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Evaluation of the antidiarrhoeal and *ex-vivo* intestinal activity of *Hymenocardia acida* Tul. (Phyllanthaceae) methanol leaf extract and chloroform fraction using animal models

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ABSTRACT

Hymenocardia acida leaves and stem-bark are used traditionally for the management of diarrhoea and dysentery. This study was therefore aimed at investigation of the leaf extract and fraction on diarrhoea and intestinal motility. The leaves were collected, powdered, macerated in methanol for 72 h, and concentrated under pressure. Liquid-liquid partitioning was additionally performed yielding chloroform and aqueous fractions. The crude methanol extract (MHA) and the chloroform fraction (CHA) were then analysed. Qualitative phytochemical analyses on MHA were performed using standard procedures. The activity of MHA (100, 200 and 400 mg/kg) on intestinal transit, castor oil-induced diarrhoea and castor-oil induced enteropooling was assessed with atropine or loperamide (5 mg/kg) as positive control. MHA and CHA were further examined on the isolated rat ileum motility. Several secondary metabolite classes were detected. MHA had no significant effect ($P > 0.05$) on intestinal transit while producing significant inhibition of castor-oil induced diarrhoea ($P < 0.001$). MHA had no significant effect ($P > 0.05$) on intestinal fluid accumulation. MHA inhibited the amplitude and frequency of spontaneous intestinal contractions; however, CHA increased the amplitude but inhibited the frequency. Both MHA and CHA inhibited acetylcholine-induced intestinal contractions though MHA showed significant inhibition ($P< 0.001$) of the frequency. This study has shown that *H. acida* leaves have anti-diarrhoea and anti-motility activities supporting its use traditionally.

Keywords: Acetylcholine; Anti-diarrhoea; *Hymenocardia acida*; Intestinal motility

INTRODUCTION

Diarrhoea is a gastrointestinal disorder in which gastrointestinal contents are rapidly passed through the intestine, and is characterized by high fluidity and high frequency of faecal evacuation, which maybe semisolid or watery, and occurs three or more times daily (Whyte and Jenkins, 2012). There is an increase in flow frequency of faeces with or without the presence of blood and mucus, and this is usually accompanied by increased secretion and decreased fluid absorption, resulting in loss of water and electrolytes (Schiller, 2012). Enteric pathogenic bacteria are often the major causes of diarrhoea in human beings (Whyte and Jenkins, 2012). Other causes may also include viruses, protozoans, helminths, intestinal disorders, immunological factor, poor hygiene and medications (Baldi et al., 2009). About 3-5 billion people annually are reported to be affected by diarrhoea worldwide with associated 5 million deaths annually (Page et al., 2011). Children below 5 years of age have the highest mortality cases due to diarrhoea (Whyte and Jenkins, 2012). Programs and different global solutions have been put in place to assist in tackling diarrhoea in developing countries (Casburn-Jones and Farthing, 2004). However, diarrhoea remains a major challenge in developing countries (Mishra et al., 2016). Search for new, effective and affordable therapies from medicinal plants are on the increase (Mishra et al., 2016). Several medicinal plants in different regions of the world have been used to cure diarrhoea (Mishra et al., 2015; Uwumarongie et al., 2016). One of such plants is *Hymenocardia acida.*

Hymenocardia acida Tul of the Phyllanthaceae family, is a small tree of about 6-10 m high, widespread in tropical Africa and widely distributed in the savannah region of Nigeria (Burkill, 1985). The plant is known as "Enache" by the Idoma people of North Central Nigeria and as "Janyaro" among the Hausas in Nigeria (Abu and Uchendu, 2011). There have been some scientific reports on the effects of the leaves on several conditions. The leaves of the plant had shown antidiabetic effects in alloxan-induced diabetic rats (Ezeigbo and Asuzu, 2012), the leaves had also shown anti-inflammatory and anti-nociceptive activities in animal models (Sofidiya et al., 2010).

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The plant has been reported to be used traditionally in some parts of Africa for the management of diarrhoea and dysentery (Irvine, 1961). This study is therefore aimed at investigating the potential of *H. acida* as a lead plant for anti-diarrhoeal agents.

