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Anti-Diarrheal Effects of Aqueous Leaf Extract of *Holarrhena floribunda* (G. Don) Schinz and Dur in Rats

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ABSTRACT

The leaves of *Holarrhena floribunda* were subjected to Soxhlet extraction with distilled water and concentrated *in vacuo*. The aqueous extract concentrate gave a yield of 10.96% (w/w) and was used to investigate anti-diarrheal activity. Studies were carried out on castor oil-induced diarrhea, intestinal secretion and small intestine charcoal meal transit in rats. The extract at 400–1000 mg/kg caused a marked inhibition of diarrhea and accumulation of intestinal fluid following the administration of castor oil. A similar dose of the aqueous extract significantly ($P < 0.05$) reduced the charcoal meal transit in a dose-dependent manner. These results suggest a potential usefulness of the leaf extract of *H. floribunda* in the control of secretory diarrhea associated with microbial pathogens like enteritis and enterocalitis

Keywords: charcoal meal, castor oil, dose dependent, fluid accumulation, Soxhlet extraction

INTRODUCTION

Medicinal plants are a reservoir for drug and lead compounds for many therapeutic agents (Akah *et al.* 2007), many of which are efficacious and contain compounds that are potential drugs that require further examination (Sofowora 1993; Abdulrahman *et al*. 1999, 2007). The usefulness of certain herbs as therapeutic agents has been known for thousands of years through the enormous contribution of primitive man worldwide who virtually ate plants *in loco* to ascertain their use (Seshadri 2008; Usman 2010). The type and concentration of phytochemicals may vary within and among species due to variables in plant growth, soil, weather conditions and the age of the plant (Abdulrahman *et al.* 2007; Natural Remedies 2008; Usman 2010). The therapeutic effects of plants have been utilized the world over and despite the influence of modern medicine still about 30% of the oral populace depends on traditional medicine for the treatment of their diseases (Ogundaini 2005).

In various parts of the world, medicinal plants constitute alternative sources of drugs for the majority of the population that has inadequate contact with orthodox health care facilities. The lack of availability of synthetic drugs to combat common ailments like diarrhea has made many communities turn to medicinal plants that can alleviate the condition or permanently put the secretory process leading to diarrhea under control (Ayinde *et al.* 2009).

H. floribunda is a member of the Apocyanacea family found mainly in Saharan and sub-Saharan Africa. It is a shrub to medium-sized tree about 4.5-15 m high. The bark is smooth, grey and pale-yellow yielding abundant latex. The medicinal value of this plant lies in some chemical substances such as phenolic compounds (tannins, flavonoids), terpenes, glycosides (cardiac flavonoids, steroidal saponins), anthraquinones, volatile-oils and gums that produce a definite physiological action on the human body (Edeoga *et al.* 2005). Various medical preparations of *H. floribunda* are used as remedies for diseases such as skin infections, malaria, dysentery, convulsion, diarrhea, gonarhoea and diabetes (Burkill 1985; Akah 1997; Fotie *et al*. 2006; Sasidharan *et al*. 2007). Secretory diarrhea caused mainly by enterotoxin (*Vibro cholerae*) and *Escherichia coli* remains one of the major causes of death, especially in developing nations and its prevalence had been on the increase a few decades ago (WHO 1990), However, it is well documented that castor oil produces diarrhea due to its most active component ricinoleic acid through a hypersecretory response (Meite *et al*. 2009), Therefore it can be assumed that the anti-diarrheal actions of *Morinda morindoides* extract were mediated by an antisecretory mechanism. These enterotoxins seem to stimulate fluid secretion from the transepithelial impetus (Charke *et al*. 1992). The increased secretion enhances the water and electrolyte content of the stool that can lead to dehydration, a decreased in blood plasma shock and cardiovascular collapse (Sasidharan *et al*. 2007; Suleiman *et al*. 2008). Oral rehydration therapy (ORT) has remained the immediate supportive treatment for secretory diarrhea using the ability of the intestine to absorb salt and water during glucose absorption but ORT can only reduce the mortality and not the morbidity of the diarrhea (WHO 1990; WHO 2006; Stanley 2007).

There is the need for drugs that can be used to reduce intestinal hyper secretion when used in combination with ORT. *H. floribunda* was chosen in this study based on its common use for treatment of diarrhea by traditional healers in Bauchi State, Nigeria.

Diarrhea is a disease condition characterized by frequent discharge of semi-solid or fluid fecal matter occasioned by uncontrolled peristalsis of the intestines. It may be acute or chronic and can be very serious in infants and elderly people because of the risk of severe, potentially fatal dehydration (Ayinde *et al.* 2009).

In addition, a preliminary qualitative photochemical investigation of *H. floribunda* revealed the presence of flavones, sterols, triterpenes, tannins saponines and alkaloids (Aska 2008). Therefore, this work sought to investigate the anti-diarrheal effect of the leaf extract to see if there is any scientific basis for it folkloric use.

