Antidiarrheal and Antispasmodic Effects of Ethanolic Stem Bark Extract of *Boswellia dalzielii* H. in Wistar Rats

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**ABSTRACT**

The modulatory potentials of the ethanolic stem bark extract of *Boswellia dalzielii* H. on diarrhea and gastrointestinal motility was investigated in rats. Antidiarrheal activity was evaluated in castor oil induced model using 25 inbreed, overnight fasted rats divided into five groups (A-E). Intestinal motility was determined using Charcoal transit time in 25 overnight fasted rats divided equally into five groups (A-E). Graded doses of the plant extract (100, 200 and 400mg/kg respectively) were given to groups B, C and D, while group A served as vehicle control (VEH) (distilled water) and group E served as Atropine control (0.1mg/kg). Antispasmodic activity of the plant extract was determined with 3cm piece of isolated rat ileum incubated in a thermostatically regulated organ bath. The set-up allows for in vitro assessment of intestinal motility with atropine and acetylcholine as standard drugs for cholinergic muscarinic receptor potentials. The ethanolic stem bark extract of *Boswellia dalzielii* H significantly (P<0.0001) and dose dependently reduced castor oil induced diarrhea and activated charcoal transit time in rats. The plant extract significantly (P<0.05) and dose dependently inhibited acetylcholine induced contractility of isolated ileac segments of rats when compared to controls. The ethanolic stem bark extract of *Boswellia dalzielii* H causes delay in gastrointestinal contractions via the inhibition of muscarinic cholinergic receptors in rats.

**Keywords:** Acetylcholine; Boswellia; Diarrhea; Intestinal; Motility

**INTRODUCTION**

Diarrhea is a change in bowel habits with decrease in stool consistency, increased stool volume, fluidity and frequency (3 or more times within 24 hours). It causes disturbance of the secretory and motor functions of the gastrointestinal tract through activation of calcium ion signaling and cyclic nucleotide pathways which stimulate Chloride ion channels in the apical membrane of intestinal enterocytes to increase intestinal fluid accumulation, inhibit Na+ transport and decrease fluid absorption (Field, 2004; Thiagarajah et al., 2015; Rao, 2019). Prolonged diarrhea prevents proper digestion, alters secretion and causes ineffective absorption of nutrients, vitamins, minerals, water and electrolytes. Diarrhea can be caused by parasites, bacteria and virus commonly spread through contaminated water. Inflammation, chemicals and hereditary disorders can also trigger diarrheal disease. Diarrhea can result in dehydration, distortion of body fluid volume, electrolyte level, cardiac output, blood pressure, heart rate and loss of energy that is detrimental to quality of life (World Health Organization (WHO), 1999). Diarrhea is among the common illnesses in clinical medicine and a global public health burden. It is a leading cause of morbidity and mortality. In children below the age of five years, diarrhea is the second foremost cause of death (525,000) yearly and with over 1.7 billion cases of diarrheal diseases globally (Igboeli et al., 2015; WHO, 2017; Lee et al., 2021).

Hypermotility of the intestine arising from diarrhea can distort good nutrition, damage mucus layer due to mucosal rubbing and enhance passage of enterobacteria into the mucosa (Takeuchi, 2018). Amelioration of secretory diarrhea involves fluid and electrolyte replacement. Adverse effects may arise from the commonly used prescription in the treatment of diarrheal diseases. The routine pharmacologic...
prescriptions are antikinetics and antidiarrheal agents like dicyclomine, hyoscymamine, 5-HT3 antagonists and α2 – noradrenergic blockers. So also, prostaglandin synthase inhibitors, μ, δ-opiod agonists are used to reduce gastrointestinal motility in diarrhea. Some of these drugs in certain individuals aggravate gastric esophageal reflux disease, ischaemic colitis, constipation, and lead to cardiac cytotoxicity, disturbance in respiratory and central nervous system functions (Lee et al., 2021).

