Analgesic and Anti-inflammatory Effects of the Aqueous Leaf Extract of Dichrostachys cinerea

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ABSTRACT: In this present study, the analgesic activity of the aqueous extract of the leaves of Dichrostachys cinerea was investigated in mice using acetic acid-induced writhing and hot plate test, while the anti-inflammatory activity was investigated in rats using the carrageenan and dextran-induced paw edema. The extract (400, 800mg/kg) exhibited a dose dependent inhibition of abdominal writhing in mice compared to control. The effects of the extract were not significantly different from that of aspirin. The extract (800mg/kg) caused a significant (p<0.01) increase in paw thickness, at 60th minute post treatment in the hot plate test and the effects of the extract were lower than that of morphine (4mg/kg). The extract (800mg/kg) and indomethacin (10mg/kg) at 1st hour and 2nd - 4th hour produced inhibitions of the carrageenan-induced paw oedema versus control. The results also showed that the extract (800mg/kg) produced inhibition of dextran-induced paw edema between 2nd hour and 4th hour compared to control. Overall, our data suggest that the extract possesses anti-inflammatory and analgesic effects, which may be mediated solely by peripheral mechanisms. ©JASEM

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Keywords: Dichrostachys Cinerea; analgesic, anti-inflammatory, hot plate, dextran, carrageenan, edema, pain.

It is common knowledge that humans have relied on herbs and other natural products for the cure and management of various ailments over the years and the use of extracts from plant materials as relief for pain and inflammation dates as far back as 30 AD (Vane and Botting, 1987). Similarly, many medicinal plants are used in developing countries for the management of several medical conditions including those characterized by pain and inflammation (Adebayo et al., 2015). While several modern medicines are effective in treating pain and inflammatory disorders, their prolonged use may cause severe adverse effects, the most common being gastrointestinal bleeding and peptic ulcers (Vernierito et al., 2010). Hence, the need for the development of more effective pain medications devoid of adverse effects has become imperative and medicinal plants and herbs are potential sources from which such medications could be developed.

D. cinerea is one medicinal plant that has been used in folkloric medicine in Nigeria for the treatment of syphilis, body pains and toothaches. It is popularly called "dundu" among the hausa speaking people of northern Nigeria and "kora" among the Yoruba speaking people of south western Nigeria (Gill, 1992). D. cinerea (fabaceae) is a spiny deciduous shrub or small tree, up to 5-10m high with compound pinnate leaves (Kuber et al., 2009), and it is found in tropical and sub-tropical conditions (Mishra et al., 2009).

The chloroform extract of the leaves of D. cinerea has been demonstrated to possess antibacterial and analgesic effect (Mishra et al., 2009) and the saponin extract of the leaves has also been demonstrated to possess anti-inflammatory activity (Hassan et al., 2012). Kuber et al., 2009 evaluated the neuropharmacolgical effects of the root of D. cinerea. These scientific evaluations, which used only one model of acute pain and inflammation, partly validate the medicinal usefulness of D.cinerea in folkloric medical management of painful and inflammatory conditions. However, the need to give more validity to its folkloric medicinal claims using more extensive model of acute pain and inflammation has become necessary. To this end, this present study was conducted to screen the aqueous extract of the leaves of D. cinerea for analgesic and anti-inflammatory activities using two acute models of pain and inflammation in mice and rats respectively.

MATERIALS AND METHODS

Drugs and chemicals: Carrageenan, indomethacin, morphine, acetic acid and acetylsalicylic acid were all obtained from Sigma chemical Co. All drugs and chemicals were freshly prepared before use.

