Stereospermum kunthianum (Cham, Sandrine Petit), family Bignoniaceae (Juss. nom. Cons.) is a genus of tropical Africa and Asia. It is native to Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, Kenya, Mozambique, Senegal, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia, and Zimbabwe. Stereospermum kunthianum is distributed from Senegal to East Africa. It is found mainly in the Sudano-Guinean savannahs on altitudes between 500 – 2400 m, mean annual temperature of up to 40°C and annual mean rainfall of 450 – 900 mm (1, 2). The genus Stereospermum is represented in Nigeria by two species: Stereospermum kunthianum (Cham) which grows on the savannah, and Stereospermum acuminatissimum (K. Schum) which grows in the forest. Stereospermum kunthianum, also referred to as pink jacaranda, in English, is known locally as sansami among the Hausa of Northern Nigeria; umana among the Tiv of Middle Belt of Nigeria, ayada among the Yoruba of South West Nigeria, and alakiriti among the Igbo of South East Nigeria (1, 3, 4). Various morphological parts of Stereospermum kunthianum are used in traditional medicine to treat an array of human ailments. The pods are chewed with salt to treat coughs and are used in treatment of ulcers, leprosy, skin eruptions and venereal diseases, while the stem bark decoction or infusion is used to cure bronchitis, pneumonia, coughs, rheumatic arthritis and dysentery. The twigs are chewed to clean teeth and to treat toothache. The roots and leaves have been found useful in treating venereal diseases, respiratory ailments and gastritis (4). The efficacy of the water extract of Stereospermum kunthianum in human complement system fixation in-vitro has been reported (5). The antiplasmodial activity of naphthoquinones and one anthraquinone from the lipophilic extract of the root bark of Stereospermum kunthianum has also been reported (6). The analgesic activity of the aqueous extract of Stereospermum kunthianum stem bark was investigated using various pain models in mice or rats in order to validate its local use to relief pain in ailments accompanied with pain.

ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF STEREOSPERMUM KUNTHIANUM (Cham, Sandrine Petit) STEM BARK

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Abstract: The analgesic activity of the aqueous extract of Stereospermum kunthianum stem bark was studied using the acetic acid-induced writhing, the hot plate test, tail flick test, and formalin pain test in mice or rats. The aqueous extract (100, 200 or 400 mg/kg) produced a significant (p<0.001) dose-dependent inhibition of abdominal writhes in mice. The results of the hot plate test showed a dose-related and time-dependent significant (P<0.001) increase in pain threshold in mice 60 minutes after treatment at all the doses used in the study. The extract (100, 200 or 400 mg/kg) showed significant (p <0.05) dose-dependent increase in tail flick latency in rats and also inhibited both phases of the formalin pain test in mice with a more intense effect on the first phase than the second phase. The results indicate that the aqueous extract of Stereospermum kunthianum stem bark possesses analgesic activity which is mediated through both central and peripheral mechanisms. This is a possible rationale for its use in traditional human medicine for pain relief.

Keywords: Stereospermum kunthianum, analgesic activity, acetic acid, formalin, hot plate, tail flick, mice, rats

― Stereospermum kunthianum (Cham, Sandrine Petit), family Bignoniaceae (Juss. nom. Cons.) is a genus of tropical Africa and Asia. It is native to Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, Kenya, Mozambique, Senegal, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia, and Zimbabwe. Stereospermum kunthianum is distributed from Senegal to East Africa. It is found mainly in the Sudano-Guinean savannahs on altitudes between 500 – 2400 m, mean annual temperature of up to 40°C and annual mean rainfall of 450 – 900 mm (1, 2). The genus Stereospermum is represented in Nigeria by two species: Stereospermum kunthianum (Cham) which grows on the savannah, and Stereospermum acuminatissimum (K. Schum) which grows in the forest. Stereospermum kunthianum, also referred to as pink jacaranda, in English, is known locally as sansami among the Hausa of Northern Nigeria; umana among the Tiv of Middle Belt of Nigeria, ayada among the Yoruba of South West Nigeria, and alakiriti among the Igbo of South East Nigeria (1, 3, 4). Various morphological parts of Stereospermum kunthianum are used in traditional medicine to treat an array of human ailments. The pods are chewed with salt to treat coughs and are used in treatment of ulcers, leprosy, skin eruptions and venereal diseases, while the stem bark decoction or infusion is used to cure bronchitis, pneumonia, coughs, rheumatic arthritis and dysentery. The twigs are chewed to clean teeth and to treat toothache. The roots and leaves have been found useful in treating venereal diseases, respiratory ailments and gastritis (4). The efficacy of the water extract of Stereospermum kunthianum in human complement system fixation in-vitro has been reported (5). The antiplasmodial activity of naphthoquinones and one anthraquinone from the lipophilic extract of the root bark of Stereospermum kunthianum has also been reported (6). The analgesic activity of the aqueous extract of Stereospermum kunthianum stem bark was investigated using various pain models in mice or rats in order to validate its local use to relief pain in ailments accompanied with pain.

