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Evaluation of the Antidiarrheal Activity of Methanol Leaf Extract of *Bombax Buonopozense* in Rats

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Abstract
Objectives: The methanolic extract of the leaves of Bombax buonopozense was screened for antidiarrheal effects. Materials and methods: The extract was evaluated for castor oil- induced diarrhoea and enteropooling as well as intestinal transit in rats. Results: Bombax buonopozense significantly ($p<0.05$) and dose-dependently reduced frequency of stooling in castor oil-induced diarrhoea, castor oil- induced enteropooling and intestinal motility in rats. The oral LD_{50} values obtained were greater than 5000mg/kg in mice. Conclusion: These findings suggest that the methanolic extract of the leaves of B. buonopozense may contain some biologically active ingredients that are active for the treatment of diarrhoea in Nigerian herbal traditional medicine.

Key words: Bombax buonopozense; Medicinal plant; Antidiarrheal activity; Castor oil.

Introduction

Diarrhea accounts for more than 5-8 million deaths worldwide each year in < age 5, especially in developing countries (1,2). To combat this problem, the world health organization (WHO) has initiated a diarrhea disease control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and prevention approaches (3,4). Plants have been a valuable source of natural products for maintaining human health for many years. More recently, there has been a greater search for natural therapies. The use of herbal drugs in the treatment of diarrhea is a common practice

in many African countries. The WHO suggested that medicinal plants would be the best source from which to develop a variety of medications. About 80% of individuals from developed countries receive traditional medicines including compounds derived from medicinal plants. Such medicinal plants can be exploited since it has been shown that they are important sources of new chemical substances with potential therapeutic effects (5,6).

Bombax buonopozense P. beauv. (Bombacacea) is a large tropical tree that grows up to 40 meters in height with large buttress roots that can spread six meters. The bark is covered with large by conical spines, especially when the tree is young, but sheds them with age in varying degrees. The branches are arranged in whorls, the leaves are compound and have five to nine leaflets and five to twenty five secondary veins. The individual leaflets have entire margins and are large. The undersides of the leaflet may be glabrous or puberclous (7). This tree is widely distributed in African countries such as Ghana, Sierra Leone, Uganda and Gabon. It is known by common vernacular names in different languages such as Vabga (Dagbani) and Kurya (Hausa) and different parts are used for different purposes (8). However, there is limited scientific evidence supporting the potential use of B.buonopozense as an antidiarrheal agent. We have therefore investigated the scientific basis for the efficacy of its anti-diarrheal properties.

Materials and Methods

Plant collection and Preparation of the Extract

The leaves of Bombax buonopozense were collected from Suleja, Niger State by an experienced ethnobotanist (Ibrahim Muazzam), identified and authenticated by a taxonomist (Mrs. Grace Ugbabe) and a voucher specimen (# 6402) was deposited in the herbarium at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The plant material was air-dried at room temperature and pulverized into a dry powder, then macerated with 70% methanol in water for 72h with constant shaking. The resultant mixture was filtered using Whatmann (No 1) filter paper and the filtrate was concentrated using a rotary evaporator and dried on a water bath to give a yield 7.7% (w/w). The extract was reconstituted in normal saline at appropriate concentrations for the various experiments conducted.

Experimental animals

Adult wistar rats (200-250g) of either sex were used for the study. The animals were maintained at the Animal Facility Centr of the NIPRD. They were fed with standard diet and had free access to water ad libitum. The animals were maintained under standard conditions of humidity, temperature and 12h light/12h darkness cycle. The animals were used in accordance with the NIH Guide for the care and use of Laboratory Animal (9).

Acute toxicity test

The acute toxicity median lethal dose (LD_{50}) of the extract was estimated p.o. in Swiss albino mice following Lorke's method (10). Dose levels used ranged from 100-5000 mg/kg of the methanolic extract. The acute toxicity LD_{50} was calculated as geometric mean of the dose that results in 100% lethality and that which caused no lethality.

Preliminary Phytochemical Screening

The crude extract of Bombax buonopozense was subjected to qualitative phytochemical screening according to standard methods (11).

Castor oil-induced diarrhea

Albino rats of either sex (200-250g) were divided into five groups of six animals each. They were fasted for 24h prior to the test, but allowed free access to water. Group 1 was treated with 0.2ml of normal saline, which served as control; Group 2 received standard drug (Loperamide 3mg/kg). Groups 3, 4 and 5 received different doses of the extract (100, 200 and 400mg/kg) respectively. All dose were administered orally. The animals were then housed singly in cages lined with transparent paper. One hour after pre-treatment with the extract, the animals were challenged with 1 ml of castor oil orally. Thereafter, they were observed for 4h for the presence of diarrhea defined as watery (wet), unformed stool.

Study of small intestinal transit

This was done according to the method previously described (12) using charcoal mean as a diet marker. Albino rats of either sex (200-250g) were randomly divided into five groups of six rats each. They were fasted for 24 hours prior to the test, but were allowed free access to water. The first group was orally administered with 0.2ml normal saline. The second group orally received atropine sulphate (5mg/kg). The third, fourth and fifth groups orally received different doses of the extract (100, 200 and 400mg/kg). Thirty minutes after drug administration, 1ml of charcoal meal (5% activated charcoal in 10% aqueous tragacanth) was administered to all the animals in the study and thirty minutes later, all the rats were sacrificed and the

abdomen opened. The small intestine was dissected out and the distance covered by the charcoal meal in the small intestine from the pylorus to the caecum was measured and expressed as a percentage of the distance traveled.

