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Research Article

ANTINOCICEPTIVE AND ANTI INFLAMMATORY ACTIVITY OF MESUOL ISOLATED FROM *MESUA FERREA* L. SEED OIL

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ABSTRACT

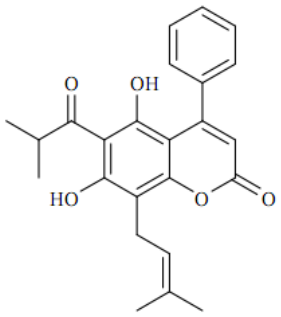
The mesuol isolated from *Mesua ferrea* seed oil was evaluated for analgesic and anti-inflammatory activity at the doses 20 and 40 mg/kg in experimental animals. The mesuol exhibited significant inhibition of acetic acid induced writhing in mice and prolonged the latency period in hot plate as well as tail immersion models. The mesuol also significantly reduced the carrageenan induced paw edema in rats. The findings of the study indicate the analgesic and anti-inflammatory activity of mesuol.

Keywords: *Mesua ferrea,* Mesuol, Analgesic, Anti-inflammatory, Carrageenan

INTRODUCTION

Pain and inflammation is a pathophysiological response of living tissue to undesirable stimuli. Inflammation leads to the local accumulation of plasmic fluid and blood cells. The pharmacology of pain has become a complex field and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. More recently, completely synthetic compounds based on natural pharmacophores have been introduced into the market but, research and medical fields still struggle with side-effect profiles from these analgesic and anti-inflammatory substances that are undesirable. Therefore, development of newer and more substantial analgesic and anti-inflammatory drugs with lesser side-effects is necessary.

Mesua ferrea (cluciaceae) is locally known as Nagakeshara[1] widely distributed in tropical countries like india, Burma, Thailand, Indochaina and New guinea[2]. Traditionally *Mesua ferrea* is used in inflammation and septic conditions, to poultice wounds and skin eruptions[3-5]. The previous studies confirmed the analgesic activity of n-hexane leaf extract[6] and anti-inflammatory activity of xanthones isolated from *Mesua ferrea* seed oil[7]. The mesuol is a 4-phenylcoumarin isolated from *Mesua ferrea* L seed oil and was exhibited antibacterial and antifungal activities in previous studies [8]. However, mesuol has not been evaluated for analgesic and anti-inflammatory activities. Therefore, an attempt was made to evaluate the analgesic and anti inflammatory activity of the mesuol.



Mesuol

MATERIALS AND METHODS

Plant material

The seeds of *Mesua ferrea* were collected in August 2010 from Shimoga, Karnataka, India and authenticated by Prof. K. Siddappa, Department of Botany, Sree Siddaganga Boy's College, Tumkur (Karnataka), India. A voucher specimen is preserved in college herbarium (SSCP11PC0010).

Drugs

Mesuol suspended in 2% Tween 80 (5 ml/kg.b.w.), Carrageenan 1% v/v, (Sigma, U.S.A.) Diclofenac 5 mg/kg i.p. (Neon laboratories limited) was used.

Experimental animals

Healthy albino mice (25-35 g) and Albino wistar rats (160-250 g) of either sex breed in animal house of Sree Siddaganga College of Pharmacy, Tumkur, Karnataka, India, were used [Ref: 123/1999/CPCSEA, dated 19-5-1999]. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC). [Ref: SSCPT/IAEC, Clear/76/2009-10 dated 30-11-2009]. The animals were housed under standard conditions of temperature (25^{o}C), 12 h/12 h light/dark cycles and fed with standard pellet diet and clean tap water *ad libitum*.

Selection of dose

The acute toxicity test was performed according to OECD-423 guidelines, 20 and 40 mg/kg.b.w. of mesuol were selected for the study.

Preparation of the extract

The seeds were sun dried and coarsely powdered by pulverization method, before subjecting to extraction. The seeds (100 g) were extracted with 500 ml of petroleum ether by soxhlet apparatus for 6 h, the residue was removed by filtration and concentrated. The concentrated extract was transferred to china dishes and allowed to dry. The final percentage yield of the extract was 70%. The extract was liquid, brownish in color and the preliminary phytochemical screening confirmed the presence of flavanoids and triterpenoides.

Isolation of mesuol

The oil expressed from seed kernels was kept in freeze for three weeks and filtered. The remaining residue was first washed with petroleum ether and then with methanol. The remaining residue was dissolved in benzene for crystallization once and then with methanol. This yielded pale yellow rectangular plates and the yield was 0.2%. The melting point was 154^{0}C and mesuol was confirmed by comparing the physical and spectral characters with published data[9].

