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Brachystegia Eurycoma Harms (Fabaceae) Stem Bark Extract Modulates Gastrointestinal Motility in Animal Models

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ABSTRACT

Brachystegia eurycoma, a plant in the Fabaceae family has been used ethno-medicinally in the softening of bulky stool, and has been associated with protection of colonic cancer. This study was therefore aimed at the evaluation of possible modulatory effect of the stem bark extract of *Brachystegia eurycoma* (BE) on gastrointestinal motility. The in vivo activity of BE on gastrointestinal motility was evaluated using the following experimental models viz: normal intestinal transit, castor oil- induced diarrhoea, and intestinal fluid accumulation tests in rodents. The in vitro activity on isolated intestinal motility was also investigated on the following parameters viz: spontaneous, acetylcholine- and high KCl-induced intestinal contractions. BE (100, 300 and 700 mg/kg p.o.) produced a moderate decrease in normal intestinal transit while significantly ($P < 0.05$) reducing the propulsive movement in castor oil-induced diarrhoea compared with the control group (5% tween 80 10 ml/kg, p.o.). The extract also produced a dose-dependent, significant increase in the onset of diarrhoea. BE (100, 300 and 700 mg/kg) additionally reduced the diarrhoea score, number and weight of wet stools. The in-vivo antidiarrheal index ($ADI_{in\text{-}vivo}$) of 37.14% produced by the extract at the dose of 300 mg/kg was lower compared to that produced by loperamide 5 mg/kg (54.74%). In vitro results revealed that the crude extract, the aqueous and chloroform fractions inhibited both spontaneous, acetylcholine and KCl-induced contractions in a concentration-dependent manner. In conclusion, the stem bark extract of *Brachystegia eurycoma* has been shown to exhibit antidiarrheal activity, possibly mediated by anti-muscarinic receptor activity or interaction with calcium channels

Keywords: *Antidiarrhea; Gastrointestinal motility; Brachystegia eurycoma; Diarrhea; Enteropooling*

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INTRODUCTION

Gastrointestinal (GI) motility is an integrated process that involves myoelectrical and contractile activity, tone, compliance and transit. These make up the entities of motility and can be produced and regulated by local and circulating neurohumoral substances (Hansen, 2003). Hormones and neurotransmitters are the dominating components involved in smooth muscle motility, and they interact either directly or indirectly on muscle cells (Hansen, 2003). Oftentimes the normal GI motility can be modified by various means which includes toxins, germs, and spicy foods leading to diarrhoea. Diarrhoea is a symptom characterized by rapid and frequent passage of semi-solid or liquid faecal material through the gastrointestinal tract. Several types of diarrhoea exist (based on their clinical manifestations), but it can be classified broadly into acute and chronic for diagnostic and therapeutic purposes. Acute diarrhoea is often the more frequently observed type of diarrhoea and it has a significant impact on morbidity and mortality in all age groups worldwide, however infants and children under the age of three are more susceptible(Hirschhorn, 1980; Muriithi, 1996; Farthing, 2004). Diarrhoea was estimated to have caused 1.1 million deaths in children of about 5years and a further1.5 million deaths in children under the age of 5 (UNICEF, 2009; Wardlaw *et al*., 2010). Etiological classification of diarrhoea can be classified as infectious or non-infectious (de Hostos, Choy and Nguyen, 2011). Infectious diarrhoea is caused by a virus, parasite or bacterium while non-infectious diarrhoea can be caused by toxins, chronic diseases or antibiotics (Reisinger *et al*., 2005; Hodges and Gill, 2010). Pharmacological models of non-infectious diarrhoea were employed in the current study.

Brachystegia eurycoma Harms is native to tropical Africa (Okwu & Okoro, 2006). It can be found growing along river banks or swamps in Western and Eastern Nigeria and has also

been found in Cameroon (Uzomah & Ahiligwo, 1999). It is a large tree and its branches are spreading in nature and are either irregular or twisted in shape. The fruit matures from September to January and is released by an explosive mechanism (Uzomah and Ahiligwo, 1999). The bark of the plant is rough and fibrous which peels off in patches and often gives out brownish buttery exudates (Keay, Onochie and Standfield, 1964; Enwere, 1998). *B. eurycoma* is consumed as a food crop in Nigeria and often used for thickening soups and as a flavouring agent (Ene-Obong and Carnovale, 1992). It is known locally in Nigeria by the following names 'okuen (Edo), 'okung' (Efik), eku (Esan), 'achi' (Igbo),The exudate is used in fast healing of wounds (Adikwu and Enebeke, 2017). The stem bark has been reported to have anti-inflammatory activities and diuretic effect (Igwe and Okwu, 2013). The seeds of *B. eurycoma* are reported to assist in softening bulky stools and have been associated with colon and rectal cancer protection (Ndukwu, 2009). This study is therefore aimed at the investigation of the methanol stem bark extract of *B. eurycoma* on GI motility in normal and diarrhoeic conditions.

