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Full Length Research Paper

Antidiarrhoeal activity of aqueous extract of *Combretum sericeum* roots in rats

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The antidiarrhoeal activity of aqueous extract of *Combretum sericeum* roots was evaluated in rats. Studies were carried out on castor oil induced diarrhea, gastrointestinal motility and castor oil induced fluid accumulation. The extract (25 and 50 mg/kg) causes a dose dependent protection against castor oil induced diarrhea and castor oil induced fluid accumulation and decreased markedly gastrointestinal transit. The intraperitoneal LD_{50} of the extract was found to be 177.48 mg/kg in mice. A preliminary phytochemical screening of the aqueous extract of *C. sericeum* roots revealed the presence of tannins, flavonoids, glycosides, anthraquinones and alkaloids. The results obtained showed that the water extract of *C. sericeum* roots may be active against diarrhea and this may be the basis for its use traditionally for gastrointestinal disorders.

Key words: *Combretum sericeum*, diarrhea, gastrointestinal tract and tannins.

INTRODUCTION

Diarrhea may be defined as a situation in which an adult daily stool exceeds 200 g and contains 60-95% water. This disease is common in the tropics. Indeed, in certain parts of the world, diarrhea produces more illness and causes death of more infants and children than all other diseases combined (Weber, 1976). Diarrhea ranges from a mild to a socially inconvenient illness to a major cause of malnourishment among children of developing countries, and causes 4-5 million deaths through out the world, annually. To combat the problem of diarrhea in developing countries, the world health organization (WHO) has constituted a diarrhea disease control programme (DDC) which includes studies of traditional medicine practices together with the evaluation of health education and prevention approaches (Syder and Merson, 1982). The use of herbal drugs in the treatment of diarrhea disease is common practice in many countries of Africa. *Combretum sericeum* (family *Combretacae*), called “Taru” in Hausa and “Nyangbimsa” in Jaba, is a shrub that is abundant in the savannah region of West Africa. The plant is used for the treatment of various ailments such as stomach disorders, conjunctivitis and to reduce fever. Smoke from burning of the plant is a remedy for cough while also roots decoction is used for diarrhea and pneumonia (Abdullahi et al., 2003).

This study aims at evaluating the traditional claim of the use of the aqueous roots extract of this plant against diarrhea.

MATERIAL AND METHOD

Animals

Swiss albino rats and mice of both sex maintained at the animal facility center of the Faculty of Pharmacy Ahmadu Bello University, Zaria were used for the experiment. The animals were fed with standard feed (Pfizer feed PLC, Lagos) and water *ad libitum*.

Plant material

The roots of *C. sericeum* were collected from ‘kurmin Bauna’ village in Jaba local government area of the southern Kaduna state in Nigeria in November 2007. The plant was identified and authenticcated by Mr. Gallah, U. S. in the department of biological sciences Ahmadu Bello University, Zaria. A voucher spacemen (Number 000135) was deposited in the departmental herbarium.

Preparation of extract

The roots were washed clean using distilled water shade dried and

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Table 1. Phytochemicals detected in *Combretum sericeum* root extract.

Test	Tannins	Alkaloids	Flavonoids	Glycosides	Saponins
Inference	+	+	+	+	-

+ Present; - absent.

pulverized using pestle and mortar. About 130.4 g of the powdered plant material was packed into a thimble and placed on a shaker for 24 h. The extract was then sieved and filtered using filter paper. This was concentrated and the last traces of the solvent were removed heating in a water bath at 40°C. The water extract yield was 28.50 g (w/v), and this was used for the pharmacological evaluations.

Phytochemical test

The freshly prepared extract was subjected to a standard phytochemical screening test for various constituents (Trease and Evans, 1993). The extract was screened for the presence of alkaloids, glycosides, saponins, tannins anthraquinones and flavonoids using conventional protocol.

Acute toxicity test

The acute toxicity study was carried out based on the method described by Lork (1983). All mice were injected intraperitoneally (i.p). The study was divided into two phases. In the first phase nine mice of both sexes were divided into three groups of three mice each. Group A received 10 mg/kg extract while Groups B and C received 100 and 1000 mg/kg extract, respectively. The animals were observed for signs and symptoms of toxicity including mortality for 24 h after treatment. In the second phase, four mice were separated, the first mice received extract at a dose of 140 mg/kg, while the second, third and fourth received the extract at doses of 225, 370 and 600 mg/kg, respectively. The mice were also observed for 24 h. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose i.e. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Castor oil induced diarrhea

Rats were fasted for 12 h prior to the commencement of the experiment and were randomly divided into four groups of five rats each. The animals in Group 1 received distilled water orally using orogastric cannulla, the animals in Groups 2 and 3 received the extract 25 and 50 mg/kg of the extract orally and Group 4 received a standard drug, loporamide (5 mg/kg). After 30 min of the drug pre-treatment, castor oil 1.0 ml/rats was administered orally. The animals were placed in individual cages over clean filter paper. Three hour after oil challenge the rat cages were inspected (by an observer unaware of the treatment) for the presence of characteristic diarrheal droppings, their absence was recorded as a protection from diarrhea (Abdullahi et al., 2003) and the percentage protection calculated.