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Short Communication

MATERIALS AND METHODS

Plant collection, identification and extraction

Fresh leaves of a *H. floribunda* were collected from a matured plant in December 2006 from Sirko village Garden/forest in Misau Local Government Area, Bauchi State, Nigeria. Prof. S.S Sanusi of the Department of Biological Sciences, University of Maiduguri, identified the plant and voucher specimen No 45BA was deposited in the Department of Chemistry Research laboratory. The leaves were air-dried for 48 hrs in the laboratory and pulverized to a powder using mortar. 500 g of the powdered sample were continuously extracted with a Soxhlet apparatus, concentrated *in vacuo* and the extract yield was determined and stored at 4°C until use. The extract concentrate was reconstituted in distilled water and used at the time of the experiment.

Animals

About 80 Wistar white albino rats of both sexes weighing between 70 and 200 g were purchased from the National Veterinary Research Institute, Vom, Nigeria. They were kept in clean plastic cages and allowed to acclimatize to laboratory conditions for 2 weeks before the experiment began. They were fed with grower's mesh (Sander's Feeds Nigeria Ltd.) and water *ad libitum*.

Castor oil-induced diarrhea

The effect of the aqueous leaf extract of *H. floribunda* on castor oil-induced diarrhea was evaluated. The method described by Offia and Chikwendu (1999) was employed. Five groups of five rats each were randomly selected and separated singly in cages lined with white blotting paper. Group A received normal saline (2 ml/rat) orally which served as control; Groups B, C and D were treated orally with 400, 700, and 1000 mg/kg body weight of the extract, respectively. Group E was treated with Diphenoxylate (5 mg/kg) intraperitoneally (i.p.) which served as the standard drug. After 60 min, (1 ml/rat) of castor oil was administered orally and observed for 6 hrs for wet or watery faeces. The faeces of each rat were counted and recorded.

Accumulation of intestinal fluid

The effect of the aqueous leaf extract of *H. floribunda* on the accumulation of castor oil-induced intestinal fluid was assessed. The method described by Robert *et al.* (1976) was employed. Five groups of five rats each were randomly selected and kept in separate cages. The rats were fasted overnight. Group A was given normal saline (2 ml/rat) orally which served as control. Groups B, C and D were given an oral dose of 400, 700, and 1000 mg/kg of the extract, respectively. Group E was given atropine (3 mg/kg body weight) i.p., which served as the standard drug. After 60 min, each rat in all the groups was given castor oil (1 ml/rat) orally.

The content of the intestine was collected by milking (squeezing) and the weight of the content and the empty intestine was recorded.

Transit of small intestine

The effect of aqueous leaf extract of *H. floribunda* on intestinal transit of charcoal meal was tested using the charcoal method (Mascolo *et al.* 1999; Chitme *et al.* 2004). Five groups of rats (n=5) fasted overnight were randomly selected and kept in separate cages. Group A received normal saline (2 ml/rat) orally which served as control. Groups B, C and D were given an oral dose of 400, 700 and 1000 mg/kg body weight of the extract, respectively. Group E was given atropine (3 mg/kg body weight) i.p., which served as the standard drug. After 10 min, each rat in all the groups was given charcoal meal (1 ml/rat) of 5% activated charcoal suspension in 10% aqueous solution of *Acacia senegalensis* powder. 30 min later, the rats were sacrificed by slaughtering and the abdomen from each rat was removed and extended on a clean glass surface. The length traveled by the charcoal marker was recorded.

Animal ethics

Animals were strictly handled according to the guidelines for biomedical research involving animals prepared by the Council for International Organization of Medical Scientist (CIOMS) as accepted by the Department of Veterinary Medicine Ethical Committee on Animals, University of Maiduguri, Nigeria.

Statistical analysis

Data were expressed as mean ± SEM (standard error of mean) and n represents the number of animals used. Where applicable, the data were compared using one way analysis of variance (ANOVA) at $P < 0.05$ using Graph pad InstantR version 2.05a software (UK).

RESULTS AND DISCUSSION

The results of this study indicate that aqueous leaf extract of *H. floribunda* significantly ($P < 0.05$) reduced the castor oil induced diarrhea in (**Table 1**). The extract at the dose of 1000 mg/kg body weight reduced the diarrhea by 67.31% and at the dose of 700 and 400 mg/kg body weight the incidence of diarrhea was reduced by 50.96 and 29.81%, respectively. The effect observed with aqueous leaf extract was dose-dependant. However, this was no protection in the rats treated with castor oil and normal saline (control). The standard drug (Diphenoxylate) used was observed to be superior in its ability to reduce the castor oil originally obtained from *Ricinus communis* L. is well known to contain ricinoleic acid which when administered induces irritation of the intestinal membrane causing increase inflammation, secretion of intestinal, fluid and increase intestinal mortality resulting in diarrhea (Piece *et al.* 1970; Donaldson 1977; Ayinde *et al.* 2009). Since the result of this study showed that the extract has the ability to reduced secretory diarrhea, then effect of the extract should include the inhibition of the intestinal mortality and the decrease in intestinal secretion, even though, diarrhea may also occur due to decreased reabsorption of substance within the intestine (Galvez *et al.* 1993; Lucas and Gilles 2003). From the results it was observed that the extract has the ability to prolong the intestinal transit period of charcoal meal (**Table 2**) which could increase reabsorption of substance in the intestine, the extract at dose of 1000 mg1/kg reduced the intestinal of charcoal meal by 27.0% compared with the standard drug (atropine) which reduced the intestinal transit by 44.4%. The re-

