw edema, sponge implantation, and pleurisy using different chemical agents such as carrageenan [3], turpentine [4], and formalin [5] to screen new anti-inflammatory molecules [6]. It has been reported that rat paw edema model using different mediators are suitable *in vivo* animal model to predict the efficacy

of anti-inflammatory agents, which mainly act by inhibiting the mediators of acute inflammation [7].

According to the International Association for the Study of Pain, pain is defined as an unpleasant feeling, which may involve tissue damage, and could have physical and emotional components [8]. However, pain sensation will protect our body from external stimuli by causing us to perform certain actions. Pain may be defined as a predictor, protector, or simply a hassle [9]. The pain can be classified as acute or chronic. Acute pain is defined as short-term but extreme pain that comes on quickly but last only for a brief period. But in usual conditions, such pain can be resolved by the body's physiologic response from the endogenous pain modulating system [10]. The pain which persist for a longer duration compared to normal time course called as chronic pain, and mostly associated with a some sort of injury or disease condition. Most common nonmalignant chronic pain syndromes are divided into categories of neuropathic and non-neuropathic ("functional"). It can be made much worse by environmental and psychological factors [11]. The major classes of analgesics include NSAIDs, COX_2 inhibitors, opiates, and morphinomimetics. The main demerit about the present synthetic drug therapy against inflammation is their toxicity and reappearance of symptoms after discontinuation. Therefore, screening and development of natural drugs for analgesic and anti-inflammatory activity are still in progress, and there is much hope for finding these drugs from indigenous medicinal plants [12].

Insomnia is a sleep disorder which mainly perception or complaint of inadequate or poor-quality sleep due to various psychological and physical disorder. Sedatives are the drugs which calm the central nervous system (CNS) and have a relaxing effect. Sedatives at higher doses usually cause sleep; they can be defines as hypnotics. The basic difference between sedatives and hypnotics is the amount of the dose; calming effect achieved at low dose while sleep at a higher dose. Recent studies have shown that herbal drugs exert good sedative and hypnotic effect on the CNS [13,14].

Our experimental laboratory and research group have been engaged in the identification of various herbal plants for the anti-inflammatory, analgesic and hypnotic activity using anti-inflammatory, analgesic and hypnotic experimental animal models. This study evaluates the anti-inflammatory, analgesic and hypnotic potentiation of MPE against various animal experimental models. These activities are important for any ulcer healing property including wounds as anti-inflammatory activity would help in decreasing wound inflammation, while analgesic and hypnotic activity would reduce the reaction to pain and cause euphoria.

MATERIALS AND METHODS

Chemicals and Instruments

The drugs used in this study include carrageenan (Sigma-Aldrich, USA), turpentine oil (Loba Chemie, India), formaldehyde (Merck Limited, India), and diclofenac sodium (Jagsonpal Pharmaceuticals, India). However, standard drugs such as pentazocine (Ranbaxy Pharmaceuticals, India), acetic acid (Merck, India), and pentobarbitone (Loba Chemie, Mumbai, India) were used for the different pharmacological studies.

The instruments used in the study include tail flick apparatus (Socrel model, DS2, Milan, Italy), plethysomometer (Ugo basile, Mila, Italy), and hot plate (Harvards Apparatus Ltd, UK).

Preparation of Plant Extract

Mallotus philippinensis fruits were procured from Botanical Garden, Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, India. Fruiting season of the plant is in the month of March to April, whole fruits were collected initially to prepare the fruit glandular hair extract. Identification and authentication were done by Professor Asthana Department of Botany, Banaras Hindu University, India with a reference voucher number RKA/BOT/Sept. 10-12. Further, the plant sample was preserved in the Department of Botany, BHU, Varanasi, India. The glandular hairs of fruit (red color powder) adhering at the surface of shade dried fruits were collected. Approximately, 500g of powder was collected and added to 1000 mL of 50% ethanol in a bottom flask and was kept at room temperature for 3 days in shade. The organic fraction was collected and concentrated *in vacuum* in a rotary evaporator, and the residue was dried in desiccators over calcium chloride powder.

