Evaluation of Anti-inflammatory and Analgesic Activity of Methanolic Extract of *Cassia auriculata* Leaves

A.A. Mali, D.D. Bandawane and M.G. Hivrale
Department of Pharmacology, PES’s Modern College of Pharmacy, Nigdi, Pune, Maharashtra 411044, India

**ABSTRACT**

**Background:** *Cassia auriculata* L. is highly valued in Indian medicines for management of painful inflammation and diabetes. The objective of this study was to evaluate the anti-inflammatory and analgesic potential of methanolic extract of *C. auriculata* leaves (MECA). **Materials and Methods:** The MECA (300 and 600 mg kg⁻¹) was subjected to anti-inflammatory, analgesic activity using carrageenan induced rat paw edema, cotton pellet induced granulomatous chronic inflammation, hot plate method and tail immersion method. The antioxidant potential of MECA was determined by using 1,1-diphenyl-2-picyrylhydrazyl (DPPH) radical scavenging, nitric oxide radical scavenging activity and reducing power assay. In addition to this the ulcerogenicity of the extract was tested using ethanol induced ulcer model. **Results:** Results showed that MECA significantly (p<0.05 and p<0.01) inhibited inflammation induced by carrageenan and cotton pellet implants. There was significant (p<0.05 and p<0.01) increase in latency periods in Eddy’s hot plate and tail immersion induced pain. Similarly, MECA at 100 μg mL⁻¹ exhibited significant (p<0.01) reducing power, DPPH free radical scavenging and nitric oxide radical scavenging activity. **Conclusion:** The results obtained indicate that MECA has dose dependant anti-inflammatory activity, central analgesic activity and lack ulcerogenicity. These activities of MECA are attributed to its antioxidant mechanism and presence of tannins and flavonoids which themselves are responsible for antioxidant potential.

**Key words:** *C. auriculata* leaves, methanolic extract, anti-inflammatory, analgesic, carrageenan


**INTRODUCTION**

The search of new agents having various pharmacological activities, obtained by screening the natural sources like different plant extracts has led to the discovery of many clinically useful drugs which are immensely useful in the treatment of various human diseases. Herbal drugs can therefore be considered as a better alternative to synthetic anti-inflammatory drugs. *Cassia auriculata* Linn. (fam. Caesalpiniaceae) is a tall, branched, bushy shrub growing wild throughout forests, along roadsides and in wastelands. Traditionally the plant has been used in ayurvedic medicine as ‘Avarai Panchaga Chooram’ and as constituent of ‘Kalpa herbal tea’. The leaves are bitter, astringent, acrid, constipating and expectorant. The qualitative phytochemical analysis of extracts from the roots and leaves of *C. auriculata* showed the presence of anthraquinone glycosides, alkaloids, flavonoids, phenolic compounds, saponins, steroids and tannins.

Previous studies has proved that the chemical constituents such as flavonoids, bioflavonoids, alkaloids, tannins and terpenoids are promising agents in treatment of inflammation. Flavonoids such as hesperidin, apigenin, luteolin and quercetin are found to be potent anti-inflammatory constituent and flavonoids like myricetin, quercetin, naringenin, apigenin and catechin have been reported to be effective antioxidant agents. High content of flavonoids and bioflavonoids in methanolic extract of *C. auriculata* flowers is reported for its anti-inflammatory activity. Preliminary phytochemical analysis of MECA shows the presence of tannins, flavonoids, steroids, alkaloids saponins and glycosides.

This, therefore, forms the basis of the current study which evaluates the anti-inflammatory effect and mechanism of MECA. Also, considering the strong association between inflammation and oxidative damage, the current study evaluates the *in vitro* antioxidant activity of the methanolic extract using DPPH radical scavenging activity, nitric oxide radical scavenging activity and reducing power assay. The main side effect and limitation of synthetic anti-inflammatory and analgesic compounds is its ulcerogenic property. Hence, we investigated the ulcerogenicity of MECA.
MATERIALS AND METHODS

Materials: The leaves of the C. auriculata were obtained from their natural habitat in the Pune region of Maharashtra in the month of August. The plant was identified and authenticated by Mr. Diwakar, Botanical Survey of India, Pune. Voucher specimen (CAAAAM5) has been deposited in the herbarium of BSI, Pune for future reference. Carrageenan was procured from Anabals fine chemicals, Mumbai. All other chemicals and reagents used were of analytical grade (SRL, Mumbai, E. Merck India).