Analgesic Activity

Eddy's hot plate method

The mice of either sex were weighed (20–30 g each) and divided into four different groups containing six animals in each group. The animals were selected previously from those presenting latency to the thermal stimulus equal to or less than 20 sec; and the cutoff point was set at 40 sec. Group I served as normal control and II, III,

were treated with mesuol 20 and 40 mg/kg i.p. respectively and IV, received diclofenac 5mg/kg i.p., was served as standard. The reaction time of animals was noted down on hot plate at 15, 30, 60, 90 and 120 minutes after the treatment. The basal reaction time was taken by observing hind paw licking or jumping response (Whichever appear first) in animals while placed on hot plate, which was maintained at constant temperature 55 ± 0.5 ◦C[10].

Tail immersion method

The mice of either sex were weighed (20–30 g each) and divided into four different groups containing six animals in each group. Group I served as normal control and II, III, were treated with mesuol 20 and 40 mg/kg i.p. respectively and IV, received diclofenac 5mg/kg i.p., was served as standard. Prior to the experiment, the animals were screened for a sensitivity test by immersing the tip of the tail gently in hot water[11-13]. The method involves immersing extreme 5 cm of rat tail in water bath containing water at a temperature of 55 ± 0.5 ◦C. The rats which withdraw the tail within 5 sec. were selected for the activity and after each determination tails were carefully dried. Each animal served as its control at 0 and 10 min interval, the average of the two values was the initial reaction time. The reaction time for the same group was taken at interval 15, 30, 60, 90 and 120 min. A cut off period 10 s was observed to avoid damage to the tail.

Acetic acid induced writhing method

Male albino mice weighing between 20-25 g were selected for the study. The animals were divided into four groups containing six animals in each group. All the animals received 0.1 ml acetic acid (0.6% v/v i.p.) and first group served as control. Group II and III were treated with mesuol 20 and 40 mg/kg i.p. respectively, 30 min prior to the administration of actic acid and group IV served as positive control and received diclofenac 5 mg/kg i.p. The writhing effect was indicated by the stretching of abdomen with simultaneous stretching of at least one hind limb. This was observed for 10 minutes and change in number of writhing in test group compared with standard treated and control treated groups[14].

Anti-inflammatory activity

Twenty-four albino wistar rats of either sex weighing between 150-200 gm were divided into four groups containing six animals in each group. Group I received Tween 80 (2%, i.p.) and served as control. Group II and III received mesuol 20 and 40 mg/kg i.p. respectively. Group IV received indomethacine 10 mg/kg i.p. and served as standard. The animals of all the groups were injected with, 0.1 ml of 1% carrageenan solution beneath the sub-plantar surface of the right hind paw after 30 min of their respective treatment.. For the assessment of the anti-inflammatory activity, the volume of the paw was measured with the help of mercury plethysmometer at 0, 15, 30, 60, 90, 120, 180 and 240 min. after the carrageenan treatment[15].

Statistical Analysis

Data were expressed as the mean standard deviation (SDE) of the means and statistical analysis was carried out employing one-way ANOVA followed by suitable post hoc test. $P < 0.05$, $p<0.01$ and $p<0.001$, are considered statistically significant.

RESULTS AND DISCUSSION

Hot-plate test is one of the most common tests of nociception that are based on a phasic stimulus of high intensity[16]. Pain induced by thermal stimulus of the hot-plate is specific for centrally mediated nociception[17] and thought to involve opioids[18]. However, all doses of the mesuol prolonged the hot-plate latency with time, (Table 1) which was not significantly different from diclofenac, indicating that the mesuol's 69.02±11.10 (20 mg/kg) and 76.75±5.594 (40 mg/kg) at 90 min. is comparable to that of diclofenac 107.7±6.333 at 90 min though it is less potent and slow acting. The ability of the mesuol to prolong the reaction latency further suggests that the mesuol is endowed with a central analgesic activity.

The tested mesuol, protected mice against a thermal induced noxious stimulus which was evidenced from hot plate method. Mesuol produced significant analgesic effect in tail immersion model (Table 2) which was assayed to characterize central analgesic activity. The extent of activity shown by the mesuol was less than that of the standard drug diclofenac but it is more than that of control group which justifies its activity.

Acetic acid-induced writhing test (Figure 1) was used for detecting peripheral analgesia[19]. 20 mg/kg mesuol reduced acetic acid-induced writhing as higher as (10.00±1.461) and moderate at 40 mg/kg (11.67±1.542), producing an effect comparable to that of the standard (21.67**±**1.838). The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins. The analgesic effect of the mesuol may therefore be due either to its action on visceral receptors sensitive to acetic acid, to the inhibition of the production/action of algogenic substances such as prostaglandins or inhibition at the central level of transmission of painful messages.

Carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs and has been frequently used to assess the anti-edematus effects of natural products. Mesuol exhibited significant reduction in paw edema volume (Table 3) in a dose dependent manner. The anti-inflammatory activity might be attributed to the inhibition of release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin. Histamine is one of the important inflammatory mediators and is a potent vasodilator substance and increases vascular permeability. Thus it can be estimated that the mesuol may exerts anti-inflammatory effects by inhibiting the synthesis, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandin involved in inflammation[20].

