Antinociceptive and Anti-inflammatory Activities of the Ethanol Extract of *Carissa edulis* Vahl. Root Bark in Rats and Mice

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Abstract: The ethanol extract of *Carissa edulis* root bark was studied for its antinociceptive and anti-inflammatory activities. Three different animal models (acetic acid-induced writhing, thermal nociception and formalin tests) were used to study the antinociceptive activity of the extract alone and with various opioid, muscarinic and dopaminergic receptor agonist and opioid receptor antagonist (naloxone). Egg albumen-induced paw edema test was used to evaluate the anti-inflammatory activity. The results showed dose-dependent antinociceptive and anti-inflammatory activities of the extract. Naloxone abrogated the antinociceptive activity of the extract during the first phase of formalin test. It was concluded that the crude ethanol extract of *C. edulis* root bark has antinociceptive and anti-inflammatory properties in rats and mice which may be mediated peripherally and centrally. Opioid receptors may be involved in the centrally mediated antinociceptive properties.

Keywords: *Carissa edulis*; root; bark; antinociception; anti-inflammation.
1. Introduction

Medicinal plants are the most common sources of remedies in traditional medicine, the most ancient way of curing diseases. These plants are also the sources of many conventional drugs such as morphine, vincristine and ergometrine (Sofowora, 2008). Despite advances in health care delivery, medicinal plants still play important role in human and animal health care and about 60% of the World’s population (and 80% of Africa’s population) depend on herbal medicine for their primary health care (WHO, 2000). Many plants have been documented to have antinociceptive and anti-inflammatory activities but out of the 250,000 - 500,000 plant species on earth, only 1% to 10% has been studied for their potential medicinal values (Verpoorte, 2000). More are being exploited with the view to developing new drugs because the conventional analgesics have some setbacks as the opioid analgesics are known to produce respiratory depression, constipation and dependence while the non-steroidal anti-inflammatory drugs (NSAID) may produce gastrointestinal and renal damage as their side effects. Also, the use of steroidal anti-inflammatory drugs (glucocorticoids) is associated with problems of immunosuppression, osteoporosis and gastrointestinal ulceration.

The plant, *Carissa edulis* Vahl., has been reported to be used in managing disease conditions such as epilepsy, headache, toothache, cough, chest complaints, rheumatism, fever, sickle cell anemia, gonorrhea, syphilis, helminthoses and rabies (Nedi et al., 2004; Ibrahim et al., 2007; Ya’u et al., 2008). The plant belongs to the family Apocynaceae and is distributed in tropical Africa and Asia. It is locally called “Carisse” in English, “Cizaki” or “Karen-kafo” in Hausa, “Behoni” in Fulfulde, “Andirim” or “Ndirim” in Babur-Bura and “Ndirma” in Marghi languages. While the literature has shown that this plant is used in many painful and inflammatory disease conditions, there is little scientific work to ascertain this. This research is therefore designed to investigate the antinociceptive and anti-inflammatory activities of the ethanol extract of *Carissa edulis* root bark in rats and mice.

2. Materials and Methods

2.1. Plants Source

The plant, *Carissa edulis* was obtained in the bush near Ngulde in Askira/Uba Local Government Area of Borno state. It was identified and authenticated by Prof. S. S. Sanusi of the Department of Biological Science and a voucher sample (Vet212K1) was preserved at the Veterinary Pharmacology Laboratory, University of Maiduguri, Nigeria.

2.2. Preparation of the Extract
The root bark of the plant was collected, cleaned, crushed and then air-dried at room temperature for one week. It was pulverized using a mortar and pestle and 350 g of the ground herb was soaked overnight in petroleum ether. The residue from defatted samples was extracted in 95% ethanol for 24 h. The sample was filtered using Whatman filter paper No. 1 and evaporated to dryness under reduced pressure using a rotary evaporator (R201D PEC Medicals, USA).

