Analgesic and anti-inflammatory properties of the leaf extracts of *Anacardium occidentalis* in the laboratory rodents

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**Summary:** *Anacardium occidentalis* (family: Anacardiaceae) is a plant of the tropical climate widely used by folklore to treat pain and inflammation. This study was conducted to evaluate the analgesic and anti-inflammatory effects of the leaf extracts in rat and mice using different models in other to confirm folkloric claims. The aqueous, hexane, dichloromethane and methanol extracts (AEAO, HEAO, DEAO and MEAO respectively) were investigated for analgesic effects in acetic acid induced pain in mice. They significantly reduced the number of writhing (p<0.001) and the highest analgesic effect was seen in DEAO extract. DEAO was further studied on various analgesic and anti-inflammatory models in graded doses. The extract significantly reduced writhing induced by acetic acid and the number and time of paw licking induced by formalin (P < 0.05) in a dose related manner. It inhibited the neurogenic and inflammatory phases of formalin (P < 0.05). Analgesia was shown in the inhibition of nociception induced by tail immersion in 55°C hot water. The extract prolonged the latencies of tail withdrawal to a similar degree as pentazocine. The extract caused significant inhibition of carrageenan induced paw oedema in rats (P < 0.05) in a dose dependent manner. These findings suggest that the leaf extracts of *Anacardium occidentalis* are highly potent analgesic and anti-inflammatory agents. Phytochemical analysis showed that the leaf extracts contain alkaloids, tannins, saponins and cardenolides.

**Keywords:** *Anacardium occidentalis*, analgesic, anti-inflammatory, rats, mice

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Manuscript Accepted: March, 2012

**INTRODUCTION**

Nociception, initiated by pain receptors, is known as the neural process of encoding and processing noxious stimuli. It is the afferent activity produced in the peripheral and central nervous system by stimuli that have the potential to damage tissue. Then, inflammation is the complex biological response of vascular tissues to harmful stimuli such as pathogens or irritants, with a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.

*Anacardium occidentalis*, commonly known as Cashew, is a plant of the tropical climate widely used by folklore to treat pain and inflammation. *Anacardium occidentalis* is a plant very abundant in the tropical rainforest while Nigeria is the second largest cashew producing country (Rosen and Fordice, 1994; UNFAA, 2006). It is a perennial tree about 10–15m tall and known to originate from Brazil. Cashew plant has numerous documented scientific and ethno-botanical uses among which are its astringent and antimicrobial (Akinpelu, 2001); antibacterial and anti-*Helicobacter pylori* (Laurens et al., 1982; Mustapha and Hafsat, 2007); antidiabetic (Kamtchouing et al., 1998); anti-malaria and anti-ulcer (Franca, 1993) activities. In Africa, a bath of the leaf decoction is usually taken to cure malaria (Taylor, 2005; Odugbemi and Akinsulire, 2006). Ethno-medicine also claims that cashew leaves is used to treat inflammatory conditions, relief pain, treat toothache, heal wounds, treat rheumatism and dysentery among many other medicinal uses (Odugbemi and Akinsulire, 2006). Samuel and co-workers reported that the plant may be used locally to relief abdominal pains (Samuel and Arlene, 1979). Plants reportedly used to relief pain are confirmed to be rich in active principles such as alkaloids, flavonoids, tannins, terpenoids and steroids (Tanko et al., 2008). The plant, *Anacardium occidentalis*, according to the database given by Duke (2005), contains these secondary metabolites as well as antioxidants which may possess analgesic and anti-inflammatory properties. Thus, *Anacardium occidentalis* may have some potent analgesic and/or anti-inflammatory metabolite. However, there seems to be a dearth of information on the scientific evaluation of *Anacardium occidentalis* for the
treatment of pain and inflammation as claimed by folklore. Thus, this study was conducted to evaluate the analgesics and anti-inflammatory properties of the leaf extract of *Anacardium occidentalis*.

**MATERIALS AND METHODS**

**Plant material**
The fresh leaves of *Anacardium occidentalis* were collected around June/July 2008 at the Amina way of the University of Ibadan, Ibadan and properly identified in the herbarium of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan. The plant was authenticated by O.A. Ugboyu and A.A. Ekundayo of FRIN Herbarium, with the voucher number: FHI-108362 deposited in the same place.

**Preparation of extracts**
The fresh leaves of *Anacardium occidentalis* were air dried and ground into fine powder. The powder was fractionated successively in hexane, dichloromethane, methanol and aqueous in a soxhlet extractor and the solvents were evaporated from each extract at 40°C in a rotatory evaporator. The percentage yields were 1.67%, 1.17%, 20% and 23.3% for the hexane, dichloromethane, methanol and aqueous extracts respectively. The extracts were stored in a refrigerator at 4°C until when needed. All doses of the extracts used in this study were expressed in terms of the dried sample prepared as suspensions with 2.5% Tween 20 in normal saline for the physiological and pharmacological experiments - fresh samples were prepared each day for each experiment.

