Analgesic activity of the aqueous seed extract of *Hunteria umbellata* (K. Schum.) Hallier f. in rodents

Olufunmilayo Olaide Adeyemi¹*, Adejuwon Adewale Adeneye¹,²*, & Tope Elizabeth Alabi¹

¹Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos State, Nigeria
²Department of Pharmacology, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria

Received 6 April 2011; revised 24 June 2011

The analgesic effect and possible mechanism(s) of action of 50-200 mg/kg of the aqueous seed extract of *H. umbellata* (HU) were investigated in different experimental models of analgesia using the tail flick, tail immersion, acetic acid-induced writhing tests and formalin-induced algesia. Oral pre-treatment with 50-200 mg/kg of HU caused significant and dose related analgesic effect in the treated rats in all the experimental models used. This analgesia was mediated via central and peripheral mechanisms. Overall, the results showed that HU possesses analgesic effect which lends support to its folkloric use in the local management of pain.

**Keywords:** Analgesic activity, Aqueous seed extract, *Hunteria umbellata*, Rodents

*Hunteria umbellata* (K. Schum.) Hallier f. (Apocynaceae) is a tropical rainforest glabrous tree having smaller flowers and fruits, and broad and elongated leaves, measuring about 10-20 cm × 3.5-10 cm and ubiquitous to the West African and Central African rainforests¹. Its fruit measures up to 5-25 cm in diameter and consists of two separate globose mericaps which are about 3-6 cm long, yellow, smooth, 8-25 seeded and embedded in a gelatinous pulp².

Different parts of the plant are employed by the African traditional herbalists in the local management of veterinary and human diseases. For example, cold water decoction made from fresh leaves of the plant is highly valued by traditional midwives in the induction and augmentation of labour while water decoction made from the seeds of its mature and ripe fruits is employed in the management of infections, stomach ulcers, diabetes, and obesity³. However, the folkloric use of the fresh leaves as an oxytocic agent was recently validated and its oxytocic action mediated via muscarinic acetylcholinergic mechanism have also been established³. The antinociceptive and antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica* have equally been reported⁴. Also, the analgesic and antipyretic effects of the aqueous extract of the fruit pulp of *H. umbellata* have been investigated and proven to be effective in the regulation of pain and fever and these effects were independent of its antibacterial activities⁵. *Hunteria umbellata* have also been shown to be very active against micro-organisms such as *Escherichia coli*, *Proteus spp* and *Staphylococcus aureus*⁵. Oral hypoglycaemic activity of 50-200 mg/kg of the aqueous seed extract of *H. umbellata* in various models of hyperglycaemia, which was mediated via inhibition of intestinal glucose uptake, inhibition of adrenergic mechanism, increased peripheral glucose uptake and improvements in insulin resistance has been recently reported⁷,⁸.

In view of its folkloric use in pain management, the present study has been designed at evaluating the analgesic property and the possible mechanism(s) of action of 50, 100 and 200 mg/kg of the aqueous seed extract of *H. umbellata* in different experimental models of analgesia. The chosen dose range (50-200 mg/kg) of the extract was made based on the results of the preliminary study conducted earlier.

**Materials and Methods**

*Collection of plant materials—*Fresh leaves, inflorescence and mature fruits of the *Hunteria umbellata* (HU) plant were collected from the deciduous forest of Odorasanyin District of

*Correspondent authors
Telephone (Mobile): +234-803-445-9618, +234-802-069-0946
E-mail: ooadey@yahoo.com; adeneye2001@yahoo.com
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Ijebu-Igbo in Ijebu North Local Government Area of Ogun State, Nigeria, in June, 2009. Botanical identification and authentication were done as previously described7.

Plant extraction process—Hunteria umbellata seeds were harvested from the collected fresh ripe and mature fruits of the plant. The seeds were gently washed in tap water and completely dried under room temperature (30±2°C) for 4 weeks protected from direct heat or sunlight. When dried, the seeds were de-coated of their light-brown thin coatings. The dried seed was then blended into fine powder using a domestic blender. Powdered sample (60 g) was dissolved in 1.5 L distilled water and left to stand in the refrigerator for 72 h. After 72 h, the mixture was rigorously shaken but at intervals for 6 h and decanted. The solution was filtered using a piece of clean, white cloth and the filtrate was completely air-dried in aerated oven preset at 40°C to obtain deep brown and sweet-smelling solid residue (yield was 40% w/w). The residue obtained was kept in air- and water-tight container and stored in the refrigerator and until required for experimentation. From the stock, a concentration of 100 mg/ml was prepared each time the extract is required for experiment.