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MATERIALS AND METHODS

Plant material

The fresh stem bark of the *Stereospermum kunthianum* was collected in Idi-Oke, Ogun State, Nigeria in March, 2006. Identification and botanical authentication were done by Mr. Usang Felix Inah of the Forestry Research Institute of Nigeria, Ibadan, where a voucher specimen (No. FHI 107277) was deposited for future reference.

Extraction

The stem bark was carefully separated from the woody part, cut into small bits, sun-dried and pulverized using a grinder (Lab. mill, serial no. 4745, Christy and Norris Ltd., England). The powdered material (400 g) was macerated in 2 L of hot distilled water (60°C) which was allowed to cold and filtered after 24 h. The filtrate was evaporated to dryness in an oven set at 40°C until constant weight was obtained. The yield was 26.4% with reference to the powdered stem bark. The extract obtained was stored in closed containers in the refrigerator at -4°C till required.

Phytochemical screening

The powdered stem bark of *Stereospermum kunthianum* was screened for the presence of bioactive constituents by the method of Odebiyi and Sofowora (7).

Animals

Approval for the use of animals for analgesic experiments had been obtained from the Ethical Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Wistar rats and 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 Mill, Plc. Ewu, Nigeria) and water.

Acute toxicity

Acute toxicity was performed according to the OECD-423 guidelines (8). Intraperitoneal acute toxicity was studied in rats and mice. The animals had free access to feed and drinking water. Adult Wistar rats (110-130 g) and Swiss mice (20–25 g) of either sex were randomly allocated into groups of five animals per group. They were administered intraperitoneally aqueous extract (1, 2, 3, 4, 5, 6, 7, or 8 g/kg) or distilled water (5 mL/kg or 10 mL/kg for rats and mice, respectively). General symptoms of toxicity and mortality were observed for 24 h, after which the animals were left for further 14 days for delayed toxicity. If mortality was not observed, the procedure was repeated until the highest dose of 8 g/kg was obtained.

Acetic acid-induced writhing in mice

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 aqueous extract (100, 200 or 400 mg/kg, i.p.). Acetylsalicylic acid or extract was administered to the animals 30 and 60 min, respectively, before intraperitoneal injection of acetic acid (10 mg/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 min following acetic acid injection and the analgesic effect determined as described by Koster et al. (9).

Hot plate test in mice

The method of Woolfe and MacDonald (10) but modified by Zimer et al. (11) was used. The responsiveness to nociceptive stimulus was measured with the hot plate analgesimeter (Harvard Apparatus Ltd., UK). The hot plate temperature was maintained at 55 ± 1°C. The licking, biting of the hind paw or jumping was taken as an indication of pain perception. Mice screened 24 h previously for suitable reaction time were used. A cut-off time of 60 s was adopted to prevent tissue damage. Animals were placed on the hot plate surface in a glass cylinder of about 20 cm in diameter. The time in seconds between the placement and licking, biting of the hind paw or jumping was recorded as the index of response latency. Each animal served as its own control, thus one hour before pretreatment, its basal latency was taken twice at 15 min intervals. The mean of these two values constituted the basal response latency prior to treatment. The animals were randomly divided into groups of five mice each. The animals were administered normal saline (10 mL/kg i.p.), extract (100, 200 or 400 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) 30 min before placement on the hot plate. Response latencies were taken at 30, 60, 90 and 120 min and analgesic effect determined as described (10, 11).
Tail flick test in rats
The analgesic effect was determined according to the method of D’Amour and Smith (12). A radiant tail flick analgesimeter (Harvard Apparatus Ltd., UK) was used. The flick responses were elicited by applying a constant infrared light beam maintained at 70% intensity to the tail end of the rat. The tail flick latency in seconds constituted the animal’s reaction time to the heat stimulus. Rats screened 24 h previously for suitable reaction time based on their tail flick latency of 10 – 15 s were used. A cut-off time of 30 s was adopted to prevent tissue damage. Each animal served as its own control, thus one hour prior to pretreatment, its basal tail flick latency was taken twice at 15 min intervals. The mean of the two values constituted the basal tail flick latency before pretreatment. Rats were divided into groups of five animals per group. Normal saline (10 mL/kg, i.p.), extract (100, 200 or 400 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) was administered 30 min before measurement of tail flick latency. Tail flick latency was recorded at 30, 60, 90, and 120 min and the analgesic effect determined (12).

