Anti-Nociceptive and Anti-Inflammatory Activities of the Hydroethanolic Extract of the Leaf of Clerodendrum polycephalum (Lamiaceae)

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ABSTRACT

Background- The mainstay of the treatment of pain and inflammation are opioids, steroids, and non-steroidal anti-inflammatory drugs. Though, they are effective and readily available with negative and unpleasant effects, more importantly, hepatotoxicity and nephrotoxicity. Thus, the need for safer and effective therapy in the management of pain and inflammation.

Objective- The work sought to investigate the anti-nociceptive and anti-inflammatory activities of the hydro-ethanolic leaf extract of Clerodendrum polycephalum (HeCP) in animals.

Methods- HeCP (100, 200 or 400 mg/kg, p.o.) given to mice, 1 h before administer of acetic acid (0.6% v/v, i.p.), formalin (1%v/v, intraplantar) or capsaicin (1% w/v, intraplantar) for nociceptive behavior in mice while carrageenan (1% w/v in saline, intraplantar) or cotton pellet (20 mg implanted into both groin) to induce acute or chronic inflammation in rats.

Results- HeCP (100 – 400 mg/kg, p.o.) reduced mean writhes number, duration of paw licking or biting in the acetic acid, formalin and capsaicin models, respectively, in mice. However, the initial treatment of mice with L-NNA (neuronal nitric oxide synthase inhibitor), naloxone (opioid receptor antagonist), or glibenclamide (ATP-sensitive K⁺ channel blocker) prevented HeCP induced anti-nociception in mice. In contrast, the initial treatment of mice with, sulpiride (dopamine D₂-receptor antagonist) failed to reverse HeCP-induced antinoceception. In the aspect of anti-inflammatory activity, HeCP caused significantly but not dose-dependent inhibition of edema development in carrageenan-induced inflammation and cotton pellet-induced granuloma formation in rats.

Conclusion- Findings from this work indicates that the hydroethanolic leaf extract of Clerodendrum polycephalum has anti-nociceptive and anti-inflammatory possibly due to its polyphenolic constituents.

Key-words: ATP-sensitive K⁺ channel, Capsaicin, Glibenclamide, Nociception, Inflammation

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Clerodendrum polycephalum (CP) belongs to the family Lamiaceae. It is found in tropical and warm temperate regions of the world with most of the species occurring in tropical Africa, South Asia, they are also present in Cameroun, Ghana, Sierra Leone and Guinea[1,2]. In Nigeria, the Yorubas commonly refer to it as “Aporo” which means it kills pain and as an antidotes to venomous stings and bites. It is also used as painkiller and medicines for the treatment of paralysis, epilepsy, convulsion[2]. Painful sensations are the reason for physician consultation and it can interfere with a person’s quality of life and general well-being[3]. Therefore, it is almost impossible to imagine a world without pain relief; we depend on pain relief drugs to an unspeakable degree. Similarly, inflammation is a protective response that involves immune cells, blood vessels and molecular mediators; the purpose of inflammation is to eliminate the initial causes of cell
injury, clear out necrotic cells, tissues damaged from the original insult and to initiate tissue repair. In spite of the ethno-medicinal importance of this plant, very little information is available on CP in literature. The present work sought to determine the effect of CP on acute and/or chronic painful inflammatory conditions as well as its putative mechanism(s) of action using validated pharmacological tools.

MATERIALS AND METHODS

Collection of C. polycephalum- Fresh leaves of Clerodendrum polycephalum were obtained from a farm land at Okeletu, Ijede in Ikorodu, Lagos State, Nigeria in June, 2016. The botanical identification and authentication of the plant was done by Mr. O.O. Oyebanji, a forestry expert at the Department of Botany Herbarium, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria, where the herbarium voucher specimen (LUH 7080) was deposited for reference.