MATERIALS AND METHODS

Plant material: The stem bark of *Brachystegia eurycoma* was collected in May, 2015 at Iwo in Osun state. The plant material was identified and authenticated by Professor B.A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria.

Experimental animals: Albino rats and albino mice of both sexes were obtained from the Animal House of the Department of Anatomy, School of Basic Medical Science, University of Benin, Nigeria. They were allowed to acclimatize for three weeks prior to the start of the experiment proper. Throughout the study, the animals were maintained in standard cages in the Animal House of the Department of Pharmacology, Faculty of Pharmacy, University of Benin and were allowed access to regular rodent feed and clean water *ad libitum*. Due ethical approval were obtained from the Ethical Committee, Faculty of Pharmacy University of Benin, Nigeria and the animals were handled in accordance to standard guidelines as stated in the Guide for Care and Use of animals (Committee, 2011).

Preparation of extract: The collected stem bark was cut into pieces and air-dried in a well ventilated laboratory at room temperature for two weeks after which the pieces were further dried between 50-60ºC (Müller, Heindl and Bogers, 2006) in the oven for 3 h and then milled to powder. The powdered stem-bark were extracted in methanol using the Soxhlet extractor for 24 h. Filtration was performed immediately after extraction and the combined filtrate was evaporated to dryness under reduced pressure in a rotary evaporator maintained at 40ºC. The percentage yield of the extract was 7.2%w/w.The solid extract obtained was kept in the refrigerator at 4°C till required for use.

Solvent-solvent partitioning of the methanol extract: The crude extract (15 g) was dissolved in distilled water and fractionated in a separatory funnel in the presence of methanol and chloroform. The resulting mixture was then shaken vigorously. The mixture was left to stand till complete separations were observed. The non-aqueous fraction (chloroform layer) was then separated from the aqueous fraction. The procedure was repeated till an almost clear lower phase of the chloroform fraction was obtained. Both fractions were then carefully evaporated to dryness over a hot water bath maintained at 80°C for the aqueous fraction (Kiathevest *et al.*, 2009; Tan *et al.*, 2014; Bafor *et al.*, 2015) and 60°C for the chloroform fraction (Michelin *et al.*, 2005; Adegoke, Opata and Olajide, 2010). The yield for the aqueous fraction was 86.7% w/w while that for the chloroform fraction was 3.0% w/w.

***In vivo* studies**

Normal intestinal transit: Modification of a previously described protocol (Hsu, 1982; Akindele *et al*., 2014) was used. Briefly, mice (18-25 g) were allotted to five groups containing four animals each. Three groups were separately treated orally with the methanol extract (100, 300 and 700 mg/kg) while distilled water (0.2 mL) and loperamide hydrochloride (5 mg/kg) were separately administered orally to the control and standard groups respectively. One hour after treatment, the mice were administered a standard charcoal meal (0.2 mL/mouse made up of 5% charcoal suspension in 5 % tween 80) orally. The mice were then sacrificed 30 min after administration of the charcoal meal and the small intestine immediately isolated. Peristaltic index for each mouse was expressed as a percentage of the distance travelled by the charcoal meal relative to the total length of the small intestine (Aye-Than, Kukarni and Tha, 1989; Akindele, Salako and Ohonbamu, 2014).

