



Original Research Article

Antidiarrhoeal Effects of Aqueous Stem Bark Extract of *Bridelia ferruginea* Benth. Using Castor Oil Diarrhoea Induction Model

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Abstract	Keywords
<p>The folkloric claim of <i>Bridelia ferruginea</i> Benth. (Euphorbiaceae) for the treatment of diarrhoea was investigated through castor oil induced diarrhoea model, gastrointestinal motility test, castor oil induced enteropooling method and isolated tissue studies using standard procedures. The bark of <i>B. ferruginea</i> was collected, air-dried, and pulverized to powder. The studies were carried out using three treatment doses 100, 200 and 400mg/kg of the aqueous extract orally respectively comparing with an orthodox drug (as positive control), Loperamide (3 mg/kg orally in castor oil-induced diarrhoea study), atropine sulphate (3mg/kg intraperitoneally in both the gastrointestinal motility assessment study and the castor oil induced enteropooling study) and an additional control (negative control) administered as 3 ml/kg of normal saline orally. The isolated tissue study made use of adult male rabbit's jejunum. A 3 cm long segment of the jejunum was mounted vertically in 25 ml organ bath containing Tyrode solution and its response to extract doses of 0.2, 0.4 and 0.8 µg/ml and acetylcholine at a dose of 0.4µg/ml with subsequent dose combination (acetylcholine and extract) after washing tissue each time before the next reading were recorded on a laptop connected to an isometric transducer via a data capsule. The results showed that the extract significantly inhibited the diarrhoea induced by castor oil and decreased the intestinal transit of charcoal at the doses employed in the albino rats and the isolated rabbit jejunum tissue study showed a complete inhibition of acetylcholine induced contraction at extract dose of 0.8µg/ml. The findings authenticate the plants folklore use in the treatment of diarrhoea.</p>	<p><i>Bridelia ferruginea</i> Diarrhoea Ethnomedicine Folkloric medicinal plants Stem bark extract</p>

Introduction

World Health Organization (WHO) advocates for the proper identification, sustainable exploitation, scientific development and appropriate utilization of herbal medicines which provide safe and effective remedies in medicare (Wambebe, 1998). It is a known fact that a sizeable number of rural populations in African and other third world countries use medicinal plants especially in treatment of diseases. *Bridelia ferruginea* Benth. Belonging to the family Euphorbiaceae is usually a shrub but can reach the size of a tree in a suitable environment. The bark is dark grey, rough and often markedly scaly (Rashid et al., 2000). The common Nigerian names for the plant include: -kizni (Hausa), Mirehi (Fulani), Ola (Igbo), Ora (Yoruba). It is a savannah plant and the tree is about 6-15 m in height and up to 1.5 m wide (Rashid et al., 2000). The bark is used as an antidote against poisons in both Ghana and Northern Nigeria (Burkill, 1994). A decoction of the leaves has been used to treat diabetes. It has also been used as a purgative and a vermifuge (Cimanga et al., 1999) and as an antiepileptic (Akubue and Mittal, 1982). Of interest is its use for the treatment of dysentery and diarrhoea (Gill, 1992).

Diarrhoea is the passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual (WHO, 2013). It is a global and endemic problem (WHO, 1990), affecting all races and age groups. It is the second leading cause of death among children under the age of 5 years globally and nearly one in five child deaths, (about 1.5 million death of children each year) is due to diarrhoea, resulting from dehydration and electrolyte imbalances following loss of fluids (WHO, 2013).

Diarrhoea is also a common cause of death in frail, elderly people and in immune compromised individuals. This study was sequel to a toxicity study where the aqueous stem bark extract of *B. ferruginea* was found to be safe for oral use in albino rats (in press). Castor oil is used for induction of diarrhoea in experimental animals (Nwinyi et al., 2004), a triglyceride of ricinoleic acid obtained from *Ricinus communis*, it is sometimes used in the treatment of constipation in humans (Boyce and Christy, 2000).

Materials and methods

Laboratory animals

Albino rats of both sexes with weights between 200-250g were obtained from the Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University, Sokoto. They were acclimatized for two weeks in the laboratory before commencement of the experiments and they experienced a natural cycle of 12 h light alternating with 12 h of darkness. They were allowed free access to water and food. Male rats were separated from females in each group in the study.

Preparation of plant extract

B. ferruginea plant was collected from a farm in Ilorin, Kwara state and it was identified by Mallam Mohammed Musa of the Department of Biological Sciences of the Ahmadu Bello University, Zaria. The bark of the plant was air-dried to constant weight under shade, pulverized into a fine powder with mortar and pestle and then stored in a plastic container for use. Aqueous extraction: Ten grams of the powdered material was subjected to exhaustive aqueous extraction using Soxhlet Extractor and the solution was then concentrated in a hot air oven at 40°C.