Castor oil-induced enteropooling

Albino rats of either sex (200-250g) were divided into five groups of six rats each. They were fasted 24h prior to the experiment, but allowed free access to water. Group 1 (controls) was treated with 0.2ml of normal saline. Group 2 was treated with standard drug (Loperamide 3mg/kg). Groups 3, 4 and 5 was treated with different doses of the extract (100, 200 and 400mg/kg) respectively. All administered by the oral route. Thirty minutes later, all the rats were challenged with 1ml of castor oil orally. After thirty minutes, each rat was sacrificed and the whole length of the intestine from the pylorus to the contents was expelled into a measuring cylinder and the volume measured.

Statistical analysis

Results were expressed as mean ± S.E.M. The significance of difference between the control and treated groups were determined, using two-way analysis of variance (ANOVA), followed by student t-test. $P<0.05$ were considered statistically significant.

Results

Phytochemical screening

Phytochemical analysis of the crude extract gave positive reaction for each of the following secondary metabolites: Tannins, Saponins, Terpens, Steroids, Alkaloids, Flavonoids and Carbohydrates.

Acute toxicity test

There was no mortality observed in the mice upon oral administration, even at doses as high as 5000mg/kg, signifying that the LD_{50} was greater than 5000mg/kg. Apart from sedation and weakness, B. buonopozense did not produce any major clinical signs of toxicity in mice during 72h observation period.

Effect castor oil-induced diarrhea

The methanolic extract of B. buonopozense produced a marked antidiarrheal effect in the rats. Both doses

Table 1. Effect of B. buonopozense and castor oil-induced diarrhea

Group	Dose (mg/kg)	Number of wet feaces in 4h	% inhibition
Control	20 ml/kg	7.7 ± 0.6	___
Loperamide	3	0	100*
B.buonopozense	100	5.3 ± 0.8	30.8*
	200	1.8 ± 1.0	76.2*
	400	0.8 ± 0.5	89.2*

Values are mean ± S E M, n = 6
*significant as compared to control p< 0.05

Table 2. Effect of B. buonopozense on intestinal transit

Group	Dose (mg/kg)	Mean distance traveled by charcoal	% inhibition
Control	20 ml/kg	74.7 ± 4.0	___
Atropine	5	40.2 ± 1.8	46.2%*
B.buonopozense:	100	62.7 ± 2.5	16.1%*
	200	51.5 ± 1.8	31.0%*
	400	41.7 ± 3.0	44.2%*

Values are mean ± S E M, n = 6
*significant as compared to control P< 0.05

Table 3. Effect of B.buonopozense extract on castor oil-induced enteropooling

Group	Dose (mg/kg)	Volume of intestinal content (ml)	% inhibition
Control	20ml/kg	2.4± 0.25	____
Loperamide	3	0.33 ± 0.14	86.3%*
B.buonopozense	100	1.27 ± 0.19	47.1%*
	200	1.0 ± 0.16	53.3%*
	400	0.67 ± 0.20	72.1%*

Values are mean ± S E M, n = 6
*significant as compared to control P < 0.05

significantly decreased the total number of wet feces produced upon administration of castor oil and this result is similar to the effect of the standard antidiarrheal drug, loperamide (3mg/kg) (Table 1).

Effect on intestinal transit

The methanolic extract of B. buonopozense also slowed down the propulsion of charcoal meal through the gastrointestinal tract when compare to the control group. Atropine part produced a marked decrease in the propulsive movement and the intestinal length travelled by charcoal meal (Table 2).

Effect on castor oil-induced enteropooling

B. bunopozense was found to possess anti-enteropooling activity. The extract significantly decreased intestinal fluid volume in rats. However, the effect of the extract was less potent in comparison to the standard drug, loperamide (Table 3).

Discussion

Diarrhea is usually considered a result of altered motility and fluid accumulation within the intestinal tract. Many antidiarrheal agents act by reducing the gastrointestinal motility and / or the secretions. Castor oil causes diarrhea due to its active metabolite, recinolic acid (13) which increases peristaltic activity in the small intestine leading to changes in the electrolyte permeability of the intestinal mucosal membrane. The precise mechanism of action of castor oil is through elevated prostaglandin biosynthesis (14,15). Prostaglandin contributes to the pathophysiological functions in gastrointestinal tract (16). Inhibitors of prostaglandin biosynthesis delay castor oil-induced diarrhea (17).

Phytochemical screening of the plant extract in the present study revealed the presence of tannins, saponins, steroids, terpenes, alkaloids and flavonoids which have all been reported to posses activity and therefore explain its antidiarrheal action (15,18). In addition, its antidiarrheal action may also be due to the presence of denatured proteins, which form protein tannates. It has been previously demonstrated that protein tannates make the intestinal mucosa more resistant and hence, reduce secretion and peristaltic movement (19,20). This may be because B. buonopozense increases the reabsorption of water by decreasing intestinal transit of charcoal meal. It is also possible that in the methanolic leaf extract may be responsible for the antidiarrheal activity. Flavonoids have been ascribed the ability to inhibit contractions induced by spasmogenics (21-23).

The antidiarrheal activity of the plant extract was not comparable to the standard drug, loperamide, which at present is one of the most efficacious and widely employed antidiarrheal drug. Loperamide effectively antagonizes diarrheal activity induced by castor oil (24), prostaglandins (25) or cholera toxin (26). Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the intestine, reduce colon flow rates and consequently any effect on colonic motility (27, 28). The antimuscarinic drug, atropine and different doses of the extract decreased the propulsive movement in the charcoal meal study. This is possible due to its anticholinergic effect (29). The significant inhibition of the castor oil-induced enteropooling in rats suggests that B. buonopozense leaf extract produces relief in diarrhea by spasmolytic activity in vivo and also anti-enteropooling effects.

In conclusion, the present study revealed that Bombax buonopozense contains pharmacologically active substances effective for management of diarrhea. Further studies are required to fully investigate the mechanisms responsible for this observed antidiarrheal activity.

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