Castor oil – induced diarrhea: The animals were divided into five groups of four mice each. Four groups were treated with the extract (100, 300 and 700 mg/kg) and loperamide hydrochloride (5 mg/kg) orally. The control group was given distilled water (0.2 mL) orally. Pre-treatment was done one hour prior to the administration of castor oil (0.3 mL/mouse), which was determined from preliminary studies). Each mouse was kept for observation under a transparent funnel, the floor of which was lined with paper and observed for four hours (Izzo *et al.*, 1992; Akindele, Salako and Ohonbamu, 2014).The following parameters were observed: time elapsed between the administration of the castor oil and the excretion of the first diarrhoea faeces (onset of diarrhoea),the total amount of hard stool, semi-solid stool and watery stool. The weight of watery stool (i.e. the semi-solid and watery stool) and the total weight of all stools (which comprises of hard stool, semi-solid stool and watery stool) were determined. A numerical score centred on stool consistency was assigned as follows: 1 (hard stool), 2 (semi-solid stool), and 3 (watery stool) (Akindele, Salako and Ohonbamu, 2014). The *in vivo* antidiarrheal index ($ADI_{in\ vivo}$) was calculated according to the formula: $ADI_{in\ vivo} = \sqrt[3]{Dfreq \times Gmeq \times Pfreq}$ (Akindele, Salako and Ohonbamu, 2014) where D_{freq} is the delay in defaecation time or diarrhoea onset (in % of control), G_{meq} is the gut meal travel reduction (in % of control), and P_{freq} is the

purging frequency, as number of stool reduction (in % of control).

Intestinal fluid accumulation: Albino rats (150 - 200 g) were divided into three groups of four rats each. Intestinal fluid accumulation was induced by oral administration of castor oil (2 mL/rat) (Robert *et al.*, 1976). The rats were fasted overnight, but had access to water prior to the experiment. The control group was administered distilled water (0.4 mL) orally and the second group was orally administered the dose of extract with the most potent activity from the normal intestinal transit model (700 mg/kg), while a third group was administered loperamide (5 mg/kg) orally. One hour later, castor oil (2 mL/rat) was administered intragastrically and 1h after that, the rats were sacrificed. The rats were dissected and the small intestines were ligated both at the pyloric sphincter and at the ileocecal junction after which they were immediately isolated. Each intestine was weighed and the intestinal contents were carefully expelled into measuring cylinders and the volume determined. Each intestine was reweighed and the difference between the full and empty intestine was determined.

***In vitro* studies**

Tissue preparation: The mice to be used were fasted for 12 h, and were humanely euthanized under anaesthesia by cervical dislocation and exsanguination. Then the intestine from duodenum to colon was gently flushed out using sodium chloride. A section of the duodenum was carefully isolated, freed from attached mesenteries, and then placed in a petri dish containing previously warmed and aerated physiological salt solution. The physiological salt solution used for the *in vitro* studies was of the following composition (in mM): NaCl 136.9, $NaHCO_3$11.9, D-glucose 5.6, KCl 2.7, $MgCl_2$ 1.1, NaH_2PO_4 0.4 and $CaCl_2$ 1.8. The duodenum was prepared with approximately 2 cm length, and put in the 10 mL organ bath containing the constantly aerated physiological salt solution, and maintained at 37°C. Tissues were mounted under an initial load of 1.5 g, and equilibrated for 30 min or till stable regular contractions were obtained. The differential amplitude of contractions generated from the longitudinal muscle layers of each tissue segment were recorded using a 7003E-isometric force transducer (UgoBasile, Varise, Italy) connected to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (UgoBasile, Varese, Italy).

Investigation on spontaneous intestinal contraction: The effect of the crude extract and fractions on spontaneous contractions of the isolated duodenum were investigated. The extract and fractions were added cumulatively and responses to concentrations of 0.0001 – 1.1110 µg/ml were investigated. A contact time of approximately 3 min was allowed following each concentration administered. At the end of the experiment, drugs were washed off and the tissues were allowed to recover. Experiments were terminated for tissues that failed to recover.

Investigation on Acetylcholine-induced intestinal contraction: To assess the extract and fractions effect of on agonist-induced stimulation, extracts and fractions were examined on the acetylcholine (ACh) – induced duodenal contractions. Non-cumulative addition of drugs were performed and final concentrations of 0.1 and 1.0 µg/ml were investigated on ACh (0.01 mg/ml) for each extract or fraction. A contact time of approximately 5 min was allowed following each concentration administered.

Investigation on high KCl-induced intestinal tonic contraction: Extract and fraction effects on depolarized duodenum were also investigated. KCl was applied to the bath containing duodenal tissue for 5 min and the effects at concentrations of 0.0049 – 4.98 µg/ml, each were investigated in the continued presence of high KCl (80 mM). The last 3 min of tissue response to potassium chloride (KCl) prior to drug addition was taken as control.

Statistical analysis: Results were expressed as mean ± SEM. Statistical analysis of the data was done using One-way ANOVA followed by the Dunnett's multiple comparison test or Unpaired Student's t-test, where appropriate. $P \leq 0.05$ was considered significant in all cases.