2.3. Drugs and Chemicals

Morphine (morphini®, Martindale Pharmaceuticals, Romford, Essex RM3 8UG, UK), naloxone (Rotexmedica, Trittau, Germany), piroxicam (feldene®, France), metoclopramide HCl injection B.P. (metoprone® Greenfield Pharmaceutical Co Ltd, Tai Zhou, China), atropine sulphate (amopin® Yanzhou Xierkangtai Pharma Co Ltd, Jiuguan Yanzhou, China), ethanol and petroleum ether (BDH Analar ® Poole, England) were used during this research.

2.4. Animals

Wistar albino rats (= 40) (124 - 220 g) and Swiss mice (= 85) (16 - 35 g) were brought from Jos, Plateau State. They were kept in plastic rat cages and allowed to acclimatize to the laboratory environment for a minimum period of one week before the commencement of the experiments. They were fed with grower’s mash (Vital Feeds Nig Ltd, Jos, Nigeria) and water was provided ad libitum. The experiments were conducted in compliance with the international guiding principles for biochemical research involving animals (CIOMS, 1985).

2.5. Determination of Antinociceptive Properties

2.5.1. Acetic acid-induced writhing test

Twenty adult mice of both sexes (weighing between 16 and 31 g) were randomly separated into four groups of five mice each. They were deprived of food for 24 h before the commencement of the experiment. Those in group A received normal saline (0.2 mL) to serve as negative control group while those in groups B and C received 50 and 100 mg/kg, respectively, of the ethanol extract of the plant, and those in group D received piroxicam 10 mg/kg to serve as positive control group. All drug and extract administrations were carried out intraperitoneally (I.P.). Thirty minutes later, 0.1 mL of 1% acetic acid solution was administered I.P. to all groups to induce writhing. Writhing response was observed as described by Turner (1965). The number of writhes was counted from five minutes after acetic acid administration for ten minutes. A reduction in the number of writhing as compared with the negative control group was considered as evidence of analgesia. The percentage protection was obtained using the formula described by Hernandez-Perez et al. (1995).
% inhibition = \frac{W_C - W_T}{W_C} \times 100

where \( W_C \) = mean number of writhes in control group, \( W_T \) = mean number of writhes in test group.

2.5.2. Thermal nociception test

Twenty rats of either sex (weighing between 128 and 176 g) were randomly divided into four groups of five rats each and the method described by Corea et al. (1996) was used. They were deprived of food for 24 h before the commencement of the experiment. Those in group A (negative control) received normal saline (0.5 mL) while those in groups B and C received 50 and 100 mg/kg, respectively, of the extract of the plant, and those in group D (positive control) received morphine 10 mg/kg. All treatment was by I.P. Thirty minutes later, they were placed on Eddy’s hot plate having a constant temperature of 50 °C. The time taken for either pad licking or jumping was recorded in seconds.

2.5.3. Formalin test

Twenty mice of both sexes weighing between 16 and 23 g were separated at random into four groups of five mice. Mice in group A received normal saline (0.2 mL I.P.) only, mice in group B received morphine 10 mg/kg while those in groups C and D received extract 50 and 100 mg/kg I.P. After 30 min each mouse in all groups was injected with 20 µL of 1% formalin at the plantar surface of the left hind paw and immediately placed in a transparent plastic chamber (Hunskar and Hole, 1987). The mouse was then observed for the first five minutes and then from 20 to 30 min after formalin injection. The time spent in licking the injected paw was recorded in seconds during these periods.

2.5.4. Evaluation of opioid receptor mediation

Fifteen mice of both sexes (weighing between 25 and 34 g) were randomly separated into three groups of five mice each. They were deprived of food for 24 h before the commencement of the experiment. Those in group A received extract 100 mg/kg, those in group B received naloxone 1.5 mg/kg followed by extract 100 mg/kg after 30 min, and those in group C received naloxone 1.5 mg/kg only (Ratnasooriya et al., 2007). All administrations were done intraperitoneally. Thirty minutes later, the formalin test was carried out on the mice.

