Analgesic activities of fractions of Stereospermum kunthianum stem bark.
E Omogbai, F Abiodun, S Okpo, C Poh

Citation

Abstract
Stereospermum kunthianum (Bignoniaceae) is a woody shrub indigenous to Africa and Asia where the plant parts are used in traditional human medicine for its analgesic properties. We have recently demonstrated the analgesic activity of its aqueous stem bark extract. Vacuum liquid chromatography (VLC) of the methanol extract produced 3 fractions A, B, and C while further column chromatography (CC) analyses of the VLC fractions yielded fractions L, S and Y respectively. The fractions were evaluated for possible analgesic activity using the acetic acid and formalin pain tests. Fractions A, B and C (100, 200, and 400 mg/kg) significantly (p<.0001) inhibited abdominal writhes in mice. While fractions L and Y (100 - 400 mg/kg) significantly (p<.0001) inhibited both phases of the formalin-induced pain in mice with a more intense effect on the late phase than the early phase. Fraction S at the same doses significantly (p<.0001) inhibited both phases but with a more marked effect on the early phase. The results indicate that the VLC and CC fractions of Stereospermum kunthianum may inhibit pain responses mediated via both central and peripherally mechanisms. The present study has confirmed that Stereospermum kunthianum stem bark contains pharmacologically active constituents which possess analgesic activity justifying its popular use in treating painful conditions.

INTRODUCTION
Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in term of such damage 1. Many pathological disorders are accompanied with painful conditions. Man from time immemorial has used various substances from animals, plants and minerals sources to alleviate pain. Many rural dwellers of the world dependent largely on herbs for the treatment of painful conditions because medicinal herbs constitute indispensable components of traditional medicine practice due to low cost, easy access and ancestral experience 2. Stereospermum kunthianum (Cham, Sandrine Petit), family Bignoniaceae is a woody shrub of the Sudano-Guinea savannah regions of Africa and Asia, where the plant parts are used to treat various ailments 3. The plant is reputed for its use in the treatment of inflammatory conditions and pain. The efficacy of the water extract of Stereospermum kunthianum in human complement system fixation in-vitro has been reported 4. Antiplasmodial activity of naphthoquinones and one anthraquinone from the lipophilic extract of the root bark of Stereospermum kunthianum has also been reported 5. We have recently reported the in-vivo antidiarrhoeal and analgesic activities of the aqueous extract of Stereospermum kunthianum stem bark 6,7. In the present study, we report the analgesic activity of the vacuum liquid and column chromatography fractions of Stereospermum kunthianum stem bark.

MATERIALS AND METHODS
PLANT COLLECTION AND PREPARATION:
The plant was sought for based on its uses in human traditional medicine by most rural dwellers in Africa. Fresh stem bark of S. kunthianum was collected in March, 2006 in Ogun state Nigeria. Botanical identification and authentication was done by Mr. Usang Felix of the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria and a voucher specimen (No. FHI 107277) was deposited at the institute for future reference. The stem bark was cut into bits, shade dried and pulverized.

EXTRACTION AND CHROMATOGRAPHIC ANALYSES:
The powdered plant (500 g) was extracted with methanol (4x5Lx48 h) by maceration at room temperature. The extract was evaporated to dryness to yield a residue (80 g) which was subjected to vacuum liquid chromatography over silica
gel F$_{254}$ using gradient solvent systems n-C$_5$H$_{11}$: CHCl$_3$ and CHCl$_3$: CH$_2$OH, gradient up to 100 % CH$_2$OH as eluting solvents to obtain 19 fractions. The 19 fractions were subjected to repeated thin layer chromatography (TLC) using MeOH: CHCl$_3$ (3 : 1) as solvent system to obtain fractions with similar spots and R$_f$ –values which were then bulked into 3 fractions [ A(27.36g), B (41.40g) and C(10.23 g)]. Each of the fractions (A, B, and C) was evaluated for analgesic activity.

