

**STUDIES ON THE ANTIDIARRHOEAL PROPERTIES OF THE ETHANOLIC
EXTRACT OF *KIGELIA AFRICANA* (BIGNONIACEAE).**

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Summary

The anti-diarrhoeal activity of the ethanolic extract of *Kigelia africana* was evaluated using Swiss albino mice based on its ethnomedicinal use in the treatment of diarrhoea. Mice were divided into five groups of five animals each. Diarrhoea was induced by administering 0.3ml of castor oil orally to mice. Group one served as control (normal saline 10 ml/kg), groups 2, 3 and 4 received the ethanolic extract (100, 200 and 500 mg/kg respectively) while group 5 received atropine (0.1 mg/kg). For the small intestinal motility, groups of overnight fasted mice (n=5) received the ethanolic extract of the bark (100, 200 and 500 mg/kg), atropine (0.1 mg/kg) and 10 ml/kg of distilled water prior to the administration of 0.2 ml of activated charcoal. *Kigelia africana* at oral doses of 100 mg/kg, 200 mg/kg and 500 mg/kg caused a marked inhibition of the diarrhoea response following castor oil administration ($P<0.05$). It also significantly ($p<0.0001$) inhibited the small intestinal motility in mice, with the 500 mg/kg dose giving the highest effect in both castor oil-induced diarrhoea and in the small intestinal motility.

When compared with Atropine, its antidiarrhoeal effect at 500 mg/kg was found to be 82 % and 62.7 % respectively on castor oil- induced diarrhoea and on small intestinal motility. The plant thus possesses anti-diarrhoea properties.

Keywords: Castor oil- induced diarrhoea; *Kigelia africana*; Mice; Small intestinal motility.

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Introduction

Secretory diarrhoea caused by enterotoxins (*Vibrio cholerea* and *Escherichia coli*) remains one of the major causes of morbidity and mortality in many tropical countries (1, 2). These toxins appear to function by stimulating transepithelial chloride secretion, thereby increasing the osmotic impetus for fluid secretion (3). The increased secretion enhances the water content and fluidity of the stool that could lead to dehydration, hyponatremia and hypokalemia. The strategy of oral rehydration which has now been available for several years, utilizing the ability of the small intestine to absorb water and salt during glucose absorption even in patients with cholera, can significantly reduce the mortality but not the morbidity of acute diarrhoea(4). Therefore, there is a need for drugs that decrease intestinal hypersecretion, to be used in combination with rehydration solution. In traditional medicine, many plants are claimed to have an antidiarrhoeal effect without scientific basis. The stem bark of the plant *Kigelia africana* Lam, Benth, Family: Bignoniaceae also called *Kigelia pinnata* has a wide reputation in folk medicine for the treatment of malaria, rheumatism, wounds, ulcers, retained placenta, venereal diseases, diarrhoea and to combat infections (5).

To our knowledge there are no available reports on the bioactivity of the ethanolic stem bark extract of *Kigelia africana* with the exception of recent reports that described the analgesic and anti-inflammatory effect of the stem bark (6), antibacterial activity of the stem bark (7), antibacterial activity of the fruits (8) and central nervous stimulant effect of the stem bark (9). In the present study, we evaluated the ethanolic extract for a possible antidiarrhoeal activity in animal models of secretory diarrhoea and its inhibitory effect on intestinal transit.

Materials and Methods

Drugs and Chemicals

Atropine sulphate(Merck, Nigeria); Castor oil (Well's Brand, Nigeria); Gum acacia (Department of Pharmaceutics and Pharmaceutical Technology, University of Benin, Nigeria); Activated charcoal (Department of Pharmaceutics and Pharmaceutical Technology, University of Benin, Nigeria).

Collection and Identification of Plant Material

The barks of *Kigelia africana* were collected in Okhoro Village, Egor Local Government Area of Edo State, Nigeria, between February and July 2005. The botanical identity of the plant and its bark were authenticated by Alhaji Alasa Abubakar (of blessed memory), a herbarium curator of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. Botanical authentication was confirmed at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen (No. FHI107654) was deposited for future reference. Immediately after collection, barks were cut into small pieces and dried under sunlight. The dried barks were pulverized into a smooth powder using impact mill, weighed and kept for further analysis.