some medicinal plants have antidiarrheal and antispasmodic potentials that is achieved through restoration of smooth muscle segmentation activity, elongate intestinal transit time and enhance consistency of faeces (Bakare, 2011; Sumi, 2015; Lee et al., 2021). Medicinal plants are generally affordable, accessible, and available and appear to exhibit less adverse effects inherent in the present day antidiarrheal and anti-motility pharmacologic agents. The plant Boswellia dalzielii H contains several secondary metabolites such as flavonoids, tannin, saponins among others and are responsible for the medicinal uses of the plant (Nwinyi et al., 2004; Danlamli et al., 2015; Kafuti et al., 2018). The plant has hypolipidemic, antioxidant, antibacterial, antifungal potentials, and promotes the healing of chronic gastric ulcer (Jaafar et al., 2017; Kafuti et al., 2017; Akinsanmi et al., 2019; Yusuf et al., 2022). The Boswellia plant is also used for the treatment of skin ailments, asthma, rheumatisms, bursitis, a tendonitis, ulcerative colitis and Crohn’s diseases, dysentery, ulcers, blood purification, several gastrointestinal disorders in the folk medicine (Kim and Neophytou, 2009; Smithson et al., 2017; Varma et al., 2021). This study investigates the effects of ethanolic stem bark extract of Boswellia dalzielii H on Castor oil induced diarrhea and on intestinal motility via muscarinic-cholinergic pathways in rats.

MATERIALS AND METHODS

Chemicals: Atropine sulphate, acetylcholine hydrochloride, ethanol, castor oil, activated charcoal, sodium chloride, potassium chloride, calcium chloride, potassium dihydrogen phosphate, magnesium chloride, sodium bicarbonate, and glucose of ANALAR grade and were either obtained from Sigma (St. Louis, MO, USA) or BDH England. Stock concentrations of acetylcholine, atropine and plant extract were prepared with distilled water and subsequently diluted serially with Tyrode solution to avoid distorting the composition of the physiologic saline in the tissue bath.

Collection and Authentication of Boswellia dalzielii H

Plant

The stem bark of Boswellia dalzielii H was obtained from Arawa area of Akko LGA of Gombe State (Latitude 10o18’ North and longitude 11o 1’ East). The stem bark was identified and authenticated by a Botanist in the Department of Biological Science, Faculty of Science, University of Maiduguri. A voucher specimen (BS/SB/04/23) was deposited in their herbarium.

Preparation of Ethanolic Stem Bark Extract of Boswellia dalzielii H

The stem bark was shade dried and pulverized with porcelain pestle and mortar. Fine powder was obtained using fine sieve of 0.05mm diameter. Standard protocol of Soxhlet extraction (50g) with absolute ethanol was followed. Filtrate obtained was evaporated with dry heat in oven at 40oC-45o C. The percentage yield was determined as:

Weight of extracted material (g) ×100
Weight of original plant material (g)

{Percentage yield: 7.35g (14.7%)}.

The dried marc was kept in an airtight dry glass container at 4oC in a refrigerator until required.

Preparation of standard drugs (Acetylcholine, atropine).

The molar concentration of the standard drugs was calculated as:

Molarity= moles of solute/liter of solution.

Distilled water was used to prepare the stock solution and the appropriate final concentration to be added to the tissue bath was diluted serially using Tyrode solution (Dubbelboer, 2019), in a ratio of 1:9. i.e. one part of the stock with 9 part of Tyrode solution.

Animals

Inbreed, healthy and pathogen free (regular monitoring of rectal temperature) Wistar rats of either sex (180-200g) obtained from the animal house of the Department of Human Physiology, College of Medical Sciences, University of Maiduguri were used in the study. The rats were kept for five days at a temperature of 250C-28°C with a cycle of 12 hours of darkness, 12 hours of day/light and humidity of approximately 46% to acclimatize with the study environment. Drinking water and commercially available feed pellets (Livestock feed© were provided to the animals ad libitum. All the animals in the study were kept and catered for in accordance with ethical requirements on Animal Experimentation.