**Experimental Animals**
Matured male Sprague Dawley albino rats (100-180g) and Swiss mice (20 – 25g) were obtained from the Central Animal house of the College of Medicine, University of Ibadan, Ibadan, Nigeria. Animals were maintained under standard nutritional and environmental conditions of normal relative humidity, room temperature, and 12 hour light and 12 hour dark cycle. The animals were provided with standard food pellets and clean water *ad libitum*. Experimental protocols complied with the ‘Principle of Laboratory Animal Care’ (NIH publication No. 85-23) guidelines (PHS, 1996).

**Phytochemical screening**
The phytochemical screening for alkaloids, anthraquinones, cardenolides, saponins and tannins was carried out on the powdered leaf using standard procedures reported (Trece and Evans, 1999). Drangenduf, Mayer and Wagner’s Tests were used to test for Alkaloid. Keller-Kalliani and Kedde’s test was used to test for cardenolides; Chloroform/ammonia was used to test for Anthraquinone; Frothing test was done for Saponins and Ferric chloride test was used to test for the presence of Tannins. Color change or frothing was an indication of the presence of these metabolites.

**Analgesic and anti-inflammatory studies**

**Acetic acid-induced writhing in mice:** Writhing was induced in the mice according to Siegmund et al., (1957) as modified by Koster et al., (1959). Twenty-four hours fasted mice were injected intraperitoneally with 0.2ml of 3% acetic acid solution 1hour after oral administration of the extracts, standard drug and normal saline. The number of writhing was observed between 5 and 15mins. A reduction in the number of writhing compared with the control group was considered as evidence for analgesia. Index of analgesia was expressed as percentage inhibition of writhing and calculated according to the following formula:

\[
\% \text{ Inhibition} = \left( \frac{\text{MW}_c - \text{MW}_t}{\text{MW}_c} \right) \times 100
\]

where \( \text{MW}_c \) = Mean number of writhing in the control group \( \text{MW}_t \) = Mean number of writhing in treated group.

**Tail flick test in rats:** About 5cm of the rats tail was immersed into hot water maintained at 55±1 °C and the tail withdrawal reflex period (latency) was taken at 0, 15, 30, 60 and 90 minutes after oral administration of extract, drug and saline (Uma - Devi et al., 1999).

**Formalin induced paw licking in rats:** Twenty microlitre of formalin was injected into the planter surface of the left hind paw of the rats 1hour after extract or drug administration. The time that the animals spent licking the injected paws (Hunskaar and Hole, 1997) and the number of times the rats licked the injected paws (Coderre and Melzack, 1992) were observed and measured as an index of pain. The response was bi-phasic, the initial nociceptive response (0-5mins) after formalin injection indicated the early phase while (15-30mins) indicated the late phase. This experiment was carried out in a transparent plastic chamber (30x30x30)cm with a mirror placed at the bottom for unobstructed view of the rats.

**Carrageenan induced paw oedema in rats:** Pedal acute inflammation was induced in male albino rats by injecting 0.1ml of 1% carrageenan into the subplantar surface of the right hind paw of the rats one hour after treatment with drug or extracts (Winter et al., 1962). Oedema was assessed by the measurement of an increase in circumference on the injected paw according to the method of Bangbose and Naomesi, (1981). The measurement was done before injection (0), 1, 2, 3, 4, 5 hours after injection. Percentage inhibition of oedema (anti-inflammatory potency) was computed using the formula:
% Inhibition = \frac{(Ct - Co)_{\text{control}} - (Ct - Co)_{\text{test}}} {(Ct - Co)_{\text{control}}} \times 100\%

\text{where } Co = \text{paw size before carrageenan injection; } Ct = \text{paw size at time t-hour after carrageenan injection.}

Statistical analysis
The results of the experiments were expressed as mean ± S.E.M. The statistical significance of differences was estimated using "Newman-Keuls Multiple Comparison Test ANOVA". The value with P ≤ 0.05 is considered to be significant.

RESULTS

Phytochemical studies
The phytochemical screening of the leaf extract of Anacardium occidentalis revealed the presence of alkaloids, tannins, saponins and cardenolides.

Acetic acid-induced writhing test in mice
The extracts AEAO, DEAO, HEAO and MEAO at 25mg/kg and 100mg/kg, demonstrated analgesia by significantly (p<0.001) inhibiting the number of writhing in acetic acid induced writhing in mice with the highest inhibition observed in DEAO extract (Tables 1 & 2). Results summarized in Figure 1 shows that DEAO inhibited writhing in acetic acid induced mice in a dose dependent manner.

