Anti-inflammatory and antinociceptive activities of *Crotalaria burhia* Buch.-Ham. whole plant

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Received 16 June 2011; Accepted 7 April 2012

*Crotalaria burhia* Buch.-Ham. (Family-Fabaceae) popularly known as *Khip* is employed in Indian folk medicine for the treatment of gout, hydrophobia, pain and swelling. In present study antinociceptive and anti-inflammatory activities were assessed using acetic-acid induced writhing and formalin induced pain in mice and acute, subacute models of inflammation in rats. These studies demonstrated that oral administration of methanolic extract of whole plant (including aerial parts and root) of *C. burhia* (MECB) (100, 200, 400 mg/kg) exhibited significant antinociceptive and anti-inflammatory activities. In acetic acid writhing reflex model MECB 400 mg/kg, p.o. had shown significant antinociceptive effect but pretreatment with naloxone blocked the protective effect of the extract. In formalin induced pain MECB 400 mg/kg significantly inhibited the inflammation-induced pain better than the pain resulting from neurogenic phase. In acute inflammation as produced by carrageenan 62.19% after 6 h, by histamine 20.00%, by 5-hydroxytryptamine 27.27%, and by prostaglandin E2 26.92% protection was observed, while in subacute anti-inflammatory model using formaldehyde-induced hind paw edema (after 1.5 h) 49.29% protection from inflammation was observed at 400 mg/kg oral dose of MECB. MECB neither showed ulcerogenic effect at different doses of MECB nor any sign of toxicity and mortality up to a dose level of 5000 mg/kg, p.o. in rats and mice. These data indicate that MECB possesses significant antinociceptive and anti-inflammatory activities without ulcerogenic effect.

**Keywords:** Acute toxicity, Anti-inflammatory, Antinociceptive, *Crotalaria burhia*, Ulcerogenic.

**IPC code; Int. cl. (2011.01)—**A61K 36/00, A61P 29/00

**Introduction**

The genus *Crotalaria* Linn. (Fabaceae) has 300 species worldwide and about 18 species are reported in India. The genus produces mainly pyrrolizidine alkaloids but some flavonoid glycosides have also been reported1. *Crotalaria burhia* Buch.-Ham. is an undershrub, fibrous plant, common in the arid parts of West Pakistan and India (Punjab, Rajasthan and Gujarat) and Afghanistan2,3. Flowering and fruiting are from March–August. It is known as *Shinio* in Rajasthan, its Hindi name is *Khip*4, in Punjab as *Bhata*, in Gujarat as *Ghughato*, in Marathi *Ghagri* and in Bengal called as *Ban sutri*. *C. burhia* is a well known medicinal plant in Ayurveda and has been used as an important drug for centuries. Its leaves, branches and roots are used as a cooling medicine3,4, fresh plant juice is applied on eczema and it is also very useful in gout, hydrophobia, pain and swellings5,6.

Phytochemical studies have revealed the presence of pyrrolizidine alkaloids7-8 as main compounds in this plant. In addition, flavonoids like quercetin9, steroids like β-sitosterol2 have also been isolated from this plant. Anticancer10, antimicrobial9,11 and antibacterial6 activities of *C. burhia* have been reported by previous authors. An ethanolic (50%) extract of root of this plant showed anticancer activity in mice10. Ethanolic (50%) and ether extract9 of this plant showed *in vitro* antimicrobial activity against *Streptococcus faecalis* and *Staphylococcus albus* due to diffusion of antimicrobial agent (Quercetin-Flavonoids) to certain extent into the medium. Methanolic extract of whole plant has been reported to possess antibacterial activity against *E. coli*, *P. vulgaris*, *S. aureus* and *P. aeruginosa*, *E. coli*, *P.
vulgaris, S. aureus by disk diffusion method and well method, respectively \(^6\). Acetone extract of root showed 45\% ACE inhibition after tannin removal but the same was not showed by water and ethanolic extract of the root of the *C. burhia* \(^12\).

In the present study, *C. burhia* was selected because it is one of the medicinal plant commonly used in remedies to treat pain, swelling and fever in Indian traditional medicine and other countries in Asia. However, till date no ethnopharmacological study has been systematically conducted to evaluate the antinociceptive and anti-inflammatory action supporting traditional uses of this plant in folklore medicine. In this work we evaluated the antinociceptive and anti-inflammatory potential of this whole plant (including aerial part and root) in experimental animals using methanol extract.

