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Anti-diarrhoea Effect of *Boswellia dalzielii* Stem Bark Extract in Albino Rats

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Abstract: This study examines the effect of aqueous extract of *Boswellia dalzielii* stem bark on castor oil induced diarrhoea in albino rats. Graded doses of the extract (100, 200 and 300 mg kg⁻¹) were administered orally to three groups of rats (n = 6) before induction of diarrhoea with castor oil. Another two groups of the animals were treated with normal saline (control) and diphenoxylate, a conventional anti-diarrhoea drug respectively. In a separate experiment, an isolated ileum of rabbit was mounted in an organ bath containing aerated Tyrode solution. The tissue was stimulated with acetylcholine (2 µg) and its responses recorded. Various concentrations of the extract (2, 5, 10 and 20 µg) were applied to the tissue and the responses recorded. The responses were similarly recorded when acetylcholine was separately combined with atropine and 10 µg of the extract. The extract produced a significant inhibition of the castor oil induced diarrhoea in the animals in a dose dependent manner. Furthermore, the extract relaxed isolated rabbit ileum and reduced the contractions induced by acetylcholine in a manner similar to atropine during the *in vitro* studies. These findings suggest that, aqueous stem bark extract of *Boswellia dalzielii* possesses anti-diarrhoea effect, which may be related to anticholinergic mechanisms.

Key words: Transit time, charcoal, castor oil, acetylcholine

Introduction

Boswellia dalzielii Hutch (family: Burseraceae) is a tree plant found in the savannah region of northwestern Nigeria. The plant is regarded as a veritable source of natural medicine (FRIN, 1994). The local inhabitants of this region have been using the decoctions from various parts of this plant for the treatment of a plethora of both animals and human diseases. The extracts of the leaf is used locally for the treatment of diarrhoea in both human and poultry (Nwude and Ibrahim, 1998). Diarrhoeal diseases cause almost three million deaths in a year globally. These deaths occur mainly among children less than five years of age (Berne *et al.*, 1992). It is the major cause of morbidity and mortality in infants in the developing countries (Das *et al.*, 1999). Diarrhoea prevalence among children was as high as 22% in the northeastern region of the country and the prevalence was associated with maternal education (Anonyms, 1999). The health problems associated with diarrhoea diseases especially in the developing countries where the standard of hygiene is poor and the level of poverty is high are enormous. The cost and availability of conventional antidiarrhoea drugs in these areas have constituted another impediment, thus raising the need to find an alternative remedy available locally for the treatment of this condition. Several scientific studies have been carried out to evaluate the anti-microbial, anti-ulcer and anti-inflammatory properties of *Boswellia dalzielii* (Nwinyi *et al.*, 2004).

But only a very limited study of its antidiarrhoeal effect has been reported to our knowledge. The present study therefore, is to examine the effects of aqueous stem bark extract of *Boswellia dalzielii* on diarrhoea induced by castor oil in albino rats and its possible mechanism of action.

Materials and Methods

Plant Collection and Preparation of the Extract

This study was conducted in the Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The plant material was collected in the month of September 2004 from its natural habitat at Zuru in Kebbi State of Nigeria. Dr. B.L. Aliero, a botanist in the Biological Science Department of Usmanu Danfodiyo University, Sokoto authenticated the plant and a voucher specimen (No: D-01 BD-4) was deposited at the department's herbarium for future reference. The stem bark was cleaned, cut into pieces and air dried to constant weight. It was pulverized in wooden mortar into a dry powder. About 200 g of the powder was macerated in 1.5 L of distilled water for 48 h. The solution was filtered and the filtrate evaporated to dryness at 50°C. The percentage yield of the extract was found to be 6.8% (w/w). On any experimental day, a fresh solution containing the required concentrations of the extract was prepared and put into use.