RESULTS

Normal intestinal transit

In control animals, the charcoal meal traversed 21.79% of the small intestinal total length. The highest dose of the crude methanolic extract used in this study (700 mg/kg) produced significant ($P < 0.001$) inhibition of intestinal transit and this was comparable to the positive control drug loperamide (5 mg/kg) with inhibition value of 27.95% (Table 1). Similarly, the crude extract (700 mg/kg) significantly decreased ($P < 0.05$) the peristaltic index in normal animals to 78.19% and this effect was also comparable to the positive control drug loperamide (5 mg/kg) which reduced peristaltic index to a value of 72.37% (Table 1).

Table1

Effect of *Brachystegia eurycoma* on normal intestinal transit

Group	Dose (mg/kg)	Peristaltic index (%)	Inhibition (%)
Control	-	91.90±4.90	-
BE	100	94.97±4.67	5.03
BE	300	93.16±1.09	6.81
BE	700	78.19±0.15*	21.79
Loperamide	5	72.37±0.23**	27.95

Values are indicated as mean ± SEM (n = 4 animals).
*P<0.05; **P<0.001 vs. Control. (One way ANOVA followed by the Dunnett's Multiple Comparison Test). BE = *Brachystegia eurycoma*.

Castor oil-induced diarrhea

As shown in Table 2, three hours after the administration of castor oil, all groups produced watery stool. However, there was a dose-dependent significant ($P < 0.001$) delay in the onset of diarrhoea produced by the methanolic extract with the peak effect produced at the dose of 300 mg/kg (216.00 min) compared with the control (32.00 min).

Table 2
Effect of *Brachystegia eurycoma* on Castor oil-induced diarrhea

Group	Dose (mg/kg)	Onset of Stooling (min)	Number of Wet Stools	Total Number of Stools	Total Weight of Wet Stools (g)	Total Weight of all Stools (g)	Diarrhoea Score	Percentage Protection	In vivo antidiarrheal index (%)
Control (distilled water)		32.00 ±8.91	2.84 ± 0.84	14.00 ±5.81	0.37 ± 0.19	0.68±5.22	2.43±1.11	-	-
BE	100	213.75 ±13.85***	0.25 ± 0.25**	5.25±2.07	0.06 ± 0.03**	0.54 ± 0.19	1.09±2.42	52.61	36.37
BE	300	216.00 ±16.49***	0.50 ± 0.5**	2.75±1.39*	0.10 ± 0.07**	0.25 ± 0.17*	1.36±0.39	40.87	37.14
BE	700	109.67±23.21*	1.38 ± 0.38*	7.30 ±3.21	0.19 ± 0.12**	0.49±0.14	1.94±0.35	15.65	29.72
Loperamide	5	198.25±25.69*	0.50 ± 0.5**	2.01 ±1.42*	0.15 ± 0.09**	0.26 ± 0.11*	1.51 ±1.82	34.78	54.47

***p < 0.05; ****p < 0.01; *****P < 0.0001 compared to control (One way ANOVA with Dunnett's multiple comparison test); n = 4 animals

Similarly, there was reduction in the number of wet stools, total number of stools (reflected in the percentage inhibition of the total number of faeces and wet faeces produced (Table 3), total weight of wet stools and total weight of all stools and diarrhoea score in the presence of the methanolic extract used when compared with control. Also , the extract produced its highest percentage protection in terms of reduction in diarrhoea score and in vivo antidiarrheal index at the dose of 300 mg/kg (40.87% and 37.14% respectively), but this was comparable to that produced by loperamide (34.78% and 54.47% respectively).

Table 3
Percentage Inhibition Values of *Brachystegiaeurycoma*on Castor oil-induced Diarrhoea in Mice

Group	Dose	Inhibition of Faeces (%)	Inhibition of Wet Faeces (%)
Control (distilled water)		-	-
BE	100	24.89	91.19
BE	300	60.51	82.39
BE	700	32.19	51.41
Loperamide	5	81.97	82.39

n = 4 animals

Table 4.
Effect of *Brachystegiaeurycoma* on Intestinal Fluid Accumulation

Group	Dose (mg/kg)	Weight of Intestinal Content (g)	Volume of Intestinal Content (ml)
Control (Distilled Water)	-	2.23 ± 0.91	1.66 ± 0.44
BE	700	1.13 ± 0.38*	1.20 ± 0.32*
Loperamide	5	1.20 ± 0.32	2.98 ± 0.36

*p < 0.05 compared to control (One way ANOVA with Dunnett's multiple comparison test)