The reason to use methanol extract in this investigation is that methanol is more nonpolar than water therefore, several substances including alkaloids and flavonoids, the major chemical constituents of *C. burhia*, would be expected to be more soluble in methanol extract than in water extract.

**Materials and Methods**

**Chemicals**

Acetic acid, formalin, carrageenan, histamine, 5-hydroxytryptamine (5-HT), prostaglandin E\(_2\) (PGE\(_2\)), bradykinin, aspirin, indomethacin, morphine and phenylbutazone (PBZ) were procured from Sigma Chemicals, St. Louis, MO, USA. Formaldehyde was purchased from Hi-Media Laboratories, Mumbai, India and the water represents the double distilled water.

**Plant material**

Whole plant (including aerial part and root) of *C. burhia* was collected in the month of February–March 2009 from Jaipur, Rajasthan, India. The plant material was authenticated by the Joint Director, Botanical Survey of India (BSI), Jodhpur (Rajasthan, India) and a voucher specimen (JNU/JPR/PC/SK-1) has been deposited in their herbarium.

**Preparation of extract**

The air-dried and powdered whole plant (including aerial part and root) of *C. burhia* (50.3 g) was extracted with methanol by using Soxhlet apparatus. The obtained extract was filtered and evaporated under reduced pressure, on a rotary evaporator at 40-45°C, to yield 7.8 g of crude methanolic extract.

**Animals**

Healthy, Wistar albino rats (180-210 g) and Swiss albino mice (30-35 g) were taken for the study and were maintained at the departmental animal house under standard environmental conditions (temperature, 22 ±2°C, and 12 h light/dark cycle). The animals had free access to standard pellet diet and water *ad libitum* and were maintained in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The Institutional Animal Ethics Committee approved the protocol of the study (Approval no.001/2009/IAEC/JNU).

**Antinociceptive activity**

**Acetic acid-induced writhings in mice**

Mice (six per group) were injected intraperitoneally with 0.6\% acetic acid at a dose of 10 ml/kg. The extract (100, 200, 400 mg/kg, p.o.), morphine (5 mg/kg, s.c.), naloxone+extract (1 mg/kg, i.p. + 400 mg/kg, p.o.) and distilled water (p.o.) were administered 30 min prior to treatment with acetic acid. The writhings induced by the acid, consisting of abdominal constrictions and hind limbs stretching, were counted for 30 min after a latency period of 5 min. The percentage analgesic activity was calculated \(^13\).

**Formalin test in mice**

Pain was induced by injecting 0.05 ml of 2.5\% formalin (40\% formaldehyde) along with distilled water in the subplantar of the right hind paw. Rats (six per group) were given extract (100, 200, 400 mg/kg, p.o.), aspirin (100 mg/kg), morphine (5 mg/kg, s.c.) and distilled water (p.o.) 30 min prior to injecting formalin. These rats were individually placed in a transparent Plexiglass cage (25 cm × 15 cm × 15 cm) observation chamber. The amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0 to 5 min (first phase) and 15-30 min (second phase) were counted after injection of formalin. These phases represented neurogenic and inflammatory pain responses, respectively \(^14\).

**Anti-inflammatory activity**

**Carrageenan-induced hind paw edema in rats**

Five groups of six animals per group were used. The plant extract was administered orally at doses
100, 200, 400 mg/kg as well as 10 mg/kg of indomethacin. Controls received distilled water. The administration of extract and drugs was 30 min prior to injection of 0.05 ml, 1% carrageenan in the right hind paw sub plantar of each rat. The paw volume was measured plethysmometrically (model 7150, Ugo Basil, Italy). Prior to injection of carrageenan, the average volume ($V_0$) of the right hindpaw of each rat was calculated from 3 readings which did not deviate more than 4%. At 1, 2, 4, and 6 h after injection of the phlogistic agent, only one reading was obtained for each rat ($V_t$). The percentage inhibition for each rat and each group was calculated.