Tail flick test in rats
Figure 2 reveals that there is prolonged latency of tail flick response in rats treated with graded doses (25, 100 and 400mg/kg) of DEAO. The highest latency was seen in 400mg/kg DEAO after 90mins.

Table 1. Effect of 25mg/kg of various extracts of Anacardium occidentalis on acetic acid-induced writhing test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Number of writhing</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10ml/kg)</td>
<td>38±1.14</td>
<td>0</td>
</tr>
<tr>
<td>AEAO (25mg/kg)</td>
<td>13±0.99**</td>
<td>65.8</td>
</tr>
<tr>
<td>HEAO (25mg/kg)</td>
<td>12.4±1.02**</td>
<td>67.4</td>
</tr>
<tr>
<td>MEAO (25mg/kg)</td>
<td>11.2±0.86**</td>
<td>70.5</td>
</tr>
<tr>
<td>DEAO (25mg/kg)</td>
<td>9.4±0.51**</td>
<td>75.3</td>
</tr>
<tr>
<td>Indomethacin (25mg/kg)</td>
<td>9.2±1.02**</td>
<td>75.9</td>
</tr>
</tbody>
</table>

The Values are expressed as mean ± S.E.M. (n = 6 mice). **P<0.05; significantly different from the control value.

Table 2. Effect of 100mg/kg of various extracts of Anacardium occidentalis on acetic acid-induced writhing test in mice.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean Number of writhing</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10ml/kg)</td>
<td>38.0±1.14</td>
<td>0</td>
</tr>
<tr>
<td>AEAO (100mg/kg)</td>
<td>6.8±0.37**</td>
<td>82.1</td>
</tr>
<tr>
<td>HEAO (100mg/kg)</td>
<td>5.4±0.4**</td>
<td>85.8</td>
</tr>
<tr>
<td>MEAO (100mg/kg)</td>
<td>4.4±0.74**</td>
<td>88.4</td>
</tr>
<tr>
<td>DEAO (100mg/kg)</td>
<td>4.2±0.37**</td>
<td>88.95</td>
</tr>
<tr>
<td>Indomethacin (5mg/kg)</td>
<td>9.2±1.02**</td>
<td>75.9</td>
</tr>
</tbody>
</table>

The Values are expressed as mean ± S.E.M. (n = 6 mice). ***P<0.05; significantly different from the control value.

Formalin induced paw licking test
Figures 3 and 4 showed that DEAO significantly inhibited both the time of paw licking and the number of paw licking respectively in both the early and late phases of Formalin induced paw licking in male albino rats.

Carrageenan induced paw oedema in male albino rats
The anti-inflammatory potency of DEAO extract on carrageenan induced paw oedema in male albino rats is shown in Figure 5. The extract strongly inhibited oedema (p<0.0001) and this inhibition seems to be dose dependent.
Figure 1. Effects of graded doses of DEAO on acetic acid-induced writhing in mice. 
Values are expressed as mean ± S.E.M. (n = 6 mice). ***P<0.05; significantly different from the control value.

Figure 2: Effects of graded doses of DEAO on tail flick latency in albino rats. 
Values are expressed as mean ± S.E.M (n = 6 rats). ***P<0.05; significantly different from the control value.

Figure 3: Effect of graded doses of DEAO on paw leaking in the formalin-induced nociception in mice. 
The values are expressed as mean ± S.E.M (n = 6 rats). ***P<0.05; significantly different from the control value.

Figure 4: Effect of graded doses of DEAO on flinching in the formalin-induced nociception in mice. 
The values are expressed as mean ± S.E.M (n = 6 rats). ***P<0.05; significantly different from the control value.

Figure 5: Effect of graded doses of DEAO on change in paw size in carrageenan-induced paw oedema in the albino rats. 
Values are expressed as mean±SEM of n=5. (P<0.05 at 0hr, P<0.0001 at 1-5hour.)

DISCUSSION

Acetic acid induced writhing test is a model of visceral pain which is a very sensitive test for analgesic drugs (Vyklicky, 1979). In the acetic acid induced writhing models, results show that the extracts significantly reduced writhing response. As intraperitoneal injection of the acid produced abdominal writhing by activating chemosensitive nociceptors but the extracts were able to protect the animals, thus exhibiting analgesia. At the low dose of 25mg/kg, there was similar analgesic effect to that of the standard drug indomethacin but at 100mg/kg and 400mg/kg, the extracts were more potent than indomethacin. The dose dependent inhibition of acetic acid induced writhing by the extracts indicated a peripheral effect and it’s suggestive of the dose...
dependent manner of medicinal plants extracts in the treatment of pain and inflammation (Oriowo, 1982; Olajide et al., 2000). The efficacy of most herbal preparations is attributed to the presence of various active principles in combination thus this may be the reason why the extracts showed higher potency than the standard drug. The inhibition of acetic acid writhing shows that the extracts may have central effects on the nervous system and depressant effect on the nervous system since central nervous system depressants have been known to inhibit or reduce the number of writhing in acetic acid pain models (Hasan et al., 2009; Stevenson et al., 2009).