Preparation of plant extract- The fresh leaves of Clerodendrum polycephalum were dried and milled into the coarse powder and 1.252 kg of the dried leaves was loaded into a percolator. Extraction was done with 2.8 L absolute ethanol in 1.2 L of water for 72h. After filtration, the residue was discarded and the final filtrate was concentrated in a rotary evaporator (40°C, under vacuum), yield was 7.65% w/w. The greenish solid extract obtained was always reconstituted in distilled water to appropriate concentrations before administration to experimental animals.

Experimental animals- Albino mice (20 – 25g) and Wistar rats (180 – 200g) of either sex used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were kept in well-ventilated and hygienic compartments, maintained under standard environmental conditions and fed with standard feed (Livestock Feed Plc, Lagos, Nigeria) and water ad libitum. The experimental procedure adopted in this study was in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [4].

Drugs and Chemicals- The chemicals used were acetic acid, formaldehyde (May and Baker Ltd, Dagenham, England); diclofenac (Total Healthcare, Parwanoo, India), carrageenan, capsaicin, and celecoxib (Sigma Aldrich, St. Louis, MO, USA).

Acute toxicity tests- Three groups of 5 mice (n=5) fasted over-night before the experiment were given doses of HeCP (1000, 2000, or 5000 mg/kg, p.o.). Animals in the different groups were observed for 2 h post-treatment for behavioral parameters such as convulsion, piloerection, hyperactivity, food and water intake, and respiratory pattern. The mice were further observed for up to 24 h and 14 days for any signs of delayed toxicity and mortality.

Test for analgesia

Mouse writhing test- Mice fasted overnight were divided into five groups (n=5). The animals were then treated with distilled water (10 ml/kg, p.o.); HeCP (100, 200, 400 mg/kg, p.o.); and diclofenac (50 mg/kg, p.o.; as a standard drug). Sixty minutes after treatment was carried out, mice were administered with acetic acid (0.6% v/v in saline, 10 ml/kg, i.p.). The number of writhes (characterized by contraction of the abdominal musculature and extension of hind limbs) was then counted at 5 min interval for 30 minutes [5].

\[
\text{Inhibition %} = \frac{\text{Number of writhes (control)} - \text{Number of writhes (treatment)}}{\text{Number of writhes (control)}} \times 100
\]

Formalin test- Mice fasted overnight was divided into five groups of five animals each. The different groups of animals were treated with distilled water (10 ml/kg, p.o.); HeCP (100, 200 or 400 mg/kg, p.o.); and diclofenac (50mg/kg, p.o.). Sixty minutes after administration, formalin (20µL of 1% solution) was injected subcutaneously into the right hind paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw, indicative of pain was recorded for each animal. The responses of the mice were observed for 5 min (first phase), 15 – 30 min (second phase) and post-formalin injection [6].

Capsaicin test- Mice fasted overnight was divided into five groups of five animals each. The different groups of animals were treated with distilled water (10 ml/kg, p.o.); HeCP (100, 200 and 400 mg/kg, p.o.); and diclofenac (50 mg/kg, p.o.). Sixty minutes after

\[\text{Number of writhes (control)} \times 100\]
administration, capsaicin was injected subcutaneous into the right hind paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw, indicative of pain was recorded for each animal. The responses of the mice were observed for 5 min (first phase), 15 -30 min (second phase) and post capsaicin injection.[7].

Elucidation of the mechanism of HeCP- induced anti-nociception in mice- To investigate the mechanism by which HeCP produces anti-nociception in acetate acid-induced writhing test, animals were pre-treated with an antagonist of receptors implicated in pain. The choice of the doses was based on previous studies [8].

Involvement of the opioidergic system- To investigate the role of the opioid system in HeCP-induced antinociceptive effect, mice were pre-treated with naloxone (5 mg/kg, i.p.) (a non-selective opioid receptor antagonist) or vehicle and after 15 min, HeCP (100 mg/kg, p.o.) was given. One hour later, acetic acid 0.6% v/v in saline (10 ml/kg, i.p.) was administered [8].

Involvement of L-arginine nitric oxide pathway- To investigate the role played by nitric oxide pathway in the antinociceptive effect of HeCP, mice were pretreated with N\textsuperscript{0}-nitro-L-arginine (10 mg/kg, i.p., neuronal nitric oxide synthase inhibitor), after 15 min, animal received HeCP (100mg/kg, p.o). One hour after treatment, acetic acid (10 ml/kg, i.p.) was given.

