Research Article

Analgesic Effect of Methanol Leaf Extract of Alstonia Boonei De Wild (Apocynaceae)

Loretta O Iniaghe*, Stephen O Okpo, Jacob E Olung and Agharese A Eguae

Department of Pharmacology & Toxicology, University of Benin, PMB 1154, Benin City 300001, Nigeria

Abstract

Purpose: To evaluate the possible analgesic properties of the methanol leaf extract of Alstonia boonei (De Wild, Apocynaceae) a locally available plant used in traditional medicine for the management of pain and other conditions.

Methods: Alstonia boonei leaves were extracted with methanol. Rodent models were employed in screening the analgesic effect of the extract. Pain indices evaluated in hot plate and tail flick tests, formalin pain test and mouse writhing assay were mean reaction time to latent heat, time spent in licking of injected paw and abdominal writhes, respectively.

Results: Oral administration of the extract caused a significant (p < 0.05) dose-dependent reduction in the number of abdominal writhes (control, 84.67 ± 9.58; 100 mg/kg, 57.86 ± 3.07; 200 mg/kg, 24.40 ± 3.92; and 400 mg/kg, 22.50 ± 2.53). The extract also produced significant (p < 0.05) but non-dose dependent increase in elevation of pain threshold in the hot plate (ranging from 27.99 to 42.26 % inhibition) and tail flick tests (ranging from 34.73 to 81.42 % inhibition) in mice and rats, respectively. All doses of the extract used produced significant (p < 0.05) inhibition of both phases of the formalin-induced pain in mice, with a more pronounced anti-inflammatory effect on the late (ranging from 46.96 to 74.12 %) phase than the early anti-neurogenic (ranging from 49.52 to 51.47 %) phase.

Conclusion: The results obtained suggest that the analgesic effect of Alstonia boonei may be mediated via both central and peripheral mechanisms.

Keywords: Alstonia boonei, Analgesia, Pain, Methanol extract.

Received: 30 April 2012 Revised accepted: 12 July 2012

*Corresponding author: Email: lo.iniaghe@uniben.edu; Tel: +234 802211 3816
INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pharmacologic management of pain requires the use of analgesic drugs. These drugs, though effective, can cause serious side effects. The search for potent analgesic agents with minimal side effects remains the goal of many scientific studies [1].

Medicines from indigenous plants form the basis of primary health care for a majority of people living in urban and rural or remote areas of the third world countries. The reason for this dependence is the perceived low cost, easy access and ancestral experience as well as the belief that these medicines are devoid of adverse effects [2].

Alstonia boonei (De Wild, Apocynaceae) is a large deciduous tree found in various parts of Africa. It is known as Awun among the Yorubas of southwestern Nigeria, Egbu-ora among the Igbos of southeastern Nigeria and Ukpukunu by the Urhobos of south central Nigeria. Parts of the plant are employed in the treatment of various ailments. While the stem bark is used as an astringent, alternative tonic and a febrifuge for relapsing fevers, the leaves and latex are used for the treatment of rheumatic and muscular pain, and hypertension [3,4].

Anti-plasmodial, antimicrobial, analgesic and anti-inflammatory properties of the aqueous and ethanol extracts of Alstonia boonei stem bark have also been reported [5,6]. In the present study, we report the analgesic activity of the methanol extract of Alstonia boonei leaves.

EXPERIMENTAL

Plant collection

The stem and leaves of Alstonia boonei were collected around the premises of University of Benin, in the month of October 2010. The plant was identified by Mr HO Uhumarogie of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin and authenticated by Mr KA Adeniyi of the Forest Research Institute of Nigeria, Ibadan where a voucher specimen (FHI 109505) was deposited for future reference. The leaves were washed, freed of debris and air-dried for a period of two weeks; thereafter, they were reduced to fine powder and stored in an airtight container.

Extraction

The powdered plant (500 g) was extracted with methanol (2 x 3L for 48 h each) by maceration at room temperature. The resulting extract was, decanted, filtered and evaporated to dryness in an oven set at 40 °C to yield a solid mass of 24 g (4.8 %)

Animals

Albino mice (25 - 30 g) and Wistar rats (180 – 230 g) of both sexes were obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. The animals were maintained under standard environmental conditions and allowed free access to feed (Bendel Feeds and Floor Mills, Ewu, Edo State, Nigeria) and water ad libitum. The animals were exposed to 12 h dark/12 h light cycle and temperature (24 - 28 °C). They were handled according to the standard protocols for the use of laboratory animals of the National Institute for Health [7]. Ethical approval was obtained from the Ethical Committee of the Faculty of Pharmacy, University of Benin (ref EC/FP/010/02).

