The Antidiarrheal Activities of Methanol extract of*Holarrhena Floribunda* on rodents

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ABSTRACT

The antidiarrheal activities of methanol extract of *H.floribunda* on rats were investigated using castor oil-induced diarrhea, small intestinal transit time and castor oil-induced fluid accumulation models. In the castor oil induced diarrhea theresults showed that the extract(110.22mg/kg)alone produced a mean fecal matter of 10.83±0.82 translating to 69.05% inhibition of the diarrhea .The percentage inhibition was significant[P≤0.01]. When Yohimbine was combined with the extract, the mean fecal matter increased from 10.83±0.82 to 25.16±1.6 (28.11%) compared to control. This shows that Yohimbie, an α_{2-} adrenoceptor antagonist, antagonized the effect of the extract. The intestinal transit time significantly[P≤0.01]decreased from 81.71±1.63 to 65.71±2.90 tranlating to 25.58%inhibition. As the dose of the extract increased from 36.74 to 110.22 mg/kg, the intestinal fluid accumulation decreased significantly[P≤0.01] from 3.26±0.14 to1.55±0.10 translating to 13.75 to 58.99% reduction. Similarly, when Yohimbine was co-administered with the extract the intestinal fluid accumulation increased from1.55±0.10 (58.99%) to 1.88±0.13 (50.26%) fluid accumulation. The fact that Yohimine has antagonized the antidiarrheal effect of the extract suggests that theα_{2-} adrenoceptor has a role to play in this antagonism. The percentage inhibition by Diphenoxylate, a standard antidiarrhea and a muscarinic blocker(67.14%) was higher than that of the combined doses of the extract (28.11%).Phytochemical analysis also shows that *H.floribunda* contain Phlobatanins, Flavonoids and alkaloids which might have also contributed to the antidiarrheal activities.

Key words: *Holarrhena floribunda*, Diarrhea, Rodents.

INTRODUCTION

Diarrhea is frequent passage of watery or loose stool up to three or more times within 24 hours (Donald 1994). It is a leading cause of morbidity and mortality in the world and is responsible for approximately three million deaths each year among children under five years of age, that is one child dies every 10 seconds(Kumar and Clark,2006).

There are estimated 1.8 billion episodes of childhood diarrhea per year and virtually all of these acute diarrhea episodes are related to infectious agents.

In developing countries, acute diarrhea results in death in malnourished infants, inconvenience and discomfort in a healthy well-nourished adult (Rang *et al* 2003).In Nigeria diarrhea is responsible for more than 5-8 million deaths each year in infants and small children less than five years(Jousilahti *et al* 1997)

In wartorn areas where malnutrition and poor sanitary conditions prevail, acute and chronic diarrhea co-exist, leaving people with the choice of oral rehydration and in an acute disease treatment (Costello*etal* 1992)

Holarrhena floribunda is a shrub tree that belongs to the family of apocyanaceae. The bark is gray, yielding copious white latex. It produces white scented flowers. The plant bears linear cylindrical follicles hanging in pairs and enclosing flattened seeds with numerous brownish silky hairs. The plant is found in the dry tropical forest regions and fringing savannah forest. It is also found in the Republic of Guinea, Angola and Nigeria (Hutchinson and Dalziel,1972).

The bark macerated in palm wine is used in the treatment of dysentery and fever. The leaf, bark and roots are taken for malaria or used as ingredients in steam bath for malaria and fevers. The root decoction is used alone or in a mixture with other herbs for the treatment of female infertility (Burkill,1997). In East and southern Africa the bark is dispensed as a febrifuge, a tonic and as remedy for snake bite and for the treatment of sexually transmitted diseases. The stem bark of *H. Floribunda* is used in Nigeria, Burkina Fasso and Cameroon to treat abdominal pains, nausea, indigestion, diarrhea. The stem bark is used as a febrifuge and could be a substitute for quinine. It showed inhibitory activity against drug resistant strains of *plasmodium falciparum*. It contains conessine that destroys amoeba without emetic effect (Bogne *et al* 2007).