MATERIALS AND METHOD

Plant materials

The leaves of *H. acida* were collected in April from Iwo Town, Osun State, Nigeria and identified by Professor B. A. Ayinde of the Department of Pharmacognosy, University of Benin. Authentication was carried out by Mr S. A. Odewo; a plant taxonomist at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria and a herbarium number FHI110465 was issued. The leaves of the plant were freed of debris, cleaned and shade-dried. As soon as the leaves were dry, they were ground into fine powder using a milling machine (Christy Turner, Suffolk, UK) and kept in air-tight containers till needed.

Animals

Albino Sprague Dawley rats (106 – 211 g) and albino mice (18 – 20 g) of either sex were purchased from the Animal Centre Ogbomosho, Oyo State, Nigeria and maintained at the Animal House Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo state, Nigeria. They were housed in plastic cages at an environmentally-controlled room temperature of approximately 27 ± 5°C and environmentally-controlled lighting conditions of approximately 11 h/13 h light and dark cycles. Relative humidity ranged from 85-88%. The animals were acclimatized to these conditions. Handling was done as much as possible according to standards of the Public Health Service policy on humane care and use of Laboratory Animals (National Research Council 2010). The animals were maintained on standard diet of animal pellets and clean tap water. Ethical approval was obtained from the Ethical Committee, Faculty of Pharmacy, University of Benin, Nigeria.

Drugs and reagents

Chloroform, methanol, and phytochemical analysis reagents were obtained from BDH chemicals, UK, acetylcholine (Sigma Aldrich, UK), castor oil (Bell, Sons & Co. (Druggists) Ltd., Merseyside, UK), activated charcoal (General Carbon Co., NJ USA), loperamide HCl (Shine Pharmaceuticals, Gujarat, India). Salts for the physiological solution were obtained from BDH chemicals, England, UK.

Preparation of plant extract

The powdered leaf material (1500 g) was macerated in methanol (3 L) for 72 h and then filtered. The filtrate obtained was concentrated to dryness with the aid of a water bath set at 70°C and the resulting extract (MHA) was weighed to give 137.55 g with a percentage yield of 9.17%w/w.

Phytochemical qualitative analysis

The plant material was analysed for phytochemical compounds using standard procedures earlier described (Harborne, 1998; Odebiyi and Sofowora, 1978; Trease and Evans, 1989). Briefly, phytochemical screening of the plant material was performed for the presence of glycosides, saponins, flavonoids, alkaloids, tannins, anthracene derivatives and triterpenoids.

Glycosides test

Dried powdered *Hymenocardia acida* leaves (0.1g) were extracted by boiling over a water bath for 3 min in distilled water (20 mL) and filtered. The filtrate was used for tests (a) and (b) below.

(a) Molisch's Test for Carbohydrate

To 2 mL of the filtrate was added 10% alcoholic solution of α – naphthol. At an inclined angle (of approximately 45°), concentrated sulphuric acid (H_2SO_4) (2 mL) was carefully poured down the sides of the tube forming a layer below the extract solution (Sofowora, 1993; Trease and Evans, 1989).

(b) Fehling's Test for Reducing Sugars (General Test for Glycosides)

The filtrate (2 mL) was mixed with Fehling's solution (A and B in equal volumes), heat was applied to boiling and the mixture observed for colour change.

An additional confirmatory test was performed by mixing dilute H_2SO_4 (3 mL) with the filtrate (5 mL) and the mixture gently heated over a water bath for 5 min and filtered. To the resulting filtrate was added sodium bicarbonate. Fehling's solution (A and B in equal volumes) was added and the mixture was heated on a water bath and observed for colour change (Trease and Evans, 1989).

Alkaloids test

An aqueous extract of *H. acida* was obtained by boiling 5 g of powdered plant in distilled water (100 mL) over a water bath set at 100°C for 15 min. The mixture was then filtered and the resulting extract solution tested with the following alkaloidal reagents:

Mayer's reagent; Dragendroff's reagent; Wagner's reagent and Hager's reagent (picric acid)

a. A methanol extract of *H. acida* was evaporated to dryness in a dish over water bath. The residue was dissolved in 1% H_2SO_4 and filtered. The resulting filtrate was tested with the alkaloidal reagents listed above.

b. A chloroform extract of *H. acida* was obtained and dried over a water bath set at 40°C. The resulting residue was dissolved in 1% H_2SO_4 and filtered. The filtrate was tested with the alkaloidal reagents listed above (Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

Saponins test

***Frothing Test*:** About 0.1g of *Hymenocardia acida* aqueous extract was shaken vigorously with distilled water in a test tube. Frothing that persisted on standing was taken as evidence for the presence of saponins (Sofowora, 1993).

