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Full Length Research Paper

Antidiarrhoeal activities of aqueous extract of *Stereospermum kunthianum* (Cham, Sandrine Petit) stem bark in rodents

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The antidiarrhoeal effect of the aqueous extract of *Stereospermum kunthianum* (Cham, Sandrine Petit) stem bark was investigated in *in vivo* experimentally-induced diarrhoeal models using mice and rats. The extract (100, 200 or 400 mg/kg) dose-dependently reduced castor oil-induced intestinal transit in mice, with the greatest effect observed with the 200 mg/kg body weight of the extract. The pretreatment of mice with the extract (100, 200 or 400 mg/kg) caused a dose-dependent and significant ($p < 0.05$) delay in the onset of diarrhoea, frequency of stooling, decreased weight of wet stools and the general diarrhoeal score in mice. The effect of the extract on the normal intestinal transit in mice was not significant. However, in the phenol red meal test in rats, the extract (100, 200 or 400 mg/kg) in a dose-related manner, significantly ($p < 0.05$) decreased the normal intestinal transit in rats, without a significant effect on the gastric emptying compared to loperamide (5 mg/kg) treated rats. The extract produced no significant effect on the castor oil-induced fluid accumulation in rats. Also, the extract produced no mortalities in mice at a maximum oral dose of 8 g/kg; it is therefore well tolerated and relatively safe by oral route. The results indicate that the aqueous extract possesses antidiarrhoeal activity. This is a possible reason for its antidiarrhoeal use in traditional medicine.

Key words: *Stereospermum kunthianum*, antidiarrhoeal activity, mice, rats.

INTRODUCTION

Acute diarrhoea constitutes a serious problem and is a leading cause of morbidity and mortality amongst the children in the developing countries (Coker et al., 1998). Despite the emergence of a number of drugs, none has found a place in the routine management of diarrhoea (Farthing, 2002). Therefore, there is need for continuous search for drugs that might inhibit the process of diarrhoea development. Medicinal plants are one of the useful areas of research in this regard. Medicinal plants which are believed to possess antidiarrhoeal activities are used in most rural areas of the developing countries in the management of diarrhoea. A number of these plants have been studied to provide pharmacological evidence for their local antidiarrhoeal use (Offiah and Chikwendu, 1999; Odetola and Akojenu, 2000; Abdullahi et al., 2001; Kumar et al., 2001; Shoba and Molly, 2001; Umukoro and Ashorobi, 2003; Adeyemi et al., 2003; Nwafor et al., 2005). *Stereospermum kunthianum* (Cham, Sandrine Petit), family *Bignoniaceae* is used by most people of the Sudano-Guinea savannah to treat various ailments which include dysentery, diarrhoea, bronchitis, pneumonia and coughs, wounds, ulcers, leprosy, respiratory ailments, venereal diseases, asthenia and exhaustion, and as a charm against witchcraft (Hutchison and Dalziel, 1963; Von Maydell, 1986; Keay et al.,1989; Gill, 1992). The efficacy of the water extract of *S. kunthianum* in human complement system fixation *in vitro* has been reported (Drissa et al., 2002). Antiplasmodial activity of naphthaquinones and one anthraquinone from the lipophilic extract of the root bark of *S. kunthianum* has also been reported (Onegi et al., 2002). No previous scientific literature was found on its antidiarrhoeal activity. The

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antidiarrhoeal activity of the aqueous extract of *S. kunthianum* stem bark was therefore investigated in mice and rats' *in vivo* experimentally-induced diarrhoeal models.

MATERIALS AND METHODS

Plant material

The fresh stem bark of the *S. kunthianum* was collected in Idi-Okpe, 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 pieces 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 distilled water at an initial temperature of 60°C, allowed to cool and filtered after 24 h. The filtrate was evaporated to dryness in an oven set at 40°C until a 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.

Animals

Approval for the use of animals for antidiarrhoeal 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 and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals maintained under standard laboratory conditions (12 h light and dark cycle) had free access to standard chow (Bendel Feeds and Flour mill Plc. Ewu, Nigeria) and drinking water. Animals were accustomed to the experimental conditions prior to the experiment.