Drugs

Castor oil: Castor oil was used for induction of diarrhoea in experimental animals (Nwinyi et al., 2004). Manufacturer: Bell Sons & Co. (Druggists) Ltd., Southport PR9 9AL, England. Batch number: 5561K1. Expiry date: 1 August 2014.

Loperamide (Imodium)®: Loperamide is a phenylpiperidine derivative used to control diarrhoea (Katzung, 2007). Manufacturer: Janssen-Cilag Ltd. 50-100 Holmers Farm way, High Wycombe, Bucks HP12 4EG, UK. Batch number BFB2000. Expiry date: May 2016.

Acetylcholine: Manufacturer-Alphachemika, India. Batch number: A0096

Atropine: Manufacturer-Laborate Pharmaceutical. Batch number: 10AC06

Activated charcoal: Manufacturer-Qualikems. Batch number: CCW170811.

Acacia powder: Manufacturer-Fischer Scientific Laboratory Company, Manufacturing Division, New Jersey. Lot number: 773126.

Castor oil-induced diarrhoea study

The effect of *B. ferruginea* aqueous stem bark extract on diarrhoea was assessed in rats using the castor oil-induced diarrhoea method (Awouters et al., 1978; Offiah and Chikwendu, 1999; Ezenwali et al., 2010). Twenty five adult rats were denied feed 12 h prior to the experiment but were allowed free access to water. They were then randomly divided into 5 groups of 5 animals each. Group I-III were treated with 100, 200 and 400mg/kg of the aqueous extract orally respectively while group IV was treated with 3mg/kg of Loperamide orally (orthodox drug treatment) and group V received 3ml/kg of normal saline orally serving as control. One hour after the treatments, all the animals were given 1 ml of castor oil orally and placed singly in cages lined with a white sheet of paper for observation of the number and consistency of faeces. The numbers of both formed and unformed stools were counted every hour and the white paper changed after each count. This was repeated for 4 h. The means of the stools passed by the treated groups were compared with that of the control. The mean of diarrhoea stools passed by the control group was considered as 100%. The level of inhibition (%) of wetness of faeces and frequency of stooling caused by the extract was calculated relative to the control using the relation:

$$\left. \begin{array}{l} \text{Inhibition of} \\ \text{defaecation (\%)} \end{array} \right\} = (\text{NFec}-\text{NFeT})/\text{NFec} \times 100$$

Where, NFec = mean number of faeces of control group; NFeT= mean number of faeces of treated group.

The level of reduction (%) in defaecation of watery faeces was calculated using the relation:

$$\left. \begin{array}{l} \text{Inhibition of} \\ \text{diarrheic faeces (\%)} \end{array} \right\} = (\text{NDFc}-\text{NDFT})/\text{NDFc} \times 100$$

Where, NDFc = mean number of diarrheic faeces of control group; NDFT = mean number of diarrhoeic faeces of treated group.

Gastro-intestinal motility study

The effect of the extract on gastrointestinal motility was assessed using the method of Chitme et al., (2004). Twenty five rats were fasted for 18 h but with free access to water. The rats were subsequently divided randomly into five groups of five rats each. The rats in groups I to III were treated orally with 100, 200 and 400mg/kg of the aqueous extract respectively. The rats in group IV (control) were treated with normal saline orally 3ml/kg while group V received atropine sulphate, 3mg/kg intraperitoneally which served as the standard treatment. After 30 min. of drug administration, each rat received 1ml of charcoal meal (5% activated charcoal suspended in 10% gum acacia) and 30 min. later, all the rats were sacrificed under chloroform anaesthesia and the abdomen opened. The small intestine was carefully dissected out from the pylorus to the caecum and the total distance travelled by the charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to the caecum. The inhibitory effect of the extract on gastrointestinal transit was calculated relative to the control.

Castor oil induced enteropooling study

The effect of the aqueous stem bark extract of *B. ferruginea* on pooling of enteric contents was assessed using the method of Roberts et al. (1976). Adult rats were fasted for 18 h with free access to water and divided into five groups of five rats each. Groups I-III were treated with 100, 200 and 400 mg/kg respectively of the aqueous extract orally while group IV received 3mg/kg of atropine sulphate intraperitoneally while group V was treated with 3 ml/kg of normal saline orally. One hour later, each rat was treated with 1 ml of castor oil orally and in the following 1 h, the rats were sacrificed. The small intestine was dissected out and weighed (full intestine). The Intestinal contents were milked into a graduated tube and their volumes measured. The intestine was re-weighed (empty intestine) and the difference between full and empty intestines calculated. The level of reduction in the volume and weight of intestinal content was calculated relative to the control. The mean weight and volume of intestinal content of the control were considered as 100%.

