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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 inbred, 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, hyoscyamine, 5-HT₃ antagonists and α_2 – noradrenergic blockers. So also, prostaglandin synthase inhibitors, μ , δ -opioid 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; Danlami et al., 2015; Kafuti et al., 2018). The plant has hypolipidemic, antioxidant, antibacterial, antifungal potentials, and promotes the healing of chronic gastric ulcer (Jaafaru 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, rheumatism, 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 10°18' North and longitude 11°01' 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 0.05 mm diameter. Standard protocol of Soxhlet extraction (50g) with absolute ethanol was

followed. Filtrate obtained was evaporated with dry heat in oven at 40°C-45°C.

The percentage yield was determined as:

$$\frac{\text{Weight of extracted material (g)}}{\text{Weight of original plant material (g)}} \times 100$$

{Percentage yield: 7.35g (14.7%)}



Figure 1: Map of Gombe state showing Akko local government area where the plant was collected

Source: <https://soluap.com/akko-local-government-area-of-gombe-state/>

The dried marc was kept in an airtight dry glass container at 4°C 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

Inbred, 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 25°C-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 Tagne 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 dalzielii* 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:

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

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

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 dalzielii* 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 dalzielii* 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 was removed from the abdomen. The intestines were carefully separated from mesentery, 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.

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 (NaH₂PO₄·2H₂O) 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 x10⁻⁴ M) were added to tissue bath with final bath concentrations of 2.72 x10⁻⁶, 5.44 x10⁻⁶, 1.09 x10⁻⁵, 1.63 x10⁻⁵ and 2.18 x10⁻⁵ M respectively. Each concentration of acetylcholine added to the bath was allowed to be in contact with the tissue for 30-45seconds 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 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 8.64x10⁻⁹ was attained and maintained throughout the investigation. The ileum was incubated with atropine for fifteen minutes and graded concentrations of acetylcholine (2.72 x10⁻⁶, 5.44 x10⁻⁶, 1.09 x10⁻⁵, 1.63 x10⁻⁵ and 2.18 x10⁻⁵ M were tested respectively. The contact time and washing process for acetylcholine was maintained.

Effect of Ethanolic Stem Bark Extract of *Boswellia dalzielii* 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 4x10⁻⁴ 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 x10⁻⁶, 5.44 x10⁻⁶, 1.09 x10⁻⁵, 1.63 x10⁻⁵ and 2.18 x10⁻⁵ 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 dalzielii* 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.

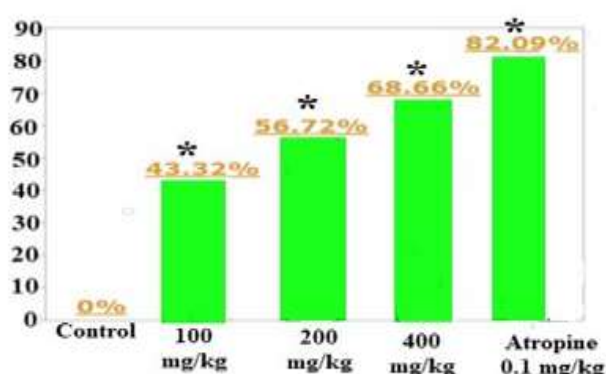


Figure 2: Percentage Inhibition of Diarrhea by Graded Doses of Ethanolic Stem Extract of *Boswellia dalzielii* in Castor Oil Induced Diarrhea in Rats

Effects of Ethanolic Stem Bark Extract of *Boswellia dalzielii* 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 dalzielii* H has remarkably reduced the frequency of stool induced in rat by Castor oil. The lowest dose (100mg/kg) of the plant extract caused 43.32% decrease in the stool emission frequency while at 400mg/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 reduction in the rate of stooling by the plant and atropine was significant ($P < 0.0001$) and dose dependent when compared to control.

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

Pre-treatment with graded doses (100, 200 and 400mg/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.

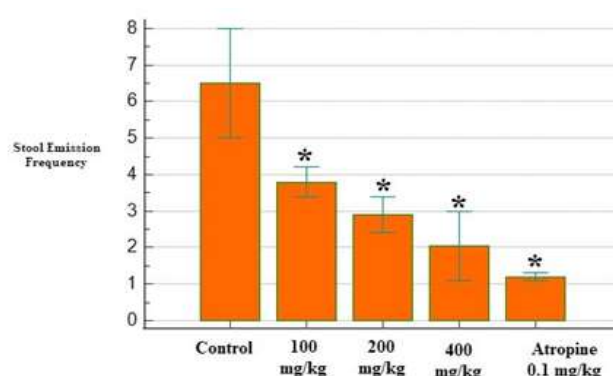


Figure 3: Effects of Ethanolic Stem Bark Extract of *Boswellia dalzielii* H. on Stool Emission Frequency in Castor Oil Induced Diarrhea in Albino Rats.