Experimental animals—Mature, white Albino Wistar rats weighing 100-150 g and Swiss albino mice weighing 16-20 g were obtained from the Laboratory Animal House of the College of Medicine of the University of Lagos, Nigeria, after the ethical approval for the use of the animals have been obtained. The animals were allowed to acclimatize to laboratory conditions and given water and food ad libitum. All experimental animals were handled according to institutional and international guidelines guiding the use of experimental animals3.

Tests for analgesic activities of HU—Tail flick method—The rat cold water tail flick test was based on a modification10 of the original method described by Pizziketti et al11. Rats were randomly allotted to 5 groups of 5 animals each. Pain was induced with cold water at 0–1°C. Distilled water (10 mL/kg, po) was given to the control group while the reference group was given morphine (3 mg/kg, sc) the remaining groups were administered 50, 100 and 200 mg/kg, po of HU. The time in seconds for tail withdrawal from the water was taken as the reaction time, with a cut-off time of 10 s. The reaction time was measured 30 min before and after administration of the drugs for two and a half hour.

Acetic acid-induced mouse writhing test—This was based on the method described by Koster et al.13 and Singh et al14. Mice were randomly allotted to 5 groups with 5 animals each. The control group received 10 mL/kg distilled water orally. The reference group received aspirin (100 mg/kg dissolved in distilled water, po) and groups III, IV and V mice were orally pre-treated with 50, 100 and 200 mg/kg HU, respectively. All drugs were administered 30 min before ip injection of 0.6% (v/v), 1 mL/kg glacial acetic acid solution. The number of writhes (extension of hind limb as a result of a wave of contraction of the abdominal muscles) immediately after injection of the acetic acid was counted for 30 min. A reduction in the number of writhes is an indication of analgesic property.

Formalin test—The method adopted is as described by Shibata et al15. Rats were divided into 6 groups of 5 rats each. They were pre-treated with distilled water (Group I, 10 mL/kg, po); morphine (Group II, 10 mg/kg, ip) and 100 mg/kg of aspirin (Group III, 100 mg/kg, ip) and HU (Group IV, 50 mg/kg, po) (Group V, 100 mg/kg, po) (Group VI, 200 mg/kg, po). After 30 min, 0.03 mL of 1% formalin was injected into the plantar aponeurosis of the right hind paw of each rats. The licking or biting of the formalin injected right hind paw was counted in two phases. The first phase was the first 5 min immediately after the injection of the formalin and the second phase was 15-30 min after the injection of the formalin.
A reduction in the number of paw licking or biting is an indication of analgesic property.

Statistical analysis—Results are presented as the mean±SE. Data comparisons between treatment groups were done by use of one-way ANOVA followed by Dunnett’s post test. Values were considered statistically significant at P<0.05, <0.01, and <0.001.

Results

Tail flick test—Oral pre-treatment with HU produced significant dose-dependent significant (P<0.05) increase in reaction time to tail flick as time progressed. The peak of the effect was recorded at 120 s compared with the control. This effect was significantly higher for 100 and 200 mg/kg of the extract compared with the control (Table 1).

Tail immersion test—Similarly, oral treatment with 50-200 mg/kg of HU produced a significant dose-related (P<0.05) prolongation of the reaction time to the tail immersion test. The peak effect of the extract was recorded at 90 s compared with the untreated control. However, this effect was lower in all extract-treated groups when compared with 10 mg/kg morphine-treated group (Table 2).

Acetic acid-induced mouse writhing test—The number of writhes obtained in the control group was 66.0 ± 1.8. Oral treatment with 50-200 mg/kg of HU caused a significant (P<0.001) and dose-dependent inhibition of the acetic acid-induced writhes (Table 3). In addition, the inhibition (76.6%) produced by 200 mg/kg the aqueous seed extract was significantly (P<0.001) higher than that of 100 mg/kg aspirin.