2.5.5. Evaluation of dopamine and muscarinic receptors mediation

Thirty mice of either sex (weighing between 19 and 34 g) were randomly separated into six groups of five mice each. They were deprived of food for 24 h before the commencement of the experiment. Those in group A received extract 100 mg/kg, those in group B received metoclopramide (dopamine receptor antagonist) 1.5 mg/kg followed by extract 100 mg/kg after 30 min, those in group
C received metoclopramide 1.5 mg/kg only and those in group D were given normal saline only (Ratnasooriya et al., 2007). Mice in group E were given atropine (muscarinic receptor antagonist) 2 mg/kg followed by extract 100 mg/kg after 10 min while mice in group F were given atropine only (Ratnasooriya et al., 2007). All the administrations were done intraperitoneally. Thirty minutes later, the formalin test was carried out on the mice.

2.6. Determination of Anti-inflammatory Activity

Twenty rats of either sex (weighing between 124 and 220 g) were randomly divided into four groups of five rats each and the method described by Mandade et al. (2010) was used. They were deprived of food for 24 h before the commencement of the experiment. Rats in group A received normal saline (0.5 mL I.P.) while animals in groups B and C received 50 and 100 mg/kg I.P., respectively, of the extract of the plant, and those in group D received piroxicam 10 mg/kg I.P. After thirty minutes, each rat was injected with 0.1 mL of fresh egg albumen under the subplantar surface of the left hind paw. Paw diameter was measured in centimeter just before albumen injection and then at 1, 2, 3, 4 and 24 h after egg albumen injection using a pair of compass and a ruler. Percentage inhibition was calculated using the formula below (Bamgbose and Noamesi, 1981).

\[
\text{% inhibition} = \frac{(C_T - C_O)_{\text{Treated}} - (C_T - C_O)_{\text{Control}}}{(C_T - C_O)_{\text{Control}}} \times 100
\]

where \(C_T\) = mean paw diameter after extract administration, \(C_O\) = mean paw diameter before extract administration.

2.7. Statistical Analysis

Data generated during the study were expressed as mean ± standard error of the mean (SEM) and analyzed statistically by one way analysis of variance (ANOVA). Graphpad InStat® (2003) computer statistical software package was used for the analysis and \(p \leq 0.05\) was considered significant.

3. Results and Discussion

3.1. Antinociceptive Activities

The effect of the crude ethanol extract of *Carissa edulis* root bark on acetic acid-induced writhing response in mice is presented in Table 1. The extract significantly (\(p < 0.05\)) reduced the number of writhes from 27.4±7.7 in the control group to 9.6±2.3 (64.9%) and 9.0±1.6 (67.1%), in 50 and 100 mg/kg treated groups, respectively. The standard drug, morphine (10 mg/kg) produced 100%
reduction in the number of writhes (p < 0.01). Likewise, in the thermal nociception test in rats (Fig. 1), the extract and the morphine significantly (p < 0.05) increased the latency of response when compared with their respective pretreatment values.

The significant activities recorded in both thermal nociception test and acetic acid-induced writhing test signifies that the extract was acting both peripherally and centrally in producing analgesia. Noxious stimuli cause release of chemicals such as prostaglandins, histamine and serotonin thereby inducing pain locally. The extract contains many pharmacologically active compounds that may be responsible for the local or central antinociceptive effect. Saponins have been reported to inhibit the release of the pain mediator, histamine, in vitro (Rao and Gurfinkel, 2000; Mandade et al., 2010). Also chromatographic analysis of the fruit water extracts of the plant indicated the presence of salicylate (Ibrahim et al., 2007). Salicylates possess antipyretic, analgesic and anti-inflammatory activities. This could be responsible for the observed effect in this study especially in the acetic acid-induced writhing test where the extract and morphine respectively produced 67.1% and 100% protection against induced writhing. The antinociceptive activity observed may also be through the effect of the extract on inflammatory process. This is further supported by the result obtained in the formalin and egg albumen-induced rat paw edema tests (Fig. 2 and Table 2).