Cardiac glycosides test

Salkowski's test for steroidal nucleus: To about 2 mL of *H. acida* chloroform extract solution, was carefully added concentrated H_2SO_4 (1 mL) and changes observed.

Kellier-killiani test for de-oxysugars: To about 2 mL of ethanol extract or chloroform extract of *H. acida* glacial acetic was added followed by a drop of ferric chloride solution and 1mL of concentrated H_2SO_4. The mixture was closely observed for changes.

Lieberman Burchard test for triterprenoid nucleus: About 2 mL ethanol extract of *H. acida* was dissolved in a few drops of chloroform, to which acetic anhydride was added followed by concentrated H_2SO_4.

Flavonoids test

To the aqueous extract of *H. acida* (3 mL) was added about 0.1 mL dilute sodium hydroxide solution and 0.2 mL concentrated hydrochloric acid solution. The mixture was closely observe for changes (Odebiyi and Sofowora, 1978; Sofowora, 1993).

Test for anthracene derivatives

To the aqueous extract of *H. acida* (3 mL) was added 2.5 mL of 15% ferric chloride solution and concentrated hydrochloric acid solution (1 mL) which was heated on a water bath for 5 min. On cooling the solution was vigorously shaken with chloroform (5 mL) and 3 mL of the chloroform layer was pipetted out and shaken with dilute ammonia solution (1.5 mL).

Test for phenolic compounds

The aqueous extract solution of H. acida (2 mL) was mixed with 0.1 mL aqueous ferric chloride and observed (Trease and Evans, 1989).

Liquid-liquid partitioning of the extract

The crude extract (75 g) was dissolved in a mixture of methanol and water (1:4) and then partitioned with chloroform (100 x 3 mL) in a separating funnel with occasional stirring. The chloroform (CHA) and aqueous phases were collected and concentrated to dryness over a water bath set at 40^0C for the chloroform fraction, and 60^0C for the aqueous fraction. The dried fractions were weighed and stored in the refrigerator at 4°C till needed.

Normal intestinal transit

A modified protocol earlier described for this model was used (Hsu, 1982; Akindele et al., 2014). The mice were randomly allotted to five groups containing five animals each. The animals were fasted 18 h prior to experiments but allowed unlimited access to water *ad libitum*. Group 1 was the positive control and received atropine (5 mg/kg p.o.). Group 2 was the negative control and received 0.2 mL distilled water. Groups 3, 4 and 5 were the treatment groups and received the crude methanol extract (100, 200 and 400 mg/kg p.o.). Oral administrations were achieved with the aid of a feeding syringe. Charcoal meal suspension (0.5 mL of 17% charcoal in distilled water) was orally administered 30 min after initial drug/treatment administration in each group. Thirty minutes (30 min) post charcoal administration, the mice were humanely killed by cervical dislocation and the small intestine was immediately isolated and the total length measured (Adeyemi et al., 2009). The distance travelled by the charcoal meal from the pylorus to the caecum was measured and percentage inhibitions of movement was calculated (Oben et al., 2006) as a function of the distilled water-treated negative control.

$$\%\boldsymbol{Inhibition} = \frac{MDC - MDT \times 100}{MDC}$$

Where MDC = Mean distance travelled in control

MDT = Mean distance travelled in test

Castor-oil induced diarrhoea

Rats were randomly allocated into five groups of 5 animals each and fasted for 18 h prior to experiments but with free access to drinking water *ad libitum*. The animals were randomly divided into five groups (1 – 5) with five rats in each. All administrations were done with the aid of a feeding syringe. Group 1 received distilled water (1 mL) and served as the negative control, group 2 received loperamide (5 mg/kg p.o.) and served as the positive control while groups 3, 4, 5 orally received 100, 200 and 400 mg/kg of the extract respectively and served as the treatment groups. One hour after administrations of drug/treatment in all groups, castor oil (20 mL/kg p.o.) was then administered to induce diarrhoea and the animals were individually placed in well-ventilated transparent cages with floor lining of clean paper (Adeyemi et al., 2009; Oben et al., 2006). The animals were observed for defecation every hour up to 3 h and the total number of dry or wet faeces as well as the total weight of faecal droplets were recorded from the pre-weighed paper which lined the cages. The paper was replaced every hour. The difference in time between castor-oil administration and the excretion of the first wet stools was calculated. The total number of faecal output, the number of wet stools excreted by each animal, and the total weight of wet stools was additionally observed and recorded.