Experimental Animals

Healthy Charles-Foster albino rats (180-200 g) of either sex were selected and collected from the Central Animal House facility (Reg.no.542/02/ab/CPCSEA), IMS, BHU, Varanasi, India. All the animals were kept in Plexiglas cages in groups of six, with free access to water and food in the animal house of pharmacology departmental with standard temperature condition as 26 ± 2°C, 44-56% of relative humidity, light and dark cycles of 10 and 14 h, respectively, during the experiments. The diet for animals was purchased as a standard rodent pellet diet (Pashu Aahar Vihar, Ramnagar, Varanasi) and water ad libitum for all the animals during the experiment. Laboratory animal care principles and guidelines (NIH publication no. 82-23, revised 1985) will be followed after approval of Institutional Animal Ethical Committee for experimental work (Notification no. Dean/13-14/CAEC/331 dated 20.11.2013).

Anti-inflammatory Activity

Dose selection and treatment protocol

Considering the significant dose of MPE, and the extract yield (≈11.6%), the fruit extract dose in the rat was calculated according to the surface area in relation to the human. Thus in this experiment, we have planned initially graded anti-inflammatory doses of MPE (100, 200, and 400 mg/kg) against

formalin-induced pedal edema. 0.5% carboxymethylcellulose (CMC) was given to all animals in the control group as a vehicle, while test extracts/standard drug, diclofenac sodium were given orally in a volume of 1 mL/100 g body weight, once daily using the orogastric tube.

Indigenously prepared graduated plethysmograph was used for the study. The mercury displacement due to the dipping of the paw was directly read from the scale attached to the mercury column. A mark was made on both the hind paw (right and left) just beyond tibiotarsal joint, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. Mercury displacement measured the initial paw volume (both right and left) of each rat in formalin and carrageenan-induced paw edema animal models.

Formalin-induced pedal edema

All animals were divided into five groups ($n = 6$ in each group). 0.1 ml of 2% v/v formalin was injected in the subplanter region on the first and third day of the experiment of each rat in hind paw region.

Control, 1st group received 0.5% CMC while the treated groups from the 2nd to 4th groups received MPE (100, 200, and 400 mg/kg, p.o.) and the 5th group received diclofenac sodium (DFC, 10 mg/kg, p.o.), respectively. The initial dose of the extract was given 60 min before the injection of formalin and was continued until 7 days. The paw thickness was measured using a plethysmograph on 0 and 3 h and the 7th day according to the standard method mentioned by Singh *et al*. [15]. Percent inhibition in paw volume between treated and control groups was calculated as follows:

$$\text{Percent inhibition} = (1\text{-}V_T/V_C) \times 100$$

Where, V_T and V_C were mean paw volume of treated and control groups, respectively, at 3 h or 7th day.

Carrageenan-induced pedal edema

Carrageenan-induced inflammation in paw was performed according to the method described in detailed by Winter *et al*. [16]. Total of three groups of rats having six animal in each were used for the study. The animals in control group were administered with 0.5% CMC, while the treated group received MPE (200 mg/kg) and DFC (10 mg/kg) orally 60 min before carrageenan administration in 18 h fasted rats. For inducing inflammation, carrageenan suspension (1.0%) in 0.9% sodium chloride solution (sterile normal saline) were administered. Paw volume in each control and treated group were measured by means of volume displacement technique using plethysmometer (Ugo Basile no. 7140) immediately after injection of carrageenan and also after 1, 2, 3, and 24 h. duration. The percent inhibition in paw volume between treated and control groups were calculated as per equation mentioned below:

$$\text{Percent inhibition} = (1-V_T/V_C) \times 100$$

Where, V_T and V_C were mean paw volume of treated and control groups, respectively, at 1, 2, 3, or 24 h.

Turpentine oil-induced granuloma pouch

Subcutaneous dorsal granuloma pouch was made by injecting 25 ml of air in anesthetized rats, followed by 0.5 ml of turpentine oil injection into it 17. Extract, the standard drug, and CMC were administered orally 60 min prior to turpentine oil injection and will further continue until seven consecutive days. After last day of the experiment (on day 7), the pouch was removed and the content of exudates was collected after 60 min of the last dose of drugs. Further, the amount of exudate (in volume) and weight of pouch were calculated and presented as g and ml per 100 body weight of the animal. The percent inhibition of exudates volume and granuloma pouch weight between control and treated groups were calculated as follows [17]:

$$\text{Percent inhibition} = (1\text{-}V_T/V_C) \times 100$$

Where, V_T and V_C were mean weight and volume of treated and control groups, respectively, at 7th day.