Experimental Animals

Adult Albino rats of both sexes (150-180 g) used in the study were acquired from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The animals were fed with standard feed (Vital Feeds, Jos), provided with free access to water under a well-ventilated condition of 12 h light cycle. They were kept in metal cages with wood shavings as bedding and were allowed to acclimatize for two weeks before the commencement of the experiments. The study was carried out in accordance with the Organization for Economic and Development (OECD) principles on Good Laboratory Practice (GLP) (OECD, 2001).

Effect of Extract on Castor Oil Induced Diarrhoea

The castor oil test was performed as described by Offiah and Chikwendu (1999). Thirty adult rats of both sexes were randomly selected, examined thoroughly to ensure that they were healthy and divided into five groups. The animals were housed singly in cages lined with white blotting paper. Those in groups 1, 2 and 3 were pretreated orally with 100, 200 and 300 mg kg⁻¹ of *Boswellia dalzielii* extract, respectively, while the rats in groups 4 and 5 received 2 mL kg⁻¹ of normal saline (Dana, Nigeria) and 5 mg kg⁻¹ of diphenoxylate (Searl, Germany) respectively through the same oral route. One hour later, all the rats were subsequently treated with 1 mL of castor oil orally. The animals were then kept and observed over a period of 6 h for the frequency of passing watery (wet) or unformed stool. Absence of such watery dropping was recorded as positive, indicating protection.

Effect of the Extract on Gastrointestinal Motility

The method of Chitme *et al.* (2004) was slightly modified and used to test the effect of *Boswellia dalzielii* extract on the gastrointestinal motility of the rats. The albino rats (5 groups; n = 6) were starved of food but given free access to water for 18 h before drug treatment. The rats' in-group 1 was treated with 2 mL of normal saline orally; that in-group 2 received 3 mg kg⁻¹ of atropine intraperitoneally, while the rats in groups 3, 4 and 5 were administered orally with 100, 200 and 300 mg kg⁻¹ of the extract respectively. About 10 min later, 1 mL of 5% charcoal suspension in 10% aqueous solution of tragacanth powder was given orally to each rat. After another 30 min, the animals were all sacrificed by cervical dislocation. The small intestine of each rat was carefully examined and removed. The distance traveled by the marker (charcoal) from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to the caecum.

Effect of the Extract on Castor Oil Induced Entero-Pooling

The intraluminal fluid accumulation test was conducted according to the method of Robert *et al.* (1976). The animals (6 rats/group) were fasted over night before pretreatment with 2 mL kg⁻¹ of normal saline orally and 3 mg kg⁻¹ of atropine intraperitoneally for the rats in groups 1 and 2, respectively. Those in groups 3, 4 and 5 were pretreated orally with 100, 200 and 300 mg kg⁻¹ of the extract, respectively. Then 1 h later, 1.0 mL of castor oil was orally administered to each rat. At the expiration of another 1 h, the rats in each group were sacrificed, the small intestine of each rat removed, its contents were collected and the weight measured.

Effect of Extract on Isolated Rabbit Ileum

The method described by Offiah and Chikwendu (1999) was adopted. An adult male rabbit, which had free access to water but starved over night prior to the experiment, was used. The rabbit was anaesthetized with chloroform and laparotomy performed immediately. Segments of the ileum, 2-3 cm were obtained and each mounted in 50 mL organ bath containing aerated Tyrode solution. The preparations were set up under a tension of 0.5 g and responses were recorded on a kymograph paper through an isotonic frontal writing lever. The tissue was equilibrated for 60 min before use. Response to acetylcholine (ACh) (2 µg) was recorded after which the tissue was washed three times with the physiological fluid and allowed to rest. Similarly, dose response to varying concentrations of the extract (2, 5, 10 and 20 µg) was recorded. The response of acetylcholine with atropine (1 µg) was also recorded. This was followed with acetylcholine in combination with 10 µg of the extract.

Statistical Analysis

All data were expressed as the mean±standard error of the mean (SEM). One-way analysis of variance (ANOVA) with subsequent Dunnet's post hoc analysis was used to detect further differences between groups. Values less than 0.01 were considered significant. All statistical analysis was carried out using the Instat statistical package (Graph pad software Inc. USA).