Intestinal fluid accumulation

Intestinal fluid accumulation was induced in rats by oral administration of castor oil, 20 mL/kg (Adeyemi and Akindele, 2008). The rats were fasted for 24 h prior to the experiment but with free access to water. They were randomly divided into five groups consisting of five rats each. Group 1 received

distilled water (1 mL), group 2 received loperamide (5 mg/kg p.o.) and groups 3, 4, and 5 received the extract (100, 200, and 400 mg/kg p.o. respectively). One hour later, castor oil (20 mL/kg) was then administered orally. All oral administrations were performed with the aid of a feeding syringe. The animals were then placed individually in separate transparent, well ventilated cages and observed. Two hours (2 h) after castor oil administration, the animals were humanely sacrificed by cervical dislocation and the small intestines immediately isolated after ligation of the pyloric sphincter and the ileo-caecal junction. Each isolated intestine was weighed, the intestinal content was collected in a dry graduated measuring cylinder and the volume determined. Each intestine was re-weighed after removal of content, the weight of the empty intestine was taken and the difference between the full and empty intestine determined.

***Ex-vivo* analyses**

Tissue preparation

The mice to be used were fasted for 12 h prior to the experiment and were humanely euthanized under anaesthesia by cervical dislocation. The intestine from duodenum to colon was gently flushed using saline solution. A section of the ileum was carefully isolated, freed from attached mesenteries, and then placed in a petri dish containing previously warmed and aerated physiological salt solution. The Tyrode's physiological salt solution used for the *ex-vivo* 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 ileum was prepared with approximately 2 mm length and placed in a 10 mL organ bath containing constantly aerated physiological salt solution and maintained at 37°C. Tissues were mounted under an initial load of 1.0 g and equilibrated for a minimum of 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).

Effect of extract and fractions on spontaneous ileal contractions

The effect of the crude extract and fractions (0.01 – 0.3 mg/mL) on spontaneous contractions of the isolated ileum were investigated. The extract and fractions were added cumulatively and responses observed. A contact time of approximately 3 min was allowed following each concentration administered. At the end of the experiment, the extract or fraction was washed off and the tissues were allowed to recover. The amplitude and frequency were assessed for each experiment. Experiments were terminated for tissues that failed to recover.

Effect of extract and fractions on ACh-induced ileal contractions

To assess the effect on agonist-induced stimulation, the extracts and fractions (0.03, 0.3 and 3.0 mg/ml) were examined on the acetylcholine (ACh) – induced ileal contractions. Non-cumulative additions were performed on ACh (0.55 nM) for each extract or fraction. A contact time of approximately 5 min was allowed following each concentration administered.

Statistical Analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical analysis of the data was done using One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. In experiments with sufficient datasets the concentration-response plots were derived according to the formula Y=Bottom + (Top-Bottom)/(1+10^((X-LogIC50))) or Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope)) as appropriate, where IC50 or EC50 is the concentration of agonist that gives a response half way between minimum and maximum response. The number of animals used were represented by n. Results were considered significant when $P \leq 0.05$ in all cases.

RESULT

Phytochemical screening

H. acida leaf was observed to yield positive results to the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins and anthracene derivatives (Table 1).

Table 1: Phytochemical constituents detected in the leaves of *H. acida*

Phytochemicals	Results
Glycosides	+
Alkaloids	+
Saponins	+
Flavonoids	+
Anthracene derivatives	-
Phenolic compounds	+

+ = present; - = absent

Normal intestinal transit

In control animals, the charcoal meal traversed 33.70 ± 0.71 cm and in the presence of atropine (the positive control), charcoal meal traversed a distance of 19.11 ± 0.82 cm significantly ($P < 0.001$) inhibiting normal intestinal transit. The doses of extract used in this study slightly reduced normal intestinal transit but produced no significant ($P > 0.05$) inhibitions. However, the extract at 100 mg/kg produced the highest inhibitions among the extract doses used (Table 2).

