Evaluation of Analgesic and Anti-inflammatory Activities of Methanol Leaf Extract of *Croton lobatus* (Euphorbiaceae) in Rodents

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ABSTRACT

*Croton lobatus* (Euphorbiaceae) is an annual herbaceous plant found throughout the West African region. The leaves of the plant are used in Senegal for treating whooping cough and to assuage the pain of scorpion sting in Nigeria. The methanol leaf extract of *Croton lobatus* (MLECL) was evaluated for possible analgesic and anti-inflammatory activities in mice and rats. Phytochemical screening and evaluation of median lethal dose (LD50) of the extract were carried out. Acetic acid-induced writhing test in mice and formalin-induced pain in rats were used to evaluate the analgesic activity using piroxicam and morphine as standard drugs, while the effect of the extract on acute inflammation was investigated using albumin-induced paw oedema in rats at 125, 250 and 500 mg/kg doses of the extract. The LD50 of the extract in mice was found to be greater than 5,000 mg/kg. Preliminary phytochemical screening of the extract revealed the presence of tannins, saponins, flavonoids, cardiac glycosides, cyanogenic glycosides, resins, steroids and carbohydrates. The extract significantly (*p* < 0.01) inhibited acetic acid induced writhing in mice and significantly (*p* < 0.01) attenuated the neurogenic phase of formalin-induced pain in rats at the highest dose tested (500 mg/kg). The extract also produced a significant (*p* < 0.05) anti-inflammatory activity at all the doses tested (125, 250 and 500 mg/kg). The results obtained in this study revealed that MLECL possesses significant analgesic and anti-inflammatory activities and supported the ethnomedicinal use of the plant in the management of pain and inflammatory conditions.

Keywords: *Croton lobatus*, Analgesic, Anti-inflammatory, Methanol extract, Euphorbiaceae

Introduction

Medicinal plants have long been used for the treatment of various ailments. An accidental discovery of some new plant food or juice that eased pain or relieved fever might have been the beginning of folk knowledge, which was passed down for generations and eventually became the foundation of medicine.1 About three-quarters of the world’s population relies on plants and its extracts for health care.2 In recent years, researchers have shown more interest to find new drugs from natural sources and medicinal plants with possibly fewer side effects in the treatment of diseases.3 This has become necessary to discover newer plants of medicinal value and to provide alternatives to synthetic drugs to combat the increasing problem of diseases. Croton is known to be one of the largest genera of flowering plants,4 with many species that widely found use in ethnomedicine including pain and inflammation. The plant *Croton lobatus* is an annual herbaceous plant recorded throughout the West African region and widespread across tropical Africa and Arabia.5 It is known in Hausa as ‘gadsayar’, in Yoruba as ‘ajofolé’; it belongs to the family Euphorbiaceae. Many studies have shown the biological activities of *Croton lobatus* including the safety profile of its aqueous extracts when administered orally in rats,6 its use traditionally for the treatment of malaria throughout malaria endemic areas,7 and the use of its species from South Africa, (*C. pseudopulchellus* Pax) for antimalarial activity.8 It has also been claimed by traditional herbal users to have some ethnomedicinal uses, for example, the extract obtained from boiled leaves serves as an enema for gynaecological conditions, and if mixed with palm-oil paste can be applied to guinea-worm sores. The heated leaves could be rubbed on areas of coastal and rheumatic pain, leaf decoction by mouth or a bark decoction by enema could be administered as a purgative.9 It has been used in the treatment of ulcers, sores and headache. In Nigeria, it is used to relieve pains from scorpion stings,8 and in the treatment of skin cancers.10 It is also used to treat body rashes and rheumatism.11 Despite all the biological properties of *Croton lobatus*, its analgesic and anti-inflammatory activities are yet to be reported. The present study, therefore, evaluated the analgesic and anti-inflammatory properties of the crude methanol extract of the dried leaf powder of *Croton lobatus* in mice and rats to validate its ethnomedicinal use.