Involvement of ATP-sensitive potassium channel pathway- To investigate the possible contribution of ATP-sensitive potassium channel pathway in the anti-nociceptive effect of HeCP, mice were pre-treated with glibenclamide (10mg/kg, i.p.) and 15 min later, they received HeCP (100 mg/kg, p.o.). One hour post-treatment, acetic-acid writhing test was carried out.

Involvement of dopaminergic pathway- The possible participation of non-selective dopaminergic pathway, particularly the D\textsubscript{2} in the anti-nociceptive effect of HeCP was evaluated, mice were pretreated with sulphiride (50 mg/kg i.p; dopamine D\textsubscript{2} receptor antagonist), after 15 min, the animal received HeCP (100mg/kg, p.o.). One hour post-treatment, acetic acid (10 ml/kg, i.p.) was given.

Anti-inflammatory activity

Carrageenan-induced paw oedema- Rats used in this experiment were divided into five groups of five animals each and the respective groups were treated with distilled water (10 ml/kg, p.o.); HeCP (100, 200, 400 mg/kg, p.o.) and diclofenac (50 mg/kg, p.o.). One hour after administration of the various agents, oedema was induced by injection of carrageenan (100 µl, 1% w/v in saline) into the sub-plantar tissue of the right hind paw [9]. The linear paw circumference was then measured using the cotton thread method of Bamgbose and Noamesi [10]. Measurements of paw circumference were done immediately before injection of the phlogistic agent and at 30 min interval for 3 hours.

Inhibition (%) = Increase in paw oedema (control) – Increase in paw oedema (treated) X 100

Cotton pellet-induced granuloma formation in rats- The study was done to know whether HeCP is able to inhibit the proliferative component of the sub-chronic and chronic inflammatory process. The pellets of adsorbent cotton wool (20 mg) were sterilized in a hot air oven (model 600, Memmert, Germany) at 100°C for 2 h. The two pellets were implanted subcutaneously onto the dorsal groin region of rats.HeCP (100, 200 and 400 mg/kg, p.o.), celecoxib (30mg/kg., p.o.) or distilled water (10 ml/kg, p.o.) were administered to rats for 7 consecutive days. On the 8\textsuperscript{th} day, animals were sacrificed, the cotton pellets were carefully removed out from the surrounding tissue and then wrapped immediately inside a foil paper which was dried inside the oven at 40°C for 24 h, after which the mean wet and dried weight for different groups were determined and compared with the vehicle-control group [11]. The transudative weight, granuloma weight and the percentage granuloma inhibition of the test substance were calculated.

Quantitative phytochemical screening- Total flavonoid, tannins, saponins, alkaloid, and steroid were determined by the method of Marcel et al. [12].

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Statistical Analysis - The results from the experiment were expressed as mean±standard error of the mean (S.E.M). Statistical comparisons between groups were analyzed by using two-way analysis of variance (ANOVA), followed by Tukeys post hoc multiple comparison test.

RESULTS

Acute toxicity test in mice - Acute oral administration of *C. polycephalum* up to 5000mg/kg neither produced toxic behaviors nor mortality.

Antinociception assay

Acetic acid-induced writhing test - As shown in Table 1, intraperitoneal injection of acetic acid elicited writhing syndrome in control mice with 106.0±14.87 writhes counted in 30 mins. However, the pretreatment of mice with HeCP (100, 200 or 400 mg/kg) produced significant reduction in mean writhes number with 57.40, 27.20 and 26.10% inhibition, respectively. Similarly, diclofenac pretreated mice had 62.50% inhibition of writhes.