Analgesic Activity

Dose selection and treatment protocol

Considering the significant dose of MPE in above experiment, authors have planned graded doses of MPE (100, 200, and 400 mg/kg) were tested to find an optimal effective analgesic dose in tail flick test. 0.5% CMC was given as vehicle to control group, while test extracts/standard drug, pentazocine (PTZ, positive control) in 0.5% CMC were administered (p.o.) using an orogastric tube of volume of 1 mL/100 g body weight of animal.

Tail flick test

The tail flick test with radiant heat is an extremely simplified and standard method used for analgesic activity assay. The mechanism behind thermal radiation model focuses on the time of tail withdrawn by a brief, vigorous movement [18]. It is the reaction time of this movement that is recorded (often referred to as "tail-flick latency"). A variation in the lengthening of the reaction time treated with extract, standard, and control vehicle are interpreted as an analgesic action.

CF rats of either sex were divided into five groups with six animals in each group. Analgesiometer was used to study the tail flick latencies study (i.e. reaction time) of the animals were recorded [19]. The basal reaction time of radiant heat was calculated by placing the last 2 cm of the tip of the tail on the source of radiant heat as per the standard manual instruction of instrument. Animal flicking response (tail withdrawn) was considered as the end point of the experiment. The rats were initially selected showing cut-off latency period was <5 s to prevent any damage to the animal tail. 0.5% CMC was administered to the control group, while treated groups (2-4th groups) received 100, 200, and 400 mg/kg graded doses

of MPE. However, last or the 5th group received the standard drug, PTZ (10 mg/kg). All the drugs (extracts/standard) were given *via* oral route just before the experiment. The tail flick latencies time as describes above was tabulated in sec at pre-drug (basal, 0 min reading) and at 30, 60, 120, 180, and 300 min after administration of CMC/MPE/PTZ. The percent increase in latency period was calculated following the formula:

$$\text{Percent increase} = (T_T/T_C\text{-}1) \times 100,$$

Where, T_C and T_T were defined as the mean basal (0 min) analgesic time and post-treatment time of 30, 60, 120, 180, and 300 min, respectively.

Hot plate test

Four groups of rats having six animals in each were used selected for the study. The hot plate assay was performed using Eddy's hot plate apparatus. All the steps were followed as per manufacturer's instructions. Paw licking and jumping responses of experimental animals were evaluated in terms of their reaction times and for this constant temperature were maintained during the experiment [20]. Both are considered to be supraspinally integrated responses. During the experiment, animals were placed on the top of the hot plate, which was kept at approximately 55 ± 0.5°C. Animals were placed for a maximum time of 10 s to avoid any thermal injury/damage in the paws. As soon as animal licked their fore- and hind-paws, and jumped from the top of hot plate, reaction time was recorded at before (basal/0 min) and after 30, 60, 120, 180, and 300 min following administration of CMC (control), MPE (Test drugs, 200 mg/kg), and PTZ (positive control, 10 mg/kg). Based on the data of reaction time, the percent increase in reaction time was calculated using the following formula:

$$\text{Percent increase} = (T_T/T_C\text{-}1) \times 100,$$

Where, T_C and T_T were defined as the mean analgesic time at basal (0 min) and post-treatment time of 30, 60, 120, 180, and 300 min, respectively.

Acetic acid-induced writhing response

For an acetic acid-induced writhing response, a total of four groups were selected having six animals in each group. The control group received CMC, and treated groups were received diclofenac (10 mg/kg) or MPE (200 mg/kg). After, thirty minutes 0.7% of acetic acid (i.e., 10 mL/kg) solution was injected intraperitoneal to all the animals in the different experimental groups. The number of writhes (abdominal constrictions) produced by each animal was observed individually under a glass jar for a period occurring between 5 and 20 min after acetic acid injection, and the same was counted. The antinociceptic response was considered as if the number of writhes was significantly reduced in the treated group compared to the control group [21]. The % protection of analgesic activity was calculated by using the formula:

$$\text{Percentage inhibition} = (1\text{-}W_T/W_C) \times 100,$$

Where, W_T and W_C were denoted as the number of writhing in the treated group and in the control group, respectively.