Materials and Methods

Experimental Animals

Swiss albino mice of either sex weighing 18-22 g and Wistar rats of either sex weighing 160-210 g were obtained from the Animal House facilities of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria. The animals were maintained on standard

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laboratory animal feed (vital feed, Jos) and water ad libitum. Principles of laboratory animal care, NIH publication No.85-23 revised in 1985, were followed and was approved by the Institutional Animal Ethical Committee of Ahmadu Bello University, Zaria-Nigeria.

Collection and Identification of the Plant
The fresh leaves of Croton lobatus Linn were collected in the morning from Sakara village along Dam road, off old Jos Road, Zaria, Kaduna state, Nigeria. It was identified and authenticated by Mallam Umar Gallah a taxonomist in the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria with a voucher No. 1803 deposited for future reference.

Preparation of plant extract
The leaves of the plant were shade dried at room temperature for 14 days. The dried leaf was powdered using pestle and mortar. Soxhlet extraction was employed, in which 100 g of the powdered material was transferred into a small bag and placed inside the column of the soxhlet apparatus. The powdered sample was extracted with methanol (70%) for 24 hours. The resulting methanol extract was evaporated to dryness over a water bath maintained at a temperature of 40°C. The crude methanol extract obtained was weighed and stored in an air-tight container until use.

Phytochemical Screening
Established methods for detecting the presence of alkaloids, tannins, saponins, carbohydrates, flavonoids, glycosides, triterpenes, steroids, anthraquinones and monosaccharides were used.1,3,14

Acute Toxicity Study
The LD50 of the extract in mice was conducted using the intraperitoneal route according to the described method.15 The experiment was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the MLECL at doses of 10, 100 and 1,000 mg/kg body weight, administered intraperitoneally and observed for signs of toxicity and any possible death for 24 h. In the second phase, 4 groups each containing one mouse was injected intraperitoneally with doses of 1,200, 1,600, 2,900 and 5,000 mg/kg based on the result obtained from phase I.

Analgic Activity
Acetic Acid-induced Writhing Test in Mice
Swiss albino mice were randomly divided into 5 groups of six mice per group. Group 1 mice were treated with 10 mL/kg normal saline i.p (control group), groups II, III and IV received 125, 250, 500 mg/kg i.p of MLECL, while the fifth group received piroxicam at the dose of 10 mg/kg, i.p. After 30 minutes of drug administration, the mice were treated with 0.6% acetic acid at 10 mL/kg body weight, i.p.16 Five minutes after acetic acid injection, mice were placed in individual cages and the number of writhes was counted for each mouse for a period of 10 minutes. Analgesia was expressed as the decrease in the number of abdominal constrictions in mice treated with MLECL as compared with the normal saline treated control. The percentage inhibition of writhing was calculated using the formula below:

\[
\% \text{ inhibition of writhing} = \frac{\text{mean of control} - \text{mean test}}{\text{mean of control}} \times 100
\]

Formalin Test in Rats
Animals were assigned into five groups each containing 6 rats which were administered with either normal saline solution (1 mL/kg, i.p), MLECL (125, 250 and 500 mg/kg, i.p). or morphine (5 mg/kg, s.c). Thirty minutes after this treatment: 0.05 mL of a freshly prepared 2.5% solution of formalin was injected subcutaneously under the plantar surface of the left hind paw of each rat. The rats were placed individually in an observation chamber and monitored for one hour. The severity of pain response was recorded for each rat based on the following scale: (0) rat walked or stood firmly on the injected paw; (1) the injected paw was partially elevated; (2) the injected paw was clearly lifted off the floor; (3) the rat licked, chewed or shook the injected paw. The reading was taken after one hour of injecting formalin.17

Anti-Inflammatory Activity:
Egg Albumin-Induced Rat Paw Oedema
Animals were assigned to 5 groups each containing 5 rats. Each group was administered with either MLECL (125, 250 or 500 mg/kg, i.p), piroxicam (10 mg/kg, i.p) or normal saline as control (1 mL/kg) 30 minutes before the induction of inflammation. Acute inflammation was produced by the sub-planter administration of 0.1 mL fresh egg albumin into the right hind paw of each rat 30 minutes after administration of different doses of MLECL. The paw volume was measured from 0 minutes to 180 minutes, at an interval of 30 minutes, using a digital plethysmometer.18 The difference between the readings at time zero minute and different time intervals were taken as the paw volume.