Table 1: Effect of Ethanolic Stem Bark Extract of *Boswellia dalzielii* H on Activated Charcoal Transit Time in Rats

Treatment	Length of Intestine (cm)	Length of meal (cm)	Peristaltic index	Inhibition (%)
Control	51.00 ± 1.68	41.40 ± 43	18.82	0
100 mg/kg	58.60 ± 1.34	36.80 ± 33	34.82	12.5
200 mg/kg	53.40 ± 1.08	27.60 ± 23*	48.31*	30.43
400 mg/kg	53.40 ± 1.66	20.00 ± 34*	62.54*	51.60
Atropine 0.1 mg/kg	56.40 ± 1.77	14.40 ± 33*	74.46*	65.21

* $P < 0.05$ was considered statistically significant when compared to control

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 dalzielii* H on the Contractility of Isolated Rat Ileum.

The ethanolic stem bark extract of *Boswellia dalzielii* H (2.0×10^{-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 (1.8×10^{-8} g/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.

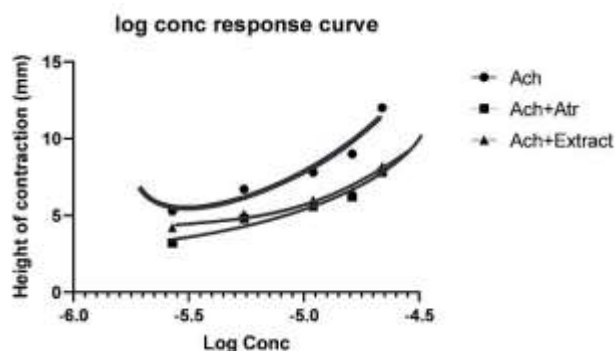


Figure 4: Effects of graded concentrations of Acetylcholine alone, acetylcholine and plant extract and, acetylcholine and atropine on contractility of rat ileum.

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 clinical values because it reflects the complex process of secretory diarrhea in humans which is characterized by intestinal hypersecretion and accelerated intestinal transit. Castor oil, a vegetable oil and laxative with hydroxyl fatty acids is hydrolyzed to ricinoleic acid by pancreatic lipase, bile and water. The ricinoleic acid stimulates the release of nitric monoxide, promotes calcium ions permeability, increase synthesis and release of prostaglandin E from intestinal mucosa. Thus, leading to increased intestinal motility and reduced reabsorption of sodium and potassium ions. As a result, excess water and salts are channeled to the colon beyond its absorptive capacity coupled with the oily quality, secretory diarrhea is facilitated (Kaur *et al.*, 2014). In this study, the ethanolic stem bark extract of *Boswellia dalzielii* H inhibits *in vivo* castor oil induced diarrhea by 43.32%, 56.72 %, 68.66%, 82.09% 12.5 %, 30.43% and 51.60% respectively when 100, 200 and 400mg/kg of the plant extract were used respectively as shown in Figure 2.

The decrease in the frequency of defecation as shown in figure 3 may be associated to its secondary metabolites (flavonoids and tannins). It has been established that flavonoids, tannin. Phenolics and other phytochemicals inhibits intestinal motility and hydro-electrolytic secretions (Palombo, 2006; Igboeli *et al.*, 2015). Tannins inhibit α -

amylase, trypsin activities and precipitates proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical irritation, alteration and reduce secretion (Ogwuru and Adamczeski, 2000; Tadesse *et al.*, 2017).

Intestinal transit time observed in this study further support the inhibitory potential of the plant extract by 12.5 %, 30.43% and 51.60% respectively when 100, 200 and 400mg/kg of the plant extract were used respectively as shown in Table 1.

The functional unit of the *Boswellia* plant is the boswellic acid and is linked to the medicinal potentials of the plant which include inhibition of 5-lipoxygenase, COX-1, NF- κ B, IL-6, IL-2, TNF- α , gamma (IFN- γ), interferon, (Yusuf *et al.*, 2022). The plant extract probably reduced the diarrheal effect of castor oil by reducing the sensitivity of intestinal mucosal epithelium or promote smooth muscle inhibition.

Atropine competitively antagonized acetylcholine induced contractile activities at muscarinic cholinergic receptors. In this study, the ethanolic stem bark extract of *Boswellia dalzielii* H delayed charcoal meal transit similar to atropine (Table 1). Cholinergic receptors are predominant in the gastrointestinal tract and are responsible for the ability of atropine to hinder the peristaltic movement of the charcoal meal in the intestine (Gandhi and Venkatakrishna-Bhatt, 1999). The ethanolic stem bark extract of *Boswellia dalzielii* H may have diminished intestinal transit time of charcoal meal due to the presence of atropine-like compound or α -adrenergic like-agonist. This delay in charcoal transit time (Table 1) supports the traditional claim of using the plant extract in the treatment of diarrhea.

Acetylcholine acting on muscarinic receptors of the metabotropic family of receptor proteins triggers signal transduction cascade that promote conformational changes in the G-protein leading to the release of calcium ions in the inositol-phospholipase pathway. Calcium ions combine with calmodulin to cause the gastrointestinal smooth muscle contraction (Sam, 2022). Results of this study on isolated rat ileum shows that atropine and ethanolic stem bark extract of *Boswellia dalzielii* H blocked graded concentration of acetylcholine-induced contractile responses (Figure 4). The antagonism exhibited by the plant extract was similar to the activity of atropine. This suggests that the plant extract mediate its inhibitory action on the intestine via muscarinic cholinergic receptor. It also possibly interferes with calcium 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 dalzielii* 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 supports 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 perform the isolated muscle study; HS, performed the gastrointestinal study; AAH and JAI conducted the diarrheal study

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