Results

Castor oil produced copious diarrhoea in all the rats within 2-3 h after the administration of the drug. The oral administration of varying doses (100, 200 and 300 mg kg⁻¹) of *Boswellia dalzielii* stem bark extract significantly ($p < 0.001$) decreased, the frequency of defecation in the rats following induction of diarrhea with castor oil in a dose dependent manner. The rats in the control group defecate averagely about 15 times within the 6 h period observed. The animals in groups 2 and 3 that received 100 and 200 mg kg⁻¹ of the extract had a mean frequency defecation of 7.2 and 1.4 times, respectively while those that were treated with 300 mg kg⁻¹ of the extract and diphenoxylate (5 mg kg⁻¹) did not defecate within the 6 hours observatory period, that is indicating 100% protection (Table1).

There was also a significant difference ($p < 0.001$) in the small intestinal transit time of the charcoal between the control and the treatment groups. The charcoal moved very rapidly along the small intestine in the control group, while the movement was markedly reduced in the rats treated with the extract dose dependently. The rats that received atropine (3 mg kg⁻¹) had the shortest distance of charcoal movement along the small intestine compared to other groups (Table 2). The intestinal transit of charcoal in the group treated with 300 mg kg⁻¹ of extract appeared to be statistically similar to the group treated with atropine (3 µg).

In the castor oil induced intestinal fluid accumulation test, there was no significant difference ($p > 0.01$) in the weight of intestinal content of rats in the control group and that of those treated with 100 mg kg⁻¹ of the extract. However the 200 and 300 mg kg⁻¹ extract treated rats showed significant decreased ($p < 0.001$) in intestinal content weight than the control group. There was a corresponding reduction of intestinal content weight in the rats treated with atropine (Table 3).

Table 1: Effects of aqueous extract of *Boswellia dalzielii* stem bark on castor oil induced diarrhea in rats (n = 6)

Treatment groups	Extract doses (mg kg ⁻¹)	Mean No. of defecation in 6 h	Protection (%)
Control	-	15.0±0.95	0.0
Diphenoxylate + CO (5 mg kg ⁻¹)	-	0.0	100.0
Extract + CO	100	7.2±0.66	52.0
Extract + CO	200	1.41±0.63	92.4
Extract + CO	300	0.0	100.0

CO = Castor Oil, p<0.001, F = 120.56, df = 24

Table 2: Effect of *Boswellia dalzielii* extract on gastrointestinal transit of charcoal in rats

Treatment (mg kg ⁻¹)	Total length of intestine (cm)	Distance traveled by charcoal (cm)	% intestinal transit
Control	99.2±4.35	98.3±4.19	99.14±0.39
Extract (100 mg kg ⁻¹)	91.3±6.20	57.3±4.01	62.76±0.69
Extract (200 mg kg ⁻¹)	92.0±4.68	50.7±2.54	55.0±0.640
Extract (300 mg kg ⁻¹)	96.8±3.37	41.5±1.60	42.88±0.80
Atropine (3 mg kg ⁻¹)	96.2±3.68	38.5±1.49	40.09±0.63

p<0.001, F = 1358.1, df = 29

Table 3: The effect of extract of *Boswellia dalzielii* on Castor oil induced entero-pooling in rats (n = 6)

Treatment groups	Weight of empty intestine (g)	Weight of intestinal content (g)	% accumulation of intestinal fluid
Control	4.3±0.210	2.46±0.05	36.47±1.61
Extract (100 mg kg ⁻¹) + CO	5.11±0.32	2.53±0.10	33.32±1.70
Extract (200 mg kg ⁻¹) + CO	5.25±0.64	1.22±0.06	19.72±2.63
Extract (300 mg kg ⁻¹) + CO	4.91±0.29	0.66±0.04	12.06±1.10
Atropine (3 mg kg ⁻¹) + CO	4.24±0.28	0.56±0.04	11.86±.290

CO = Castor oil, p<0.001, F = 44.358, df = 29

The application of acetylcholine (2 µg) resulted in the contraction of the rabbit ileum. The various concentrations (2, 5, 10 and 20 µg) of the extract produced relaxation of the rabbit ileum. The degree of contractions produced by the application of acetylcholine (2 µg) was however diminished by the application of atropine (1 µg) and the extract (10 µg).