Autocoid-induced hind paw edema in rats
The autacoids, viz., histamine (1 mg/ml), 5-hydroxytryptamine (1 mg/ml), prostaglandin E$_2$ (1µg/ml) and bradykinin (20µg/ml) were employed as phlogistic agents. The effect of MECB (100, 200 and 400 mg/kg, p.o.) was tested individually against each autocoid. Right hind paw edema was induced by the sub plantar injection of 0.1 ml of respective phlogistic agent. Test compounds were administered 1 h prior to the inflammatory insult. The pedal volume was measured just before 0 h and 3 h after the phlogistic challenge. Phenylbutazone (100 mg/kg, p.o.) was employed as reference standard.

Formaldehyde-induced hind paw volume
Pedal inflammation was induced by injecting 0.1 ml of 4% formaldehyde solution below the plantar aponeurosis of the right hind paw of the rats. The paw volume was recorded immediately prior to compound administration (0 h) and then at 1.5, 24 and 48 h after formaldehyde injection. Vehicle (1 ml/kg, p.o.), MECB (100, 200 and 400 mg/kg, p.o.) and standard drug, aspirin (300 mg/kg, p.o.) were administered 1 h prior to formaldehyde injection.

Ulcroergic activity
Acute ulcerogenic activity
The ulcerogenic potential of MECB at three different doses (100, 200 and 400 mg/kg, p.o.) was tested in overnight fasted male rats. The control group was administered vehicle (1 ml/kg, p.o.), while the other group received standard drug, aspirin (300 mg/kg, p.o.), respectively. All the animals were killed with anesthetic ether 5 h after the administration of test compounds. The stomach of animals was dissected out, incised along the greater curvature and then put in diluted formaldehyde solution (2.5%). A few minutes later, mucosa of the stomach was observed for petechial hemorrhages and ulcers, if any. The degree of ulceration was graded according to the arbitrary scale.

Chronic ulcerogenic activity
The experiment was carried out using male rats with free access to feed and drinking water throughout the period of experiment. The rats were administered vehicle (1 ml/kg, p.o.), MECB (100, 200 and 400 mg/kg, p.o.) and aspirin (300 mg/kg, p.o.), once daily for 14 consecutive days. All the animals were sacrificed in 24 h after the administration of the last dose of the drug and the stomach was removed and examined as in the acute experiment.

Acute toxicity
The up-and-down method for acute toxicity testing was carried out for acute toxicity study. In this procedure, animals are dosed one at time. If an animal died the dose for the next is decreased while if it survives, the dose for the next is increased. Each animal is then observed for 1 or 2 days before dosing the next animal. In this study, the first dose was begun at 300mg/kg and adjusted by a constant multiplicative factor of 1.5 up to 5 g/kg. The MECB was orally administered to a group of mice and rat both male and female. Behavior parameters observed after administration were convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased or decreased respiration during a period of 8 h and 7 days. Food and water were provided ad libitum.

Statistical analysis
All the data were presented as mean ± S.E.M. of measurements made on six animals in each group. Comparisons across treatments were made using one-way ANOVA with Tukey’s post hoc test or repeated measures two-way ANOVA with Bonferroni’s post hoc test, when appropriate. All data were analyzed using the Prism 4 computer software (Graph Pad, San Diego, USA). Statistical differences were considered to be significant at $P < 0.05$.

Results
Acetic acid writhing reflex
*C. burhia* significantly reduced writhings and strechings induced by 0.6% acetic acid at a dose of

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10 ml/kg (Figure 1). The significant protective effect was dose dependent with 15.31% ($P < 0.01$) reduction observed for 100 mg/kg and 44.72% ($P < 0.001$) seen for 400 mg/kg dose. Aspirin (100 mg/kg) had only 45.50% ($P < 0.001$) inhibition and morphine (a centrally acting analgesic) had 58.75% ($P < 0.001$) inhibition. Pretreatment with naloxone blocked the protective effect of the extract.

**Formalin-induced pain**

The extract had analgesic effects on both first (0-5 min) and second phases (15-30 min) of formalin test as shown on Table 1. These phases corresponded to neurogenic and inflammatory pains, respectively. Extract did not show any significant effect on neurogenic-induced pain blockade, whereas beginning from 200 mg/kg, the extract significantly blocked pain emanating from inflammation. The extract 400 mg/kg was found to inhibit the inflammation-induced pain (24.64%, $P < 0.001$) better than the pain resulting from neurogenic phase. Indomethacin was significantly active (33.31%, $P < 0.001$) only on the second phase.