Hypnotic Activity

The sedative and hypnotic activity of the glandular hairs of *M. philippinensis* fruit extract is mainly depends on the nature of CNS depressant drugs, as they potentiate a subhypnotic dose of the standard drug, pentobarbitone. It mainly concludes the mild sedatives action, along with gives results with other depressants such as drugs which are analgesic, anticonvulsants, and anxiolytics. Sodium pentobarbitone was basically used for sleep induction. For the assessment, righting reflex was the useful measure to study whether or not animals are asleep.

The sleeping time in rats was studied by the method of Dandiya and Columbine [22]. Three groups having six male rats in each group were selected for the study. CMC was administered to the experimental control group, while MPE (200 mg/kg) was given to the treated groups. Pentobarbitone (20 mg/kg, intraperitoneal) was administered as subhypnotic dose before thirty minutes, to each animal of all the defined groups. Pentobarbitone-induced hypnosis and its potentiation was observed in each animal. The time between the administration of pentobarbitone to start losing the righting reflex is denoted as onset of sleep, while the time between the onset of sleep to start regaining of righting reflex is denoted as the duration of sleep, which was recorded in each animal and then compared with respect to the control group.

Statistical Studies

All the data of control and treated animals were analyzed using ANOVA, further the significance difference was calculated between mean values. The values expressed after the analysis were average ± standard error of means (SEM). The data were also subjected to correlation coefficient using Sigmastat version 3.1 statistical analysis software for further correlation between the samples. The correlation of the data was calculated using Pearson's test. *P* values <0.01, 0.05, and 0.001 were considered as statistically significant.

RESULTS

Anti-inflammatory Activity

Formalin-induced paw edema

Formalin-induced paw edema model was studied using dose-dependent inhibition at 3 h and 7th day was observed after the oral administration of MPE (100, 200, and 400 mg/kg). The percent inhibition ranged from 23.4% to 45.2% at 3 h and 15.5% to 41.5% at 7th day with MPE at different doses. The MPE at all doses showed a significant inhibition of paw edema at the third hour as compared to reference drug. 200 mg/kg dose of extract produced a significant reduction in paw volume in comparison with the control group, which was utilized as optimal dose for further studies [Table 1].

Carrageenan-induced paw edema

In the carrageenan-induced edema test, the subplantar injection of carrageenan-induced edema in rats, the paw volumes and percentages of inhibition by the alcoholic extract of *M. philippinensis* fruit and standard drugs are shown in Figure 1. MPE (200 mg/kg) was found to inhibit carrageenan-induced paw edema by 21.9% ($P < 0.05$), 32.5% ($P < 0.01$), 55.3 and 65.8% ($P < 0.001$) at 1st, 2nd, 3rd, and 24th h which was comparable with the effect of diclofenac sodium (DCF).

Turpentine oil-induced granuloma pouch

The subcutaneous dorsal granuloma pouch weight and exudates volume results showed a significant decrease in percent inhibition as compared to control; results are summarized in Table 2. MPE after their 7 days administration, reduced weight of turpentine-induced granuloma pouch by 29.6% and volume of exudates by 26.1% when expressed per 100 g body weight of rats as compared with the standard anti-inflammatory drug, DCF.

Analgesic Activity

Tail flick test

The results of MPE at different doses (100, 200 and 400 mg/kg), for analgesic activity using tail flick method are summarized in Table 3 and found statistically significant ($P < 0.001$) elongation of reaction time at the dose of 200 mg/kg body weight as compared to the control. MPE showed dose-dependently (significant dose, 200 mg/kg) effect, i.e. it increased the latency time during first 180 min during the study and showing peak analgesic effect at 120 min. After 30 min, the extract in doses of 100, 200, and 400 mg/kg ($P < 0.001$) body weight showed a significant elongation. After 120 min, MPE at the doses of 100, 200, and 400 mg/kg showed no significant increase in reaction time. Reaction time data showed a significant increase in case of tail flick method, which indicates the analgesic effect depicting possibly the involvement of central mechanism in analgesic action.