Study Design

Determination of the Activity of Ethanolic Stem Bark Extract of Boswellia dalzielii H on Castor Oil Induced Diarrhea in Rats. Diarrhea was induced in rats with castor oil as described by Tague et al. (2019), with some modifications in the fasting duration to extend up to 24 hours. Twenty-five (25) overnight
fasted rats of either sex was assigned to five treatment groups with five rats in each group. Rats in group A received distilled water (vehicle) 5ml/kg. Rats in groups B, C, and D received 100, 200 and 400 mg/kg of the ethanolic stem bark extract of Boswellia dalzielli H through intragastric intubation respectively. Rats in group E were injected (intraperitoneal) with atropine, 0.1mg/kg. One hour after the treatments, rats in all the groups received Castor oil, 1ml/200g. Each rat was housed singly and placed on absorbent white plain sheets of papers. Time for onset of diarrhea was recorded for all the groups. The absorbent white plain sheets of papers in all the groups were changed after every one hour. The stool mass, and stool count i.e. diarrheal droppings on the papers were recorded after each hour for a period of four hours. The inhibition of diarrhea in percent and the stool emission frequency (SEF) were calculated as follows:

\[
SEF = \frac{\text{Total number of stool}}{\text{Time (4hours)}}
\]

\[
I (%) = \frac{\text{SMDC} - \text{SMDT}}{\text{SMDC}} \times 100
\]

Where I= inhibition;

SMDC = stool mass of diarrheal control;

SMDT = stool mass of diarrheal test (extract).

**Determination of the Activity of Ethanolic Stem Bark Extract of Boswellia dalzielli H on Gastrointestinal Transit Time in Rats.**

Charcoal transit time was determined in rats by the modified method of (Bakare, et al., 2011). Twenty-five (25) overnight fasted rats of either sex was divided equally into five groups (A-E). Rats in group A received distilled water (vehicle) 5ml/kg by intragastric intubation. Similarly, rats in groups B, C, and D received 100, 200 and 400 mg/kg of the ethanolic stem bark extract of Boswellia dalzielli H respectively. Rats in group E received injection of atropine sulphate 0.1 mg/kg intraperitoneally. One hour after the treatments above, rats in all the groups received 1 ml of 10% activated charcoal by gastric intubation. The rats were later euthanized with over dose of anaesthetic ether (by inhalation) at the end of one hour. The stomach along with intestines were removed from the abdomen. The intestines were carefully separated from mesentry, cut at the pylorus and ileocolonic junction. The distance travelled by charcoal meal was measured in centimeter. Peristaltic index was determined and expressed as percentage of the distance travelled by the charcoal relative to the total length of the intestine.

\[
\text{Peristaltic index (PI)} = \frac{\text{Distance travelled by charcoal meal}}{100} \times \text{Length of small intestine}
\]

\[
\text{Percentage (%) inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100\%
\]

**Preparation and Recording of Contractile Activity of Isolated Rat Ileum**

Overnight fasted rats (5) were euthanized by cervical dislocation. The abdomen was excised and 2-3cm piece of the ileum proximal to the ileocecal junction was detached from each rat (n=5) and the luminal contents flushed with Tyrode solution. Cotton thread ligatures were placed on either side of the ileal segment. One end was attached to a high-performance isometric FT-302 force transducer. The second ligature was fixed to a stainless-steel bent needle at the base of a tunnel-shaped 25 ml tissue bath filled with Tyrode solution (Sodium chloride (NaCl) 8.0g; Potassium chloride (KCl) 0.2g; Magnesium chloride (MgCl2) 0.1g; Sodium phosphate monobasic dihydrate (NaH2PO4H2O) 0.05g; Calcium chloride (CaCl2) 0.2 g; sodium hydrogen carbonate (NaHCO3) 1.0g; glucose-1.0g in a litre of distilled water) and continuously bubbled with air, maintained at 37°C and pH 7.4. One gram of (1g) of resting tension was applied which stabilized within thirty minutes. Normal contractile activities of the intestine were recorded with IWX/214 data acquisition unit (data capsule, Ugo basile) (Kim et al., 2013; Jespersen et al., 2015).