H. Florobunda contains 0.1% alkaloid of glucocosteroid type, including conessine, durchine, holacurtenin and Holacurtin. The stem barkalkaloids are derived from pregnane. The plant also contains non-steroidal trialanthine and phenolic acids and flavonoids, kaemferol and quercetin(Sofowora,1993).

In Nigeria the ethno-pharmacology of the root of *H. Floribunda* is employed in the treatment of malaria, diarrhea, dysentery, fever, pains, female sterility, skin infections, sexually transmitted diseases and snake bite (Chukwurah, 1997). The use of the root of this plant in the treatment of these diseases have not been evaluated hence the need for this study.

MATERIALS AND METHODS

Experimental Animals:

Thirty six Albino rats (male) weighing 130-200g, obtained from the University of Uyo Animal Housewas maintained under standard conditions (12hours light / 12 hours dark cycle and temperature 22-25^{0}C). The animals were fed with standard feeds (Bendel feeds and Flour Mills Ltd; EdoState) and water *ad libitum.*

Extraction of root of *H. floribunda*

The root were washed, cut into pieces and shade dried.The dried root bark was pulverized to powder using mortar and pestle. The pulverized powderweighing 1.5kg

was successively extracted with n-hexane, chloroform,ethyl acetate and methanol to obtain their different extracts. These extracts were separately concentrated to dryness in vacuo at 40^0C. These were weighed, stored in separate bottles and kept at temperature of -4^0C until used.

Phytochemical screening

Phytochemical tests were carried out according to the methods of (sofowora 1993, Trease and Evans, 2009).

Acute toxicity testing

Eighteen mice weighing between 20 to 30g were randomized and divided into six groups of three mice each. They were fasted for 24 hours and water 2 hours before the commencement of the experiment. The extracts (dose) were administered intraperitonially. Signs of acute toxicity were observed within 24 hours.

Effect of Extract on Castor oil induced Diarrhea in rats

Diarrhea was induced in rats using castor oil.(Nwafor *et al* 2005, Nwodo and Alumanah 1991). Animals were fasted for 24 hours but allowed free access to water. The animals were randomized into six groups of six rats each and treated as shown below.

Group 1 : Received distilled water(10ml/kg) orally (control).

Group 2-4 : Received the extract (36.74, 73.48 and 110.22mg/kg)respectively.

Group 5: Received 5.0mg/kg of Diphenoxylate

Group 6: Received 1.0mg/kg of Yohimbine + 73.48mg/kg middle dose extract.

One hour after treatment each animal received 2ml of castor oil per oral, then observed for consistency of fecal matter and the frequency of defecation for 3 hours. The faecal droppings were counted and recorded.

Effect of extract on small intestinal transit time in rats

The effect of methanol extract of *H. floribunda* on intestinal propulsion in rats was tested using charcoal method (Nwafor *et al* 2005.) Animals were fasted for 24 hours but were allowed free access to water. They were randomized into six groups of six rats each and treated as shown below.

Group 1 : Received distilled water(10ml/kg) orally (control).

Group 2-4 : Received the extract (36.74, 73.48 and 110.22mg/kg)respectively.

Group 5: Received 1.0mg/kg of Yohimbine

Group 6: Received 1.0mg/kg of Yohimbine + 73.48mg/kg middle dose extract.

One our later, 1ml charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth) wasadministered orally to each rat. The rats were sacrificed after 30 minutes by cervical dislocation and the small intestine was immediately ligated at the charcoal head. The small intestine was removed and placed on a clean surface, carefully inspected, the distance traveled by the charcoal meal from the pylorus was measured, and then the full length of the small intestine from the pylorus to the ileocaecal junction was also measured. This distance traveled by the charcoal meal from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

Effect of extract on castor oil-induced intestinal fluid accumulation in rats

Fluid accumulation was induced in rats using the method of Nwafor *et al* 2005 and Robert *et al* 2006.