Table 2: Effect of *H. acida* on normal intestinal transit

Group	Dose (mg/kg)	Distance travelled by charcoal (cm)	Full length of intestine (cm)	Inhibition (%)
Control (distilled water)	0.2 mL	33.70 ± 0.71	49.74 ± 0.21	32.21 ± 1.52
Atropine	5	19.11 ± 0.82	44.84 ± 0.92	57.43 ± 1.33***
Extract	100	28.23 ± 1.31	42.82 ± 0.62	34.10 ± 1.62
Extract	200	31.52 ± 1.61	46.81 ± 0.61	33.34 ± 2.00
Extract	400	31.50 ± 1.13	45.13 ± 0.54	30.21 ± 1.53

Values are indicated as mean ± SEM (n = 5 animals). ***P<0.0001 vs. Control. (One way ANOVA followed by Dunnett's Multiple Comparison Test).

Castor oil-induced diarrhoea

The *H. acida* leaf extract at all doses employed in this study (100, 200 and 400 mg/ml) produced a significant dose-dependent inhibition of diarrhoea induced by castor oil. However, loperamide the positive control drug was observed to produce a more significant inhibition ($P < 0.001$) at the dose used in this study when compared to the inhibitory effect of the extracts. It was also observed that the extracts decreased the weight and number of the faecal droplets in the 3 hour period compared to the distilled water group (negative control group). Loperamide was observed to delay the onset of diarrhoea, while the extract appeared to exert no effect on the onset of stooling compared to the distilled water group (Table 3).

Intestinal fluid accumulation

The extract produced varying results. At 400 mg/mL the extract slightly reduced the volume of the intestinal content compared to the control and a slight increase was observed on the weight of the intestinal content which was statistically insignificant ($p>0.001$). The extract at 100 and 200 mg/mL was observed to increase the volume of the intestinal content compared to the control (Table 3). Loperamide on the other hand, reduced both the volume and weight of intestinal content (Table 4).

Effect on spontaneous ileal contractions

In order to determine the effects of the extract and fraction on intrinsic intestinal contractions, MHA and CHA were tested on spontaneous ileal contractions. MHA inhibited the intrinsic ileal contractions in a concentration-dependent manner (Fig. 1) at concentrations used in this study. The IC_{50} of MHA on amplitude of spontaneous contractions was calculated as 0.02 ± 0.54 mg/mL while the IC_{50} for the frequency was calculated as 0.02 ± 0.16 mg/mL. CHA on the other hand increased the amplitude of spontaneous contractions (Fig. 2) with an EC_{50} of 0.05 ± 0.37 mg/mL. MHA was also observed to inhibit the frequency of spontaneous intrinsic intestinal contractions (Fig. 3) with an IC_{50} of 0.02 ± 0.16 mg/mL. Similarly, CHA was found to inhibit the frequency of spontaneous intestinal contractions (Fig. 4) with a calculated IC_{50} of 0.02 ± 0.05 mg/mL.

Table 3: Effect of *H. acida* on castor-oil induced diarrhoea

Group	Dose (mg/kg)	Diarrhoea onset (min)	Total weight faecal droplet (3 h)	Number faecal droplet (3 h)	Inhibition (%)
Control (distilled water)	0.2 mL	30.00 ± 0.52	7.07 ± 3.12	13.02 ± 4.24	-
Loperamide	5	60.00 ± 0.39	2.32 ± 3.61	3.36 ± 2.33	69.13 ± 1.80***
Extract	100	30.00 ± 1.02	5.80 ± 3.74	9.33 ± 4.01	25.40 ± 1.82***
Extract	200	29.08 ± 1.22	3.80 ± 2.82	5.31 ± 2.15	39.01 ± 2.00***
Extract	400	26.08 ± 0.93	3.47 ± 2.54	6.53 ± 2.62	51.76 ± 1.93***

Values are indicated as mean ± SEM (n = 5 animals). ***P<0.0001 vs. Control. (One way ANOVA followed by Dunnett's Multiple Comparison Test).