Table 1: Effect of HeCP on acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Total number of writhes</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>106.0±14.87</td>
<td></td>
</tr>
<tr>
<td>HeCP</td>
<td>100</td>
<td>45.20±5.22**</td>
<td>57.40</td>
</tr>
<tr>
<td>HeCP</td>
<td>200</td>
<td>77.20±8.26</td>
<td>27.20</td>
</tr>
<tr>
<td>HeCP</td>
<td>400</td>
<td>78.33±6.64</td>
<td>26.10</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>50</td>
<td>39.80±13.15**</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5); **P<0.01 versus vehicle treated, control

Formalin-induced nociceptive test - As shown in Table 2 below, injection of formalin into the right hind paw produced a marked biphasic response. The first phase occurs 5 mins post formalin injection while second phase seen 15-30 min after formalin administration with 88.00±20.29 and 146.00±26.41 duration of biting and licking in vehicle-control treated. However, the pretreatment of mice with HeCP dose dependently and significantly reduced the duration of licking by 83.90 and 86.00% at 400 mg/kg, significantly higher than the effect of diclofenac 50 mg/kg (37 and 49.30%, respectively).

Table 2: Effect of HeCP on formalin-induced nociception in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Total number of writhes</th>
<th>Inhibition (%)</th>
<th>Nociceptive reaction(s)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>88.00±20.92</td>
<td></td>
<td>146±26.41</td>
<td></td>
</tr>
<tr>
<td>HeCP</td>
<td>100</td>
<td>76.6±25.49*</td>
<td>12.95</td>
<td>110.6±28.92**</td>
<td>24.20</td>
</tr>
<tr>
<td>HeCP</td>
<td>200</td>
<td>140.0±20.81</td>
<td>-59</td>
<td>84.40±26.12**</td>
<td>42.20</td>
</tr>
<tr>
<td>HeCP</td>
<td>400</td>
<td>14.2±9.51***c,+</td>
<td>83.90</td>
<td>21.60±10.66***c,+</td>
<td>86.20</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>50</td>
<td>55.40±14.09**</td>
<td>37</td>
<td>74.00±17.96**</td>
<td>49.30</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=5); *P<0.05; **P<0.01; ***P<0.001 versus vehicle treated, control; *P<0.05 versus HeCP (100 mg/kg); **P<0.05 versus diclofenac (50 mg/kg)
**Capsaicin-induced nociceptive test**- To investigate the role of vanilloid receptors, capsaicin-induced nociceptive test was carried out. Table 3 shows that the intraplantar injection of capsaicin induced licking or biting reaction (22.40±5.93) in vehicle control mice. The pretreatment of mice with HeCP (100, 200 or 400 mg/kg) produced non-dose related decrease in nociceptive behavior with peak effect 62.50% inhibition at 100 mg/kg, which was comparatively similar to the effect of diclofenac (64.30% inhibition).

**Table 3: Effect of HeCP on capsaicin induced nociception in mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Response duration (s)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>22.40±5.93</td>
<td>-</td>
</tr>
<tr>
<td>HeCP</td>
<td>100</td>
<td>8.40±3.54**</td>
<td>62.50</td>
</tr>
<tr>
<td>HeCP</td>
<td>200</td>
<td>18.80±7.56</td>
<td>16.10</td>
</tr>
<tr>
<td>HeCP</td>
<td>400</td>
<td>15.00±6.08</td>
<td>33</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>50</td>
<td>8.00±1.52**</td>
<td>64.30</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=5); **P<0.01 versus vehicle treated, control

**Explanation of mechanism of antinociceptive effect of HeCP in mice**- To determine the role of nitric oxide/cyclic-guanosine monophosphate (NO/cGMP) pathway in HeCP-induced anti-nociception, Mice were pretreated with L-arginine (750 mg/kg, nitric oxide precursor). The pretreatment of mice with L-arginine did not affect the antinociceptive action of HeCP in the mouse writhing assay. However, the pretreatment of mice with L-NNA (10mg/kg, i.p. neuronal nitric oxide inhibitor) prevented the anti-nociceptive effect of HeCP. In another experiment to evaluate the involvement of opioidergic system in HeCP-induced anti-nociception, mice were pretreated with naloxone (2 mg/kg, s.c. non-selective opioid receptor antagonist). Naloxone pretreatment prevented the antinociceptive effect of HeCP (Fig. 1). In contrast, the pretreatment of mice with sulpiride (50 mg/kg, i.p. dopamine D₂ receptor antagonist) did not affect the anti-nociceptive effect elicited by HeCP in mouse writhing test. Interestingly, the pre-treatment of mice with glibenclamide (10 mg/kg, i.p. ATP-sensitive K⁺ channel blocker), prevented HeCP-induced anti-nociception in the mouse writhing assay (Fig. 1).