Acute toxicity

Acute toxicity was performed according to the OECD-423 guide lines (Ecobichon, 1997). Swiss mice (20 – 25 g) of either sex were used. The animals were administered with distilled water (10 ml/kg), the vehicle or aqueous extract (1 - 8 g/kg) of *S. kunthianum* stem bark orally (p.o). The animals were observed for 6 h within the first 24 h, then for further 14 days for deaths and manifestation of toxic effects. The toxic effects observed included agility, muscular tonus, tremors, and convulsions, breathing patterns, water and food intake. When no mortality was observed, the procedure was repeated up to the highest dose of 8 g/kg (Note: breathing patterns, water and food intake are not overt effects).

Intestinal transit time in mice

The effect on normal intestinal propulsion in mice was tested using the charcoal meal method (Aye -Than et al., 1989). Overnight mice of either sex (5 per group) were administered with distilled water (10 ml/kg, p.o), extract (100, 200 or 400 mg/kg, p.o.) or morphine (10 mg/kg, sc.). 30 min later, the animals were administered orally with a freshly prepared standard charcoal test meal (0.2 ml per mouse of a 10% activated charcoal suspension in gum acacia). After 30 min the animals were sacrificed. The small intestine was isolated and the distance traversed by the charcoal meal from the pylorus to the ileocaecal junction was measured. The length of the entire small intestine was also measured. The peristaltic index for each mouse was then expressed as a percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine.

In the castor oil-induced intestinal transit measurements, five groups of mice (5 per group) were administered with distilled water (10 ml/kg, p.o), extract (100, 200 or 400 mg/kg, p.o.) or morphine (10 mg/kg, sc.) 30 min before oral administration of castor oil (0.2 ml per mouse). 30 min later, a standard charcoal meal prepared as described above was given to each mouse by oral route. After 30 min the animals were sacrificed. The effect on the intestinal transit was assessed (Aye -Than et al., 1989).

Castor oil–induced diarrhoea in mice

Adult Swiss mice (5 per group) were administered with distilled water (10 ml/kg, p.o), extract (100, 200 or 400 mg/kg, p.o.) or morphine (10 mg/kg, sc.), 30 min before oral administration of castor oil 0.2 ml per mouse (Izzo et al., 1992). Each mouse was placed under a glass funnel, the floor of which was lined with weighed filter paper (Whatman No.1) and was observed for 4 h. The parameters observed included the onset of diarrhoea stool (first wet stool that leaves a halo on the filter paper), number of wet stools, weight of wet stools and total weight of faecal output. A numerical score based on stool consistency was assigned as follows: normal stool = 1, mild or semi solid stool = 2 and copious or watery stool = 3. Calculations were made for the delay diarrhoeal onset and purging index by comparison with the control group. The *in vivo* antidiarrhoeal index ($ADI_{in\ vivo}$) was then expressed according to the formula (Aye-Than et al., 1989):

$$ADI_{in\ vivo} = \sqrt[3]{D_{freq} \times G_{meq} \times P_{freq}}$$

where: D_{freq} = Delay in defaecation time or diarrhoeal onset (in % of control), G_{meq} = Gut travel reduction (in % of control) and P_{freq} = purging frequency as number of stool reduction (in % of control).

Intestinal fluid accumulation

Adult Wistar rats (6 per group) were administered orally with distilled water (5 ml/kg), extract (200 mg/kg) or indomethacin (10 mg/kg), 1 h before they received 2 ml each of castor oil by oral route (Robert et al., 1976). After 1 h the animals were sacrificed. The entire small intestine was isolated after ligation at the pyloric end and the ileocaecal junction respectively, and weighed. The intestinal contents were expelled into a graduated tube and the volume determined. The intestine was re-weighed and the difference between full and empty intestine was obtained.