36 rats were fasted for 24 hours but allowed free access to water. They were randomized into six groups of six rats each and treated as shown below.

Group 1 : Received distilled water(10ml/kg) orally (control).

Group 2-4 : Received the extract (36.74, 73.48 and 110.22mg/kg)respectively.

Group 5: Received 5.0mg/kg of Diphenoxylate

Group 6: Received 1.0mg/kg of Yohimbine + 73.48mg/kg middle dose extract.

One hour later, 2ml of castor oil was administered to each rat orally using orogastric cannula.30 minutes later the rats were sacrificed by cervical dislocation.The rats were dissected and the small intestine ligated immediately at both the pyloric sphincter and the ileocaecal junction.The entire small intestine was dissected out,its content expelled into a graduated measuring cylinder and the volume of the contents recorded.

STATISTICAL ANALYSIS

The results obtained from these investigations were subjected to statistical analysis expressed as multiple comparisons of means ± SEM. Significance was determined using one way ANOVA followed by Turkey-Kramer multiple comparison test. A probability level of less than 5% was considered significant.

RESULTS

Table 1: Effect of *H.Floribunda* Extract on Castor Oil-Induced Diarrhea in Rats

Group	No. of Rats	Treatment/Routeof Administration (per Oral)	Dose (mg/Kg	Mean fecal matter	% inhibition
A	6	Distilled Water (control)	10ml/kg	35.00±2.26	--
B	6	Extract	36.74	25.16±0.91[a]	28.11
C	6	Extract	73.48	15.16±0.52[a]	56.68
D	6	Extract	110.22	10.83±0.82[a]	69.0.5
E	6	Diphenoxy/ate	0.5	11.5±0.65[a]	67.14
F	6	Yohimbine+Extract	1.0+73.48	25.16±1.6	28.11

Data are represented as mean ± SEM Significant at P≤0.001, when compared to control (n=6).

Table 2: Effect of *H. Floribunda* Root Bark Extract on Intestinal transit time in Rats.

Group	No. of Rats	Treatment/Route of Administration (per Oral)	Dose (Mg/Kg	Intestinal transit time	% inhibition
A	6	Distilled Water (control)	10ml/kg		--
B	6	Extract	36.74	81.71±1.63[a]	16.77[a]
C	6	Extract	73.48	68.0±1.84[a]	19.58[b]
D	6	Extract	110.22	65.71±2.90[b]	25.58[b]
E	6	Yohimbine	1.0	87.72±2.29[b]	12.90
F	6	Yohimbine+Extract	1.0+73.48	71.05±1.97[b]	13.05

Data are represented as mean ± SEM Significant at aP≤0.001, bP≤0.01when compared to control (n=6).

Table 3:Effect of *H.Floribunda* Extract on Castor Oil – Induced Fluid Accumulation in Rats

Group	No.of Rats	Treatment/Route of Administration (per Oral)	Dose (mg/Kg	Mean volume of intestinal fluid (ml)	% reduction
A	6	Distilled Water (control)	10ml/kg	3.78±0.16	-
B	6	Extract	36.74	3.26±0.14	13.75
C	6	Extract	73.48	1.95±0.23[a]	48.41
D	6	Extract	110.22	1.55±0.10[a]	58.99
E	6	Diphenoxylate	5.0	1.42±0.13[a]	62.43
F	6	Yohimbine+Extract	5.0+73.48	1.88±0.13[a]	50.26

Data are represented as mean ± SEM Significant at P≤0.001, when compared to control (n=6).

DISCUSSION

Phytochemical analysis:

The phytochemical analysis showed that the root bark extract of *Holarrhena floribunda* contain alkaloids, tannins, saponins, flavonoids, steroids and cardiac glycosides. Flavonoids and tannins may have contributed to the antidiarrheal activities(Sofowora,1993.,Trease and Evans, 2009).