Experimental animals: Wistar rats (180-200 g) and Swiss albino mice (25-30 g) of either sex were used for the study. The animals were procured from the National Institute of Biosciences (NIB), Pune. The animals were housed at 25°C, under a 12:12 h light-dark cycle, with free access to standard pellet diet and water ad libitum. The study was approved by Institutional Animal Ethical Committee (IAEC) and experimental procedures were conducted in accordance with the regulations of CPCSEA (884/PO/ac/05/CPCSEA).

Extraction: The extraction was performed using maceration technique. The coarse powder of C. auriculata leaves (100 g) was subjected to maceration for 72 h at room temperature using 500 mL methanol. The extract was filtered and the solvent was evaporated under vacuum to obtain powdered residue.

Acute toxicity study: The MECA was tested for its acute toxicity in rats. Acute toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) guidelines OECD 423. To determine short term toxicity, the adult female Wistar rats were starved overnight and were administered with extract orally in increasing dose levels of 300, 500, 2000, 5000 and 8000 mg kg⁻¹ body weight. The mortality and general behavior of rats were observed periodically for 48 h. The animals were observed individually after dosing periodically for 24 h with special attention during first two hours and then intermittently thereafter, for a total period of 14 days. The animals were observed for the signs of toxicity which include changes in eyes and mucous membrane, skin and fur and behavior pattern. Attention was given to parameters like grooming, hyperactivity, convulsions, tremors, salivation, lethargy, diarrhea, loss of righting, reflex, sleep and coma.

Preliminary phytochemical screenings: The preliminary phytochemical analysis of MECA was performed as per the standard methods given by Trease. A series of chemical tests were carried out viz. Molisch’s, Fehling’s, Benedict’s test for carbohydrates; Biuret and Million’s test for proteins; Salkowski and Liebermann-Buchard’s test for steroids; Borntrager’s test for anthraquinone glycosides; foam test for saponins; Shinoda and alkaline test for flavonoid glycosides; Dragendorff’s, Mayer’s, Hagger’s and Wagner’s test for alkaloids; ferric chloride, lead acetate and potassium dichromate test for tannins and phenolics.

Anti-inflammatory activity in carrageenan induced rat paw edema: Wistar rats (180-200 g) of either sex were randomly divided into four groups of six animals each. In all the treated groups, rats were orally pre-treated with the MECA 1 h before inducing acute inflammation with single sub-planter injection of 0.1 mL of % w/v of carrageenan suspension in 0.9% normal saline.

Treatment of rats is as described below:

- **Group I**: Normal saline + 0.1 mL of % w/v of carrageenan suspension
- **Group II**: 10 mg kg⁻¹ indomethacin + 0.1 mL of % w/v of carrageenan suspension
- **Group III**: 300 mg kg⁻¹ of MECA + 0.1 mL of % w/v of carrageenan suspension
- **Group IV**: 600 mg kg⁻¹ of MECA + 0.1 mL of % w/v of carrageenan suspension

MECA was dissolved in distilled water and indomethacin suspension was made in 10% carboxymethyl cellulose in distilled water. The paw volume upto the tibio-tarsal articulation of all animals was measured using a plethysmometer. The increase in paw volume was determined up to 6 h. The % inhibition in paw volume was calculated by using following formula:

\[
\text{Inhibition in paw volume (\%)} = \frac{V_t - V_c}{V_c} \times 100
\]

Where:
- \(V_t\) = Mean paw volume in the drug treated group
- \(V_c\) = Mean paw volume in control group

Cotton pellet induced granulomatus chronic inflammation: The cotton pellet induced granuloma in rats was evaluated using the method of Winter. The cotton pellets weighing 25 ±1 mg were sterilized in an autoclave for 30 min at 120°C under 15 lb pressure. One pellet was implanted subcutaneously in the groin region of each rat under thiopental sodium (25 mg kg⁻¹) anesthesia. The vehicle, indomethacin (10 mg kg⁻¹), MECA (300 and 600 mg kg⁻¹) were given orally to the Group I, II, III and IV, respectively for seven consecutive days from the day of cotton pellet implantation. On
8th day, the animals were anaesthetized and blood was collected by cardiac puncture. The animals were sacrificed and pellets together with the granuloma tissue were carefully removed and made free from extraneous tissue.