The fractions (A, B and C) were further subjected to column chromatography as previously described $^{4,6}$. The column was eluted with solvents of increasing polarity consisting of n-C$_5$H$_{11}$: CHCl$_3$ (9 : 1) and CHCl$_3$: CH$_2$OH (1 : 4) mixtures. The column chromatography of fractions A, B and C yielded 99, 75 and 85 fractions, respectively, which were subjected to thin layer chromatographic analyses and subsequently bulked based on the R$_f$ – values and TLC profile, to further produced 5, 6, and 4 fractions respectively, with yields as follows:

A [J(0.21g); K(1.24g); L(9.20g); M(5.39g); N(1.17g)]; B[Q(1.08g); R(1.70g); S(7.39g); T(6.82g);V(6.70g); W(6.19g), and C[U(0.25g)]; X(0.50g); Y(3.36g); Z(2.30g)]. Fractions L, S and Y were evaluated for analgesic activities.

ANIMALS

The use of animals for analgesic experiments was according to international guidelines and approved experimental protocols. Swiss mice of either sex obtained from the Animal House unit of the Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals maintained under standard laboratory conditions (12 h light and dark cycles) had free access to standard chow (Bendel Feeds and Flour Mills, Plc. Ewu, Nigeria) and drinking water.

MOUSE WRITHING ASSAY

The acetic acid-induced abdominal writhing test was performed according to the procedure described previously $^{11}$. Mice were randomly allocated into groups of five animals per group. The animals received normal saline (10 ml/kg, i.p), acetylsalicylic acid (100 mg/kg, sc. suspended in 5% tragacanth in normal saline), or each of the vacuum liquid chromatography fractions A, B, and C (100, 200 or 400 mg/kg, i.p.), thirty minutes before intraperitoneal injection of acetic acid (10 ml/kg, 1% v/v in normal saline). The animals were observed for writhes which consisted of constriction of the abdominal muscles together with stretching of the hind limbs. The writhes were cumulatively counted for 30 minutes following acetic acid injection and the analgesic effect determined as described $^{11}$.

FORMALIN TEST IN MICE

The method of Dubuisson and Dennis was used $^{11}$. Mice were randomly allotted into groups of five animals each. The animals were given normal saline (10 ml/kg i.p), morphine (10 mg/kg, i.p) or each of the column chromatography fractions L, S and Y (100, 200 or 400 mg/kg, i.p), thirty minutes before injection of twenty microlitres of 1% formalin into the right hind paw of mice. The behavioral responses of nociception including biting and licking of the injected paw were noted and the time spent was recorded for up to 30 minutes. The first 5 minutes was considered as the early phase (neurogenic phase) and the last 15 minutes as the late phase (inflammatory phase) of the nociceptive response. Analgesic effect was expressed as a reduction in the time spent in licking or biting of the injected paw.

STATISTICAL ANALYSIS

Data were presented as mean ± SEM and analyzed using the Student’s t-test. Results were considered significant at p<0.05, p<0.0001. Pain inhibition is expressed as simple percentage.

RESULTS

The present study was designed to assess the analgesic activity of the fractions of Stereospermum kunthianum, a medicinal plant used in traditional medicine practice for its analgesic properties. It was evaluated on chemical-induced nociception using acetic acid and formalin that so both peripherally and centrally mediated effects were investigated. Table 1 shows the effect of the vacuum liquid chromatography fractions A, B and C on the acetic acid-induced mouse writhing assay. The fractions significantly (p<0.0001) inhibited the writhes compared with the normal saline treated animals. The percentage inhibition for the various fractions were A (78.9; 85.0; 87.0), B (76.5; 78.5; 85.7) and C (81.6; 81.6; 82.0) at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively. At equivalent doses of 100 mg/kg, the analgesic effects of the fractions were comparatively more pronounced than that of acetylsalicylic acid. The effect of fraction C was found to have peaked at the dose of 100 mg/kg. Tables 2, 3 and 4 show the effects of fractions L, S and Y respectively in the formalin pain test. The fractions significantly reduced behavioral responses of nociception in both phases of the formalin-induced pain, however, to varying degrees. Fraction L produced a dose-
dependent and significant (p<0.0001) inhibition of both phases of the formalin-induced pain with a more pronounced effect on the late phase than the early phase at all the tested doses (Table 2). Its effect at the highest dose (400 mg/kg) was similar to that produced by morphine (10 mg/kg) in the early phase. Similar results were observed with fraction Y except that it showed more activity on the late phase than the early phase at the lower doses (Table 4). Although fraction S significantly inhibited both phases of the formalin-induced pain in mice, it showed a much greater effect on the early phase than on the late phase (Table 3). Its effect was also found to have peaked at a dose of 200 mg/kg.

Values are mean ± SEM. *P<0.0001 significantly different from the normal saline treated animals, student’s t-test (n=5). Inhibition of pain is expressed as a percentage in parentheses.