Results and Discussion
Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is caused by physical, chemical, thermal or emotional stimuli. In the present study, the MLECL was evaluated for anti-inflammatory and analgesic activities using experimental animal models including acetic acid-induced writhing test, formalin-induced rat paw oedema and egg albumin-induced rat paw oedema. The median lethal dose (LD50) of MLECL via the intraperitoneal route in mice was found to be greater than 5,000 mg/kg using Loke’s method. These results suggest a wide safety margin of the extract at the analgesic doses used. It was earlier reported that substance with the LD50 value of about 5,000 mg/kg should be considered as relatively safe.19 The preliminary phytochemical screening of MLECL revealed the presence of tannins, saponins, flavonoids, cardiac glycosides, cyanogenic glycosides, resins, steroids and carbohydrates but no anthraquinone and alkaloids were found (Table 1). The phytochemical constituents are active secondary metabolites shown to be responsible for some important pharmacological activities of medicinal plants.19,20,21 The phytochemical constituents present may be responsible for the observed analgesic and anti-inflammatory activities of the extract in the present study.

In the acetic acid-induced writhing test in mice, MLECL caused significant reduction (p < 0.05) in mean number of writhes for all doses of the extract used when compared with normal saline treated control. The effect of the extract was slightly more potent than the standard drug piroxicam and showed the highest potency at 250 mg/kg (Table 2). This effect may be attributed to the possible peripheral analgesic effect of the extract in alleviating pain threshold caused by acetic acid. The acetic acid-induced writhing test is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods.22 It has been reported to be useful as a chemical pain model.23 Prostaglandins (PG) have been implicated in hyperalgesia by affecting the transducing property of free nerve endings so that stimuli that normally do not elicit pain are able to do so.24 Increased level of these PGs especially the PGE2 and PGF2α, as well as lipoxigenase products from leukotriene biosynthesis,25 have been found in the peritoneal fluid after intraperitoneal injection of acetic acid.26 thereby causing inflammatory pain characterized by extension of hind limbs and contraction of abdominal muscles in mice. The analgesic effect of MLECL may, therefore, be due to its action on visceral receptors sensitive to acetic acid. The activity of MLECL is comparable to that of piroxicam (standard drug), a non-selective reversible inhibitor of cyclooxygenase responsible for PG synthesis. In formalin induced pain in rats, MLECL at 300 mg/kg caused significant (p < 0.01) reduction in mean pain score after one hour when compared with normal saline control group (Table 3). The observed effect may be due to the inhibition of release of several mediators such as histamine, serotonin and kinins. Formalin test is a well-established valid model for the study of central sensitization events at the spinal level after peripheral inflammatory state.27 The formalin test is due to direct effects of formalin on nociception and due to inflammation with the release of serotonin, histamine, bradykinins and prostaglandins,28 and at least to some degree, the sensitization of central nociceptive neurons.29 Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain.30 The ability of the extract to inhibit the formalin-induced pain suggests that it may possess central analgesic activity and this could play a major role in ameliorating the pain associated with rheumatism and other inflammatory diseases. In anti-inflammatory activity study, MLECL at all doses tested caused significant (p < 0.05) inhibition of inflammation induced by egg albumin in rat's paw after 30, 60 and 180 minutes of induction but at 60 minutes, the extract inhibited the induced inflammation at 125 and 500...
Table 1: Phytochemical Constituents of Methanol Leaf Extract of Croton lobatus

<table>
<thead>
<tr>
<th>CONSTITUENTS</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + present, - absent

Data presented as mean ± SEM, *p < 0.05; **p < 0.01 as compared to normal saline control group (Dunnet’s post hoc test), n = 6, MLECL = Methanol Leaf Extract of Croton lobatus.