Formalin test in mice
The test was performed according to described standard procedure (13, 14). Mice were randomly allotted into five groups of five animals each. The animals were given normal saline (10 mL/kg i.p.), extract (100, 200 or 400 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) 30 min before subcutaneous injection of 20 microlitres of 1% formalin into the right hind paw of mice. The time (in seconds) spent on licking and biting of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min immediately after formalin injection (first phase) and 15-30 min after formalin injection (second phase), representing the neurogenic and inflammatory pain response, respectively. Analgesic effect was expressed as a reduction in the time spent in licking or biting of the injected paw.

Table 1. Effect of aqueous extract of Stereospermum kunthianum stem bark on acetic acid-induced writhing test in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of writhes (per 30 min)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>59.0 ± 3.50</td>
<td>-</td>
</tr>
<tr>
<td><em>S. kunthianum</em> (100 mg/kg)</td>
<td>14.75 ± 2.32*</td>
<td>75.0</td>
</tr>
<tr>
<td><em>S. kunthianum</em> (200 mg/kg)</td>
<td>13.75 ± 2.17*</td>
<td>76.7</td>
</tr>
<tr>
<td><em>S. kunthianum</em> (400 mg/kg)</td>
<td>11.5 ± 6.65*</td>
<td>80.5</td>
</tr>
<tr>
<td>Acetylsalicylic acid (100 mg/kg)</td>
<td>23.5 ± 1.55*</td>
<td>60.2</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. \*p < 0.001, significantly different from the normal saline treated group. Student’s t-test (n = 5 per group). Inhibition of pain is expressed as a percentage.

Table 2. Effect of aqueous extract of Stereospermum kunthianum stem bark on the latency time in the hot plate test in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latency time (seconds) at time post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Normal saline (10 mL/kg)</td>
<td>28.82 ± 0.48</td>
</tr>
<tr>
<td><em>S. kunthianum</em> (100 mg/kg)</td>
<td>31.56 ± 1.18</td>
</tr>
<tr>
<td><em>S. kunthianum</em> (200 mg/kg)</td>
<td>28.84 ± 0.54</td>
</tr>
<tr>
<td><em>S. kunthianum</em> (400 mg/kg)</td>
<td>30.64 ± 0.66</td>
</tr>
<tr>
<td>Morphine (10 mg/kg)</td>
<td>31.46 ± 0.91</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. \*p < 0.05; significantly different from the normal saline treated group. Student’s t-test (n = 5 per group). Values in parentheses are percentage pain inhibition.
**RESULTS**

Table 1 shows the effect of the extract on the acetic acid-induced mouse writhing. The extract (100, 200 or 400 mg/kg, i.p.) caused a dose-dependent and significant (p < 0.001) inhibition of the writhes. The percentage inhibitions were 75.0, 76.7, 80.5 and 60.2 for extract (100, 200 or 400 mg/kg, i.p.) and acetylsalicylic acid (100 mg/kg, sc.), respectively. Comparatively, the extract at equal doses (100 mg/kg) produced a higher effect than acetylsalicylic acid. The extract (100, 200 or 400 mg/kg, i.p.), elicited a dose-related increase in the latency response in mice in the hot plate test (Table 2). This effect (analgesic effect) was significant (p < 0.001) compared to the distilled water treated mice. Morphine (10 mg/kg, i.p.) produced a significant (p < 0.0001) increase in the latency response compared to the distilled water treated mice. The increases in the latency elicited by the extract (100, 200 or 400 mg/kg) were significantly (p < 0.05) lower compared to morphine-administered group. In the tail flick test the extract (100, 200 or 400 mg/kg, i.p.) exhibited a dose-dependent increase in the tail flick latency in rats (Table 3). The increase was significant (p < 0.05) for 100 and 200 mg/kg and (p < 0.001) for 400 mg/kg, respectively, at 60 min post treatment time point compared to the distilled water treated rats. Morphine (10 mg/kg, i.p.) elicited a significant (p < 0.001) increase in the tail flick latency at 60 min post treatment time point. The increases in the tail flick latency elicited by the extract (100, 200 or 400 mg/kg, i.p.) were significantly (p < 0.05) lower compared to morphine-administered group. The effect of the extract on formalin test is shown in Table 4.