The outcome of the tail flick test showed that the extracts inhibited the thermally induced nociceptive spinal reflex in the rats in a dose and time dependent manner with the maximum analgesic effect being reached at 90 minutes p<0.0001. The analgesic effect of the extracts is closely related, and similar to that seen in Pentazocine, a morphine analogue, and may be due to the presence of alkaloids in the extract which acts through opioid receptors (Farouk et al., 2006).

From the tail withdrawal/flick test it may be inferred that the extract acts on the spinal cord (spinal reflex) and that a higher dose of the extract may be given for a higher analgesic effect while the low dose may be sufficient for moderate analgesic effects.

The formalin induced paw licking test is a model devised to evaluate analgesic effect of new drugs (Hunskaar et al., 1985). This test is sensitive to commonly used analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). The pain stimulus is continuous rather than transient and may bear some resemblance to some kinds of clinical pain. The chemical irritant formalin produced a bi-phasic response. The first phase reflects a direct effect of formalin on nociceptors i.e. a neuropathic pain while the second phase reflects an inflammatory phase mediated by the release of several inflammatory agents including prostaglandins (Tjolsen et al., 1992; Hunskaar and Hole, 1997). The extracts reduced both the duration and the number of paw licking in both phases. It may be deduced that the extract offered protection against the activation of chemoreceptors and the activities of chemo-irritants and inflammatory agents. The anti-nociceptive effect of the extract was probably mediated via both neurogenic and inflammatory mechanisms.

Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory drugs and to predict the value of anti-inflammatory agent acting by inhibiting the mediators of acute inflammation (Dirosa et al., 1971; Mossa et al., 1995). The inflammatory condition induced by carrageenan involves stepwise release of vasoactive substances such as histamine, bradykinin and serotonin in the early phase and prostaglandins in the acute late phase.

Thus carrageenan oedema is bi-phasic (Dirosa et al., 1971; Heller et al., 1998). The first two hours of the oedema is the non-phagostic phase of carrageenan inflammation when the mast cells release serotonin, histamine and cytoplasmic enzymes (Vinegar et al., 1969; Crunkhon and Meacock, 1971) after this is the second phase which is as a result of liberation of prostaglandins, lysosome, bradykinin and proteases. These chemical substances produced increase in vascular permeability thereby promoting the accumulation of fluid in tissues that account for the oedema (Williams and Morley, 1973; White 1999). The results show that the extracts may contain substances which protects against the actions of histamine, serotonin and enzymes produced in the first phase of the oedema. The second phase of the oedema is sensitive to most clinically active anti-inflammatory drugs. The ability of the extract to almost completely inhibit oedema in the second phase (4th and 5th hour) indicate that it contains bioactive components which are active against the liberation of prostaglandins and other inflammatory agents usually released in the second phase of carrageenan oedema. According to Mossa et al., (1995) agents that inhibit carrageenan induced paw oedema significantly must have anti-inflammatory agents which act by inhibiting the mediators of acute inflammation.

The presence of flavonoids, alkaloids, cardenolides and saponins in plant products with analgesic and anti-inflammatory properties have been established (Fernanda et al., 2002). The presence of flavonoids in the leaves and whole plant of Anacardium occidentalis has been reported likewise (Duke, 2005; Gordian and Godswill, 2007). Gordian identified the flavonoid to be Keampferol (Gordian and Godswill, 2007). Also, tannins has been isolated from the bark of the plant (Mota, 1985). These compounds are known to possess analgesic and anti-inflammatory properties. In addition, the phytochemical screening in this study reveals the presence of tannins, alkaloids, saponins and cardenolides. These active ingredients may be responsible for the analgesic and anti-inflammatory properties of Anacardium occidentalis.

Present findings of analgesic activity of Anacardium occidentalis are similar to those reported for pentazocine and indomethacin in more than 2 decades ago (Di-stasi et al., 1988). Since non-steroidal analgesic and anti-inflammatory drugs such as indomethacin produce their therapeutic effects through the inhibition of prostaglandin synthesis and the extracts produced anti-inflammatory effects similar to indomethacin, therefore Anacardium occidentalis may inhibit prostaglandin synthesis by inhibiting cyclo-oxygenase 2 (COX-2).

In conclusion, this study has demonstrated that fractions from the leaves extract of Anacardium occidentalis exhibited highly potent and dose related
analgesic and anti-inflammatory activities. These findings justify the folkloric use of the plant to treat pain and inflammatory conditions, and shows that it contains highly potent analgesic and anti-inflammatory agents.

REFERENCES