**Fig 1: Mechanism of HeCP-induced anti-nociception in mouse writhing assay**

Values are expressed as mean±SEM (n= 5). ***P<0.01 versus vehicle treated control; **P<0.05; ##P<0.01 versus HeCP (100 mg/kg) treated

**Carrageenan-induced paw edema**- Intraplantar injection of carrageenan into the right hind paws induced time course increase in paw size suggestive of oedema, which peaked at 2 h (3.02±0.07cm) in vehicle control. The time course increase in paw size was significantly reduced by HeCP treatment but not dose related with peak effect 61.60% inhibition of oedema at 2h by HeCP (100 mg/kg) which was comparatively similar to the effect of control standard, diclofenac 50 mg/kg (63.90% inhibition).

**Effect of HeCP on cotton pellet-induced granuloma in rats**- Implantation of the cotton pellet (20 mg) on each groin region induced granuloma formation (Fig. 2). The granuloma formation was significantly ameliorated by the pretreatment of rats with HeCP (100, 200 or 400 mg/kg) with 60, 65 and 62% inhibition, respectively. Interestingly, COX-2 inhibitor, celecoxib significantly reduced granuloma formation by 80%.

**Quantitative phytochemical screening**- The results showed that HeCP is rich in alkaloid (9.33±0.37), flavonoid (87.48±0.84), tannin (27.92±0.1), saponins (44.39±0.49) and steroids (25.9±0.22) in 100 mg of dry extract.

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Fig. 2: effect of HeCP on granuloma formation in rats. Values are expressed as mean ± SEM (n=5); ***$P<0.001$; ****$P<0.0001$ versus vehicle treated, control

Table 4: Effect of *Clerodendrum polycephalum* on carrageenan induced paw oedema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>dose (mg/kg)</th>
<th>30mins</th>
<th>60mins</th>
<th>90mins</th>
<th>120mins</th>
<th>150mins</th>
<th>180mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>1.27±0.07</td>
<td>2.46±0.22</td>
<td>2.67±0.15</td>
<td>3.02±0.07</td>
<td>2.33±0.21</td>
<td>2.45±0.25</td>
</tr>
<tr>
<td>HeCP</td>
<td>100</td>
<td>0.66±0.15</td>
<td>0.96±0.09d</td>
<td>1.45±0.20d</td>
<td>1.16±0.21d</td>
<td>1.48±0.17a</td>
<td>1.59±0.14b</td>
</tr>
<tr>
<td>%Inhibition</td>
<td></td>
<td>48%</td>
<td>60.90%</td>
<td>45.70%</td>
<td>61.60%</td>
<td>36.50%</td>
<td>35.10%</td>
</tr>
<tr>
<td>HeCP</td>
<td>200</td>
<td>0.52±0.17a</td>
<td>0.93±0.23d</td>
<td>1.37±0.19d</td>
<td>1.4±0.28d</td>
<td>1.6±0.18a</td>
<td>1.26±0.25c</td>
</tr>
<tr>
<td>%Inhibition</td>
<td></td>
<td>59.10%</td>
<td>62.20%</td>
<td>48.70%</td>
<td>53.64%</td>
<td>31.30%</td>
<td>48.60%</td>
</tr>
<tr>
<td>HeCP</td>
<td>400</td>
<td>0.8±0.1</td>
<td>1.26±0.14d</td>
<td>1.55±0.18c</td>
<td>1.43±0.17d</td>
<td>1.85±0.09</td>
<td>1.41±0.16c</td>
</tr>
<tr>
<td>%Inhibition</td>
<td></td>
<td>37%</td>
<td>48.80%</td>
<td>41.90%</td>
<td>52.60%</td>
<td>20.60%</td>
<td>42.45%</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>50</td>
<td>0.79±0.1</td>
<td>1.12±0.11d</td>
<td>1.2±0.22d</td>
<td>1.09±0.16d</td>
<td>1.42±0.16b</td>
<td>0.97±0.13d</td>
</tr>
<tr>
<td>%Inhibition</td>
<td></td>
<td>37.80%</td>
<td>54.50%</td>
<td>55%</td>
<td>63.90%</td>
<td>39%</td>
<td>60.40%</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5); a$P<0.05$; b$P<0.01$; c$P<0.001$; d$P<0.0001$ versus vehicle treated, control