Extraction and Preparation of the Extract

The powdered material (500 g) was macerated with absolute alcohol (2.5 litres) and left for 72 hours. The mixture was stirred at six-hourly intervals using a sterile glass rod. The extract was filtered and the filtrate concentrated with the aid of a vacuum pump and rotavapour at 40°C, giving a yield of 3.78%. The concentrated extract was stored in air tight containers, labelled and refrigerated at 4⁰ C prior to use.

Animals

Albino Swiss mice weighing between 20-30 g of either sex were obtained from the Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were maintained on a standard diet (Ladokun feeds, Ibadan, Oyo State, Nigeria) and had access to food and water *ad libitum*. Animals were exposed to natural lighting conditions and were handled according to standard experimental protocols approved by the Faculty of Pharmacy Animal Ethics Committee, University of Benin.

Castor-oil induced diarrhoea

Mice were divided into five groups of five animals each. Diarrhoea was induced by administering 0.3 ml of castor oil orally to mice as described (10). Group one served as control (normal saline 10 ml/kg orally), groups 2, 3 and 4 received the ethanolic extract (100, 200 and 500 mg/kg respectively orally) while group 5 received atropine sulphate (0.1 mg/kg I.p). This was done 30 minutes before castor oil administration.

The following parameters were observed for a period of 4 hours, the onset of diarrhoea, the total number of dry and wet diarrhoea droppings and the total weight of diarrhoeal stools in that period of time. The onset was measured as the time interval (minutes) between the administration of castor oil and the appearance of the first diarrhoeal stool.

Small intestinal motility in mice

Groups of overnight fasted mice were allotted to five groups of five animals each. Groups A, B and C were orally administered the ethanolic extract at doses of 100, 200 and 500 mg/kg respectively. Group D received atropine sulphate (0.1 mg/kg intraperitoneally) while group E was administered normal saline (10 ml/kg orally). Thirty minutes later, the

charcoal meal (10 % charcoal suspension in 5 % gum acacia solution) was given at a dose of 0.2 ml/mouse. Thirty minutes after charcoal meal administration, the animal from the respective groups was sacrificed by ether inhalation. The stomach and small intestine removed and extended on a clean surface (11). The distance traversed by the charcoal marker relative to the total length of the small intestinal (mm) was expressed as a percentage.

Statistical analysis

All data were expressed as mean \pm SEM. Where applicable, the data were analysed statistically by Student's t-test using Graph pad instant version 2.05a. The level of significance was $p < 0.05$. n represents five per group.

Results

The extract produced a dose-dependent inhibition of castor oil-induced diarrhoea with the 500 mg/kg dose giving the highest effect (Table 1).

Kigelia africana significantly ($p < 0.05$) decreased the total number of stools passed (5.6 ± 0.98) as compared to the castor oil treated control groups. (12.2 ± 1.90). Atropine at a dose of 0.1mg/kg (I.p) produced a marked anti-diarrhoeal effect (5.2 ± 0.74). Hence the effect of the extract at 500 mg/kg can be said to be comparable to that of atropine.

On its effect on small intestinal motility (Table 2), there was a dose-dependent reduction in the percentage distance travelled by the charcoal meal with the 500 mg/kg dose giving the least percentage of 61.5 ± 3.0 . When compared with the control (normal saline treated group), its effect was significant ($p < 0.0001$). The onset of diarrhoea (mins), is the time interval between the administration of the cathartic agent and the first diarrhoeic stool.

Table 1 Antidiarrhoeal activity of the ethanol extract of the stem bark of *K. africana*, distilled water and atropine on castor-oil induced diarrhoea in mice.