**Carrageenan-induced oedema**

The average right back paws volumes and percentages of oedema are presented in Table 2. The percentages of inhibition are reported in Fig. 2. For the control group, the injection of the phlogistic agent caused localized oedema, 30 min after. The swelling increased progressively after 4 h to a maximum volume of 1.07±0.03 ml (44.59%) and remained 6 h after injection. Rats pretreated with *C. burhia* significantly decreased the carrageenan-induced oedema 1h post-dosing beginning with 100 mg/kg and in a dose related manner. At 100 mg/kg, the

![Fig. 1: Effect of MECB on writhing response in mice induced by acetic acid injection](image)

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**Table 1- Effect of MECB on the reaction time of mice in the formalin test**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>Early Phase (Inhibition %)</th>
<th>Late Phase (Inhibition %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>64.67 ± 3.3</td>
<td>134.52 ± 3.5</td>
</tr>
<tr>
<td>MECB 100</td>
<td>100</td>
<td>61.80 ± 2.50</td>
<td>132.91 ± 1.92</td>
</tr>
<tr>
<td>MECB 200</td>
<td>200</td>
<td>58.06 ± 2.40</td>
<td>119.84 ± 2.01</td>
</tr>
<tr>
<td>MECB 400</td>
<td>400</td>
<td>61.71 ± 2.80</td>
<td>101.37 ± 0.93</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>57.44 ± 2.51</td>
<td>89.71 ± 3.1</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for six mice. *$P < 0.01$, **$P < 0.001$ compared to respective control group.

**Table 2- Effect of MECB on carrageenan induced hind paw edema in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0h</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>6h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.74±0.1</td>
<td>0.90±0.02</td>
<td>0.98±0.02</td>
<td>1.07±0.03</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21.62)</td>
<td>(32.43)</td>
<td>(44.59)</td>
<td>(29.73)</td>
<td></td>
</tr>
<tr>
<td>MECB 100</td>
<td>100</td>
<td>1.09±0.01</td>
<td>1.22±0.01*</td>
<td>1.26±0.02*</td>
<td>1.30±0.01*</td>
<td>1.22±0.01**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11.9)</td>
<td>(15.60)</td>
<td>(19.27)</td>
<td>(11.90)</td>
<td></td>
</tr>
<tr>
<td>MECB 200</td>
<td>200</td>
<td>0.89±0.01</td>
<td>0.97±0.02**</td>
<td>0.99±0.01**</td>
<td>1.04±0.02**</td>
<td>0.98±0.02**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9.00)</td>
<td>(11.24)</td>
<td>(16.85)</td>
<td>(10.11)</td>
<td></td>
</tr>
<tr>
<td>MECB 400</td>
<td>400</td>
<td>0.95±0.02</td>
<td>1.03±0.02**</td>
<td>1.06±0.01**</td>
<td>1.07±0.01**</td>
<td>1.04±0.01**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8.42)</td>
<td>(11.58)</td>
<td>(12.63)</td>
<td>(9.47)</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.84±0.03</td>
<td>0.87±0.03**</td>
<td>0.89±0.02**</td>
<td>0.89±0.02**</td>
<td>0.90±0.01**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for six rats. *$P < 0.01$ and **$P < 0.001$ compared to respective arthritis control group. Percentages of oedema are in parentheses.
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extract showed significant inhibition of oedema formation after 2 h (28.75%, \( P < 0.01 \)) and continued for 6 h (40.91%, \( P < 0.001 \)). At 200 and 400 mg/kg, the extract achieved its maximal inhibitory effects of 60.09% (\( P < 0.001 \)) and 62.19% (\( P < 0.001 \)), respectively, at 6 h. Indomethacine (81.25%, \( P < 0.001 \)) attained its effects 1 h post-dosing. Although both the extract and indomethacine reduced the swellings, they still remain significantly visible 6 h after.