Gastric emptying and normal intestinal transit in rats

The extract produced no significant effect in the normal intestinal transit in mice. It was therefore decided to test this effect in rats. The method used was a modification of those already described (Reynell and Spray, 1956; Droppleman et al., 1980). Adult Wistar rats of either sex (previously fasted for 24 h prior to experiment) were randomly allotted to groups of five animals each. The rats were administered orally with distilled water (5 ml/kg), extract (100, 200 or 400 mg/kg) or loperamide (5 mg/kg). 1 h later (30 min for distilled water group) each rat received 1.5 ml (1.57 g) of the phenol red test meal prepared freshly by mixing 0.07% phenol red in 2% carboxymethylcellulose solution. After 30 min, the rats were sacri-

Table 1. Effect of aqueous extract of *S. kunthianum* stem bark on normal intestinal transit in mice.

Treatment	CML(cm)	IL(cm)	Peristaltic index (%)
Distilled water (10 mg/kg)	26.92±3.06	45.57±2.86	60.25±7.40
S. kunthianum (100 mg/kg)	34.70±3.12	50.52±1.81	69.27±7.32
S. kunthianum (200 mg/kg)	33.93±3.03	50.82±2.84	68.18±7.36
S. kunthianum (400 mg/kg)	28.60±1.93	48.66±1.85	59.18±4.94
Morphine (10 mg/kg)	10.92±1.93	47.54±0.83	23.16±4.36[a]

Values are mean ± SEM.
[a]P < 0.05 significantly different from the control, Student's t-test (n = 5 - 6 per group).
CML = Charcoal meal length; IL = intestinal length.

Table 2. Effect of aqueous extract of *S. kunthianum* stem bark on castor oil-induced intestinal transit in mice.

Treatment	CML(cm)	IL(cm)	Peristaltic index (%)	Inhibition (%)
Distilled water (10 ml/kg)	29.5±5.3	50.25±4.07	58.27+8.69	-
S. kunthianum (100 mg/kg)	22.88±1.53	47.63±1.05	48.10±2.22	17.45
S. kunthianum (200 mg/kg)	22.0±2.74	49.63±0.24	44.30±5.40	23.97
S. kunthianum (400 mg/kg)	23.06±1.97	49.70±1.59	45.82±4.23	21.37
Morphine (10 mg/kg)	17.5±0.87	52.25±1.93	33.60±1.93[a]	42.34

Values are mean ± SEM of at least five experiments.
[a]P < 0.05, significantly different from the control, Student's t-test .
CML = Charcoal Meal Length; IL = Intestinal Length.

ficed. The abdominal cavity was dissected; the full stomach and the entire small intestine were isolated. The full stomach was weighed after which it was gently opened and the content removed. Excess moisture was blotted off and the empty stomach re-weighed. The difference between the full and empty stomach was determined. The difference between the weight of 1.5 ml (1.57 g) of the test meal and the weight of the stomach content constituted the quantity emptied from the stomach during the test period. The isolated intestine was carefully inspected and the distance travelled by the phenol red test meal from the pyloric end to the ileocaecal junction was measured. The length of the entire small intestine was also measured. The peristaltic index was expressed as a percentage of the distance traveled by the phenol red test meal relative to the total length of the small intestine (Aye-Than et al., 1989).

Statistical analysis

Data are Mean ± S.E.M. and analyzed using the Student's t-test. Results were considered significant when $p < 0.05$.

RESULTS

Intestinal transit

The extract (100, 200 or 400 mg/kg) produced no significant effect on the normal intestinal transit in mice. However, morphine (10 mg/kg) significantly reduced the intestinal transit time in the mice (Table 1). Table 2 shows the effect of the extract on castor oil-induced intestinal transit in mice. The extract (100, 200 or 400 mg/kg) caused a dose-dependent reduction in the distance traversed by the charcoal meal compared to the distilled water treated mice. The highest effect of 23.97% inhibition was obtained with a dose of 200 mg/kg body weight of the extract.

Castor oil-induced diarrhoea

4 h after castor oil administration, all the mice in the control group produced copious diarrhoea. Pretreatment of mice with the aqueous extract (100, 200 or 400 mg/kg) caused a dose-dependent and significant ($p < 0.05$) delay in the onset of diarrhoea, frequency of stooling (reduction in number of wet stools and total stools), decrease in weight of wet stools and the general diarrhoea score (Table 3). The highest antidiarrhoeal index of 19.76% was obtained with the dose of 200 mg/kg body weight of the extract which was comparatively less than 63.04% produced by morphine (Table 4).