Discussion

The results of the present study demonstrate that aqueous stem bark extract of *Boswellia dalzielii* possesses antidiarrhoeal activity in castor oil-treated rats. This finding is in contrast to that of Nwinyi *et al.* (2004). In the previous study, 25-100 mg kg⁻¹ of the extract administered intraperitoneally to rats did not offer any protection against castor oil induced diarrhoea but antimotility effect of the extract was demonstrated. The present study used a higher concentration of the extract (100-300 mg kg⁻¹) and oral route as practiced locally instead of intraperitoneal route was adopted. The extract protected the animals against diarrhoea as well as reduced the volume of intestinal fluid secretion induced by castor oil. Additionally, it increased the intestinal transit time of charcoal in a dose dependent manner. Castor oil contains ricinoleic acid which when liberated induced irritation of the gastrointestinal mucosa resulting in inflammation enhanced secretion of fluid and electrolytes and increased motility of the gastrointestinal tract resulting in diarrhoea (Pierie *et al.*, 1971; Jafri and Pasricha, 2000). Castor oil is also thought to induce diarrhoea by releasing prostaglandin from the colonic cells (Capasso *et al.*, 1986; Mascolo *et al.*, 1994). Reports from several studies have shown that *boswellia* species extracts possess potent anti-inflammatory properties (Kiela *et al.*, 2005). It is possible that the anti-diarrhoea action exerted by this extract may be related to the inhibition of prostaglandin formation. However, confirmation through further studies is needed before such assertion is made. Umukoro and Ashorobi (2003) reported that the seed extract of *Aframomum melegueta* inhibited diarrhoea induced by castor oil in rodents through prostaglandin dependent mechanism.

Diarrhoea also may occur because of decreased reabsorption of substances within the intestine (Galvez *et al.*, 1993). Diphenoxylate an opioid substance is known to inhibit gastrointestinal secretion and motility (Jafri and Pasricha, 2000). The extract in this study was observed to significantly increase the intestinal transit period of charcoal and reduced the intestinal content weight of the rats. The decrease in gastrointestinal motility will allow the intestinal content more time to be exposed to the absorptive surface of the intestinal tract (Friedman and Isselbacher, 1998). Also the result of the *in vitro* study with isolated rabbit ileum showed that, the extract relaxed the tissue and significantly reduced the contractions induced by acetylcholine on the smooth muscles of the ileum. This effect was comparable to atropine, a conventional anticholinergic agent. Since the intestinal motility factor has been investigated in this study, the antidiarrhoea effect of *Boswellia dalzielii* stem bark extract may be related to anticholinergic mechanisms.

Also, in an earlier study carried out by Ojerinde and Alemia (2004), the aqueous extract of *Boswellia dalzielii* was reported to contain alkaloids, tannins, saponins and anthraquinones. Alkaloids have been known to have, analgesic, anti-inflammatory and antidiarrhoea effects (Gupta, 1994). Furthermore, tannins are known to have astringent properties and therefore could be used to treat diarrhoea (Mota *et al.*, 1985). The proteins precipitated by tannins cover the surface of the cell or tissue and act as a barrier between the tissue and irritants, with the underlying tissue soothed and protected from damage. This process could reduce intestinal mucous membrane secretions (Tripathi, 1994). Thus, the chemical constituents of the extract may contribute to its anti-diarrhoea properties.

In conclusion, the results of the current study has shown that *Boswellia dalzielii* stem bark aqueous extract possesses anti-diarrhoea effect and provide evidence that support the traditional use of the plant extract in the treatment of diarrhoeal diseases in both human and animals.

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