Gastrointestinal motility

To test the effect o the extract on the gastrointestinal motility, the animals were randomly divided into four groups of five rats each. The test rats were starved for 24 h prior to the experiment but were allowed access to water. Rats in group I were given 3 ml/kg normal saline orally while those in Groups II and III were given extract 25 and 50 mg/kg. respectively, and Group IV was given standard drug, atropine (0.1 mg/kg). Thirty minutes later after drug administration, 0.5 ml of a 5% activated charcoal suspension in a 10% suspension of tragacanth powder was administered to each rat. All rats were killed 30 min later, the abdomen opened and the distance moved by the plug from the pylorus to the caecum was determined and expressed as percentage of the total length of the small intestine (Akah, 1989).

Castor oil induced fluid accumulation

This study was done as described by Robert et al. (1976) and modified by Dicarlo et al. (1994). The rats were fasted for 24 h but allowed access to water. They were randomized and placed in four cages with five rats each. Group i was administered with normal saline (5 ml/kg), Groups ii and iii were given 25 mg and 50 mg/kg of extract, respectively, and group iv was given a standard drug diphenoxylate at a dose of 5 mg/kg (Dahiru et al., 2006). Thirty minutes after drug administration, each rat was administered 2 ml of castor oil. The rats were anesthetized 30 min later by inhalation of chloroform. The small intestine from the pylorus to caecum was extracted and its content expelled into a measuring cylinder to determine the volume of the fluid (Adzu et al., 2003).

Statistical analysis

Results are presented as mean ± SEM. The student t-test was used to determine the significant difference between two groups ($P<0.05$).

RESULTS AND DISCUSSION

The percentage yield of the aqueous extract of *C. sericeum* was 21.86%. The result of the phytochemical screening revealed the presence of alkaloid, tannins, glycosides flavonoids and anthraquinones (Table 1). The LD_{50} was estimated to be 177.48 mg/kg (ip) in mice. The effect of the aqueous extract of *C. sericeum* on castor oil induced diarrhoea (Table 2) revealed that the extract decreased the number of faecal matter pass by the animals. At 25 and 50 mg/kg extract, a significant ($P<0.05$) reduction in diarrhoea was observed representing 58.54 and 94.91% inhibition, respectively. Loporamide 3 mg/kg inhibited the castor oil induced diarrhoea by 100%. The effect of the extract on gastrointestinal transit (Table 3) revealed that 25 mg and 50 mg/kg of the extract decreased the intestinal transit length by 62.72 and 53.38%, respectively. Atropine 0.1 mg/kg caused a significant ($P<0.05$) reduction in the gastrointestinal transit by 35.63%. Studies on intestinal fluid accumulation (Table 4)

Table 2. Effect of *Combretum sericeum* on castor oil induced diarrhoea in rats.

Treatment	Dose (mg/kg)	No. of watery diarrhea	% Inhibition
Control (saline)	5	23.60±1.33	-
Extract	25	9.80±2.78*	58.47
Extract	50	1.20±0.49*	94.91
Loperamide	3	0.00±0.00*	100

Results are mean ± SEM, n = 5.
*Significant relative to control (p<0.05).

Table 3. Effect of *combretum sericeum* on gastrointestinal transit in rats.

Treatment	Dose (mg/kg)	Motility #
Control (saline)	5	87.58±3.77
Extract	25	62.72±1.21*
Extract	50	53.38±6.50*
Atropine	0.1	35.63±2.83*

Results are mean ± SEM, n = 5.
* Significant relative to control (p<0.05).
#: Distance moved by charcoal meal as percentage of intestinal length.

Table 4. Effect of *combretum sericeum* on castor oil induced fluid accumulation.

Treatment	Dose (mg/kg)	Fluid volume (ml)	% Inhibition
Control (saline)	5	6.88±0.56	-
Extract	25	3.88±0.55*	43.60
Extract	50	3.65±0.62*	46.94
Diphenoxylate	2.5	3.42±0.43*	50.29

Results are mean ± SEM, n = 5.
* Significant relative to control (p<0.05).

revealed that both 25 mg and 50 mg/kg of the extract significantly (P<0.05) inhibited fluid accumulation at the levels of 46.60 and 46.94%, respectively. Diphenoxylate 2.5 mg/kg exhibited the highest inhibition of 50.29%.

The results revealed that the aqueous roots extract of *C. sericeum* appears to contain substances that possess significant anti-diarrheal activity. The extract significantly protects the rats against diarrhea evoked by castor oil in a dose dependent manner. The maximal effect of the extract was similar to loperamide, which is at present one of the most efficacious and widely employed anti-diarrheal drug. Loperamide effectively antagonized the diarrhea induced by castor oil (Neimegeer et al., 1974), prostaglandin (Karim and Adaikan, 1977) or cholera toxin (Farack et al., 1981). The therapeutic effect of the loperamide is believed to be due to its antimotility and antisecretary activity (couper, 1987).

From this study it is likely that the extract mediates its effect through similar mechanism. Suppression of fluid accumulation by the extract also suggests inhibition of gastrointestinal function (Nwafor et al., 2000). Several constituents are present in the extract, and the compound responsible for the observed action is unknown. Flavonoids, abundant in the extract, have been demonstrated to inhibit contraction caused by spasmogenes (Macauder, 1986), inhibit intestinal secretion and small intestinal transit (Viswanathan et al., 1994; Dicarlo et al., 1993). The antidiarrrheal activity of tannins and alkaloids has been described by Mukherjee et al. (1998) and (Gricilda and Molly, 2001), respectively. Properties such as these may underlie the observed antidiarrheal activity of *C. sericeum*. There is need for study to ascertain the mechanism of action of the extract and its antimicrobial effect against diarrhea causing microorganisms.

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