In conclusion, the mesuol isolated from Mesua ferrea seed oil exhibited significant analgesic and anti-inflammatory activity in experimental animals. Further study is required to establish its exact mechanism of action and toxicity profile.

Table 1: Hot Plate Method in Mice

			Hot-plate latency in sec. at different time intervals in minutes					
Group N=6	**Treatment mg/kg i.p.**	**Dose**	**0**	**15**	**30**	**60**	**90**	**120**
I`	Tween 80,2%	5	10.0 ±1.98	9.0 ±1.64	9.1 ±1.69	8.23 ±1.56	10.95 ±1.28	12.08 ±1.31
II	Mesuol	20	9.96 ±1.58	37.48 ±7.88	57.48 ±12.37[b]	57.48 ±12.3[b]	69.02 ±11.10[a]	62.88 ±6.27[b]
III	Mesuol	40	16.13 ±2.90	19.38 ±2.15	59.80 ±8.60[b]	86.47 ±14.33[a]	76.75 ±5.59[a]	62.87 ±9.07[b]
IV	Diclofenac	5	13.30 ±1.07	16.95 ±0.81	28.20 ±0.90[c]	40.57 ±5.33[a]	107.7 ±6.33[a]	112.6 ±4.62[a]

a = p<0.001, b = p<0.01and c = p<0.05, when compared with normal control when compared with normal control (Group 1) statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison test

Table 2: Tail Immersion Method in Mice

			Average tail withdrawing time in second					
Group N=6	**Treatment mg/kg i.p.**	**Dose**	**0**	**15**	**30**	**60**	**90**	**120**
I`	Tween 80,2%	5	1.30 ±0.08	1.33 ±0.05	1.35 ±0.08	1.35 ±0.07	1.33 ±0.04	1.33 ±0.08
II	Mesuol	20	1.33 ±0.07	1.66 ±0.09	1.78 ±0.04	3.0 ±0.23[a]	4.56 ±0.17[a]	5.11 ±0.12[a]
III	Mesuol	40	1.56 ±0.08	1.76 ±0.09	1.86 ±0.10	4.10 ±0.13[a]	5.33 ±0.18[a]	5.56 ±0.12[a]
IV	Diclofenac	5	1.46 ±0.10	1.63 ±0.19	2.50 ±0.11[a]	3.98 ±0.13[a]	5.68 ±0.11[a]	6.45 ±0.16[a]

a = p<0.001, when compared with normal control when compared with normal control (Group 1) statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison test

Table 3: Anti-Inflammatory Activity of Mesuol in Rats

			Mean edema Volume (ml) at different intervals in minutes							
GroupN=6	**Treatment mg/kg i.p.**	**Dose**	**0**	**15**	**30**	**60**	**90**	**120**	**180**	**240**
I`	Tween 80,2%	5	1.52 ±0.04	1.53 ±0.03	1.64 ±0.02	1.65 ±0.03	1.62 ±0.09	1.51 ±0.05	1.51 ±0.08	1.73 ±0.04
II	Mesuol	20	1.65 ±0.04	1.46 ±0.07	1.45 ±0.02	1.43 ±0.06	1.45 ±0.05	1.42 ±0.05[c]	1.40 ±0.05[c]	1.40 ±0.05[c]
III	Mesuol	40	1.55 ±0.05	1.52 ±0.08	1.34 ±0.06	1.36 ±0.06	1.44 ±0.04	1.29 ±0.03[c]	1.06 ±0.04[a]	0.75 ±0.03[a]
IV	Indomethacin	10	1.18 ±0.08	1.03 ±0.06	0.77 ±0.03[a]	0.83 ±0.03[a]	0.71 ±0.03[a]	0.66 ±0.01[a]	0.59 ±0.03[a]	0.50 ±0.01[a]

a = p<0.001and c = p<0.05, when compared with normal control when compared with normal control (Group 1) statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison test

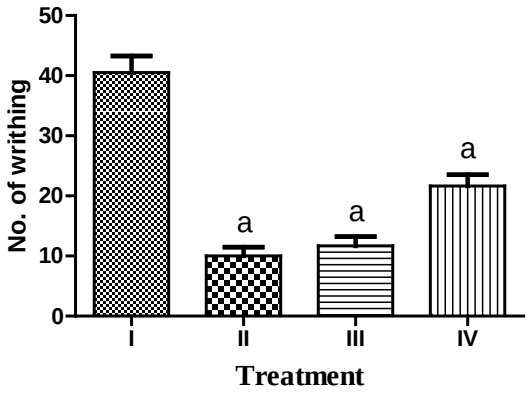


Fig. 1: Writhing Test in Mice

a = p<0.001, when compared with normal control when compared with normal control (Group 1) statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison test

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