Mouse writhing assay

The method of Koster et al [8] was used. The mice were randomly allotted into 5 groups of 6 animals per group. Alstonia boonei (100, 200 and 400 mg/kg) and acetylsalicylic acid (ASA, 100 mg/kg) were administered via the oral route to the different test groups while the control group received 0.2 ml of 1 %
Tween-80 orally 30 min before intraperitoneal injection of acetic acid (0.2 ml of 0.6 %w/v). Abdominal writhes (which consisted of constriction of the abdominal muscles along with stretching of the hind limbs) were cumulatively counted for 30 min following acetic acid injection. Acetyl salicylic acid (ASA) served as the positive control.

**Hot plate test**

The method of Woolfe and MacDonald [9] was used. The mice were divided randomly into 5 groups of 6 animals each. Each animal was trained on the hot plate (Ugo Basile Model no 35100-001 Comerio, Italy) prior to drug administration; latency time spent on the hot plate prior to drug administration was also determined. The extract (100, 200 and 400 mg/kg) and morphine (2 mg/kg, positive control) were administered orally and subcutaneously, respectively, to mice in the test groups. Tween 80 (0.2 ml, 1 %) was used as the control. Thirty minutes post-treatment (15 min for the morphine group), the animals were dropped gently on the hot plate maintained at 55 ± 0.5 °C, with the cut off time set at 60 seconds. The time (in seconds) for the mouse to either jump or lick its hind paw was taken as the reaction time.

**Formalin test in mice**

The mice were randomly allotted into five groups of 6 animals each. They were given Tween-80 (0.2 ml of 1 %w/v, p.o.), Morphine (2 mg/kg, s.c.) and the extract (100 - 400 mg/kg, p.o.) 30 min prior to injection of formalin (20 µL, 1 %) into the right hind paw [10]. Nociceptive responses were recorded as the time (in seconds) spent in biting and licking of the injected paw. Responses were measured for 5 min after formalin injection (first phase) and 15 - 30 min after formalin injection (second phase). Analgesic effect was expressed as a reduction in the time spent in licking or biting of the injected paw. The positive control was the morphine group.

**Tail flick test in rats**

The method of D’Amour and Smith was used [11]. The tail flick unit (Ugobasile, model 37360) was set at an intensity of 52 lumens, with a cut-off time of 30 s. A sensitivity test was performed by placing the tail of each rat on the radiant heat source. Animals which did not react by withdrawal of the tail in 30 s were considered insensitive and excluded from the study. Sensitive rats were randomly distributed into 5 groups of 6 animals each. Animals in group 1 (control) received Tween 80 (0.2 ml, 1 %) while groups 2, 3 and 4 received, by oral intubation, 100, 200 and 400 mg/kg of the extract, respectively, while group 5 received morphine (2 mg/kg, s.c.). Measurement of tail-flick responses were repeated 1 h post-drug administration (30 min for the morphine group which was the positive control).

**Statistical analysis**

The statistical software package, GraphPad Instat, version 3, was used to analyze the data obtained. The results are expressed as mean ± S.E.M (standard error of mean). Comparison of data between (and within) groups was carried out using the Student t-test or analysis of variance (ANOVA), followed by Dunnet’s test. The data were considered significant when p < 0.05.

**RESULTS**

**Acetic acid-induced mouse writhing**

Administration of the methanol extract of *A. boonei* prior to acetic acid administration caused a dose-dependent reduction in the number of writhes compared to control. The greatest inhibition was achieved at the highest dose (400 mg/kg) of the extract but this was significantly lower (p < 0.05) than inhibition with acetylsalicylic acid (Table 1).

**Hot plate reaction time**

The extract caused a significant (p < 0.05), but non-dose dependent increase in the
mean reaction time on the hot plate (Table 2). The greatest effect was obtained at the lowest dose (100 mg/kg) of the extract but this was significantly lower than the inhibition obtained with the standard drug (morphine).