Table 4
Table showing the IC_{50} values for the extract and fractions of *Brachystegia eurycoma* (BE) on spontaneous contractions of the isolated mouse duodenum

Extract/Fraction	IC_{50} (µg/ml)
Crude methanol extract	0.021 ± 0.69
Aqueous Fraction	0.014 ± 0.31
Chloroform fraction	0.028 ± 0.19

Values are expressed as mean ± standard error of mean (SEM). n = 5 animals

Intestinal fluid accumulation
In the fluid accumulation test, the extract at 700 mg/kg significantly (P < 0.05) reduced both the weight and volume of intestinal content (1.13 ± 0.38 g and 1.20 ± 0.32 ml respectively) compared with that of control (2.23 ± 0.91 g and 1.66 ± 0.44 ml respectively) as shown in Table 4

Effect on spontaneous intestinal contractions: The extract and fractions produced a concentration-dependent inhibitory effect on intrinsic contractions with the fractions producing greater inhibitions of spontaneous intestinal contractions than the crude extract (Fig. 1). The concentration of the extract and fractions showing 50% inhibition (IC50) of intrinsic or spontaneous contractions from concentrations used for the in vitro studies (0.0001 – 1.1110 µg/ml) are shown in Table 4. The aqueous fraction of BE achieved 50% inhibition at the lowest concentration value, this was followed closely by the chloroform fraction and then the crude extract (Table 5).

Effect on ACh-induced intestinal contractions: The extract and fractions inhibited acetylcholine (Ach)-induced contractions at concentrations used in this study. The crude extract significantly ($P < 0.05$) inhibited ACh- induced contractions at both concentrations used. The aqueous fraction significantly inhibited ($P < 0.01$ and $P < 0.05$) ACh – induced contractions at 0.1 and 1.0 µg/ml respectively while the chloroform fraction significantly inhibited ($P < 0.01$) ACh – induced contractions at both concentrations of 0.1 and 1.0 µg/ml (Fig. 2).

Effect on high KCl-induced intestinal contractions

The crude BE extract significantly inhibited ($P < 0.05$) high KCl contractions at 0.1 and 1.0 µg/ml (Fig. 3A). The aqueous fraction significantly inhibited ($P < 0.01$) high KCl at 1.0 µg/ml (Fig. 3B). The CHCl3 fraction also depressed high KCl-contractions with significant effects seen at 0.1 and 1.0µg/ml ($P < 0.05$) (Fig. 3C).

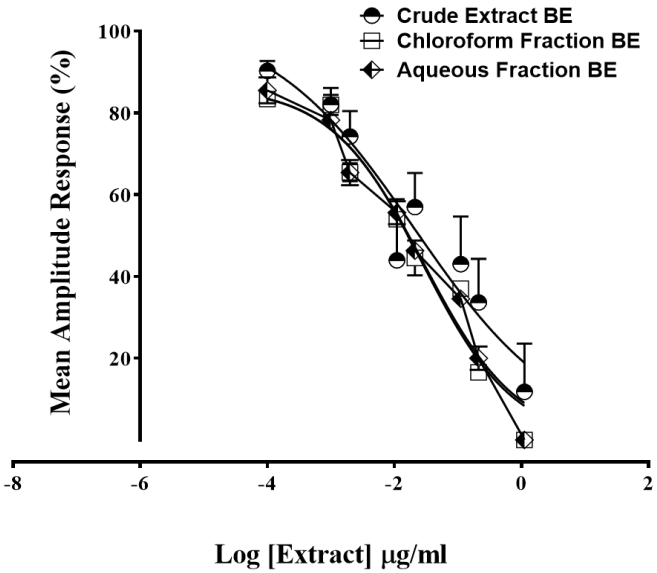

Figure 1.
Concentration-response curves showing the effects of the crude extract, chloroform and aqueous fraction of *Brachystegiaeurycoma* (BE) on spontaneous contraction of the isolated mouse duodenum. Concentrations used in all cases were 0.0001 – 1.1110 µg/ml. The extracts and fractions produced a concentration-dependent inhibition of spontaneous contractions. n = 5 animals.