**Effects of Graded Concentrations of Acetylcholine on the Contractility Rat Ileum**

On the stabilized rat ileum, graded concentrations of acetylcholine (0.1, 0.2, 0.4, 0.6 and 0.8ml) from a stock concentration (6.8 x 10^-6 M) were added to tissue bath with final bath concentrations of 2.72 x 10^-6, 5.44 x 10^-6, 1.09 x 10^-5, 1.63 x 10^-5 and 2.18 x 10^-5 M respectively. Each concentration of acetylcholine added to the bath was allowed to be in contact with the tissue for 30-45 seconds before the Tyrode was drained out from the tissue bath and refilled with fresh Tyrode from a reservoir. Three successive washings were done before addition of the next concentration of acetylcholine. Contractile responses for each concentration was recorded and measured from the baseline to the peak of response. The ceiling response attained was confirmed by testing with higher concentration of acetylcholine until diminished response was obtained.

**Effect of Atropine on Acetylcholine induced Contraction of Rat Ileum**

Atropine was added to the Tyrode in the reservoir. Final bath concentration of 8.64 x 10^-9 was attained and maintained throughout the investigation. The ileum was incubated with atropine for fifteen minutes and graded concentrations of acetylcholine (2.72 x 10^-6, 5.44 x 10^-6, 1.09 x 10^-5, 1.63 x 10^-5 and 2.18 x 10^-5 M were tested respectively. The contact time and washing process for acetylcholine was maintained.

**Effect of Ethanolic Stem Bark Extract of Boswellia dalzielli H on Acetylcholine induced Contraction of rat Ileum**

The isolated rat tissue was thoroughly washed by regular drain and refill with fresh Tyrode until it stabilized. Final...
bath concentration of $4 \times 10^{-4}$ mg/ml of the plant extract was maintained. The ileal tissue was incubated with the plant extract for 15 minutes followed by addition of graded concentrations of acetylcholine $2.72 \times 10^{-4}$, $5.44 \times 10^{-4}$, $1.09 \times 10^{-5}$, $1.63 \times 10^{-5}$ and $2.18 \times 10^{-5}$ M respectively.

All ileal responses due to acetylcholine alone, acetylcholine in the presence of atropine and acetylcholine induced contraction in the presence of the plant extract were recorded and measured in millimetre (mm).

**Statistical Analysis**

Values are expressed as mean ± SEM. Mean concentration response curve to contraction rate were analyzed by using one-way ANOVA with Dunnett’s Control test to assess for significant changes associated with the height and frequency of contractions. Analyses were considered significant at a P value of <0.05. The data were analyzed using JMP version 11 software (SAS Institute Inc, Cary, NC).

**RESULTS**

**Effect of Ethanolic Stem Bark Extract of *Boswellia dalzielli H* on Castor Oil Induced Diarrhea in Rats.**

Oral administration of 100, 200 and 400 mg/kg respectively of the extract, dose dependently and significantly (P<0.05) reduced castor oil induced diarrhea when compared to control. The graded doses of 100, 200 and 400 mg/kg of the plant extract gave 43.32%, 56.72% and 68.66% inhibition of diarrhea respectively. The standard drug atropine inhibits the diarrhea by 82.09% as shown in figure 2 below.

**Effects of Ethanolic Stem Bark Extract of *Boswellia dalzielli H* on Stool Emission Frequency in Castor Oil Induced Diarrhea in Albino Rats.**

Intragastric administration of graded doses of ethanolic stem bark extract of *Boswellia dalzielli H* has remarkably reduced the frequency of stool induced in rat by Castor oil. The lowest dose (100 mg/kg) of the plant extract caused 43.32% decrease in the stool emission frequency while at 400 mg/kg, the plant extract led to 68.88% decrease in the stool emission frequency. The muscarinic cholinergic antagonist atropine used as standard caused the highest delay in the stool emission frequency. The

**Effect of Ethanolic Stem Bark Extract of *Boswellia dalzielli H* on Activated Charcoal Transit Time in Albino Rats.**

Pre-treatment with graded doses (100, 200 and 400 mg/kg body weight of rat) of the plant extract dose dependently and significantly (p<0.05) decreased activated charcoal transit time in rat when compared to control. 400 mg/kg of the plant extract reduced the meal transit by 62.54% while atropine reduced it by 74.46%. These decreases were significant (p<0.0001) when compared to control as shown in table 1.