Plant collection, identification and extraction: The fresh plant was collected from the premises of the
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University of Benin, Benin City, Nigeria. The plant was duly identified and authenticated by Mr. Shasanya Olufemi of the Forestry Research Institute of Nigeria, Ibadan (FRIN, Ibadan) where a herbarium specimen (FHI NO: 109986) was deposited for future reference. The young leaves were then carefully separated from the stem; air dried for 7 days and pulverized using an electric mill. The powered plant material (500g) was macerated in 4.5 litres of distilled water at room temperature and filtered after 24h. The filtrate was concentrated to dryness over a water bath. The dried extract obtained (Yield=12.83%) was stored in airtight glass containers in the refrigerator at 4°C till required.

Animal: Swiss albino mice (25-35g) and wistar rats (190-300g) of either sex kept at the Animal Unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals maintained under standard environmental conditions were allowed to acclimatize for two weeks and had free access to standard diet (Bendel Feeds and Flour Mill, Ewu, Edo State, Nigeria) and water ad libitum. Animals were exposed to natural lighting conditions and handled in accordance with the international principles guiding the use and handling of experimental animals (National Institute of Health, USA, 2002).

Mouse writhing assay: The method described by Koster et al. (1959) was used. Mice were randomly divided into four groups of five animals per group. The extract (400-800mg/kg), normal saline (10ml/kg) or acetylsalicylic acid (100mg/kg) was administered orally to the mice according to their group 30 minutes before intraperitoneal injection of acetic acid (0.6%v/v in normal saline, 10ml/kg). Number of writhes, which consisted of constriction of the abdominal muscle together with a stretching of hind limbs, was cumulatively counted for 30 minutes following acetic acid injection. Inhibition of pain was expressed as a percentage of protection using the formula:

\[
\text{Inhibition of pain} = \left( \frac{\text{Mean Writhes (control)} - \text{Mean Writhes (treated)}}{\text{Mean Writhes (control)}} \right) \times 100
\]

Where mean writhes (control) is the mean writhes of the normal saline treated animals and mean writhes (treated) is the mean writhes of the animal given acetylsalicylic acid or each dose of aqueous leaf extract of D. cineraria.

Hot plate assay: Swiss albino mice of either sex screened for suitable reaction time, 24h before the experiment were used. The animals were randomly divided into four groups of five mice each. One hour before treatment the baseline hot plate latency of each mouse was taken. This represented the baseline latency before treatment. The extract (400-800mg/kg, orally), normal saline (10ml/kg, orally) or morphine (4mg/kg, i.p) were administered to the mice according to their group. One hour and thirty minutes after treatment with the extract, normal saline and morphine respectively, the response to nociceptive stimulus was measured with the hot plate analgesiometer (Ugo Basile, Italy) with the temperature maintained at 55 ± 1°C (Woolfe and Macdonald, 1944). A cut-off time of 60 seconds was adopted to prevent tissue damage. The time between the placements of the mice and licking or biting of the hind paw or jumping was recorded as the index of response latency. Response latencies were taken at 30 minutes intervals till 120 minutes post treatment.

Carrageenan-induced paw edema: Wistar rats were allotted into four groups of five rats each. Using a venier caliper, baseline measurement of the right hind paw thickness of each rat was taken. The rats were pretreated, orally, with extract (400,800mg/kg), indomethacin (10mg/kg) or normal saline (10ml/kg) according to their group. One hour later, edema was induced in the rats by injection of freshly prepared carrageenan (0.1ml, 1%w/v in normal saline) into the plantar aponeurosis of the right hind paw of each rat (Winter et al, 1962). Measurement of the thickness of the injected paw was repeated at hourly intervals for a maximum of four hours after carrageenan injection with the aid of a vernier caliper.

Dextran- induced paw edema: Wistar rats were allotted into four groups of five rats each. Using a plethysmometer the exact baseline right hind paw volume of each rat was measured. The rats were pretreated, orally, with normal saline (10ml/kg), extract (400,800mg/kg) or diphenhydramine (60mg/kg) according to their group. One hour later, edema was induced in the rats by injection of freshly prepared dextran (0.1ml, 1.5%w/v in normal saline) into the plantar aponeurosis of the right hind paw of each rat (Glauze et al., 1998). Measurement of the volume of the injected paw was repeated at hourly intervals for a maximum of four hours after dextran injection using a plethysmometer.
Statistical analysis: Statistical analysis was done using graph pad prism version 4. The data were analysed using one and two way analysis of variance (ANOVA) followed by turkey post hoc test. Data represent mean± SEM. Statistical significance was considered at p<0.05.