The wet pellets were weighed for wet weight and dried in an incubator at 60°C to a constant weight, after that the dried pellets were weighed again. The granulation tissue formation and exudate formation was calculated using following formula:

\[ \text{Measure of granuloma tissue formation} = \text{Constant dry weight-initial weight of pellet} \]

\[ \text{Measure of exudate formation} = \text{Wet weight-Constant dry weight of pellet} \]

**Hot plate method:** The method was performed as given by Vogel\(^{20}\). The heated hot plate, maintained at 55°C was used to induce pain. Mice (six per group) were individually placed on the hot plate into a transparent glass cylinder (diameter 20 cm). Each mouse acted as its own control. Before the treatment, the reaction time of each animal (paw licking or jumping) was recorded. The reaction time was recorded at 1, 2, 3 and 4 h following the administration of MECA (300 and 600 mg kg\(^{-1}\), p.o.), diclofenac (9 mg kg\(^{-1}\)) and distilled water\(^{20,21}\).

**Tail immersion method:** Tail immersion test was performed as described by Nikajoo\(^{20,22}\). This involved immersing tail of mice up to 5 cm length in a water bath maintained at 58°C. Reaction of mice by withdrawing the tail was considered as the reaction time. The reaction time for each animal was recorded with a stopwatch. Each mouse acted as its own control. MECA (500 and 600 mg kg\(^{-1}\), p.o.), diclofenac (9 mg kg\(^{-1}\)) and distilled water (10 mL kg\(^{-1}\)) were administered to mice (4 groups). The reaction time of treated animals was taken at 1, 2, 3 and 4 h after administration of tested products. The cut off time to prevent tissue damage was put at 20 sec\(^{20,22}\).

**Determination of DPPH scavenging activity of MECA:** The effect of MECA was estimated by using the method described by Dasgupta\(^{23}\). A solution of 4 mg 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in methanol (0.04%\%) was prepared and 3 mL of solution was mixed with 0.1 mL methanolic solution of MECA each from concentrations 200-1000 \(\mu\)g mL\(^{-1}\). The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30 min. Absorbance of the mixture was determined spectrophotometrically at 517 nm. Ascorbic acid prepared at same concentration was used as the reference drug. The experiment was conducted in triplicate. The percent inhibition in scavenging DPPH radicals was calculated using the formula:

\[ \text{Inhibition} (\%) = \frac{A_0-A_e}{A_0} \times 100 \]

Where:

\(A_0\) = Absorbance without extract.

\(A_e\) = Absorbance with extract\(^{26}\).

**Determination of nitric oxide scavenging activity of MECA:** Nitric oxide scavenging activity of MECA was determined by slightly modified procedure of Saha\(^{25}\). Butylated Hydroxy Tolune (BHT) was used as the standard drug.

**Determination of reducing power of MECA:** Reducing power of MECA was evaluated by method of Kumaran\(^{24}\).

**Anti-ulcerogenic activity in ethanol induced ulcer model:** Male Wistar rats were divided into four groups six animals in each as follows: Group I served as control which received vehicle, p.o., Group II was administered with omeprazole 30 mg kg\(^{-1}\) p.o. while Group III and Group IV were test groups which received MECA 300 mg kg\(^{-1}\) p.o. and 600 mg kg\(^{-1}\) p.o., respectively. The animals were fasted for 16 h and the respective treatment was orally administered. The 99.5% ethanol was given orally to induce gastric ulcers and animals were sacrificed after 1 h. The stomachs were removed and cut opened along the greater curvature and the gastric mucosa were washed with normal saline and scored according to the scale. The scoring of ulcer was performed using magnifying hand lens.

The following scale was used:

0 = Normal stomach
0.5 = Red coloration
1 = Spot ulcer hemorrhagic
1.5 = Streak and
2 = Ulcers

The results were expressed in % protection:

\[ \text{Protection} (\%) = \frac{\text{Ulcere index of control-Ulcere index of test}}{\text{Ulcere index of control}} \times 100 \]

**Statistical analysis:** Experimental results were presented as Mean±SEM of six animals. Analysis of variance was performed by ANOVA followed by Dunnet's multiple comparison test. p-values less than 0.05 were considered to be statistically significant.

**RESULTS**

**Extraction:** The yield of methanolic extract of tamarind seeds was 11.2 % w/w.
Phytochemical screening of *Cassia auriculata*: Phytochemical analysis revealed the presence of tannins, flavonoids, anthraquinone glycosides, alkaloids, phenolic compounds, saponins and steroids in the MECA.