**Effects of Acetylcholine on the Contractility of Isolated Rat Ileum.**

Graded concentrations of Acetylcholine dose dependently increased the contractile activities of the isolated rat ileum. Amplitude of contractions increased as shown in figure 4.

**Effects of Graded Concentration of Acetylcholine in the Presence of Ethanolic Stem Bark Extract of *Boswellia dalzielli H* on the Contractility of Isolated Rat Ileum.**

The ethanolic stem bark extract of *Boswellia dalzielli H* ($2.0 \times 10^{-6}$ g/ml) competitively reduced acetylcholine induced increase in intestinal motility of rat. The nature of antagonism is similar to the one exhibited by atropine in figure 4.
Effects of Graded Concentrations of Acetylcholine in the Presence Atropine on the Contractility of Isolated Rat Ileum.

Atropine (1.8 x 10^-4g/ml) competitively antagonized acetylcholine induced contractile responses on the rat intestine as shown in figure 4. The antagonism was significant (P<0.05) when compared to control. The log concentration curve for Acetylcholine in the presence of atropine and plant extract respectively were parallel and shifted to the right of Acetylcholine.

[Diagram: log conc response curve]

**Figure 4:** Effects of graded concentrations of Acetylcholine alone, acetylcholine and plant extract and, acetylcholine and atropine on contractility of rat ileum.

**DISCUSSION**

Ingestion of contaminated food or drinking water, inadequate environmental sanitation, unhygienic lifestyle and failure of the neural, paracrine or hormonal balance associated with the gastrointestinal tract can cause diarrheal diseases (Seeley, 2004, Rahman et al., 2015). In many parts of Northern Nigeria, aqueous stem bark extract of *Boswellia dalzielii* is used as a traditional remedy for diarrheal diseases for decades with little or no scientific scrutiny. In order to validate these claims, animal diarrheal disease models of castor oil, transit time and isolated intestinal motility were used. In this study, ethanol was used as solvent for extraction contrary to the aqueous extraction used in the traditional herbal practice. Ethanol has the ability to provide higher recovery yield of antioxidants, phenolics, flavonoid, tannins and other bioactive contents responsible for the medicinal use of plants in gastrointestinal disorders. Stem bark was selected for the study due to its higher contents of phytochemicals when compared to leaf or root (Dandashire, et al., 2019).

The castor oil induced diarrheal model used in this study has compared to leaf or root (Dandashire, 2004). In many parts of Northern Nigeria, the ethanolic stem bark extract of *Boswellia dalzielii* is used as a traditional remedy for diarrheal diseases for decades with little or no scientific scrutiny. In order to validate these claims, animal diarrheal disease models of castor oil, transit time and isolated intestinal motility were used. In this study, ethanol was used as solvent for extraction contrary to the aqueous extraction used in the traditional herbal practice. Ethanol has the ability to provide higher recovery yield of antioxidants, phenolics, flavonoid, tannins and other bioactive contents responsible for the medicinal use of plants in gastrointestinal disorders. Stem bark was selected for the study due to its higher contents of phytochemicals when compared to leaf or root (Dandashire, et al., 2019).

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ion oscillations to prevent acetylcholine from binding to its receptor leading to reduced amplitude and frequency of contraction of the isolated rat ileum.

Conclusion
The ethanolic stem bark extract of Boswellia dalzielli H used in this study significantly reduced intestinal transit, inhibits castor oil induced diarrhea, and relaxes ileal smooth muscle contractility and competitively antagonized acetylcholine-induced contraction of the small intestine in rats. This support the folkloric claim that the plant is used to treat diarrhea and other gastrointestinal disorders.

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Conflict of Interest
The authors have no conflict of interest to declare.

Author’s Contribution
YAH designed and coordinated the research activities; LI and LA conducted the literature search/statistical analysis; PLM performed the isolated muscle study; HS, performed the gastrointestinal study; AAH and JAI conducted the diarrheal study

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