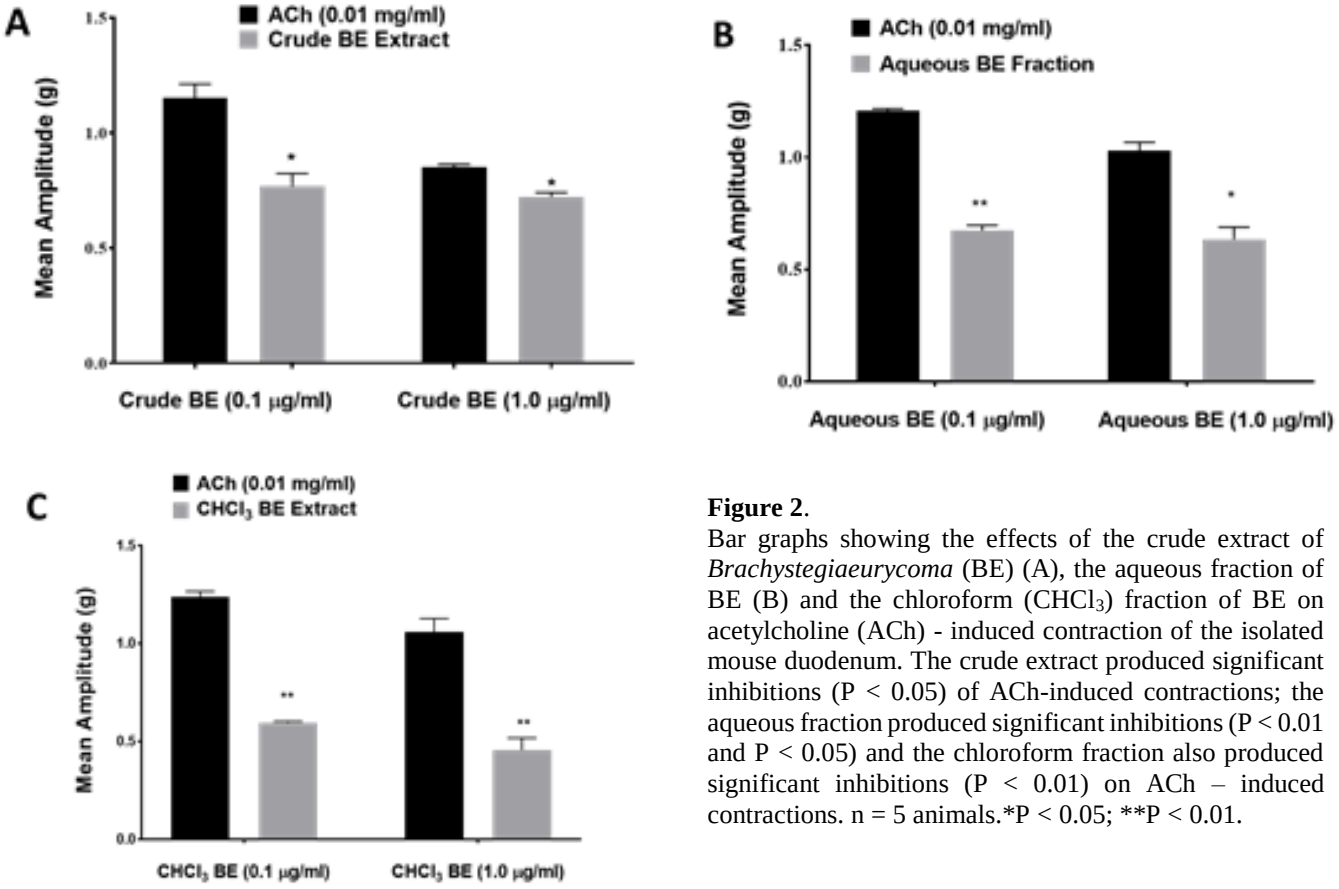

Figure 2.
Bar graphs showing the effects of the crude extract of *Brachystegiaeurycoma* (BE) (A), the aqueous fraction of BE (B) and the chloroform ($CHCl_3$) fraction of BE on acetylcholine (ACh) - induced contraction of the isolated mouse duodenum. The crude extract produced significant inhibitions ($P < 0.05$) of ACh-induced contractions; the aqueous fraction produced significant inhibitions ($P < 0.01$ and $P < 0.05$) and the chloroform fraction also produced significant inhibitions ($P < 0.01$) on ACh – induced contractions. n = 5 animals.*$P < 0.05$; **$P < 0.01$.