Hot plate test

The result of hot plate test showed no significant difference in mean pre-drug pain reaction time among the groups but after administration, the extract generally significantly ($P < 0.001$) increased the post drug pain reaction time. MPE (200 mg/kg) increased the pain reaction time ($P < 0.001$) similar to the standard antinociceptive agent, PTZ [Figure 2].

Acetic acid-induced writhing

MPE showed a significant antinociceptive activity in terms of acetic acid induced writhes which was decreased significantly with inhibitory rates of 45.7%, while the standard drug, DCF showed a significant antinociceptive activity with the inhibitory rate of 76.2% [Table 4].

Table 1: Anti-inflammatory effect of MPE against formalin-induced paw edema in rats

Oral treatment (mg/kg)	Paw volume (ml)		Inhibition (%)*	
	3 h	7 day	3 h	7 day
Control (0.5% CMC)	1.37±0.09	1.42±0.06	-	-
MPE (100)	1.05±0.08[a]	1.20±0.05[a]	23.4	15.5
MPE (100)	0.80±0.04[c]	0.88±0.02[c]	41.6	38.0
MPE (100)	0.75±0.04[c]	0.83±0.03[c]	45.2	41.5
DCF (10)	0.68±0.03[c]	0.76±0.06[c]	50.4	46.5

Values expressed as mean±SEM (n=6), *Percent inhibition=(1−VT/VC)×100. [a]$P<0.05$, [c]$P<0.001$ compared with respective h/day control group, SEM: Standard error of mean, CMC: Carboxymethylcellulose, MPE: *Mallotus philippinensis* extract

Table 2: Effect of MPE on turpentine oil-induced granuloma pouch in rat

Oral treatment (mg/kg×7 days)	Granuloma pouch		Exudate	
	Weight (g/100 g bw)	*Percent inhibition	Volume (ml/100 g bw)	*Percent inhibition
Control (0.5% CMC)	2.94±0.15	–	0.78±0.04	–
MPE (200)	2.07±0.16[b]	29.6	0.57±0.05[b]	26.1
DCF (10)	1.84±0.12[c]	37.4	0.48±0.03[c]	38.5

Values expressed as mean±SEM ($n=6$) *Percent inhibition=$(1-V_T/V_c)\times100$. [b]$P<0.01$, [c]$P<0.001$ compared to respective control group, CMC: Carboxymethylcellulose, MPE: *Mallotus philippinensis* extract, SEM: Standard error of mean

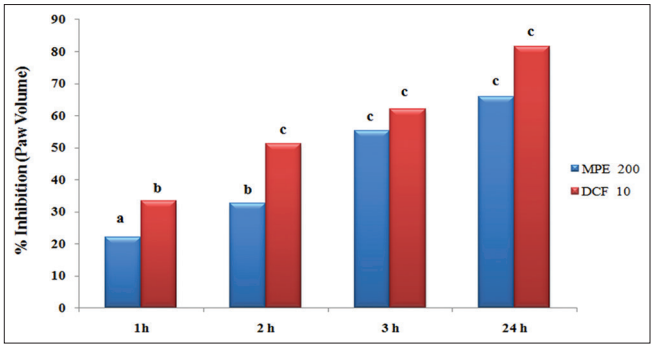

Figure 1: Anti-inflammatory effect of *Mallotus philippinensis* extract against carrageenan-induced paw edema in rats, values expressed as % inhibition paw volume, *P* values: [b]<0.01 and [c]<0.001 compared with respective h control group

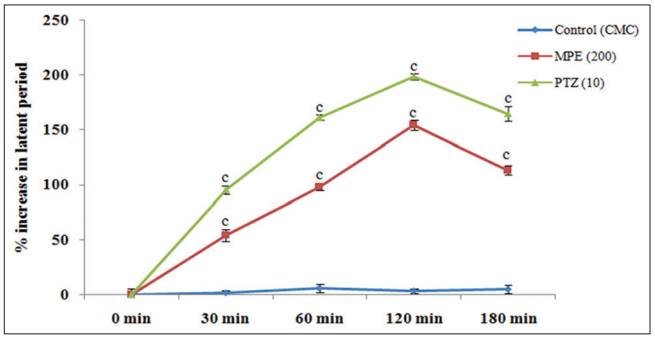

Figure 2: Analgesic activity of *Mallotus philippinensis* extract by hot plate method in rats, values expressed percent mean ± standard error mean of control values. *P* value: [c]<0.001 compared with respective h control group