Table 2: Effect of Methanol Leaf Extract of Croton lobatus on Acetic Acid-induced Writhing in Mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Mean No. of Writhing</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>20.08 ± 3.9</td>
<td>~</td>
</tr>
<tr>
<td>MLECL 125</td>
<td>9.7 ± 2.7*</td>
<td>53.4</td>
</tr>
<tr>
<td>MLECL 250</td>
<td>6.8 ± 1.3**</td>
<td>67.3</td>
</tr>
<tr>
<td>MLECL 500</td>
<td>8.7 ± 1.4**</td>
<td>58.2</td>
</tr>
<tr>
<td>Piroxicam (10)</td>
<td>10.7 ± 1.6*</td>
<td>48.6</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM, *p < 0.05; **p < 0.01 as compared to normal saline control group (Dunnet’s post hoc test), n = 6, MLECL = Methanol Leaf Extract of Croton lobatus.

Table 3: Effect of Methanol Leaf Extract of Croton lobatus on Formalin-induced Pain in Rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Mean Pain Scores After 1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>MLECL 125</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>MLECL 250</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>MLECL 500</td>
<td>0.33 ± 0.2*</td>
</tr>
<tr>
<td>Morphine 5</td>
<td>1.4 ± 0.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM, *p < 0.01, as compared to normal saline control group (Dunnet’s post hoc test), n = 6. MLECL = Methanol Leaf Extract of Croton lobatus.

Table 4: Effect of Methanol Leaf Extract of Croton lobatus on Egg Albumin-induced Paw Oedema in Rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Mean Paw Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (min)</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>N/S (1 mL/kg)</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>MLECL (125)</td>
<td>2.6 ± 0.2**</td>
</tr>
<tr>
<td></td>
<td>(29.7)</td>
</tr>
<tr>
<td>MLECL (250)</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(13.5)</td>
</tr>
<tr>
<td>MLECL (500)</td>
<td>2.6 ± 0.2**</td>
</tr>
<tr>
<td></td>
<td>(29.7)</td>
</tr>
<tr>
<td>PRC (10)</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>(18.9)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM, n = 5, *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to normal saline control group (Dunnet’s post hoc test), values in parenthesis represent percentage inhibitions (%), MLECL = Methanol Leaf Extract of Croton lobatus, PRC = Piroxicam. N/S = Normal saline.

mg/kg only when compared to the control. The highest protection was observed at 180 minutes at a dose of 250 mg/kg (94.4%). The reason for the observed highest activity at 250 mg/kg was not clear. Unpredictably, piroxicam which was used as the standard drug only inhibited inflammation at 90 minutes to 180 minutes (Table 4). This effect suggests that the anti-inflammatory activity of the extract was higher than that of piroxicam. This is a demonstration of the effect of the extract in inhibiting inflammation by blocking the release of histamine and 5-HT, two mediators that are released by egg albumin. However, Egg albumin-induced inflammation model is a significant predictive test for anti-inflammatory activity. These results indicate the possible effect of Croton lobatus in acute inflammatory disorders. Plants with phytochemical constituents like flavonoids and tannins have been observed to possess analgesic and anti-inflammatory activity. Some studies have demonstrated the presence of various flavonoids like quercetin which is known to be effective in inflammation and saponin known to have analgesic effects. Therefore, the analgesic and anti-inflammatory effect observed may, therefore, be due to the presence of these phytochemical constituents. This may probably be by inhibiting the release and synthesis of inflammatory cytokines and mediators. The absence of alkaloids in the present study suggests that the extract has no activity against pyrexia since alkaloids have been implicated in pyrexia. However, further studies should be carried out to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanisms of action for the analgesic and anti-inflammatory activities of the MLECL.

Conclusion

The findings from this study suggest that the MLECL contain bioactive constituents that have analgesic and anti-inflammatory activities and this further support the ethnomedicinal claim of the use of the plant in the management of pain and inflammatory conditions.

Conflict of interest

The authors declare no conflict of interest.

Authors’ declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References