Isolated tissue study

An adult male rabbit was used for the experiment. This was starved of food overnight and was exsanguinated by severing the jugular vein after which the intestine was removed *via* a midline incision on the abdomen. The intestine was placed inside a beaker containing Tyrode solution. A length of 3 cm segment of the jejunum was cut and mounted vertically in 25 ml Organ bath containing Tyrode solution. One end of the tissue was tied to a fixed support inside the Organ bath (Ugo Basil model 4050, Italy) and the other end to an isometric transducer (Ugo Basil Model 7005E) which was connected to a Laptop computer *via* a data capsule.

An equilibrium period of 30 min. was observed and the viability of the tissue was tested by recording its response to acetylcholine at a dose of 0.4 µg/ml. The tissue was then washed three times and allowed to rest, before being subjected to the extract at doses of 0.2, 0.4 and 0.8 µg/ml. Afterwards, the tissue was again washed and 0.4 µg/ml of acetylcholine plus 0.2 µg/ml of the extract were added and the response noted. Subsequently, after further washing of the tissue, its response to 0.4 µg/ml and 0.8

µg/ml respectively of the extract in the presence of 0.4 µg/ml of acetylcholine was recorded.

Statistical analysis

Graphpad instat was used for the analysis of data and all the data were summarized as the Mean ± Standard Deviation (S.D). The significance of differences among the groups was assessed using the one way analysis of variance (ANOVA) followed by Tukey-Kramer's post test. *P*-values less than 0.05 were considered significant.

Results

Effect of Aqueous extract of *Bridelia ferruginea* on castor oil induced diarrhoea

The aqueous stem bark extract of the plant significantly (*p*<0.05) reduced the frequency of stools and increased the consistency of the watery stools passed at all doses tested in a dose dependent fashion. Percentage inhibition of defaecation was 73% at 100mg/kg, 87% at 200mg/kg, 97% at 400mg/kg of extract and 100% for Loperamide (Table 1).

Table 1. Effect of *Bridelia ferruginea* Benth. on castor oil induced diarrhoea.

Treatment	Dose (mg/kg)	Mean number of stools	Mean number diarrhoea stools	Inhibition of defaecation (%)	Inhibition of diarrhoea stools (%)
<i>B. ferruginea</i> aqueous stem bark extract	100	3.4 ± 1.82*	0.2 ± 0.45*	73	97
	200	1.6 ± 2.30*	0.2 ± 0.45*	87	97
	400	0.4 ± 0.55*	0 ± 0.00*	97	100
Loperamide	3	0 ± 0.00*	0 ± 0.00*	100	100
Normal saline	3 ml/kg	12.6 ± 2.88	6.8 ± 1.30	0	0

Values are Mean ± SD; n = 5; **p*<0.05 compared with control (ANOVA, Tukey-Kramer Post Test).

Table 2. Effect of aqueous stem bark extract of *Bridelia ferruginea* Benth. on small intestinal transit in rats.

Treatment	Dose (mg/kg)	Intestinal Transit (%)	Inhibition (%)
<i>B. ferruginea</i> aqueous stem bark extract	100	52.67*	45.92
	200	59.00*	39.43
	400	41.81*	57.07
Atropine	3	31.04*	68.13
Normal saline	3 ml/kg	97.41	-

Values are Mean ± SD; n = 5; **p*<0.05 compared with control (ANOVA, Tukey-Kramer Post Test).

Effect of aqueous extract of *Bridelia ferruginea* on small intestinal transit

The stem bark aqueous extract of *B. ferruginea* significantly (*p*<0.05) reduced the small intestinal

transit of charcoal in rats induced by castor oil in a non-dose related manner. Percentage inhibition small intestinal transit in rats by *B. ferruginea* extract was 46% at 100 mg/kg, 39% at 200 mg/kg, 57% at 400 mg/kg and 68% for atropine (Table 2).

Effect aqueous extract of *Bridelia ferruginea* on castor oil induced enteropooling in rats

The extract significantly ($p < 0.05$) reduced the volume and weight of intestinal content in a dose related manner. Volume of intestinal contents was 2.90 ± 0.18 ml at 100 mg/kg, 2.60 ± 0.11 ml at 200 mg/kg, 1.45 ± 0.10 ml at 400 mg/kg, 0.70 ± 0.40 ml for atropine, 3.80 ± 0.29 ml for normal saline (Table 3).

