EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF CURCUMA CAESIA ROXB. RHIZOMES IN LABORATORY ANIMALS

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ABSTRACT
Objective: The objective of present study was to evaluate analgesic and anti-inflammatory activity of methanolic extract of Curcuma caesia (MECC) rhizomes.

Methods: In the present study MECC at 100, 200 and 400 mg/kg body weight was studied for analgesic and anti-inflammatory activities in different animal models. Analgesic activity was carried out by using acetic acid induced writhing model and hot plate test in Swiss albino mice. Anti-inflammatory activity was carried out by using carrageenan induced paw edema model and cotton pellet induced granuloma model in wistar rats.

Results: The results suggested that MECC possess analgesic activity in mice as well as anti-inflammatory activity in acute as well as sub acute models of inflammation in rats. MECC (200 and 400 mg/kg) showed significant (p < 0.001) activity in acetic acid induced writhing model as well as in hot plate test which suggested that MECC is peripherally as well as centrally acting analgesic. MECC (200 and 400 mg/kg) also showed significant (p < 0.001) anti-inflammatory activity by reducing the paw edema volume in carrageenan-induced paw edema in rats in the late phase (3 to 5 h) regulated by prostaglandins and leukotrienes and in cotton pellet induced granuloma model MECC decreased dry weight of granuloma.

Conclusion: The observed pharmacological activity may be due to presence of phytochemical compounds present in the extract like alkaloids, flavonoids, phenols, saponins and tannins.

Keywords: Analgesic, Anti-inflammatory, Curcuma caesia Roxb.

INTRODUCTION

Pain is sensorial modality representing in many cases the only symptom for diagnosis of several diseases [1]. While inflammation is a complex defensive mechanism which consists of highly sequential events provoked by number of stimuli like pathogens, noxious mechanical and chemical agents, and autoimmune responses. The subsequent cascade of events which takes place in inflammation is characterized by various signs and symptoms like redness, swelling, heat, and pain. A regulated response protects against further injury and clears damaged tissue in physiological conditions while in pathological condition inflammation may result in tissue destruction and lead to organ dysfunction [2]. All the steroid and non steroid anti-inflammatory drugs (NSAID’s) available in market cause undesired and serious side effects during their clinical use. so, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs. The currently used analgesics and anti-inflammatory drugs may not be useful in all cases so there is increased focus on plant research and their active constituents [1].

Curcuma caesia Roxb (family- Zingiberaceae) commonly known as kari haldi is a perennial herb belonging to Genus Curcuma. The plant is distributed throughout tropical and subtropical regions of the world. In India it is found in North-East and Central part and also sparsely found in Papi Hills of East Godavari, West Godavari and Khammam Districts of Andhra Pradesh [3]. Curcuma caesia is widely used in India as anti-inflammatory and antasthmatic in Ayurvedic medicine [4]. The plant is used in sprains and bruises by ethnic communities of Tinsukia district of Assam (India) [5] and by rural people in Gohpur of Sonitpur district Assam (India) [4]. The Khanti tribe of Lohit valley forest in eastern Arunachal Pradesh use fresh rhizome juice for its anti-inflammatory action [7].

Previous workers reported the essential oil composition from C. caesia rhizome and its antifungal activity [8], anti-asthmatic and smooth muscle relaxant effects in guinea pig trachea [4], antimicrobial activity [9], phenolics content and in vitro antioxidant activity [10, 11], bronchodilating activity [12], analgesic and anti-pyretic activity [13], anxiolytic and CNS depressant activities [14], locomotor, anticonvulsant and muscle relaxant property [15] and anti-cancer activity [16]. Presence of curcuminoids, phenolics, flavonoids, volatile oil, protein, amino acids and alkaloids were reported in the rhizomes of Indian C. caesia [17]. A survey of literature revealed that there is no scientific data published to confirm ethno pharmacological claim of anti-inflammatory activity. The objective of present study was to evaluate and justify the traditional claim of analgesic and anti-inflammatory activity of methanolic extract of Curcuma caesia rhizomes.

MATERIALS AND METHODS

Collection and authentication of rhizomes
Curcuma caesia Roxb. rhizomes were collected from local market. The plant was identified and authenticated at Department of Botany, Agharkar Research Institute, Pune, India and voucher specimen (R129) was deposited at that Institute.

Drugs and chemicals
Carrageenan (Sigma-Aldrich, St. Louis, MO, USA), dicyclofenac (gift sample from Emcure pharmaceuticals Ltd., Pune) and pentazocine (Fortwin, Ranbaxy), acetic acid (Pure Chem. Ltd., India), ethanol (Qualigens, Mumbai, India), carboxy methyl cellulose (CMC) (Research- Lab, India) were purchased from respective vendors.