In the formalin test presented in Fig. 2, the extract produced significant (p < 0.05) reduction while morphine recorded highly significant (p < 0.001) decrease in the time spent in licking of the formalin injected limb during both first and second phases of response. At the dose of 50 mg/kg of the extract, the period of licking decreased significantly (p < 0.05) from 100.4±10.3 seconds in the control group to 63.0±8.3 seconds (37.3% reduction) in the treated group. However there was no significant (p > 0.05) decrease during the second phase at this dose. At the higher dose of 100 mg/kg, the licking time was significantly reduced during both phases from 100.4±10.3 to 53.0±7.6 seconds (47.2% reduction) during the first phase (p < 0.01) and from 67.4±5.4 to 42.4±5.4 seconds (37.0% reduction) during the second phase (p < 0.05). The significant (p < 0.01) reduction of licking time observed with the extract was inhibited (5.8% reduction) when there was co-administration of naloxone with extract during the first phase of formalin test but this was not impaired (44.2% reduction) in the second phase. Also in Fig. 2, administration of extract with atropine and with metoclopramide did not reverse the response produced by the extract during both phases of formalin test. However the group treated with both extract and metoclopramide, though the latter did not impair the response of the extract during the first phase, had significantly (p < 0.001) potentiated the extract’s response (59.9% reduction) as there was significant (p < 0.01) difference from the control group and also from the groups treated with the extract alone and with metoclopramide alone.

In formalin test method, the first phase response was due to direct effect of formalin on nociceptors (neurogenic pain) and the second phase was due to release of inflammatory mediators
(inflammatory pain) sequel to formalin injection (Hunskar et al., 1985; Murray et al., 1988). It was envisaged that centrally-acting analgesics inhibit both phases of formalin induced pain whereas peripherally acting analgesics inhibit only the second phase (Santos et al., 1998). The crude ethanol extract of C. edulis root bark significantly reduced the period of licking formalin injected limb in both phases at the dose of 100 mg/kg in mice. Thus, the antinociceptive effect of the extract could be mediated both through neurogenic and inflammatory processes acting locally and centrally. Antinociception can be mediated through different mechanisms in the body such as through cholinergic, opioid and dopaminergic receptor mechanisms (Rang and Dale, 1991). However, administration of atropine (a muscarinic receptor antagonist) and metoclopramide (a dopamine receptor antagonist) along with the extract could not reverse the effect of the antinociceptive activity of the extract. Therefore the induced antinociception is not mediated through cholinergic and dopaminergic mechanisms. On the other hand, naloxone which is an opioid receptor antagonist, abrogated the extract induced nociception during the first phase but there was no significant (p > 0.05) difference between groups treated with extract alone and extract along with naloxone during the second phase of formalin pain response (Fig. 2). This supports partial involvement of opioid receptor mechanism by the extract. The significant reduction in licking time following administration of naloxone alone during the second phase (inflammatory pain) and not during the first phase (neurogenic pain) is unclear.

It was also observed in this study that metoclopramide significantly (p < 0.001) potentiated the extract antinociceptive activity during the inflammatory pain period. The extract may be acting centrally with mechanism of action predominated through receptors other than opioid receptors such as serotonin receptor. The effect of metoclopramide on the extract may be through serotonin receptors since metoclopramide has mixed actions not only on dopamine but also on 5-HT3 receptor (Pasricha, 2006). In addition, serotonin is among the chemicals released during inflammatory process, thus co-administration of metoclopramide and extract would have more effect during the second phase or inflammatory pain than the first phase or neurogenic pain of formalin induced pain.

**Table 1.** Effect of crude ethanol extract of Carissa edulis root bark on acetic acid-induced writhing response in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. writhes (mean±SEM)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline 0.2 mL</td>
<td>27.4±7.7</td>
<td>-</td>
</tr>
<tr>
<td>Extract 50 mg/kg</td>
<td>9.6±2.3*</td>
<td>64.9</td>
</tr>
<tr>
<td>Extract 100 mg/kg</td>
<td>9.0±1.6*</td>
<td>67.1</td>
</tr>
<tr>
<td>Morphine 10 mg/kg</td>
<td>0.0±0.0**</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Note: **Significantly (p < 0.01) lower than control, * Significantly (p < 0.05) lower than control, n = 5.
Figure 1. Effect of crude ethanol extract of *Carissa edulis* root bark on thermal nociception test in rats.

* Significant (p < 0.05, n=5) difference from pretreatment at the same dose values in percentages refer to percentage increase from pretreatment at the same treatment.