**Statistical analysis**

Data were expressed as the mean ± SEM of at least five experiments and analyzed using the Student’s t-test. Results were considered significant when p < 0.05.
Table 4. The extract inhibited both phases of the formalin test with a more intense effect on the first phase than the second phase. The extract significantly (p < 0.0001) and dose dependently inhibited the first phase of the formalin-induced pain in mice. The effect of the extract on the second phase of formalin-induced pain was significant (p < 0.05, p < 0.001) at 200 mg/kg and 400 mg/kg, respectively. Morphine inhibited both phases of the formalin-induced pain with 48.4 and 100% inhibition in the first and second phase, respectively. The effect of morphine in the first phase was not significantly different from that produced by 400 mg/kg body weight of the extract.

The phytochemical screening of the powdered stem bark of Stereospermum kunthianum revealed the presence of alkaloids, tannins, phlobatannins, saponins, cardiac glycosides, anthraene derivatives and reducing sugars. The extract caused reduced agility, lower food and water intake in the rats and mice within 24 h following intraperitoneal injection of the extract in the acute toxicity study. These symptoms disappeared after 24 h. No deaths were recorded even at the highest dose of 8 g/kg body weight within the 14 days observation period.

DISCUSSION

Several acute and chronic pain models in rodents were employed in evaluating the analgesic effect of the aqueous extract of Stereospermum kunthianum. It is necessary to apply tests which differ with respect to stimulus quality, intensity and duration, to obtain as complete a picture as possible of analgesic properties of a substance using behavioral nociceptive tests (15). The results obtained indicate that the extract possesses a moderate dose-dependent analgesic effect on the various pain models used. Acetic acid causes inflammatory pain by increasing capillary permeability (16). Writhes induced by noxious chemicals injected intraperitoneally is due to sensitization of nociceptors by prostaglandins. This test is useful for evaluation of mild analgesic non-steroidal antiinflammatory compounds (17). The aqueous extract of Stereospermum kunthianum stem bark caused a significant and dose-dependent inhibition of writhes in mice. The effect of the extract was comparable to that of acetylsalicylic acid, a cyclooxygenase inhibitor. This suggests that the extract may have a peripheral analgesic action. The extract showed significant effects in the hot plate and tail flick tests. Centrally acting analgesic drugs elevate pain threshold of animals to heat and pressure. The hot plate induced pain indicates narcotic involvement (18), whereas the tail flick test is considered selective for opioid-like analgesic compounds. The results obtained indicate a significant, dose and time related analgesic activity of the extract in both the hot plate and tail flick assays. The effect of the extract on these pain models indicate that it might be centrally acting. The extract inhibited both phases of the formalin-induced pain, with a more intense effect at the lower doses on the first phase than the second phase. However, at the highest dose (400 mg/kg) the extract exhibited an equipotent effect on both phases. Formalin exhibits neurogenic, inflammatory and tonic pain as in clinical pain situations. Drugs which act mainly centrally such as narcotic analgesics inhibit both phases of pain in this model while peripherally acting drugs, such as aspirin or indomethacin, only inhibit the late phase (19). The inhibitory effect of the extract on both phases of the formalin pain model confirms its analgesic activity via central and peripheral mechanisms. The phytochemical screening indicates the presence of various bioactive constituents in the powdered Stereospermum kunthianum stem bark. Bioactive constituents in plants suggest possible drug basis (20). The aqueous extract did not caused mortality even at the highest acute toxicity dose level of 8 g/kg used in the study and hence was considered to be relatively safe for administration at these doses.

With the results of the present study taken together, it is concluded that the aqueous extract of Stereospermum kunthianum stem bark possesses analgesic activity, which may be mediated via peripheral and central mechanisms. This provides evidence for its use locally in human medicine to relief pain in the treatment of ailments accompanied with pain.

REFERENCES


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