RESULTS AND DISCUSSION
The extract (400mg and 800mg) produced a significant (p<0.01) and dose-dependent inhibition of acetic acid induced writhes compared to normal saline control group. The inhibition of writhes produced by the extract (400mg/kg and 800mg/kg) was not significantly different from that of acetylsalicylic acid (100mg/kg) (Table 1). Acetic acid injected intraperitoneally is believed to act indirectly by inducing the release of prostaglandins and lipoxygenase products into the peritoneum which in turn stimulate the nociceptive neurons to produce writhes (Hunskaar and Hole, 1987). Acetic acid induced mouse writhing test is useful for the evaluation of mild analgesic non-steroidal anti-inflammatory compounds for peripheral anti-nociceptive or analgesic activity (Berkenkopf et al., 1988). Thus, the significant inhibitory effect exerted by the extract on the acetic acid induced mouse writhing suggests that the extract has a peripheral analgesic effect. This finding is in consonance with earlier report which demonstrated the analgesic effect of the chloroform extract of *D. cinerea* leaves using the acetic acid induced mouse writhing model (Mishra et al., 2009).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Mean writhes</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>10ml/kg</td>
<td>43.80±9.2</td>
<td>-</td>
</tr>
<tr>
<td><em>D. cinerea</em> 400</td>
<td>19.0 ± 5.6**</td>
<td>56.6</td>
<td></td>
</tr>
<tr>
<td><em>D. cinerea</em> 800</td>
<td>13.40 ± 2.7**</td>
<td>69.4</td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>18.80 ± 2.9**</td>
<td>57.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM **p<0.01 significantly different from control group (n=5)

The extract only produced a significant (p<0.01) increase in latency time at a dose of 800mg/kg at the 60th minute compared to the control group. Morphine (4mg/kg) produced a significant (p<0.0001) increase in latency time from the 30th to the 90th minute (Table 2). Centrally mediated nociception is often modeled using the thermal stimulus produced by the hot plate. Consequently, the hot plate test is often used to screen substances for central analgesic activity and centrally active analgesics increase reaction latency time of laboratory animals to thermally induced pain by the hot plate (Heidari et al., 2009). Thus, the significant elevation of the reaction latency time of the mice, 60 minutes following administration of the extract (800mg/kg) suggests that the extract, at high doses may have a weak and short-lived central analgesic activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>0min (baseline)</th>
<th>30min</th>
<th>60min</th>
<th>90min</th>
<th>120min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline control</td>
<td>-</td>
<td>3.96±0.25</td>
<td>3.42±0.44</td>
<td>5.70±2.35</td>
<td>5.10±1.05</td>
<td>4.30±1.54</td>
</tr>
<tr>
<td><em>D. cinerea</em> 400</td>
<td>4.32±0.36</td>
<td>7.96±1.78</td>
<td>7.94±1.33</td>
<td>5.68±1.22</td>
<td>4.68±0.62</td>
<td></td>
</tr>
<tr>
<td><em>D. cinerea</em> 800</td>
<td>4.36±0.88</td>
<td>7.82±1.05</td>
<td>13.42±1.80***</td>
<td>10.52±2.56</td>
<td>5.84±1.72</td>
<td></td>
</tr>
<tr>
<td>Morphine 4</td>
<td>5.20±0.88</td>
<td>14.56±1.84****</td>
<td>20.40±3.55****</td>
<td>16.28±2.84****</td>
<td>11.80±2.16*</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean time (Seconds) ± SEM *p<0.05, **p<0.01, ****p<0.0001 versus control group (n=5).