Table 4: Effect of *H. acida* on intestinal fluid accumulation

Group	Dose (mg/kg)	Volume intestinal content (mL)	Weight intestinal content (g)
Control (distilled water)	1.0 mL	1.02 ± 0.61	1.89 ± 0.44
Loperamide	5	0.85 ± 0.18	1.50 ± 0.49
Extract	100	1.21 ± 0.73	1.65 ± 0.69
Extract	200	1.56 ± 0.69	1.84 ± 0.47
Extract	400	0.90 ± 0.45	1.93 ± 0.46

Values are indicated as mean ± SEM (n = 5 animals).

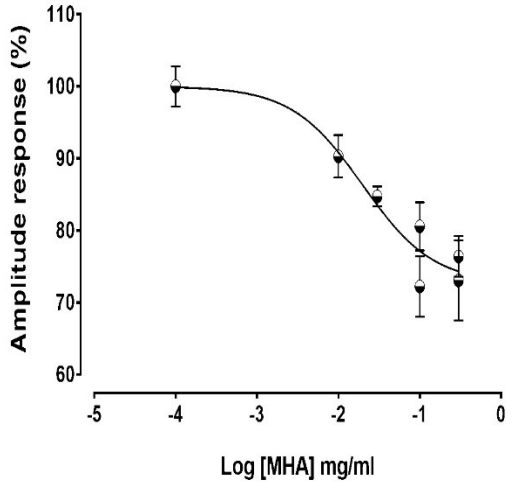

Figure 1. Concentration-response curves showing effect of MHA on the amplitude of spontaneous intestinal contractions. n= 5 animals.

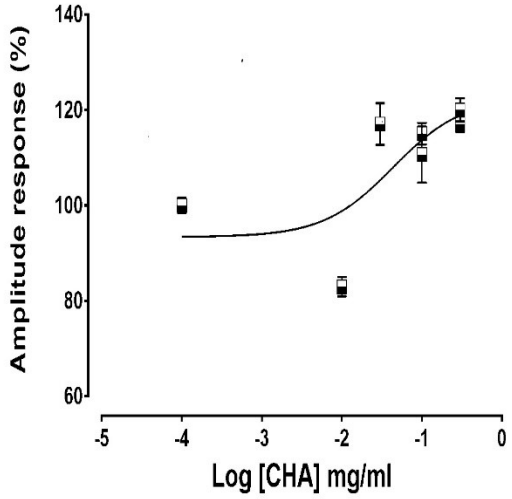

Figure 2. Concentration-response curves showing effect of CHA on the amplitude of spontaneous intestinal contractions. n=5 animals

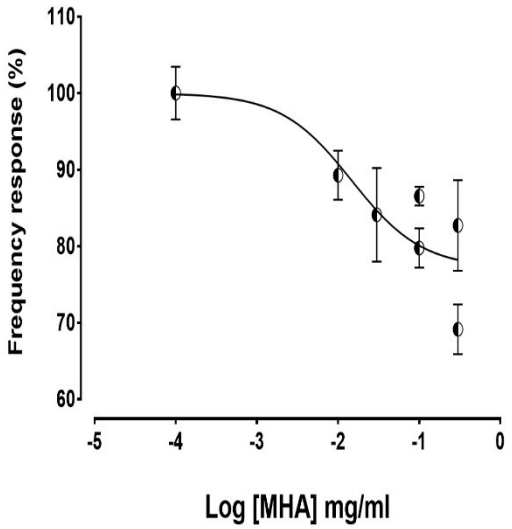

Figure 3. Concentration-response curves showing effect of MHA on the frequency of spontaneous intestinal contractions. n= 5 animals.

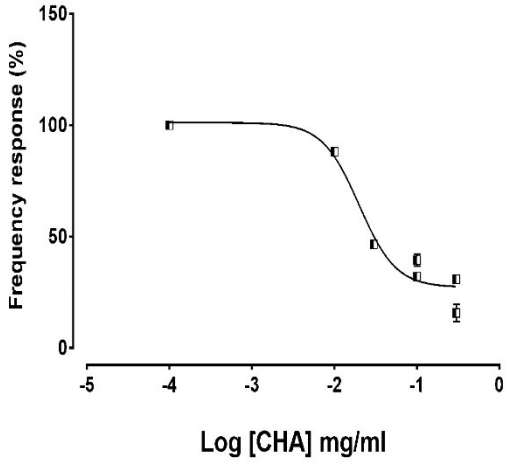

Figure 4. Concentration-response curves showing effect of CHA on the frequency of spontaneous intestinal contractions. n= 5 animals.