Preparation of Methanolic Extract of Curcuma caesia rhizomes (MECC)
Fresh rhizomes of Curcuma caesia Roxb were cut, dried in sun and powdered in grinder. The known quantity of powdered rhizomes was extracted with methanol using soxhlet apparatus for 16 hours and then filtered at room temperature. The filtrate was dried on tray dryer at 40°C (yield ~ 9.2% w/w). The dried extract was dissolved in distilled water containing 2% gum acacia to prepare the drug extract (MECC) and used for pharmacological studies.

Experimental animals
Female Wistar rats (180–220 g) and female Swiss albino mice (25–30 g) were purchased from Bharati Vidyapeeth Central Animal House, Pune, India. They were maintained at a temperature of 24 ± 2°C and relative humidity of 45 to 55% under 12-h light: 12-h dark.
cycle with free access to food pellets (Pranav Agro Industries Ltd., Sangli, India) and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPSEA), India (No: CPCSEA32/12)

**Preliminary Phytochemical Screening**

Qualitative preliminary phytochemical screening was carried out for evaluation of tannins, alkaloids, flavonoids, saponins, etc using standard procedures and tests [18].

**Acute Toxicity Study**

Acute toxicity study was carried out as per OECD guidelines- 425. Five female Swiss albino mice were used for studies which were carried out in the morning, providing free access to water. The test extract was administered orally at one dose level of 2000 mg/kg body weight and animals were observed continuously for the first 4 h and then periodically up to 24 h for toxicity and mortality.

**Analgesic activity**

**Acetic acid induced writhing in mice**

Female Swiss albino mice were divided into five groups (n=6);

- Group 1 - Vehicle control,
- Group 2 - Acetyl salicylic acid (100 mg/kg, p.o.)
- Group 3 - MECC (100 mg/kg, p.o.),
- Group 4 - MECC (200 mg/kg, p.o.),
- Group 5 - MECC (400 mg/kg, p.o.).

Before 60 min of administration of acetic acid solution at a dose of 10 ml/kg (0.6%, ip) the mice were pretreated orally with MECC or acetylsalicylic acid. The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 15 min. The analgesic activities were expressed as mean number of writhes and percentage inhibition was calculated by the following formula:

\[
\% \text{ Inhibition} = \left(1 - \frac{W_t}{W_c}\right) \times 100
\]

Where, \(W_c\) and \(W_t\) are mean number of writhes observed in vehicle control group and treatment group respectively [19].

**Hot plate method**

The Swiss albino mice were first screened by placing them on hot plate (UGO Basile, Italy model no.-DS-37) maintained at 55 ± 1°C and the reaction time was recorded in seconds. The pain threshold was considered to be reached when animals showed the signs of paw licking or jumping. Only those mice which reacted within 15 s and which did not show large variations when tested on four separate occasions, each 15 min apart, were used for the test. The time for paw licking or jumping on the hot plate was selected as a reaction time.

The mice were divided into five groups (n=6);

- Group 1 - Vehicle control,
- Group 2 - Pentazocine (30 mg/kg, p.o.),
- Group 3 - MECC (100 mg/kg, p.o.),
- Group 4 - MECC (200 mg/kg, p.o.),
- Group 5 - MECC (400 mg/kg, p.o.).

Various responses such as paw licking or jumping were recorded before and after 30, 60, 90, 120 and 150 min following oral administration of MECC or Pentazocine [20]. A cut-off time of 15 s was used to avoid harm to the animals.

**Anti-inflammatory activity**

**Carrageenan-induced paw edema in rats**

The wistar rats were divided into five groups (n = 6);

- Group 1 - Carrageenan control,
- Group 2 - Diclofenac (10 mg/kg, p.o.),
- Group 3 - MECC (100 mg/kg, p.o.),
- Group 4 - MECC (200 mg/kg, p.o.),
- Group 5 - MECC (400 mg/kg, p.o.).

Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% lambda Carrageenan (Sigma Chemical Co., USA) suspension in sterile normal saline in the left hind paw of each rat. Rats were pretreated orally with MECC and diclofenac (10 mg/kg p.o.) 1 h before carrageenan injection. The rat paw volume up to the ankle joint was measured using Plethysmometer (Ugo Basile, Italy) from 0-6 h at an interval of 1 h. The mean changes in injected paw volume with respect to initial paw volume were calculated. Percentage inhibition of paw volume between treated and control group was calculated using following formula

\[
\% \text{ Inhibition} = \left(1 - \frac{V_t}{V_c}\right) \times 100
\]

Where, \(V_c\) and \(V_t\) represent mean increase in paw volume in control and treated groups, respectively [21].