Table 3: Analgesic activity of MPE by tail flick method in rats

Oral treatment (mg/kg)	Latent period (sec)					
	Basal (T_c)	30 min (T_T)	60 min (T_T)	120 min (T_T)	180 min (T_T)	300 min (T_T)
Control (CMC)	4.51±0.21 (0.0)	4.54±0.21 (0.8)	4.58±0.22 (1.5)	4.51±0.26 (0.0)	4.59±0.20 (1.8)	4.65±0.19 (3.1)
MPE (100)	4.75±0.24 (0.0)	6.05±0.14[c] (27.4)	6.50±0.20[c] (36.8)	6.95±0.18[c] (46.3)	6.36±0.15[c] (33.9)	5.63±0.20[b] (18.5)
MPE (200)	4.78±0.17 (0.0)	7.55±0.21[c] (57.9)	7.80±0.18[c] (63.2)	8.06±0.19[c] (68.6)	7.52±0.23[c] (57.3)	6.75±0.23[c] (41.2)
MPE (400)	4.47±0.16 (0.0)	8.23±0.18[c] (84.1)	8.41±0.24[c] (88.1)	8.68±0.23[c] (92.2)	8.61±0.17[c] (92.6)	7.30±0.19[c] (63.3)
PTZ (10)	4.38±0.19 (0.0)	7.76±0.21[c] (81.3)	8.95±0.25[c] (109.1)	9.41±0.19[c] (119.8)	8.78±0.25[c] (105.2)	7.53±0.22[c] (75.9)

Values expressed as mean±SEM (n=6). Results in brackets represents percentage increase in analgesic time from respective basal value.
[c]P<0.001 compared to respective min control group, SEM: Standard error of mean, MPE: *Mallotus philippinensis* extract, PTZ: Pentazocine, [b]P<0.01

Table 4: Effects of MPE and DCF on acetic acid induced writhing and pentobarbitone (PENTO)-induced sleeping time in rats

Acetic acid-induced writhing (mean number of writhing in 15 min)			Pentobarbitone-induced hypnosis (PENTO, 20 mg/kg; intraperitoneal)		
Treatment	Number of writhing	% inhibition*	Treatment**	Sleep latency (min)	Sleeping time (min)
Control (CMC)	53.8±2.8	-	Control (CMC+PENTO)	3.86±0.3	47.2±3.5
MPE (200)	29.2±3.0[c]	45.7	MPE (200+PENTO)	3.53±0.4	65.8±2.2[b]
DCF (10)	12.8±2.5[c]	76.2	-	-	-

Values expressed as mean±SEM (n=6). *Percentage inhibition=$(1-W_T/W_C)\times100$. **Male rats only. [b]P<0.01, [c]P<0.001 as compared to respective control group, CMC: Carboxymethylcellulose, MPE: *Mallotus philippinensis* extract, DCF: Diclofenac sodium, SEM: Standard error of mean

Hypnotic Activity

The hypnotic effect was evaluated by sleep latency and time of duration induced by the subhypnotic dose of pentobarbitone. MPE (200 mg/kg) showed no change in the time of onset of sleep but increased sleeping time induced by pentobarbitone ($P < 0.01$) [Table 4].

DISCUSSION

The process of acute inflammation is mainly initiated by dendritic cells, macrophages, kuppfer cells, histiocytes, and mastocytes. These cells will be activated at the onset of any type of infection, or other injuries, and will release the inflammatory mediators, which are responsible for the clinical signs of inflammation. The aim of the inflammation is to repair the damage or at least to limit it and also to remove the cause [23]. Carrageenan-induced rat paw edema is an acute inflammatory model which involves several mediators released in sequence. It was reported that during the first phase, i.e., 1.5 h, most of the histamine and serotonin were released; while the second phase, i.e., 1.5-2.5 h is mediated by bradykinin and the third phase, i.e., 2.5-6 h after carrageenan injection, in which the mediator is possible as prostaglandin. The carrageenan-induced hind paw edema in rats is known to be sensitive to cyclooxygenase inhibitors, but not to lipoxygenase inhibitors [24]. In this study, MPE significantly decreased the rat paw edema induced by carrageenan in all phases, suggesting the possible mechanism of action of the MPE may involve inhibition of these inflammatory mediators release in all phases.