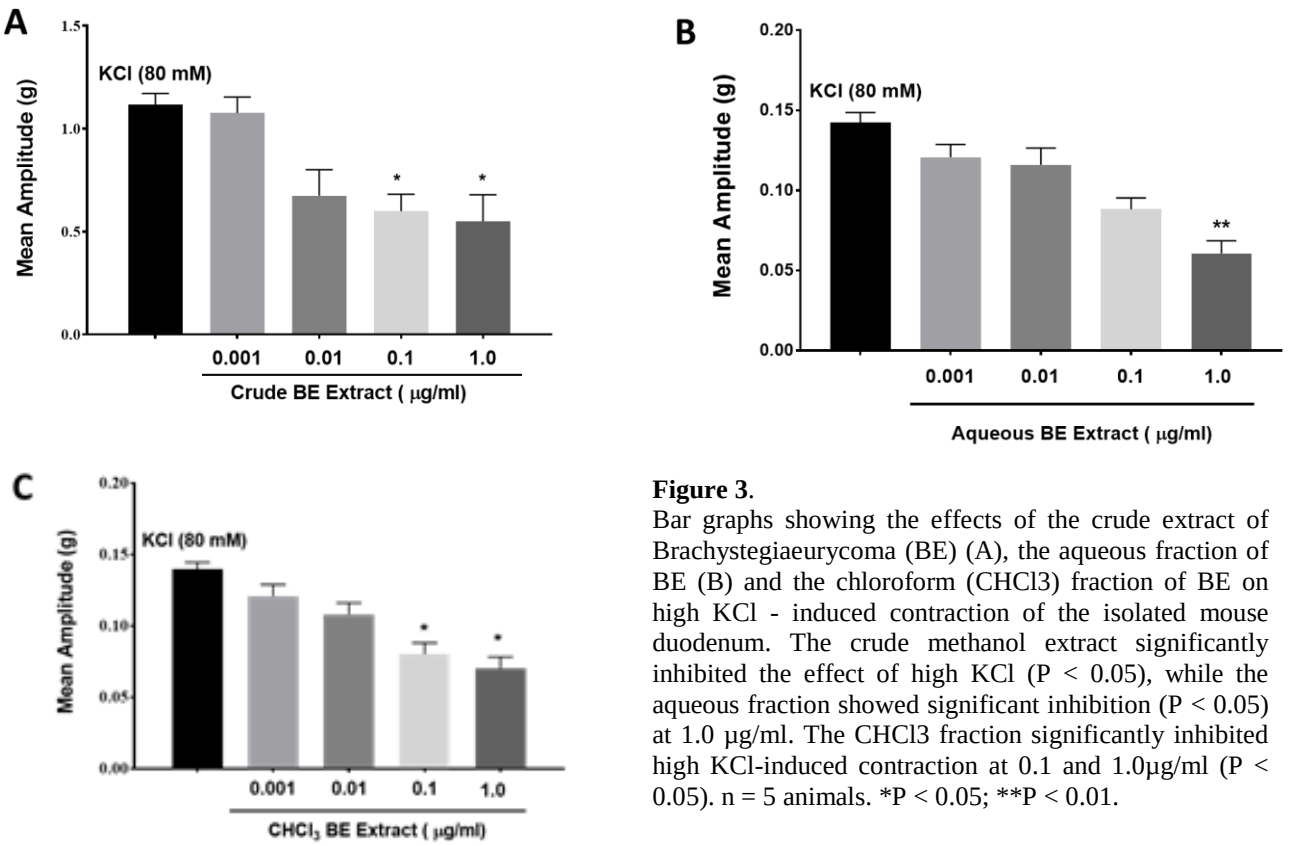

Figure 3.
Bar graphs showing the effects of the crude extract of Brachystegiaeurycoma (BE) (A), the aqueous fraction of BE (B) and the chloroform (CHCl3) fraction of BE on high KCl - induced contraction of the isolated mouse duodenum. The crude methanol extract significantly inhibited the effect of high KCl ($P < 0.05$), while the aqueous fraction showed significant inhibition ($P < 0.05$) at 1.0 µg/ml. The CHCl3 fraction significantly inhibited high KCl-induced contraction at 0.1 and 1.0µg/ml ($P < 0.05$). n = 5 animals. *$P < 0.05$; **$P < 0.01$.