**Cotton pellet-induced granuloma in rats**

The effect of MECC in sub acute inflammation was assessed using cotton pellet granuloma in rats [22, 23]. The rats were divided into five groups (n = 6);

- Group 1 - Vehicle control
- Group 2 - Diclofenac (10 mg/kg, p.o.),
- Group 3 - MECC (100 mg/kg, p.o.),
- Group 4 - MECC (200 mg/kg, p.o.),
- Group 5 - MECC (400 mg/kg, p.o.).

Autoclaved cotton pellets weighing 35 ± 1 mg each were implanted subcutaneously through small incision made along the axilla or flank region of the rats anesthetized with anesthetic ether. MECC and diclofenac (10 mg/kg p.o.) were administered once daily for seven consecutive days from the day of cotton pellet insertion. On the eighth day all rats were sacrificed and the cotton pellets covered by the granulomatous tissue were excised from animal body and dried in hot air oven at 60°C for 24 h and weighed.

**Statistical Analysis**

Values were expressed as mean ± SEM and statistically analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying One Way ANOVA with Dunnett's test or Two Way ANOVA with Bonferroni test, p< 0.05 was considered to be significant.

**RESULTS**

**Phytochemical analysis**

Preliminary phytochemical qualitative analysis of MECC showed the presence of alkaloids, saponins, flavonoids, tannins, phenol compounds in the extract.

**Acute oral toxicity**

The MECC did not exhibit any toxic symptoms and mortality when given orally at dose of 2000 mg/kg b.w. Hence, the extract was found to be safe at the dose of 2000 mg/kg b.w. Therefore three doses 100, 200 and 400 mg/kg b.w. were selected for pharmacological studies.
Analgesic activity

Effect of MECC in acetic acid induced writhing in mice

Administration of acetic acid produced 67 ± 1.9 writhes in mice. Administration of MECC at 100 mg/kg significantly (p < 0.01) & also at 200 and 400 mg/kg significantly (p < 0.001) decreased the number of writhings by 11.94%, 22.38% and 31.34%, respectively when compared to vehicle control group. Acetylsalicylic acid (100 mg/kg) significantly (p < 0.001) reduced the number of writhings by 61.19% when compared to vehicle control group (Table 1).

Effect of MECC on Hot plate method

 Pretreatment of mice with MECC at 200 and 400 mg/kg significantly (p < 0.001) increased the pain latency, there was dose dependent increase in latency time in response to thermal stimulation, whereas pentazocine (30 mg/kg, i.p) also significantly (p < 0.001) increased the response latency at 30, 60, 90, 120 and 150 minutes of administration (Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Paw Withdrawal Latency (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Min</td>
<td>30 Min</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>-</td>
<td>3.35 ± 0.22</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>30</td>
<td>3.57 ± 0.37</td>
</tr>
<tr>
<td>MECC</td>
<td>100</td>
<td>3.62 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>3.93 ± 0.66</td>
</tr>
<tr>
<td>MECC</td>
<td>400</td>
<td>3.85 ± 0.74</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for six animals and analysed by One way ANOVA followed by post hoc Dunnett’s test *p< 0.05, **p< 0.001 when compared to vehicle control.

Table 2: Effect of MECC in Carrageenan induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Change in paw volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>3 h</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>-</td>
<td>0.81 ± 0.08</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>0.41 ± 0.06* (49.18)</td>
</tr>
<tr>
<td>MECC</td>
<td>100</td>
<td>0.72 ± 0.12 (11.48)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.63 ± 0.09 (23.16)</td>
</tr>
<tr>
<td>MECC</td>
<td>400</td>
<td>0.48 ± 0.07 (40.57)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, *p< 0.05, **p< 0.001 when compared to healthy control.

Table 3: Effect of MECC on cotton pellet-induced granuloma in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Dry weight of granuloma (mg)</th>
<th>Percent Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>-</td>
<td>132 ± 4.6</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>64 ± 2.5**</td>
<td>51.52</td>
</tr>
<tr>
<td>MECC</td>
<td>100</td>
<td>118 ± 5.1</td>
<td>10.61</td>
</tr>
<tr>
<td>MECC</td>
<td>200</td>
<td>104 ± 4.6**</td>
<td>21.21</td>
</tr>
<tr>
<td>MECC</td>
<td>400</td>
<td>86 ± 4.2**</td>
<td>34.85</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for six animals and analysed by One way ANOVA followed by Dunnett’s test, **p< 0.001 when compared to vehicle control.