Table 1 Effect of aqueous leaf extract of *Holarrhena floribunda* on castor oil induced diarrhoea in rats.

(n=5) Group	Treatment mg/kg	Mean number of defecation in 6 hrs	% protection
A	Control	20.8 ± 1.79	0
B	400	14.60 ± 1.14 [b]	26.81
C	700	10.2 ± 1.09 [b]	50.96
D	1000	6.80 ± 2.398 [b]	67.31
E	5[a] (Diphenoxylate)	0.20 ± 0.45 [b]	99.04

Mean ± SD based on five observations
[a] Diphenoxylate (standard drug).
[b] Significantly different ($P < 0.05$) compared with control group.
N = number of rats in each group.

Table 2 Inhibitory effect of aqueous leaf of *Holarrhena floribunda* on intestinal transit in rats.

Group n=5	Treatment (mg/kg)	Total length of intestine (cm)	Movement of charcoal meal (cm)	% intestinal transit
A	Control	109.38 ± 7.49	89.24 ± 7.22	81.6
B	400	104.85 ± 9.51	66.80 ± 9.04 [a]	63.7
C	700	103.98 ± 8.40	61.64 ± 6.73 [a]	59.3
D	1000	104.10 ± 6.24	56.84 ± 7.80 [a]	54.6
E	Atropine (3)	102.10 ± 7.41	37.98 ± 3.79 [a]	37.2

Mean ± S.D based on five observations
n = number of rats in each group
[a] Significantly different ($P < 0.05$) compared with control 1 group.

Table 3 Inhibitory effect of aqueous leaf extract of *Holarrhena floribunda* on intestinal fluid accumulation.

Group n = 5	Treatment (mg/kg)	Weight of intestine + content (g)	Weight of empty intestine (g)	Weight of contents (g)	% fluid accumulation
A	Control	6.96 ± 0.70	3.14 ± 0.50	3.78 ± 0.42	54.3
B	400	6.48 ± 0.63	3.44 ± 0.49	2.88 ± 0.24 [a]	46.3
C	700	5.82 ± 0.48	3.38 ± 0.41	2.22 ± 0.29 [a]	38.1
D	1000	5.52 ± 0.54	3.56 ± 0.21	1.64 ± 0.50 [a]	29.7
E	3 (Atropine)	4.42 ± 0.29	3.62 ± 0.32	0.76 ± 0.2 [a]	17.2

n = number of rats in each group
Mean ± SD based on five observations.
[a] Significantly different ($P < 0.05$) compared with control group.

duction in movement of charcoal meal and intestinal fluid accumulation help in re absorption of substances from the intestine (Lawrence 1997; Abdulrahman *et al*. 2007; Seshadri 2008).

The result of the effect of the extract on castor oil induced intestinal fluid accumulation showed that there was gradual reduction in intestinal fluid accumulation with increasing dose of the extract (**Table 3**). The standard drug (atropine) reduced the intestinal fluid accumulation by 37.1% of white the extract of the dose of 1000 mg/kg reduces the intestinal fluid accumulation by 24.4%. The increase in the intestinal fluid accumulation could be caused by electrolyte and water secretion and by increase intestinal motility. The time needed for the intestinal content to be absorbed of the absorptive surface of the intestinal tract depends largely on the gastro intestinal motility, to give enough time for the reabsorption of the intestinal content which content which subsequently reduce diarrhea (Salgado *et al*. 2006).

In the experimental model of castor induced intestinal secretion, the aqueous leaf extract of *Holarrhena floribunda* conferred a dose dependant protection in the rats treated with the various doses of the extract compared with the control and atropine, a drug known for its anti secretory and anti-muscarinic effects (Reynolds 1989). The rats treated with normal saline and castor oil had significant increase of intestinal fluid accumulation compared to rats treated with the aqueous leaf extract. The extract was able to reduce castor oil induced increased in intestinal fluid accumulation dose dependently.

CONCLUSION

The results of this study showed that the leaf extract of *Holarrhena floribunda* has some anti diarrheal activity providing a scientific basis for its used in treatment of diarrhea by traditional healers in Bauchi State, Nigeria. However, further work should be done in order to establish the mechanism of anti diarrheal effects.

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