**DISCUSSION**

In this work, the hydroethanolic leaf extract of *Clerodendrum polycephalum* (Lamiaceae) subjected to antinociceptive and anti-inflammatory assays using the acetic acid abdominal constriction test, formalin and capsaicin-induced paw licking models. The results showed antinociceptive effect of *C. polycephalum*. Hence, the mechanism of action was elucidated. HeCP inhibited the capsaicin-induced paw licking test suggesting the involvement of vanilloid receptors, naxoxone (a non-selective opioid antagonist) reversed HeCP anti-nociceptive activity indicating involvement of opioid receptor system, and L-NNA (an inhibitor of NO synthase), but not L-arginine (a nitric oxide precursor), reversed the anti-nociceptive activity of HeCP suggesting the involvement of NO-mediated/cGMP-independent pathway. Glibenclamide (K'ATP sensitive channel blocker) prevented anti-nociceptive effect of HeCP indicating role for K'ATP sensitive pathway. Interestingly, *C. polycephalum* leaf extract inhibited carrageenan-induced paw oedema and cotton pellet-induced granuloma indicative of its potential as anti-inflammatory drug.

The acetic acid-induced abdominal constriction test has been associated with the activation of peripheral nociceptive processes [13]. Agents that inhibit the action
of COX are good antinociceptive agents as seen with the peripherally acting non-steroidal anti-inflammatory drugs (NSAIDs), diclofenac. *C. polycephalum* leaf extract made less effective the acetic-acid-induced peripheral nociception indicating the presence of analgesic principles with ability to attenuate inflammatory-mediated pain. However, the abdominal constriction test is considered a non-specific test due to its inability to provide information on the peripheral and/or central nociceptive level inhibited by HeCP and to have poor specificity as it can give false positive results when used to test certain non-analgesic drugs such as muscle relaxants. Thus, the applications of other nociceptive models are necessary before the final conclusion on the possible mechanisms of action adopted by HeCP could be drawn. In this work, the formalin-induced paw licking test was adopted to further determine the antinociceptive activity of HeCP. The leaf extract inhibits both phases of the formalin-induced nociception, thus, further suggesting its ability to block the central nociceptive center. To further ascertain the mechanism of action of HeCP, it was assessed for pain induced by capsaicin, a natural product that specifically and directly activates TRPV1 receptor. Here, HeCP showed a significant anti-nociceptive effect on capsaicin-induced pain that was maintained for up to 4 h, strengthening the hypothesis that the antinociceptive effect of this extract is at least partially mediated by the inhibition of TRPV1 channel. Overall, findings obtained from the three nociceptive assays implied that HeCP contains bioactive compound(s) with ability to modulate the central and peripheral nociceptive mechanisms.