Anti-inflammatory activity

Carrageenan induced paw edema in rats

Treatment with MECC at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg exhibited a significant decrease in paw volume. MECC at 100 mg/kg showed significant (p< 0.05) and at 200 & 400 mg/kg also showed significant (p< 0.001) decrease in paw volume at 3rd and 5th h. Diclofenac (10 mg/kg) exhibited a significant (p< 0.001) reduction in paw volume at 3rd and 5th h as compared to vehicle control. The percentage inhibition of change in paw volume of MECC at 100 mg/kg, 200 mg/kg and 400 mg/kg was found to be 21.12%, 35.60% and 55.13% respectively at 3h. However the maximum percentage inhibition was found to be at 5th h 23.33%, 57.86% and 82.28% for 100 mg/kg, 200 mg/kg and 400 mg/kg of MECC respectively. The percentage inhibition of diclofenac (10 mg/kg) was found to be 75.64% and 92.49 % at 3rd & 5th h respectively when compared with carrageenan control animals (Table 3).

Cotton pellet-induced granuloma in rats

In cotton pellet granuloma, the MECC (200 and 400 mg/kg) significantly (p< 0.001) inhibited the granuloma formation when compared to vehicle control group. The degree of inhibition was dose dependent. The MECC at 100, 200, and 400 mg/kg inhibited the granuloma formation by 16.61%, 21.21% and 34.85% respectively. Diclofenac (10 mg/kg) significantly (p< 0.001) inhibited the granuloma formation by 51.52% (Table 4).

**DISCUSSION**

The use of traditional medicine is widespread and plants still serve as leads for development of novel drugs [24]. The present...
investigation was carried out to scientifically evaluate the traditional claim of Curcuma caesia Roxb rhizomes as analgesic and anti-inflammatory. On acute oral toxicity the extract was found to be safe up to 2000 mg/kg. Phytochemical screening showed the presence of saponins, phenols, flavonoids, alkaloids and tannins.

Analgesic activity of MECC was evaluated using acetic acid induced writhing test and hot plate model to characterize peripheral and central analgesic activity. MECC exhibited significant and marked analgesic actions in both the models. Acetic acid is very sensitive method for screening analgesic effect and causes increase in PGE2 and PGF2α in peritoneal fluid [25]. MECC produced significant inhibition of writhes at the dose of 200 mg/kg (p<0.01) and 400 mg/kg (p<0.01) compared to vehicle control. Central analgesic activity of Curcuma caesia has been reported in tail flick test [15] but not by using hot plate method.

The hot plate test has been found to be suitable for evaluation of centrally acting analgesics [26]. The hot plate test measures the response to a brief noxious stimulus thus bears a closer resemblance to clinical pain [19]. The increase in reaction time in the hot plate test suggests the central analgesic effect of Curcuma caesia. The ability of MECC in analgesic activity may be due to the involvement of endogenous prostaglandins. This means that MECC exerted both peripheral and central analgesic activity for the transmission of painful message in mice.

Carrageenan induced paw oedema which is a classical model of acute inflammation has been widely used in the study of steroid and non-steroid anti-inflammatory drugs [27]. Carrageenan-induced inflammation has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation [25]. Carrageenan is a family of linear sulphated polysaccharides extracted from the red seaweed marine alga Chondrus crispus. Lambda carrageenan is used in animal models of inflammation to test anti-inflammatory activity because dilute carrageenan solutions (1-2%) induce oedema and pain [28]. The edema produced by subplantar injection of carrageenan in rat hind paw is biphasic over 4 or more hours. The early phase is attributed due to release of serotonin and histamine while later phase is sustained by prostaglandins and leucotrienes [29] and continuity between two phases is provided by kinins [30]. The second phase is sensitive to most clinically effective anti-inflammatory drugs. The MECC was found to significantly inhibit carrageenan induced rat paw edema in the late phase regulated by prostaglandins and leucotrienes.

Cotton Pellet induced granuloma in rats is a chronic model of inflammation which has been widely used to assess activity of anti-inflammatory drugs on proliferative phase of inflammation [27]. Proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels which are the basic sources of highly vascularized reddish mass is termed as granulation tissue is seen during repair process of inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed [31]. In the present study significant activity of MECC was seen against cotton pellet induced granuloma in rats indicating ability of MECC in reducing number of fibroblasts and synthesis of collagen and mucopolysaccharide, natural proliferative events of granulation tissue formation. The presence of phenolic compounds in the extracts may be responsible for the anti-inflammatory activities in both the models [32]. Therefore analgesic and anti-inflammatory activity of MECC may be attributed to its phytochemical compounds present in the extract.

CONCLUSION

MECC showed analgesic activity in acetic acid induced writhing model (peripheral) and hot plate test (central). It also showed anti-inflammatory activity in carrageenan (acute) and cotton pellet induced granuloma (sub acute). This activity can be contributed to the phytochemicals present in the extract like alkaloids, phenolic compounds, flavonoids and tannins.

REFERENCES