**Autacoid-induced hind paw edema**

The mean increase in paw edema volume produced at 3 h after injection of different autacoids, viz. histamine, 5-HT, PGE2 and bradykinin was 0.25±0.01, 0.44±0.01, 0.26±0.01 and 0.28±0.04 ml, respectively. MECB (200 and 400 mg/kg, p.o.) significantly inhibited (\( P < 0.05 \), \( P < 0.001 \)) hind paw edema induced by histamine, 5-HT and PGE2 but not that of bradykinin. However, PBZ (100 mg/kg, p.o.) significantly (\( P < 0.001 \), \( P < 0.05 \)) inhibited all autacoids including bradykinin-induced hind paw edema with the percent inhibition greater than that produced by MECB (400 mg/kg, p.o.) as shown in Table 3.

**Formaldehyde-induced hind paw edema**

MECB (100 and 400 mg/kg, p.o.) significantly diminished the mean paw edema volume at 1.5 (\( P < 0.001 \)) and 24 h (\( P < 0.05 \), \( P < 0.01 \)). The maximum per cent inhibition of edema volume produced by (400 mg/kg, p.o.) was almost comparable to that of Aspirin (300 mg/kg, p.o.) (49.29 vs 46.47 at 1.5 h). Interestingly, the effect of MECB up to a period of 24 h in contrast to Aspirin, the effect of which was significant only at 1.5 h (Table 4).

**Ulcerogenic activity**

Rats administered MECB as a single dose or chronically for 14 days were found to be devoid of gastric lesions in contrast to the standard anti-inflammatory agent, aspirin that significantly (\( P < 0.05 \); \( P < 0.01 \)) induced the gastric lesions with the mean score of severity of 1.80±0.30 in acute and 2.10±0.25 in chronic studies, as compared with the vehicle treated rats.

**Acute toxicity**

MECB at the dose of 5 g/kg, p.o. given to two groups (10 males and 10 females) of mice and rat had no affect on their behavioral responses during the observation periods of 8 h and 7 days after administration. No mortality was observed up to 7 days of monitoring. The LD\textsubscript{50} value of the extract in mice was therefore, estimated to more than 5 g/kg, p.o. As the highest dose used in the present study (400 mg/kg, p.o.) was 1/12th of the dose used in this acute toxicity test it was safe to assume that the normal doses of 100, 200 and 400 mg/kg, p.o. given

### Table 3- Effect of MECB on autacoids-induced hind paw edema in rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>Autacoid-induced paw edema volume (ml; mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Histamine</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.25±0.01</td>
</tr>
<tr>
<td>PBZ</td>
<td>100, p.o.</td>
<td>0.12±0.02***</td>
</tr>
<tr>
<td></td>
<td>(52.00)</td>
<td>(53.53)</td>
</tr>
<tr>
<td>MECB</td>
<td>100, p.o.</td>
<td>0.23±0.05</td>
</tr>
<tr>
<td></td>
<td>(8.00)</td>
<td>(2.27)</td>
</tr>
<tr>
<td>MECB</td>
<td>200, p.o.</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td></td>
<td>(12.00)</td>
<td>(13.63)</td>
</tr>
<tr>
<td>MECB</td>
<td>400, p.o.</td>
<td>0.20±0.02*</td>
</tr>
<tr>
<td></td>
<td>(20.00)</td>
<td>(27.27)</td>
</tr>
</tbody>
</table>

\( n=6 \) in each group; *, \( P < 0.05 \); **, \( P < 0.01 \); ***, \( P < 0.001 \) vs. control group. The figures in parentheses indicate the percent inhibition of edema volume in comparison to control group.
Discussion
The purpose of this work was to establish the scientific basis for the folk use of *C. burhia* Buch.-Ham. The present study demonstrates, for the first time, that systemic administration of the MECB, at doses that did not produce any toxicity and ulcerogenic effect, produced consistent antinociceptive and anti-inflammatory effects in different models of pain and inflammation. The antinociceptive effect of MECB was observed in acetic acid-induced writhing and formalin test. Moreover, MECB produced anti-inflammatory effect on various models i.e., acute exudative (carrageenan-induced rat paw edema), subacute (formaldehyde), and chronic inflammatory models (formalin test). The early and late phases of formalin test have obvious differential properties, and therefore this test is useful not only for assessing the analgesic substances but also for elucidating the mechanism of analgesia. The early phase, named as neurogenic pain, is a result of direct stimulation of nociceptors and reflects centrally mediated pain; the late phase, named inflammatory pain, is caused by local inflammation with a release of inflammatory and hyperalgesic mediators. In the present study, MECB produced antinociception against both neurogenic and inflammatory phase of formalin. The effect of MECB was more pronounced against the inflammatory phase. It is widely agreed that the nociceptive behaviors manifested during the acute first phase may be caused by the direct effect on peripheral nociceptors activating primary afferent fiber. This is followed by the tonic second phase that most probably results from the tonic inflammatory nociception responses due to inflammatory mediators like histamine, prostaglandins, serotonin and bradykinin. Additionally, considering the inhibitory property of *C. burhia* on the second phase of formalin, we might suggest an anti-inflammatory action of the whole plant extract. Because MECB is effective against the formalin test, the antinociceptive activity of MECB is most likely to be mediated peripherally.