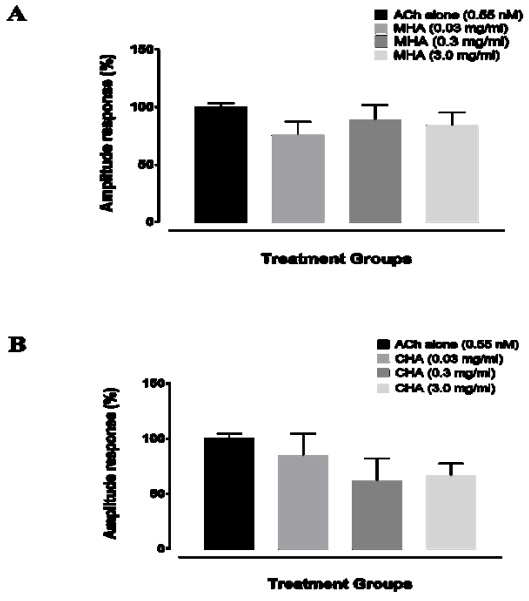

Figure 5. Bar plots showing the effect of MHA and CHA on the amplitude of ACh-induced intestinal contractions. n = 5 animals.

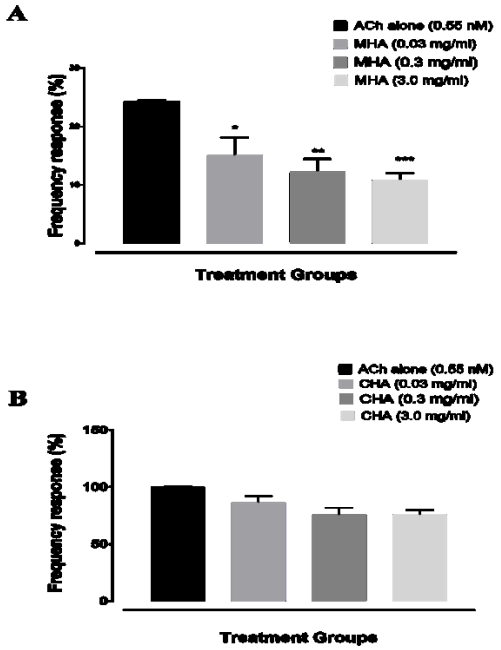

Figure 6. Bar plots showing the effect of MHA and CHA on the frequency of ACh-induced intestinal contractions. n = 5 animals.

Effect on ACh-induced ileal contractions

In an effort to examine the effect on agonist-induced intestinal contractions, the extracts and fraction were evaluated on acetylcholine (ACh)-induced intestinal contractions. MHA and CHA were observed to inhibit the amplitude of ACh-induced contractions, though it was not statistically significant (Fig. 5). However, MHA produced significant inhibition ($P < 0.001$) of the frequency of ACh-induced intestinal contractions (Fig. 6A). CHA was also observed to inhibit the frequency of ACh-induced contractions but this was not statistically significant (Fig. 6B).

DISCUSSION

Phytochemical analysis revealed the presence of some secondary metabolite classes which have been known to exert anti-diarrhoea activity (Bais et al., 2014; Kavitha et al., 2004; Taiwo and Igbeneghu, 2014; Teke et al., 2010). Anthracene derivatives on the other hand have been reported to exhibit calcium channel blocking activities (Bova et al., 2009) which may also contribute to the effect of the extract in this study. It was observed in this study that the crude leaf extract had only mildly reduced normal intestinal transit. Intestinal motility is an essential function required for propelling intestinal content, mixing with digestive fluids and enzymes and preparing the contents for excretion (Chang and Leung, 2014). The underlying factor for intestinal motility is due to increased cytosolic calcium (Chang and Leung, 2014). That the crude extract lacked pronounced effect on normal peristalsis suggests possible preference for diarrhoeic states. The extract was however found to potently inhibit diarrhoea induced by castor-oil at all doses used in this study, with the highest effect seen with the largest dose of 400 mg/kg which compared favourably with the positive control drug, loperamide. Castor oil (ricinoleic acid) affects electrolyte transport and intestinal smooth muscle contractility (Capasso et al., 1994; Gullikson et al., 1977). Castor oil inhibits intestinal Na^+, K^+-ATPase activity (Phillips et al., 1965), interferes with oxidative metabolism (Nakao, 1963) and has effects on adenylate cyclase or mucosal adenosine 3': 5'-cyclic monophosphate (cyclic AMP) content (Simon and Kather, 1980). Castor oil is cytotoxic to intestinal epithelial cells (Bretagne et al., 1981), and causes histological abnormalities with enhanced mucosal permeability (Saunders et al., 1978). These effects may be related to the release of eicosanoids (Beubler and Juan, 1979) and platelet activating factor (PAF) by the intestinal mucosa (Pinto et al., 1992). Accumulation of all these factors leads to diarrhoea. It is therefore suggested that the extract may either affect motility directly through receptor interaction and/or may interact with one of the aforementioned processes altered by castor oil. Loperamide is an orally-active drug which reduces gastrointestinal motility in animals and man through interaction with opiate receptors (Heel et al., 1978). Loperamide was observed in this study to distinctly inhibit diarrhoea induced by castor oil.