The extract (400mg/kg) administered one hour prior to carrageenan injection caused a significant (p<0.01) inhibition of paw edema from the third hour following carrageenan injection compared to control. On the other hand 800mg/kg of the extract caused a significant (p<0.01 and p<0.0001 respectively) inhibition of paw edema at 1 hour and 2-4 hours following carrageenan injection and this was comparable to the inhibition of paw edema produced by standard anti-inflammatory drug- indomethacin (Figure 1). Carrageenan as a phlogistic agent is non-antigenic and has no apparent systemic effect (Chakraborty et al., 2006). Carrageenan induces acute inflammation and it is a suitable model for screening for non-steroidal anti-inflammatory drugs (Vinegar et al., 1969). Carrageenan-induced oedema is believed to involve three phases; the first phase occurs within one hour of carrageenan injection and it is attributed to the release of mast cell autacoids like histamine and serotonin. The second phase which occurs after 1.5 hours following carrageenan injection is on account of the release of prostaglandins, arachidonic acid, and leukotrienes.
acid byproducts and the continuity between phases one and two is due to the release of kinins (Vinegar et al., 1987). Also, neutrophil migration, release of oxygen free radicals and proteolytic enzymes are believed to be contributory to the development of the second phase (Boughton-smith et al., 1993). The extract (800mg/kg) significantly inhibited carrageenan-induced paw oedema in the first and second phases. Given that histamine induced microvascular leakage is believed to be responsible for the first phase of carrageenan induced inflammation (Kuriyama et al., 2000), the inhibition of the first phase of carrageenan induced inflammation by the extract (800mg/kg) suggests anti-histamine activity. The observation that the extract at a dose of 400mg/kg did not inhibit the first phase of carrageenan induced inflammation indicates that the extract may only possess anti-histamine activity at higher doses. Furthermore, the inhibition of the second phase of carrageenan induced inflammation, which corresponds to the phase of prostalgladin release, by the extract (400 and 800mg/kg), suggests an inhibitory effect on prostalgladin activity and activity of other cycloxygenase products because carrageenan inflammatory model essentially reflects the actions of prostalgdins (Ferreira et al., 1974).

![Graph](image1)

**Fig.1:** Effect of aqueous extract of Dichrostachys cinerea on carrageenan induced paw edema in rats. Values are mean ± SEM *p<0.01, **p<0.0001 versus control. (n=5).

The extract (800mg/kg) caused a significant inhibition of dextran induced paw edema between the second hour and fourth hour following dextran injection compared to control. On the other hand, the extract at 400mg/kg produced no significant inhibition of dextran induced paw edema (Figure 2). Given that dextran induced paw edema is mediated by histamine and serotonin released from the mast cells which in turn caused marked vasodilation, increased permeability and slowing of blood flow in the vascular system (Pearce, 1986), the inhibition of dextran induced paw edema produced by the extract indicates that the extract may possess anti-histamine anti-serotonin activities.

![Graph](image2)

**Fig.2:** Effect of aqueous extract of *Dichrostachys cinerea* on dextran induced paw edema in rats. Values are mean ± SEM *p<0.01, **p<0.0001 versus control. (n=5).

On the basis of the findings from this study, it is concluded that the aqueous extract of *D. cinerea* possesses analgesic effects probably mediated, solely, by peripheral mechanisms via the inhibition of prostagladin activity and anti-inflammatory effects probably mediated by peripheral mechanisms via the...
inhibition of cyclooxygenase and histaminergic/serotonergic activities. These results therefore further support the folkloric claim of the effectiveness of the leaf extract of *D. cinerea* for the treatment of conditions involving pain and inflammation.

**REFERENCES**


Misra, US; Behera, SR; Murphy, PN; Manish, K; Kumar, D (2009). Antibacterial and analgesic effects of the leaves of Dichrostachys Cinerea. *International Journal of Pharmacy and Pharmaceutical Sciences.* 1(2).