**Acute toxicity study:** As suggested by OECD guidelines, the test animals were observed individually, after dosing at once during first 30 min, periodically during the first 24 h with special attention during first 2 h. The test animals did not exhibit any visible change and survived beyond recommended duration of observation with 8000 mg kg⁻¹. Hence, MECA was safe up to 8000 mg kg⁻¹.

**Anti-inflammatory activity in carrageenan induced rat paw edema:** The average volumes of inflamed rat paw are depicted in Table 1. Sub-planter injection of carrageenan produced edema in rat paw which increased progressively with time. MECA showed significant (p<0.05) decrease in paw edema from 1 h of carrageenan injection. However, the effect was more prominent (p<0.01) at 2, 3 and 4 h. The % inhibition of paw volume of MECA 300 mg kg⁻¹ was more prominent than MECA 600 mg kg⁻¹ at 4 and 6 h and was comparable to that of standard (Table 1).

Cotton pellet induced granulomatous chronic inflammation: The suppressive effect of MECA (300 and 600 mg kg⁻¹) on granuloma formation was shown in Table 2. Oral administration of MECA 300 mg kg⁻¹ showed more significant inhibition in transudative granuloma formation (38.08%) than MECA 600 mg kg⁻¹ (22.01%), compared to toxic control and significant inhibition in proliferative weights of cotton pellets by 26.68 and 22.21%, respectively. Indomethacin (10 mg kg⁻¹) showed significant inhibition in transudative granuloma formation and proliferative weights of cotton pellets by 56.29 and 36.4%, respectively.

**Histopathology:** Figure 1 reveals the histopathological findings of granulomatous tissue. Section from control group shows fibromuscular tissue comprising of neutrophils, lymphocytes, macrophages, giant cell and few plasma cells with proliferating blood vessels and dense infiltration by acute inflammatory cells. Rats treated with indomethacin (10 mg kg⁻¹) showed absence of necrosis and presence of diffusely arranged lymphocytes, macrophages and few plasma cells. MECA treated groups (300 and 600 mg kg⁻¹) showed diffused infiltration by lymphocyte, macrophages, few plasma cells with multinucleated giant cells and congested vessels. No evidences of necrosis were found.

**Hot plate method:** Table 3 shows the results for hot plate method. The pre-treatment of mice with MECA

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Dose (mg kg⁻¹)</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>0.10±0.04</td>
<td>0.14±0.03</td>
<td>0.17±0.13</td>
<td>0.18±0.11</td>
<td>0.16±0.11</td>
<td>0.18±0.12</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>1.30±0.03</td>
<td>1.28±0.05*</td>
<td>1.28±0.04**</td>
<td>1.26±0.05**</td>
<td>1.08±0.02**</td>
<td>1.59±0.05**</td>
</tr>
<tr>
<td>MECA 300</td>
<td>110</td>
<td>1.10±0.02</td>
<td>1.02±0.03**</td>
<td>1.42±0.06*</td>
<td>1.53±0.1*</td>
<td>1.23±0.06**</td>
<td>1.48±0.06**</td>
</tr>
<tr>
<td>MECA 600</td>
<td>115±0.05</td>
<td>1.16±0.05*</td>
<td>1.43±0.11**</td>
<td>1.60±0.05</td>
<td>1.27±0.03**</td>
<td>1.61±0.04*</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n = 6); *p<0.05, **p<0.01 compared to control. Data analysed by One-way ANOVA test followed by Dunnett’s multiple tests for comparison, MECA: Methanolic extract of *C. auriculata* leaves

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Dose (mg kg⁻¹)</th>
<th>Weight of moist cotton pellet (mg)</th>
<th>Weight of dried cotton pellet (mg)</th>
<th>Inhibition (%)</th>
<th>Transudative weight (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>268.75±0.49</td>
<td>77.67±0.25</td>
<td>-</td>
<td>221.30±9.91</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>146.5±14.15**</td>
<td>49.95±2.84**</td>
<td>30.4</td>
<td>97.67±14.15**</td>
<td>56.29</td>
</tr>
<tr>
<td>MECA 300</td>
<td>196.15±8.18**</td>
<td>56.93±1.36**</td>
<td>26.68</td>
<td>139.22±7.44**</td>
<td>38.08</td>
<td></td>
</tr>
<tr>
<td>MECA 600</td>
<td>232.98±13.44**</td>
<td>60.4±4.13**</td>
<td>22.21</td>
<td>172.58±9.01**</td>
<td>22.01</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n = 6); *p<0.05, **p<0.01 compared to control. Data analysed by One-way ANOVA test followed by Dunnett’s multiple tests for comparison, MECA: Methanolic extract of *C. auriculata* leaves