Figure 2. Effect of crude ethanol extract of *Carissa edulis* root bark and various drugs on formalin test in mice, n = 5. *, #, @ Significant (p < 0.05, p < 0.01, p < 0.001 respectively) difference from control during the same phase ‘a’, and ‘b’ are different significantly (p < 0.05) during the same phase values in percentages refer to percentage reduction from the control.
3.2. Anti-inflammatory Activity

The effect of the crude ethanol extract of Carissa edulis root bark on egg albumen-induced rat paw edema test is presented in Table 2. The result showed that at the dose of 50 mg/kg of extract, there was no significant effect at 1 h and 2 h post egg albumen injections but there was significant (p < 0.01) effect at 3 h post injection. At the dose of 100 mg/kg of the extract significant effect was observed at 1, 2 and 3 h post injections, where there were highly significant (p < 0.001) reductions in the paw edema. The result at this dose is comparable with that of the standard drug, piroxicam (10 mg/kg) at 1, 2 and 3 h post injection. However, piroxicam possessed significant (p < 0.01) effect 4 h post injection. The highest paw edema reduction was observed in all treatment groups 3 h post albumen injection where the piroxicam, extract (50 mg/kg) and extract (100 mg/kg) produced 71.8%, 43.7% and 68.7% decrease in edema, respectively. Paw size returned to normal within 24 h of paw injection with albumen in all the rats including those in the control group.

The anti-inflammatory activity was observed to be dose-dependent. The inflammatory process was resolved within 24 h in all groups including the negative control group. The initial phase of inflammation which lasted for about 90 min is due to release of histamine, serotonin and kinnins whereas prostaglandins are implicated in the second phase of inflammation which lasted for about 5 h (Di Rosa et al., 1971). The presence of secondary metabolite in the extract may be responsible for the anti-inflammatory activities of the extract (Ismaili et al., 2002; Khan et al., 2011). The anti-inflammatory activity of the extract may be due to decreased release of the mediators such as histamine, serotonin, polypeptides and prostaglandins or may be due to antagonistic effect of the extract on their receptors.

Table 2. Effect of crude ethanol extract of Carissa edulis root bark on egg albumen-induced rat paw edema test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean±SEM of paw diameter in cm ( % inhibition)</th>
<th>Pretreatment</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>4.4±0.1</td>
<td>7.7±0.4</td>
<td>8.0±0.3</td>
<td>7.6±0.1</td>
<td>6.6±0.1</td>
<td>4.7±0.1</td>
</tr>
<tr>
<td>Piroxicam 10 mg/kg</td>
<td></td>
<td>4.9±0.1</td>
<td>6.3±0.1**</td>
<td>6.4±0.2***</td>
<td>5.8±0.1***</td>
<td>5.7±0.1**</td>
<td>4.9±0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>57.5%</td>
<td>58.3%</td>
<td>71.8%</td>
<td>63.6%</td>
<td>100%</td>
</tr>
<tr>
<td>Extract 50 mg/kg</td>
<td></td>
<td>4.8±0.1</td>
<td>7.8±0.2</td>
<td>7.4±0.2</td>
<td>6.6±0.1**</td>
<td>6.9±0.2</td>
<td>4.8±0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.0%</td>
<td>27.7%</td>
<td>43.7%</td>
<td>4.5%</td>
<td>100%</td>
</tr>
<tr>
<td>Extract 100 mg/kg</td>
<td></td>
<td>4.5±0.2</td>
<td>6.4±0.2**</td>
<td>5.9±0.1***</td>
<td>5.5±0.1***</td>
<td>6.0±0.1</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42.4%</td>
<td>61.1%</td>
<td>68.7%</td>
<td>31.8%</td>
<td>66.6%</td>
</tr>
</tbody>
</table>

Note: *** Significant (p < 0.001) difference from control along the same column, ** Significant (p < 0.01) difference from control along the same column, n = 5.
4. Conclusions

The crude ethanol extract of Carissa edulis root bark has antinociceptive and anti-inflammatory properties in rats and mice that may be mediated peripherally and centrally. Opioid receptors may be involved in the centrally mediated antinociceptive properties.

References


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