The effectiveness of opioid analgesics (such as morphine) has been overshadowed by many adverse side effects (e.g. respiratory depression, vomiting, nausea, constipation, tolerance, and dependence). Hence, the need for the more efficacious drug, pretreatment of mice with naloxone prevented the antinociceptive effect of HeCP indicative of opioidergic system in its mechanism of action, HeCP anti-nociception was reversed by LNNA (neuronal nitric oxide synthase inhibitor) but not L-Arg. The production of nitric oxide (NO) from l-arginine is catalyzed by NO synthase (NOS), which exists as the following three isoforms: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). Romero et al. have shown that local injection of analgesic drugs activates nNOS to release NO and induce peripheral antinociception. The involvement of dopaminergic system was also investigated. Lateral hypothalamus (LH) involves in modulation of tonic pain regarding the direct and indirect neural connections between the LH and nucleus accumbens (NAC). Moreover, blockade of accumbal dopamine receptors attenuated analgesia induced by carbachol injection into the lateral hypothalamus during both phases of formalin test. Effect of blockade of D1- and D2-like dopamine receptors on reduction in anti-nociception was more during the late phase. The contribution of D2-like dopamine receptors to mediation of antinociception during the late phase was greater than the early phase. In this study, pretreatment of mice with sulpiride (D2 receptor antagonist) did not affect HeCP-induced anti-nociception.

Experimental data have indicated a link between the activation of the NO-cGMP pathway and the opening of the ATP-sensitive K⁺ channels. From the results observed, the ATP-sensitive K⁺ channel is suggested to be involved in the mechanism of action of HeCP. Pre-treatment with glibenclamide, an ATP-sensitive K⁺ channel blocker significantly reversed anti-nociceptive effect of HeCP. HeCP possibly acts by modulating K⁺ currents through the efflux of K⁺ ions permeating the membrane. Increase in K⁺ ion efflux alters the membrane potential to avert from action potential generation, which results in the decrease of neurotransmitter release. Other than that, the effect of HeCP through the activation of the NO-dependent pathway is similar to some pharmacological studies that have evaluated NO/cGMP activation and the opening K⁺ channels, which relates to the opioidergic pathway. The anti-inflammatory activity of *C. polycephalum* was evaluated in this study using the carrageenan induced paw oedema and cotton wool implantation tests. Inflammation induced by carrageenan is acute, non-immune, and highly reproducible and can be quantified by the increase in paw size. It is widely used to access the anti-inflammatory effect of natural products. Carrageenan-induced edema is a biphasic event, with the involvement of several inflammatory mediators: In the first phase (during the first 1hr after carrageenan injection), chemical mediators such as histamine and serotonin play a role, whereas, in the second phase (3-5 hours after carrageenan injection), kinnins and...
prostaglandins are involved. In this study, *C. polycephalum* showed the significant inhibitory effect on rat paw edema development in the early and late phase of carrageenan and inflammation, suggesting possible inhibition of serotonin, histamine, kinins and prostaglandin release. The cotton pellet-induced granuloma formation is an established chronic inflammatory model. The responses to subcutaneously implanted cotton pellet in rats have been divided into transudative and proliferative phases. The transudative phase is defined as increase in the wet weight of the granuloma whereas the proliferative phase is defined as the increase of dry weight of the granuloma. In the present study HeCP produced a significant reduction on the granulomatous tissue formation with 65% inhibition as compared with celecoxib (30 mg/kg) which produced significant inhibition of 80%. The migration of leukocytes to the injury site occurs during chronic inflammation. Leukocytes accumulation leads to the release of lysosomal enzymes and oxygen radicals at inflammatory site. The quantitative phytochemical screening of HeCP demonstrated the strong presence of flavonoids, triterpenes, tannins, saponins and steroids. The potent antinociceptive and anti-inflammatory properties of HeCP could be attributed to the presence of these phytochemicals.

**CONCLUSIONS**

In conclusion, findings from this study showed that the hydroethanolic leaf extract of *C. polycephalum* possesses antinociceptive effect possibly through K+ATP sensitive/NO/opioidergic pathway and anti-inflammatory action through inhibition of release of inflammatory mediators. Thus, could be a potential phytotherapeutic agent in the management of painful inflammatory conditions. Therefore, the result obtained justifies the use of the plant in Traditional African Medicine for the treatment of pain and inflammatory conditions.

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**CONTRIBUTION OF AUTHORS**

Drs Amole, Akinyede and Ishola designed the work, Data collection, analysis and interpretation was done by Dr Amole and Mr Adewale. Drafting and critical revision of the article for intellectual content and final approval of the version to be published was done by all the authors.

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