Table 1: Effect of methanol leaf extract of *Alstonia boonei* on acetic acid-induced mouse writhing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No of writhes</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>84.67 ± 9.58</td>
<td>-</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>57.86 ± 3.07*</td>
<td>31.66</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>24.40 ± 3.92*</td>
<td>71.18</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>22.50 ± 2.53*</td>
<td>73.43</td>
</tr>
<tr>
<td>Acetyl salicylic acid (ASA)</td>
<td>100</td>
<td>7.71 ± 2.29*</td>
<td>90.89</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. * significantly different from control (p < 0.05); n = 6

Table 2: Effect of methanol leaf extract of *Alstonia boonei* on hot plate reaction time in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean reaction time (s)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>9.11 ± 1.77</td>
<td>-</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>12.96 ± 2.96*</td>
<td>42.26</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>12.25 ± 1.52*</td>
<td>34.47</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>11.66 ± 1.00*</td>
<td>27.99</td>
</tr>
<tr>
<td>Morphine</td>
<td>2</td>
<td>16.68 ± 1.84*</td>
<td>83.10</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; * significantly different from control (p < 0.05); a significantly different from morphine (p < 0.05)

Formalin-induced pain

Table 3 shows the effect of methanol extract of *A. boonei* on formalin-induced pain. There was a significant but non-dose dependent inhibition at both phases by the extract (Table 3). The three doses of the extract used produced similar inhibitions of the first phase but the highest dose showed the greatest effect on the second phase. Morphine also inhibited both phases of the pain response but its effect on the first phase was not significantly different (p>0.05) from that produced by 400 mg/kg of the extract.

Tail flick reaction time

The effect of the extract on the reaction time in the tail flick test in rats is summarised in Table 4. Maximum inhibitory effect was observed at the highest dose of the extract and this was comparable to the effect observed with morphine (2 mg/kg).

Table 3: Effect of methanol leaf extract of *Alstonia boonei* on formalin-induced pain in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Time spent in licking and biting responses (s) 0-5 min</th>
<th>Inhibition (%) 0-5 min</th>
<th>15-30 min</th>
<th>Inhibition (%) 15-30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>111.88± 11.84</td>
<td>-</td>
<td>134.45 ± 12.83</td>
<td>-</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>54.30 ± 4.86*</td>
<td>51.47</td>
<td>66.19 ± 8.53*</td>
<td>50.77</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>60.74 ± 3.99*</td>
<td>45.71</td>
<td>71.31 ± 10.12*</td>
<td>46.96</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>56.48 ± 4.52*</td>
<td>49.52</td>
<td>34.80 ± 5.40*</td>
<td>74.12</td>
</tr>
<tr>
<td>Morphine</td>
<td>2</td>
<td>51.19 ± 8.26*</td>
<td>54.25</td>
<td>4.30 ± 3.21*</td>
<td>96.70</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (at least n = 6); * significantly different from control (p < 0.05)
a significantly different from morphine group (p < 0.05)
Table 4: Effect of methanol leaf extract of *Alstonia boonei* on tail flick reaction time in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean reaction time (s)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>9.04 ± 1.24</td>
<td>-</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>13.38 ± 2.84*</td>
<td>48.45</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>12.23 ± 2.36*</td>
<td>34.73</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>16.40 ± 2.84*</td>
<td>81.42</td>
</tr>
<tr>
<td>Morphine</td>
<td>2</td>
<td>16.77 ± 1.37†</td>
<td>85.51</td>
</tr>
</tbody>
</table>

*p < 0.05 from control, †30 min; mean ± SEM (n = 6)

The increased reaction time of the mice pre-treated with the extract in the hot plate model suggests that it might be centrally acting since centrally acting analgesic drugs are known to elevate the pain threshold of animals towards heat and pressure [13].

The tail flick test measures complex responses to acute nociceptive activity and is one of the models used for studying centrally acting analgesics [14]. The extract showed a potent activity on this model, similar to the effect of morphine. The effect of the extract in the tail flick and hot plate models indicates a central analgesic effect.

The extract inhibited both phases of formalin-induced pain in mice with a more pronounced effect on the second than the first phase. Injection of formalin into the plantar aponeurosis induces biphasic nociceptive behaviour in animals which seem to involve two distinctly different stimuli. The first (neurogenic) phase, due to the release of substance P and bradykinin is followed by a second (inflammatory) phase, which is characterized by the release of serotonin, histamine, bradykinin, and prostaglandins [15]. Non-steroidal anti-inflammatory drugs such as indomethacin are known to inhibit only the second phase of this pain model, while drugs which mainly act centrally, such as narcotic analgesics, inhibit both phases [16]. The significant inhibition of both phases by the extract suggests that it possesses central as well as peripheral analgesic activity.

**CONCLUSION**

The results obtained in this study indicate that the methanol leaf extract of *Alstonia boonei* possesses analgesic activity which is mediated via central and peripheral mechanisms and this could form the basis for its folkloric use in the management of pain and inflammatory disorders.

**REFERENCES**

1. Mukherjere KP. Quality control of herbal drugs: An approach to evaluation of botanicals. 2000; p 34, 106