Table 4- Effect of MECB on formaldehyde induced hind paw edema in rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>1.5h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.71±0.04</td>
<td>0.95±0.06</td>
<td>0.55±0.06</td>
</tr>
<tr>
<td></td>
<td>300, p.o.</td>
<td>0.38±0.04***</td>
<td>0.84±0.05</td>
<td>0.54±0.08</td>
</tr>
<tr>
<td></td>
<td>100, p.o.</td>
<td>0.54±0.03**</td>
<td>0.87±0.10</td>
<td>0.54±0.07</td>
</tr>
<tr>
<td></td>
<td>200, p.o.</td>
<td>0.46±0.03***</td>
<td>0.73±0.06*</td>
<td>0.51±0.05</td>
</tr>
<tr>
<td></td>
<td>400, p.o.</td>
<td>0.36±0.03***</td>
<td>0.71±0.04**</td>
<td>0.47±0.06</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for six rats. *P < 0.05, **P < 0.01 and ***P < 0.001 compared to respective arthritis group. The figures in parentheses indicate the percent inhibition of edema volume in comparison to control group.

To mice or rats in this study were safe.
The Carrageenan induced paw edema model in rats is known to be cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents. In autacoid-induced inflammations, MECB produced significant inhibitory activity against histamine; 5-HT and PGE$_2$ induce hind paw edema in rats. Inflammation induced by formaldehyde is biphasic, an early neurogenic component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved. In the formaldehyde-induced inflammation, the MECB demonstrated significant anti-inflammatory activity that lasted up to 24h in contrast to aspirin, which was effective only at 1.5 h, suggesting its long duration of action. Unlike the other tests, MECB treatment was started 18h after the injection of phlogistic agent, i.e., the activity of MECB was tested against the already ‘established’ inflammation that further substantiates its potential in curative purposes of various inflammatory diseases.

In autacoid-induced inflammation, MECB exhibited a significant inhibitory action against histamine, 5-HT and PGE$_2$ induced hind paw edema, which indicates that the extract exhibits its anti-inflammatory action by means of inhibiting the synthesis, release or action of inflammatory mediators, viz., histamine, 5-HT and prostaglandins involved in inflammation. The main side effect of non-steroidal anti-inflammatory drugs is their ability to produce gastric lesions. During the acute and chronic ulcerogenic studies, MECB did not induce any adverse effect on gastric mucosa, indicating non-ulcerogenic activity. From the acute, subacute and chronic studies, it is obvious that MECB possesses good anti-inflammatory activity, interestingly without any ulcerogenic activity.

Conclusion
The present study clearly showed that methanolic extract of whole plant (including aerial part and root) of C. burhia possessed good antinociceptive and anti-inflammatory activity and also scientifically validated the traditional use of this plant for treating inflammatory and pain disorders in the folk medicine. The advantages of this extract, viz. better and safer antinociceptive and anti-inflammatory profile without ulcerogenic activity deserves further studies to identify the possible mechanism of action as well as establishing the therapeutic value in the treatment of pain and inflammatory diseases.

Acknowledgement
Authors are thankful to the local inhabitants from Kukas village of Jaipur (Rajasthan) for their willingness to share their valuable knowledge and for providing the ethnobotanical information. We thank Shri Vinod ji, Botany Department of Rajasthan University, Jaipur for his encouragement and advice. Authors acknowledge Mr. Devendra Bhardwaj (Range forest officer, Eco tourism, Jaipur) and other people of Kapoor Chand Kulish Smriti-Van, Jaipur, Rajasthan for providing the local logistics.

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