Acute Toxicity Testing:

Signs and symptoms of acute toxicity ranging from decreased motor activity, increased respiratory rate, restlessness, gasping for breath to death were observed (Lorke,1983). The LD_{50} was therefore calculated to be 367.42mg/kg body weight.**Diarrhoea:** Diarrhoea is the frequent passage of watery stool up to three or more times within 24 hours (Donald,1994). This increased passage of loose and watery stools relative to the persons usual bowel habit is caused by hyper propulsive motility of the gastrointestinal tract and hypersecretion throughout the intestinal mucosa. Many medicines, particularly broad-spectrum antibiotics such ampicillin, erythromycin and neomycin, induce diarrhea secondary to therapy. The use of Castoroil to induce diarrhea is an age long practice (Walker and Wittlesea, 2002).Castor oilon its own does not induce diarrhea but when acted upon by lipases, intestinal digestive enzymes is converted to glycerol and ricinoleic acid. The ricinoleic acid is a hydroxylated unsaturated fatty acid. This acid induces strong laxative effect by causing changes in electrolyte and water transport leading to contractions in transverse and distal colon thereby producing permeability changes in the intestinal mucosa membranes which finally result in watery luminal content that flows rapidly through the small and large intestines(Kagbo and Eyaru,2012). In acute diarrhea, antimotility agents such as loperammide, diphenoxylate and codeine are occasionally useful for symptomatic control in adults who have mild to moderate diarrhea and require relief from associated abdominal cramps. Symptomatic treatment of diarrhea require the use of substances that coates the gut such as activated charcoal, substances that decrease the intestinal propulsion (antimotility drugs) and substances that reduce fluid accumulation in the intestinal lumen(Farthing,1996).

Effect of extract on castor oil induced diarrhoea in rats

In this study the extract significantly($p \leq 0.01$) decreased the number of fecal matter passed. Diphenoxylate caused 67.14% inhibition of castor oil induced diarrhea. Diphenoxylate is asynthetic opioid available as a co-phenotropein combination with a sub-therapeutic dose of atropine. Diphenoxylate, an alpha-2-adrenoceptor couples with G-1 protein which causes a decrease in cAMP activity, resulting in smooth muscle contraction which help to decrease the diarrheal state. In the presence of Yohimbine, a muscarinic blocker the antidiarrheal effect of the extract was antagonized as seen in the reduction of the percentage inhibition to 28.11%. A muscarinic receptor antagonist is an agent that blocks the activity of the muscarinic acetylcholine receptor (Walker and Whittlesea, 2002).

Effect of extract on small intestinal transit time in rats

The effect of the extract was investigated by measuring the intestinal transit time in rats. The extract caused a dose-dependent decrease in transit time translating to 16.77-25.58% inhibition for low and high doses of the extract respectively. Yohimbine alone increased the transit time causing 12.90% inhibition while co-administration of Yohimbine with the extract further decreased the transit time leading to13.05% inhibition. This shows that in the presence of Yohimbine, α_2-adrenoceptor antagonist, the effect of the extract was antagonized.

Effect of extract on castor oil-induced intestinal fluid accumulation in rats

The extract caused a dose-dependent decrease in intestinal fluid accumulation. The extracts significantly ($p \leq 0.001$) inhibited fluid accumulation by 13.75,48.41 and 58.99% for low, middle and high doses respectively relative to control. In the presence of Yohimbine the effect of the extract was antagonized as seen in the increase in fluid accumulation translating to50.26% fluid reduction.

CONCLUSION

This study shows that the methanol extract *Holarrhena floribunda* has significant antidiarrheal activity which may have been facilitated by the blockade of muscarinic receptors. The study provide a scientific basis for the ethnomedicinal use of the plant to treat diarrhea.

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