The formalin-induced paw edema involves the infiltrations of neutrophils, macrophages and proliferation of fibroblasts and this methods resembles with human arthritis and preferred as one of the most suitable assays to identify the antiarthritic and anti-inflammatory agents [25]. The results suggest that MPE significantly decreased the formalin-induced edema, which might be useful for the treatment of chronic inflammatory disease like arthritis. The turpentine oil-induced granuloma pouch model basically evaluates the study with respect to exudative type of inflammation. Kinin is considered as the main mediator of granuloma, as it is responsible for vasodilatation and can further increase the vascular permeability in early stages of inflammation [26]. The MPE showed a significant reduction in the exudative fluid. The anti-inflammatory activity exhibited by MPE showed results similar with the standard drug, which suggests that cyclooxygenase inhibition may partly mediate the plant's activity.

Pain is felt because of inflammation, infection, ischemia, tissue necrosis, chemical, or burn. When an injury occurs, pain is first evoked by stimulation of the nociceptor (Aδ and C fibers), which will release of the kinins and potassium from the injured cells. These stimulate the receptor directly resulting in the release of the neuropeptides such as substance P from nociceptive terminals and the release of the histamine from the mast cells with the production of the platelet-activating factor which in turn releases serotonin from the platelets. Histamine is also released from the mast cells, starting an inflammatory reaction leading to vasodilatation and edema [27]. Opioid receptors of the μ-, δ-, and κ-subtypes mediate the potent analgesic and addictive actions of opioid drugs [28].

Acetic acid test (chemical stimuli) elucidates the peripheral and central mediated analgesic action, while hot plate and tail flick tests (thermal stimuli) elucidate the central mediated analgesic mechanism. The tail flick model is considered as the specific test for evaluation of the central pain at spinal levels. Tail flick model depicts the results of analgesic effect as centrally acting opioid like [29,30], while the hot plate animal test is suggest the central analgesic activity or supraspinal analgesia of any compound [31]. Thermic painful stimuli are known to be selective to centrally, but not peripherally acting analgesic drugs. In the writhing test, acetic acid is used to screen both peripherally and central acting analgesic activity [32, 33]. The writhing test could suggest the possible effective analgesic doses for any test substance

that can be used in humans [33]. Acetic acid cause pain by liberating endogenous substance that includes prostaglandins, serotonin, substance P, histamine, bradykinin, etc. mostly excite the pain nerve ending leading to the abdominal writhing [29]. MPE markedly exhibited a dose-related both peripheral and central mediated analgesic activity, and the potency of MPE (200 mg/kg) was comparable to reference standards, PTZ, and diclofenac. It is possible that MPE might be producing analgesic effects by mechanisms affecting the production of endogenous opioids, prostaglandins, and other mediators important for pain production and sensitizers. *M. philippinensis* is rich in phytochemicals such as phenols, flavonoids, alkaloids, with significant antioxidant activity reported [34] which might be responsible for above biological activities.

MPE was seen to potentiate pentobarbitone induced hypnosis indicating CNS depressant property which could be mediated through gamma-aminobutyric acid or some other mechanism unknown to us. As we have not done the detailed study regarding the involvement of neurotransmitters/modulators so we cannot definitely express their involvements. However, a proven sedative effect could be boon whenever we use the extract for wound healing or for its anti-inflammatory effects. So keeping the diverse ethnopharmacological profile of *M. philippinensis* in view [35-39], we have made an effort to further validate scientifically and found that MPE possessed a significant anti-inflammatory and antinociceptive activity.

CONCLUSION

Thus from the above investigation, it was observed that *M. philippinensis* fruit extract showed significant anti-inflammatory and analgesic activity against all the rat experimental models which indicates its use in the traditional system of medicine in order to support to reduce inflammation and pain in different pathological condition but further studies are required to evaluate the mechanism of action and activity directed bioassay for the new source of drug development. Further isolation and characterization of other active constituents from the extract and the most potent one can be carried further for exploration.

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