To further investigate the potential of the extract to inhibit fluid retention caused by castor oil as a possible mechanism of activity, the extract effect on intestinal fluid accumulation was investigated. It was however observed that the crude extract did not

significantly alter the volume of intestinal content neither did it affect the weight of the intestine, instead slight increases in intestinal content were observed as opposed to loperamide which showed a decrease in both parameters. It may therefore seem that while blocking the diarrhoeal effect of castor oil, the extract may actually worsen the injury to the mucosa induced by castor oil. It has been reported that preventing diarrhoea and preventing injury damage to the mucosa are two unrelated effect and can occur independently of each other (Capasso et al., 1994). Nitric oxide (NO) has been reported to produce a similar dual action on the intestinal mucosa by promoting diarrhoea while protecting against mucosal damage (Capasso et al., 1994). Increased intestinal epithelial permeability is associated with intraluminal fluid accumulation in response to ricinoleic acid (Dobbins & Binder, 1976). Mucosal damage induced by castor oil results in the release of NO as a protective mechanism in this instance. However, NO has been shown to itself contribute to diarrhoea, an effect related to interference with cellular metabolism, and not due to gross mucosal damage (Capasso et al., 1994). It may therefore be that the extract in this study is unable to protect against mucosal damage and therefore unable to prevent water and electrolyte loss which may occur in secretory diarrhoea (Skadhauge et al., 1997) but may act solely to decrease intestinal smooth muscle motility and therefore reduce diarrhoea.

To determine possible interaction with intestinal smooth muscle motility, the extract and chloroform fraction were examined on the isolated ileum. The fraction was included in the *ex-vivo* stage in order to assist in guiding a hypothesis for future fractionation and characterization studies. It was observed that the crude extract inhibited both the amplitude and frequency of spontaneous (intrinsic) intestinal contractions. The chloroform fraction on the other hand resulted in a mild increase in the amplitude of spontaneous contraction while decreasing the frequency. Both the crude extract and the fraction shared equipotent effect in inhibiting the frequency of intrinsic contractions. The crude extract and chloroform fraction were found to inhibit ACh-induced intestinal motility, however the crude extract produced greater inhibitory effect. Intestinal contractions often occur, in the presence of excitatory neurotransmitters, one of the most prominent being acetylcholine (Chang and Leung, 2014). Acetylcholine opens calcium channels during the maximum of the pacesetter potentials, so that influx of calcium into the smooth muscle cells occurs. The influx of calcium induces the electro-mechanical coupling. It is associated with the occurrence of *spike potentials* (Chang and Leung, 2014). It therefore appears that the crude methanol extract inhibits diarrhoea through direct inhibition of intestinal motility and the effect of the crude extract appears more potent than the fraction. This effect on motility observed supports the effect observed with in vivo studies where the extract fails to prevent intestinal fluid accumulation. That the crude methanol extract produced a stronger anti-motility effect suggests that the plant is efficacious when consumed in its crude form and may therefore act either by a complement of the interacting secondary metabolites or as a prodrug.

Conclusion

This study has shown that the methanol leaf extract of *Hymenocardia acida* is active against acute diarrhoea and may exert this effect as an anti-motility agent. This therefore supports the traditional use of the plant in the management of diarrhoea. However, caution is advised in its use due to possible mucosa injury.

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