Treatment (mg/kg)	Onset time of diarrhoea (mins)	Total number of faeces	Total weight of faeces (mg)
Control(10ml/kg normal saline)	35.8 \pm 8.69	12.2 \pm 1.90	336 \pm 9.79
<i>K.africana</i> (100)	72.4 \pm 15.2	6.6 \pm 1.08 ^a	200 \pm 14.83
<i>K.africana</i> (200)	72.8 \pm 9.1 ^a	5.8 \pm 0.86 ^a	196 \pm 30.10
<i>K.africana</i> (500)	91.6 \pm 1.12 ^c	5.6 \pm 0.98 ^a	182 \pm 29.22
Atropine(0.1)	107.6 \pm 6.68 ^c	5.2 \pm 0.74 ^b	178 \pm 8.00

Values are mean \pm SEM, (n=5 mice per group). *K.africana* significantly increased the onset and inhibited the frequency of diarrhoea, (^aP<0.05, ^bP <0.005 and ^cP<0.001) different from the control group. The weight of the faeces was for both the wet and dry in milligrams.

Discussion

In controls, copious diarrhoea was evident in 100 % of the mice in the first 2 h following oral administration of castor oil. The pretreatment of mice with the ethanolic extract of *Kigelia africana* delayed the onset of diarrhoea with a significant (p<0.05) inhibition during the first 1 hour period at all doses tested. The extract afforded protection against the castor oil- induced diarrhoea. Its effect was discernible at about the 2nd hour when 50 % of the animals were protected and at the fourth hour 20 % protection was observed.

Table 2: The inhibitory effects of the ethanol extract of the stem bark of *K. africana*, distilled water and atropine on small intestinal motility in mice.

Treatment (mg/kg)	Percentage distance traveled by the charcoal.	Percentage inhibition
Control(10ml/kg normal saline)	86.2±0.45	—
<i>K.africana</i> (100)	65.8±2.39 ^c	23.7
<i>K.africana</i> (200)	67.4±4.30 ^b	21.8
<i>K.africana</i> (500)	61.5±4.35 ^a	28.7
Atropine(0.1)	46.7±3.0 ^c	45.8

Values are expressed as mean percentages travelled by the charcoal meal in relation to the full intestinal length \pm SEM, (n = 5 mice per group). The distance travelled was dose-dependently inhibited by the extract, ^aP<0.001, ^bP<0.005, ^cP<0.0001, significantly different from the control group.

The ethanolic extract at doses of 100, 200 and 500 mg/kg and the reference drug, atropine (0.1 mg/kg) produced significant inhibition of normal transit. Besides producing an antisecretory effect, the bark extract was found to inhibit the intestinal motility in mice providing 28.7 % inhibition at 500 mg/kg. The present study demonstrates that the ethanolic stem bark extract of *Kigelia africana* inhibited both castor oil-induced secretory diarrhoea and small intestinal motility in mice. Clinically, diarrhoea may result from disturbed bowel function in which case there is impaired intestinal absorption, excessive

intestinal secretion of water and electrolytes and a rapid bowel transit. The induction of diarrhoea is a well known action of castor oil attributed to its active ingredient ricinoleic acid (12) which stimulates the production of several mediator substances that includes prostaglandins, nitric oxide, platelet activating factor, and tachykinins (13). In addition the cathartic effect of castor oil, a potent laxative or purgative is associated with the liberation of prostaglandins by colonic cells (14,15). Inhibitors of prostaglandins synthesis such as acetylsalicylic acid or indomethacin significantly reduced both PGE release and the volume of fluid loss induced by castor oil (16,10). The inhibition of diarrhoea induced by castor oil has been described as an in vivo model for evaluating novel agents capable of inhibiting prostaglandin formation (17, 10). This inhibitory effect of such novel compounds seems to account for its effectiveness in treating bowel inflammation and associated diarrhoea (18). The model was therefore used to establish the antisecretory effect of the ethanolic stem bark extract of *Kigelia africana*. The results suggest that the ethanolic extract of *Kigelia africana* has a potential antidiarrhoeal effect that can be explored for therapeutic advantage in the treatment of diarrhoea.

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