Formalin test—In the formalin-induced pain test, the extract caused a reduction in hind paw licking in both early and late phases of algesia. There was a dose-dependent significant (P<0.001) inhibition in phase two of the formalin-induced pain in extract pre-treated rats. Percentage inhibition of the extract at 50 and 200 mg/kg were 59.5 and 56.0%, respectively in the early phase of formalin test and

Table 1—Effect of 50-200 mg/kg of the aqueous seed extract of *H. umbellata* (HU) on rat tail flick test [Values are mean ± S.E. from 5 rats in each group. Figures in parentheses are % increase over control]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reaction time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0 30 60 90 120 150</td>
</tr>
<tr>
<td>II</td>
<td>28.8 ± 1.4 31.4 ± 1.0 33.4 ± 1.5 34.8 ± 1.3 36.8 ± 1.5 37.2 ± 1.8</td>
</tr>
<tr>
<td>III</td>
<td>27.6 ± 1.8 68.2 ± 6.6 76.4 ± 4.8 87.0 ± 1.6 89.0 ± 0.8 48.2 ± 4.6</td>
</tr>
<tr>
<td>IV</td>
<td>26.8 ± 1.9 42.8 ± 3.4 49.4 ± 3.5 54.8 ± 3.5 58.6 ± 3.3 44.6 ± 3.4</td>
</tr>
<tr>
<td>V</td>
<td>25.4 ± 1.0 50.8 ± 2.4 62.4 ± 6.0 69.4 ± 6.3 78.4 ± 6.2 44.8 ± 7.0</td>
</tr>
</tbody>
</table>

Table 2—Effect of 50-200 mg/kg of the aqueous seed extract of *H. umbellata* (HU) on tail immersion test [Values are mean ± SE from 5 rats in each group. Figures in parentheses are % increase over control]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reaction time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0 30 60 90 120 150</td>
</tr>
<tr>
<td>II</td>
<td>108.0 ± 12.0 108.0 ± 12.0 84.0 ± 12.0 84.0 ± 12.0 84.0 ± 12.0 72.0 ± 12.0</td>
</tr>
<tr>
<td>III</td>
<td>108 ± 12.0 300.0 ± 30.0 300.0 ± 30.0 300.0 ± 18.0 156.0 ± 12.0 144.0 ± 12.0</td>
</tr>
<tr>
<td>IV</td>
<td>120.0 ± 0.0 144.0 ± 12.0 156.0 ± 12.0 156.0 ± 12.0 120.0 ± 0.0 96.0 ± 12.0</td>
</tr>
<tr>
<td>V</td>
<td>97.2 ± 12.0 204.0 ± 12.0 216.0 ± 12.0 216.0 ± 12.0 144 ± 12.0 132 ± 12.0</td>
</tr>
</tbody>
</table>

a, b and c represent significant increases at P<0.05, <0.01, and <0.001 respectively when compared to the control value. Group I= 10 mL/kg distilled water; Group II = 10 mg/kg morphine; Group III = 50 mg/kg HU; Group IV = 100 mg/kg HU; Group V = 200 mg/kg HU
these inhibitions were higher than that of 100 mg/kg aspirin (Fig. 1). The extract also produced a dose dependent reduction in hind paw licking in the phase two of the formalin-induced pain.

Table 3—Effect of 50-200 mg/kg of the aqueous seed extract of *H. umbellata* on acetic acid-induced mouse writhing test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>No. of writhing per 30 min</th>
<th>Inhibition of writhing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10 mL/kg distilled water</td>
<td>66.0 ± 1.8</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>100 mg/kg aspirin</td>
<td>29.0 ± 0.9*</td>
<td>56.5</td>
</tr>
<tr>
<td>III</td>
<td>50 mg/kg HU</td>
<td>25.0 ± 0.8*</td>
<td>62.5</td>
</tr>
<tr>
<td>IV</td>
<td>100 mg/kg HU</td>
<td>20.4 ± 0.4*</td>
<td>69.4</td>
</tr>
<tr>
<td>V</td>
<td>200 mg/kg HU</td>
<td>15.6 ± 0.5*</td>
<td>76.6</td>
</tr>
</tbody>
</table>

*represents a significant decrease at *P*<0.001 when compared to the control value

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**Discussion**

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is defined as a subjective, unpleasant, physical and psychological experience observed as a result of the stimulation of identifiable nerve fibres with defined pathways to the brain via the spinal cord. Pain often results from tissue damage that stimulates nociceptive receptors (nociceptive pain) but pain may also occur without nociception, here it could be as a result of damage to neural structures (neuropathic pain or neuralgia). While the former is often acute, self-limiting after healing and responds easily to analgesics, the latter is very difficult to treat, there may or may not be evidence of injury, causes chronic pain and will persist long after the initial injury has healed.