DISCUSSION

The present study provides evidence that BE inhibits intestinal motility, a response which may contribute to use of this plant in normalizing motility changes associated with inflammatory bowel disease. In the normal intestinal transit model, the crude extract of BE produced a dose-dependent decrease in the propulsive movement of the charcoal meal in the small intestine. The effect was observed to peak at the dose of 700 mg/kg (21.79% inhibition). This inhibitory action of the extract on intestinal transit will delay the passage of gastrointestinal contents through the intestine therefore allowing faeces to become dehydrated, resulting in further retardation of movement through the colon (Akindele, Salako and Ohonbamu, 2014). The castor oil test, which is extensively employed as a basic pharmacological test to screen antidiarrheal drugs (Aye-Than, Kukarni and Tha, 1989; Longanga Otshudi, Vercruysse and Foriers, 2000; Nworgu *et al.*, 2012; Akindele, Salako and Ohonbamu, 2014; Henry, Enike and Bafor, 2016) was used in this study to determine the potential antidiarrheal effect of BE. One of the strong points of this model is that the evacuation of unformed stools 1-2 h after laxative administration is very reproducible. It was observed in this study that BE reduced castor oil-induced diarrhoea to a greater degree than in the normal intestinal transit. This is a relevant finding which suggests that BE administration is not associated with severely constipating effects under physiological conditions. One of the major adverse effects associated with ingestion of opiates is their constipating effect (Kaser, Zeissig and Blumberg, 2010; Menees, Saad and Chey, 2012). The results obtained in the castor oil-induced diarrhoea test, revealed that BE effectively delayed the onset of diarrhoea which led to decreased purging frequency (reduction of wet stools), weight of wet stools and diarrhoea severity (diarrhoea score). In the intestinal fluid accumulation test, the extract at 700 mg/ kg markedly reduced the volume and weight of the intestinal content. The effect of BE on all the diarrhoea indicators was measured and summed up by the calculation of the *in vivo* antidiarrhoeal index. The higher the $ADI_{in\ vivo}$ value, the greater the effectiveness of the extract in the treatment of diarrhoea (Akindele, Salako and Ohonbamu, 2014). The extract produced an increase in $ADI_{in\ vivo}$ with a maximum of 37.14% at the dose of 300 mg/kg, though the value elicited by loperamide 5 mg/kg was higher (54.74%).

It was also shown that BE produced a concentration-dependent inhibition of both spontaneous, ACh and high KCl-induced intestinal contractions. BE inhibited the contractions induced by ACh (which are due to a direct interaction with muscarinic receptors located on smooth muscles) and also inhibited spontaneous intrinsic contractions which are often mediated by ACH release from myenteric nerves (Borrelli *et al.*, 2006). These results indicate that BE could interfere with endogenous substances release which have an excitatory effect at prejunctional level from neural or non-neural sources and also, that it can exert an antispasmodic effect by a direct action on intestinal smooth muscles (Borrelli *et al.*, 2006). Motility of the gastrointestinal tract is dependent on the presence of Ca^{2+}. Agonists-induced contractions may be related to the release of intracellular Ca^{2+} from sarcoplasmic stores in addition to the influx, mainly through L-type Ca^{2+} channels of extracellular Ca^{2+} (Scintu *et al.*, 2001).

Consequently, smooth muscle contraction can be abolished by antispasmodic drugs through Ca^{2+} entry or release inhibition into or from the cells. It is noteworthy that an involvement of L-type Ca^{2+} channels in the mode of action of BE is consistent with the ability of verapamil to reduce diarrhoea during small intestinal inflammation (Lee *et al.*, 1997). The effect of BE could be either due to a direct effect on L-type channel activity or to an indirect effect on cellular membrane which reduce the activity of these channels (e.g. a change of the membrane potential). This was also supported by the effect of BE on high KCl-induced contractions.

It is well known that drugs which inhibit intestinal transit in pathophysiological states may be effective in alleviating diarrhoea. In addition, because diarrhoea is a major pathophysiological feature in patients with inflammatory bowel disease, the antidiarrheal effect of BE observed in the *in vivo* studies gives credence to the effect observed *in vitro*.

Collectively, the antidiarrheal effect of the aqueous fraction appeared to exhibit a more potent activity compared to other fractions and extract studied and is therefore recommended for future studies.

In conclusion, the results obtained in this study suggest that the stem bark extract of *Brachystegia eurycoma* possesses antidiarrheal property through antimotility and antisecretory effects possibly mediated by activity on muscarinic receptors and/or interaction with calcium channels. Further studies on the plant extract are however recommended and antimicrobial studies are additionally suggested as well as further investigation of mechanism(s) of activity. This study also showed that BE prevented experimental diarrhoea without slowing transit (causing constipation), which is of potential clinical interest particularly due to the constipating effects associated with currently used antidiarrhoeal agents.

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Declaration of conflict of interest

The authors declare no conflict of interest.

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