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Dose (mg kg⁻¹)</th>
<th>Latency period (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>9.55±1.50</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>56.01±5.63</td>
</tr>
<tr>
<td>MECA 300</td>
<td>51.40±17.92</td>
<td>67.97±12.33**</td>
</tr>
<tr>
<td>MECA 600</td>
<td>44.04±5.43</td>
<td>83.25±18.55**</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n = 6); *p<0.05, **p<0.01 compared to control. Data analysed by One-way ANOVA test followed by Dunnett’s multiple tests for comparison, MECA: Methanolic extract of *C. auriculata* leaves
Fig. 1(a-d): Effect of MECA on cotton pellet induced granuloma model in rats. (a) Control, (b) Standard (indomethacin) 10 mg kg⁻¹, (c) MECA 300 mg kg⁻¹ and (d) MECA 600 mg kg⁻¹

Table 4: Effect of MECA (300 and 600 mg kg⁻¹) on tail immersion method in mice

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Dose (mg kg⁻¹)</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>2.95±0.56</td>
<td>2.39±0.54</td>
<td>3.73±0.82</td>
<td>5.07±0.82</td>
<td>2.39±0.17</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>4.36±0.53</td>
<td>6.99±1.10*</td>
<td>6.45±1.39</td>
<td>12.83±1.25*</td>
<td>9.37±1.00*</td>
</tr>
<tr>
<td>MECA</td>
<td>300</td>
<td>2.91±0.78</td>
<td>9.50±0.95**</td>
<td>7.1±2.09</td>
<td>12.53±2.64*</td>
<td>10.74±1.97**</td>
</tr>
<tr>
<td>MECA</td>
<td>600</td>
<td>4.44±0.34</td>
<td>6.94±1.31*</td>
<td>8.50±2.28</td>
<td>11.59±1.87</td>
<td>11.10±2.16**</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n = 6); *p<0.05, **p<0.01 compared to control. Data analysed by One-way ANOVA test followed by Dunnet’s multiple tests for comparison. MECA: Methanolic extract of C. sanctula leaves

(300 and 600 mg kg⁻¹) and diclofenac (9 mg kg⁻¹) significantly (p<0.05 and p<0.01) increased the reaction time of mice to thermal stimulation compared to control (Table 3).

Tail immersion method: Effect of MECA on tail immersion test is shown in Table 4. In this test, administration of MECA (300 and 600 mg kg⁻¹) as well as diclofenac (9 mg kg⁻¹) significantly (p<0.05 and p<0.01) increased the latency time, compared to the control.

Determination of DPPH scavenging activity of MECA: Using DPPH, the free radical scavenging activities of MECA and ascorbic acid were observed to be concentration dependant (Fig. 2). At 1000 µg mL⁻¹, the scavenging effect of MECA was calculated to be 65.21% whereas, the scavenging effect of ascorbic acid was 94.2%. Determination of Nitric oxide scavenging activity of MECA: MECA and BHT significantly (p<0.01) scavenged nitric oxide radicals at variable concentrations (20-100 µg mL⁻¹), shown in Fig. 3. At 100 µg mL⁻¹, % inhibition of nitric oxide radicals by MECA was calculated to be 74.55% while by BHT it was found to be 60.42%.
Table 5: Effect MECA (300 and 600 mg kg⁻¹) on ethanol induced gastric ulcers in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>20.15 ± 0.92</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>1.33 ± 0.16 **</td>
<td>93.40</td>
</tr>
<tr>
<td>MECA</td>
<td>300</td>
<td>6.60 ± 1.50 **</td>
<td>70.29</td>
</tr>
<tr>
<td>MECA</td>
<td>600</td>
<td>4.00 ± 1.80 **</td>
<td>80.15</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n = 6); *p<0.05, **p<0.01 compared to control. Data analysed by One-way ANOVA test followed by Dunnet’s multiple tests for comparison. MECA: Methanolic extract of C. auriculata leaves.