**Figure 1**
Table 1: Effect of vacuum liquid chromatography fractions of stem bark on acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of writhes in 30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Normal saline (30 ml/kg)</td>
<td>61.5 ± 2.63</td>
</tr>
<tr>
<td>S. kunthianum (100 mg/kg)</td>
<td>61.5 ± 2.63</td>
</tr>
<tr>
<td>S. kunthianum (200 mg/kg)</td>
<td>61.5 ± 2.63</td>
</tr>
<tr>
<td>S. kunthianum (400 mg/kg)</td>
<td>61.5 ± 2.63</td>
</tr>
<tr>
<td>Acetylsalicylic acid (300 mg/kg)</td>
<td>61.5 ± 2.63</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, **P<0.0001 significantly differently from the normal saline treated animals, Student’s t-test (n=5). Pain inhibition is presented as percentage.

**Figure 2**
Table 2: Effect of column chromatography fraction L of Stereospermum kunthianum stem bark on formalin-induced pain in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (sec) spent in licking or biting of the injected paw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early phase 0–5 mins late phase 15–30 mins Percentage</td>
</tr>
<tr>
<td>Normal saline (5 ml/kg)</td>
<td>115.5 ± 0.73                                 134.8 ± 0.59</td>
</tr>
<tr>
<td>S. kunthianum (100 mg/kg)</td>
<td>67.50 ± 0.37**                        41.5</td>
</tr>
<tr>
<td>S. kunthianum (200 mg/kg)</td>
<td>62.4 ± 0.18**                        45.9</td>
</tr>
<tr>
<td>S. kunthianum (400 mg/kg)</td>
<td>54.6 ± 0.21**                        52.7</td>
</tr>
<tr>
<td>Morphine (10 mg/kg)</td>
<td>54.6 ± 0.21**                        100</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *P<0.05, **p <0.0001, significantly differently from the normal saline treated animals, Student’s t-test (n = 5). Pain inhibition is presented as percentage.

**Figure 3**
Table 3: Effect of column chromatography fraction S of stem bark on formalin-induced pain in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (sec) spent in licking or biting of the injected paw</th>
</tr>
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<tbody>
<tr>
<td></td>
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Values are mean ±SEM, *P<0.05,**P <0.0001, significantly differently from the normal saline treated animals, Student t-test (n = 5). Pain inhibition is presented as percentage.
DISCUSSION

The results of the present study demonstrated that the fractions of Stereospermum kunthianum stem bark possessed analgesic activity evident in the two analgesic models, which is suggestive of the presence of both centrally and peripherally mediated mechanisms. In the acetic acid-induced abdominal constriction test, the fractions of Stereospermum kunthianum stem bark dose-dependently and significantly reduced the abdominal writhing. Acetic acid is believed to act indirectly by inducing the release of prostaglandins as well as lipoxygenase products into the peritoneum which stimulate the nociceptive neurons sensitive to the non-steroidal anti-inflammatory drugs hence the test is useful for the evaluation of mild analgesic non-steroidal anti-inflammatory compounds. Therefore, the result of the acetic acid-induced writhing strongly suggests that the mechanism of this action may be linked partly to inhibition of lipoxygenase and/or cyclooxygenase in the peripheral tissues, thereby reducing prostaglandin synthesis and interfering with the mechanism of transduction in primary afferent nociceptors. In the formalin test which is sensitive for various classes of analgesic drugs, administration of the fractions of Stereospermum kunthianum demonstrated significant inhibition in both the early and late phases. Similarly, morphine produced marked inhibition of both phases. The centrally acting drugs, such as narcotics, inhibit both phases equally, while peripherally acting drugs such as aspirin and indomethacin only inhibit the late phase. Inhibition of both phases of pain as observed with the fractions in this study suggests that they contain active analgesic principles acting both centrally and peripherally. Peripheral action in the formalin test is supported by the results recorded in the acetic acid-induced writhing test. The present findings corroborate our earlier findings in the aqueous stem bark extract of Stereospermum kunthianum. However, the fractions showed more pronounced analgesic activity compared to the aqueous stem bark extract, particularly in the formalin – induced pain model.

CONCLUSION

It can be concluded that the fractionation of Stereospermum kunthianum stem bark led to increased analgesic activity which is mediated via peripheral and central mechanism.

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References

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