Intestinal fluid accumulation

The results of the effect of the aqueous extract of *S. kunthianum* stem bark in intestinal fluid accumulation are presented on Table 5. Oral administration of castor oil to rats produced intestinal fluid volume of 2.05 ± 0.35 ml. The aqueous extract (200 mg/kg), when given 1 h before castor oil, reduced the fluid volume to 1.97 ± 0.50 ml which was not significantly different from that of distilled water treated rats. Similarly, there was no significant reduction in the weight of the intestinal content of the

Table 3. Effect of aqueous extract (oral) of *S. kunthianum* stem bark on castor oil-induced diarrhoea in mice.

Treatment (mg/kg)	Onset of diarrhoea (min)	Number of wet stools	Total number of stools	Total weight of wet stools	Total weight of stools	Total diarrhoeal score	Protection (%)
Distilled water (10 ml/kg)	72.0±5.60	6.40±0.98	9.0±1.48	0.84±0.16	0.84±0.16	24.6±3.93	-
S. kunthianum(100)	77.80±12.56	6.40±1.50	7.40±1.57	0.55±0.06[a]	0.58±0.07[a]	21.20±4.59	13.82
S. kunthianum (200)	110.5±14.92[a]	3.33±0.56[a]	4.0±0.58[a]	0.35±0.03[a]	0.42±0.03[a]	11.83±1.71[a]	51.91
S. kunthianum.(400)	105.5±18.14[a]	5.33±0.67[a]	7.17+0.95[a]	0.63±0.13[a]	0.63±0.13[a]	19.67±2.39[a]	20.04
Morphine (10 mg/kg,sc)	201.83±17.90[a]	1.50±0.67[a]	2.0±1.0[a]	0.34±0.19[a]	0.35±0.20[a]	5.5±2.62[a]	77.64

Values are mean ± S.E.M. of at least five experiments.
[a]P < 0.05, significantly different from the control, Student's t-test.

Table 4. *In vivo* antidiarrhoeal index of aqueous extract of *S. kunthianum* stem bark in mice.

Treatment	Delay in defaecation time or onset (Dfreq)	Gut meal travel distance(Gmeq)	Purging frequency in no. of tools(Pfreq)	*In vivo* antidiarrhoeal Index (%)
Distilled water (10 ml/kg)	72.0±5.60	58.27±8.69	6.40±0.98	-
S. kunthianum (100 mg/kg)	77.8±12.56	48.10±2.22	6.40±1.50	0.0
S. kunthianum (200 mg/kg)	110.5±14.92	44.30±5.40	3.33±0.56	29.76
S. kunthianum (400 mg/kg)	105.5±18.14	45.82±4.23	5.33±0.67	19.24
Morphine (10 mg/kg)	201.83±17.91	33.60±1.80	1.50±0.67	63.04

Values are mean ±SEM of five experiments.

Table 5. Effect of aqueous extract of *S. kunthianum* stem bark on castor oil-induced intestinal fluid accumulation in rats.

Treatment	Weight of intestinal content (g)	Volume of intestinal content (ml)
Distilled water (5 ml/kg)	2.96±0.27	2.05±0.35
S. kunthianum (200 mg/kg)	2.75±0.55	1.97±0.50
Indomethacin (5 mg/kg)	1.91±0.28[a]	1.15±0.22[a]

Values are mean ± SEM of six experiments.
[a]P < 0.05,significantly different from the control, Student's t-test.

extract-treated animals compared to that of distilled water-treated rats. It was however, observed that the secretions obtained in the animals pretreated with the extract were more viscous compared to that of the distilled water-treated rats.

Gastric emptying and normal intestinal transit

In the rats administered only distilled water, the phenol red test meal traversed 89.12 ± 2.55% of the total length of the small intestine. The aqueous extract (100, 200 or 400 mg/kg) produced a dose-dependent and significant ($p < 0.05$) decrease in the small intestinal transit with the greatest effect observed with the 200 mg/kg body weight of the extract (Table 6). The effect of the extract (100, 200 or 400 mg/kg) on the normal gastric emptying in the rats was not significant. However, a dose of 5 mg/kg of loperamide significantly ($p < 0.05$) reduced the gastric emptying in the rats (results not presented).