Isolated tissue study

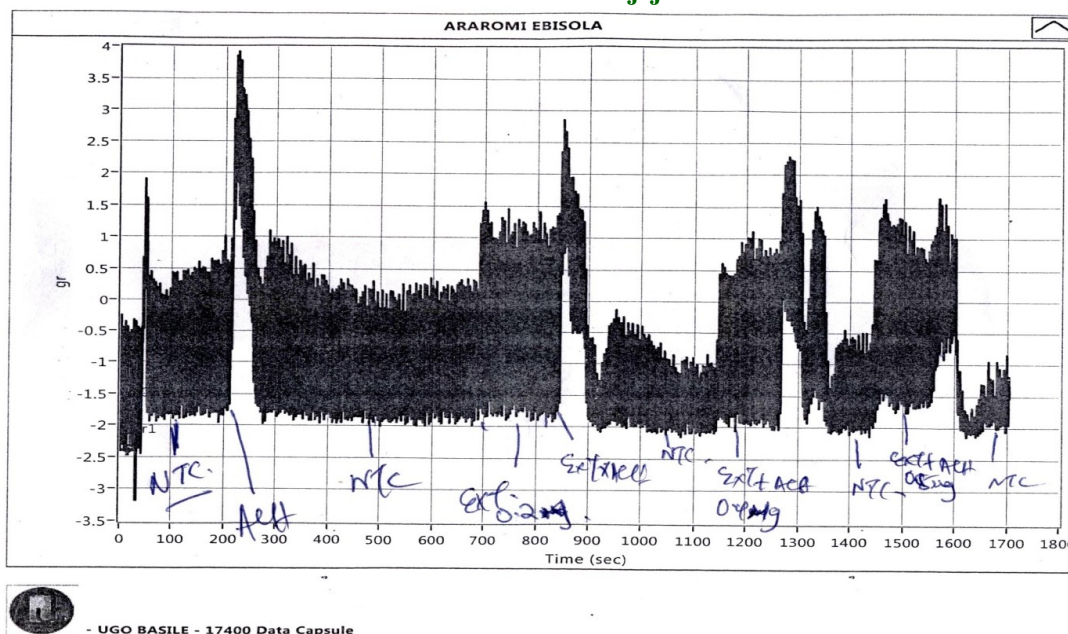
The aqueous stem bark extract of the plant stimulated spontaneous contraction of the rabbit jejunum at all the doses tested and reduced the acetylcholine evoked response in a dose dependent fashion. At a dose of $0.8 \mu\text{g/ml}$, the effect of acetylcholine was completely blocked by *B. ferruginea* stem bark aqueous extract as can be seen in Fig. 1.

Table 3. Effect of aqueous stem bark extract of *Bridelia ferruginea* Benth. on castor oil induced enteropooling in rats.

Treatment	Dose (mg/kg)	Weight of intestinal content (g)	Volume of intestinal content (ml)
<i>B. ferruginea</i> aqueous stem bark extract	100	4.44 ± 1.36	$2.90 \pm 0.18^*$
	200	3.44 ± 1.04	$2.60 \pm 0.11^*$
	400	3.06 ± 1.05	$1.45 \pm 0.10^*$
Atropine	3	2.82 ± 1.32	$0.70 \pm 0.40^*$
Normal saline	3 ml/kg	4.78 ± 1.36	3.80 ± 0.29

Values are Mean \pm SD; n = 5; * $p < 0.05$ compared with control (ANOVA, Tukey-Kramer Post Test).

Fig. 1: Effect of aqueous stem bark extract of *Bridelia ferruginea* Benth. on isolated rabbit jejunum



Discussion

The fact that the aqueous stem bark extract of *B. ferruginea* plant significantly ($p < 0.05$) reduced the frequency of stools (Table 1) as supported by the high percentage inhibition of defaecation (73% at 100mg/kg, 87% at 200 mg/kg, 97% at 400 mg/kg) and percentage inhibition of diarrhoea stools (97% at 100mg/kg, 97% at 200mg/kg, 100% at 400mg/kg) makes the plant at high doses to be in the class of

loperamide (orthodox drug) in the treatment of diarrhoea diseases.

This finding authenticates the plants folklore use in the treatment of diarrhoea (Gill, 1992). This effect can be attributed to the phytochemical content of the plant tannins, alkaloids, flavonoids, terpenes, saponins and anthraquinones and plants that have a high level of tannins are useful in the treatment of diarrhoea and dysentery (Dharmananda, 2003).

Owing to the fact that the extract significantly inhibited diarrhoea induced by castor oil in rats and caused a reduction of the castor oil induced enteropooling also in rats, and knowing that castor oil is believed to induce diarrhoea through increased secretion of the intestinal mucosa, the mechanisms for inhibition of diarrhoea by the extract may be antisecretory. This is supported by the fact that there was a significant ($p < 0.05$) reduction in the small intestinal transit of charcoal in rats induced by castor oil (though dose independently) further causing an increase in the absorption of water and electrolytes from the intestinal content. It suffices to note that percentage inhibition for atropine was better than that of the aqueous stem bark extract of *B. ferruginea* plant (Table 2). Also, owing to results of the isolated rabbit jejunum tissue study which showed a complete inhibition of acetylcholine induced contraction at extract dose of 0.8µg/ml, the extract may be considered to be anti cholinergic.

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