Fig. 2: DPPH radical scavenging activity of methanolic extracts of C. auriculata leaves. MECA: Methanolic extract of C. auriculata leaves, ASC: ascorbic acid.

Fig. 3: Nitric oxide scavenging activity of methanolic extract of C. auriculata leaves. MECA: Methanolic extract of C. auriculata leaves, BHT: Butylated Hydroxy Toluene.

Fig. 4: Reducing power of methanolic extracts of C. auriculata leaves. MECA: Methanolic extract of C. auriculata leaves, ASC: ascorbic acid.

**DISCUSSION**

In the present study, the anti-inflammatory, analgesic and antioxidant activities of MECA were evaluated using different standard methods. Carrageenan induced paw edema has been widely used to screen natural products with anti-inflammatory potentials. Inflammation induction with carrageenan involves the activation of platelet activation factor and release of pro-inflammatory mediators such as prostaglandins, kinins, tumor necrosis factor and nitric oxide. Sub-planter injection of carrageenan in the rat hind paw induces inflammation in two distinct phases namely: the first phase (0-2 h) which involves release of histamine and 5-hydroxytryptamine and second phase (2-6 h) which involves release of the inflammatory mediators like prostaglandins, leukotrienes, polymorphonuclear cells and bradykinins. These two phases are linked with kinin release. However, synthetic anti-inflammatory agents such as aspirin, indomethacin and diclofenac are known to mediate their anti-inflammatory action via inhibition second phase of inflammatory response. Since MECA showed maximum reduction in paw edema during second phase, it may be stated that MECA might have mediated its anti-inflammatory action by inhibiting the release of mediators like prostaglandins, leukotrienes, polymorphonuclear cells and bradykinins. Moreover,
these results indicate that the anti-inflammatory activity of MECA can be attributed to its tannins and flavonoids.

In the present study, cotton pellet granuloma model was used to evaluate the transudative and proliferative components of the chronic inflammation\(^5\). The decrease in the size of the granuloma observed by MECA may be via events inhibition of granulocyte infiltration, preventing generation of collagen fibres and by suppression of mucopolysaccharides. Histopathology of granulomatous tissue further confirmed the anti-inflammatory potential of MECA. MECA treated groups showed absence of necrosis, reduction in dense infiltration inflammatory cells and exudate compared to toxic control.

It is well known that antioxidant potential plays an important role in the anti-inflammatory activity\(^,\(^6,\(^7\). It has been reported that the neutralization of free radicals by antioxidants and radical scavengers eases inflammation\(^8,\(^9\). Bearing this strong association between inflammation and oxidation, it is possible that anti-inflammatory activity of MECA is partly mediated via its antioxidant mechanism. Tannins and flavonoids have been reported to exhibit significant inhibitory activities on nitric oxide implicated in physiological and pathological process as chronic inflammation\(^10\).

In the present study, Eddy’s hot plate method and tail immersion methods were used to evaluate analgesic activity of MECA. The validity of these tests has been shown even in the substantial impaired motor performances of animals. Hot plate method is based on the mice paw sensitivity to heat at temperatures not damaging the skin which is observed as jumping, paw licking and paw withdrawal\(^11\). While in case of tail immersion method, nociceptive response is noted as withdrawal of tail from hot water. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas, peripherally acting analgesics do not generally affect these responses\(^12\). In the present study, MECA was found to affect jumping, withdrawal or paw licking response which makes it evident that it is centrally acting. This suggests implication of \(\mu\) receptors in the analgesic effect. The significant analgesic activity exhibited by MECA suggests an advantage in the use of the extract as an anti-inflammatory agent.

Reports from previous studies suggest that tannins and flavonoids inhibit prostaglandin synthesis\(^13\). The presence of tannins and flavonoids in the MECA as results of our preliminary phytochemical analysis, appears to inhibit prostaglandin synthesis and exerts the anti-inflammatory effect. Also tannins are reported to possess protein precipitating and vasoconstricting effect\(^14\). But inhibition of prostaglandin synthesis is always associated with ulcerogenic activity. The major limitation of most of the NSAIDs is the ulcerogenic activity which is due to inhibition of prostaglandin synthesis. MECA had shown significant anti-inflammatory and analgesic activity probably mediated through inhibition of prostaglandin synthesis which might be attributed to presence of tannins and flavonoids. While absence of ulcers by MECA might be due to presence of tannins which prevented damage to gastric mucosa through its astringent action.

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