Acute toxicity

The aqueous extract at the doses of 1 - 8 g/kg showed no signs of toxicity in the mice within and after 24 h following oral administration. No deaths were recorded even at the highest dose of 8 g/kg body weight.

DISCUSSION

Diarrhoea may result from disturbed bowel function, in which case there is increased bowel transit, excessive intestinal secretion of water and electrolytes as well as decreased intestinal reabsorption (Gurgel et al., 2001). The results of this study revealed that the aqueous extract of *S. kunthianum* stem bark possessed anti-

Table 6. Effect of aqueous extract of *S. kunthianum* stem bark on normal intestinal transit in rats.

Treatment	Length of test meal (cm)	Length of intestine (cm)	Peristaltic Index (%)	Inhibition (%)
Distilled water (10 ml/kg)	104.0±2.34	117.0±3.27	89.12±2.55	-
S. kunthianum (100 mg/kg)	86.96±4.81	116.14±2.92	74.8±3.08[a]	16
S. kunthianum (200 mg/kg)	80.63±4.07	111.73±1.49	72.18±3.84[a]	19
S. kunthianum (400 mg/kg)	84.2±5.60	113.8±2.06	73.95±4.17[a]	17
Morphine (10 mg/kg)	68.03±8.09	105.8±2.62	64.1±7.22[a]	28

Values are mean ± SEM.
[a]$P < 0.05$ significantly different from the control, Student's t-test (n = 5 - 6 per group).

diarrhoeal activity in castor oil treated animals. The extract dose-dependently inhibited castor oil-induced transit in mice and this indicates possible antispasmodic activity. The frequency and severity of castor oil-induced diarrhea in the mice was inhibited in a dose-related manner by the aqueous extract. There was delay in onset of diarrhoea and the total number of stools, number of wet stools, and weight of wet stools were all dose-dependently decreased, with the highest effect observed with the 200 mg/kg body weight of the extract. These effects were, however, comparatively less than those produced by morphine. The antidiarrhoeal index (ADI) is a measure of the combined effects of the various components of diarrhoea such as onset of diarrhoeal stools, purging frequency, and intestinal frequency. The fact that the extract produced a significant antidiarrhoeal index reinforces its protective action in diarrhoea. Castor oil-induced diarrhoea is related to the release of prostaglandins by the colonic cells (Capasso et al., 1986; Mascolo et al., 1994). Also the delay in castor oil-induced diarrhoea and inhibition of intestinal fluid secretion is known to characterize non-steroidal antiinflammatory drugs (Awounters et al., 1978). Our unpublished data indicate that the aqueous extract of *S. kunthianum* stem bark inhibits inflammation caused by carrageenan. It is therefore likely that the antidiarrhoeal action exerted by the extract may also be attributed to the inhibition of prostaglandin biosynthesis or release. Castor oil is hydrolyzed to ricinoleic acid which causes irritation on gastric mucosa inducing changes in mucosa fluid, electrolytes transport and diarrhoea (Ammon et al., 1975). The extract did not significantly inhibit the castor oil-induced intestinal fluid accumulation and this suggests that its antidiarrhoeal activity may not be related to antisecretory action. The intestinal contents of rats pretreated with the extract were, however, observed to be comparatively more viscous than in the distilled water treated rats and this may be attributed to the significant inhibition of intestinal transit in the phenol red test (Reynell and Spray, 1956; Droppleman et al., 1980). The extract did not remarkably inhibit normal gastric emptying which was significantly inhibited by loperamide. This suggests that its antidiarrhoeal action may not be related to inhibition of gastric emptying. The oral administration of the extract up to 8 g/kg caused no mortalities. The extract is better tolerated and relatively safe at these doses.

In conclusion, the results of the present study provide evidence that support the use of aqueous extract of *S. kunthianum* stem bark in diarrhoeal conditions among the rural dwellers of the Sudano-Guinea savannah. However, it is difficult at this time to clarify the mechanism(s) involved. Further studies are required in his regard.

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