The present study was aimed at investigating the anti-nociceptive property of the aqueous seed extract of *H. umbellata*, using the tail flick, tail immersion and the acetic acid-induced mouse writhing tests while the formalin-induced pain method was adopted so as to establish its mechanism(s) of action. In the tail immersion and tail flick tests, oral pre-treatment with the extract caused a profound and dose related analgesia in the treated rats although the analgesic effect of the extract was less effective than morphine. The above two procedures consists of behavioural methods that have been developed to study nociception in animals. The animal response in these tests is usually integrated at the lower levels in the central nervous system, thus, giving information about the pain threshold. They are, therefore, used to detect narcotic and non-narcotic analgesics. It is well established that thermal nociceptive tests are more sensitive to opioid µ-agonists and non-thermal tests to opioid κ-agonists. The data generated in the present study suggest that the involvement of both κ and µ opioid receptor in the analgesic activity of HU, from which the central involvement of the extract could be deduced. The formalin-induced pain as an experimental model of analgesia is useful for elucidating mechanism of pain and analgesia since it measures the response to a long-lasting nociceptive stimulus and, therefore, resembles clinical pain.

Subcutaneous injection of dilute formalin into mouse hind-paw produces biphasic nociceptive response namely: the first transient phase is caused by the direct effect of formalin on sensory C-fibers, and the second prolonged phase is associated with
the development of the injury induced spinal sensitization, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron occurs during inflammatory pain\textsuperscript{23-25}. Drugs that act centrally, such as the narcotics inhibit both phases of formalin-induced pain, while peripherally acting drugs such as aspirin only inhibit the late phase\textsuperscript{25-27}. Results of the present study showed that HU inhibit both the early and late phases of formalin induced pain, thus, suggesting its central and peripheral anti-nociceptive actions. Aside this, HU produced greater inhibition of the early phase than the late phase and was more efficacious than the standard drug, aspirin, in this phase. Earlier studies have shown that substance P participates in the early phase, while histamine, serotonin, excitatory amino acids and prostaglandins are involved in the late phase of formalin test with bradykinin affecting both phases\textsuperscript{23-28}. The abdominal contraction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics and such a response is thought to involve local peritoneal receptors. Significant protection was observed in the extract-treated groups of animals and it compared favourably with the standard drug. Aspirin is one of the most frequently used drugs in the treatment of mild to moderate pain, including that of migraines and fever\textsuperscript{29}. It is often combined with other non-steroidal anti-inflammatory drugs and opioid analgesics in the treatment of moderate to severe pain\textsuperscript{30}. Aspirin produces analgesia through inhibition of prostaglandin synthesis although the mechanism underlying this action is complex\textsuperscript{31}.

Secondary metabolites such as the alkaloids, saponins, tannins, flavonoids and glycosides have been reported to be present in HU\textsuperscript{31,32}. Alkaloids have been found to be responsible for both analgesic and anti-inflammatory actions in some natural products\textsuperscript{33}. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception\textsuperscript{34,35}. Also, there are few reports on the role of tannins and saponins in anti-nociceptive and anti-inflammatory activities\textsuperscript{36-38}. Saponins have also been reported to inhibit histamine release in vitro\textsuperscript{39}. Thus, the presence of these active biological principles in HU could have accounted for its analgesic activity.

In conclusion, the aqueous seed extract of *H. umbellata* demonstrated promising anti-nociceptive property in the various animal models used in this study, confirming its efficacy in the treatment of pain in its users. However, both central- and peripheral mediated analgesic mechanisms have been postulated. Further studies, especially at the molecular level may provide better understanding of its exact mode and site(s) of actions. In addition, isolation and characterization of the suspected analgesic phytocomponents will be worthwhile in the nearst future.

References


