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RESEARCH ARTICLE

ANTI-DIARRHEIC PROPERTIES OF THE AQUEOUS METHANOLIC EXTRACT OF *PALISOTA HIRSUTA* LEAVES AND ITS FRACTIONS IN MICE

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ABSTRACT

The anti-diarrheic properties of methanolic leaf extract of *P.hirsuta* and its fractions were evaluated using castor oil-induced diarrhea model and charcoal meal to evaluate small intestinal transit. The extract yielded seven fractions after column chromatography. The extract (100 mg/kg) significantly ($P<0.05$) decreased total number of fecal output 3 h post-administration of castor oil compared with 10 ml/kg distilled water and caused 46% inhibition of diarrhea compared with diphenoxylate (5 mg/kg). Comparing the ratio of solid to watery stools showed that the extract (100 mg/kg) produced 61:39% against 41:59% by diphenoxylate (5 mg/kg). The small intestinal transit was significantly ($P<0.05$) decreased by all doses of extract used (50, 100, 150 mg/kg) when compared with distilled water and caused 4% inhibition of intestinal fluid accumulation at 50 mg/kg compared with diphenoxylate. Fraction 2 (50 mg/kg) caused significant ($P<0.05$) decrease in small intestinal transit compared with distilled water and caused 2% inhibition of intestinal fluid accumulation compared with diphenoxylate. The major phytochemical constituents of fraction 2 were tannins.

The extract of *P. hirsuta* showed promising anti-diarrheic properties, possibly through retardation of gastrointestinal motility and mediated by tannins, justifying its traditional use for treatment of diarrhea among the Igbo tribe of Eastern Nigeria.

INTRODUCTION

Diarrhea is a major health problem in developing countries especially sub-Saharan Africa due to a number of different social, political, and economic factors which contribute to the constant morbidity from acute and persistent diarrhea, as well as intermittent epidemics of cholera and dysentery (Hamer, 1998). The use of antibiotic in the treatment of certain types of diarrhea especially in people infected with *Escherichia coli* O157:H7 could be counterproductive due to development of haemolytic uremic syndrome (Wong *et al*., 2000). There are also concerns for antibiotic resistance (WHO, 2009). It is necessary to identify and evaluate the commonly available natural drugs for efficacy and safety as alternative to the currently available allopathic anti-diarrheic drugs, which are expensive and have serious side effects. Various plant preparations have been used as sources of drugs for treatment of various ailments including diarrhea. The leaves of *Palisota hirsuta* have been used among the Igbo tribe of Eastern Nigeria for the treatment of stomach aches, dyspepsia and diarrhea (Oliver, 1960). Roots are used to treat dysentery, anemia and rheumatism while a leaf decoction is used to treat colic, (Abbiw, 1990; Mshana *et al.*, 2000). The plant, *Palisota hirsuta* K. Schum (family Commelinanceae) is variously known as "*thunb"* in English, "*ikpere aturu"'* or *"ikpere anukwu"* in various parts of Igbo land, Eastern Nigeria. *P. hirsuta* is a robust herb of forest regions, about 2 – 4 cm high reproducing from seeds (Okezie *et al*., 1987). Antiinflammatory, antipyretic, analgesic, antimicrobial, anxiolytic and sexual stimulant effects have been reported of this plant (Boakye-Gyasi *et al*., 2008; Benson *et al*., 2008; Anaga *et al*., 2009; Wood *et al*., 2010). There has been no scientific report on the anti-diarrheic effects of *P. hirsuta*. We therefore report on the anti-diarrheic effect of *P. hirsuta*

MATERIALS AND METHODS

Animals

Mature white albino mice of both sexes (23 – 34g) were procured and housed in stainless steel cages. They were fed *ad-libitum* with a standard laboratory animal feed (vital feed®, Grand cereal and oil meals Ltd. Nigeria), except were fasting was necessary. They were maintained in accordance with the recommendation in the guide for the care and use of laboratory animals (DHH, NIH publication No. 85: 23, 1985). All animal experiments were conducted with the permission of the institution's Animal Ethics Committee.

Plant Material

Fresh green leaves of *Palisota hirsuta*, were collected from Obukpa in Nsukka Local Government Area of Enugu state, South Eastern Nigeria and were confirmed as *P. hirsuta* by a plant taxonomist.

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The leaves were dried under laboratory conditions at the temperature range of 25-27°c for about 10 days and pulverized to a coarse powder (mesh size1.00 mm) using hammer mill. 382g of the plant material was extracted by cold maceration using 70% methanol (Sigma-Aldrich Laborchemikallen GMBH, Germany) with intermittent shaking for 48hours. The extract was filtered with Whatmann No.1 filter paper and concentrated *in vacuo* to dryness using rotary evaporator (Buchi Labotechnik, Switzerland) and was referred to as leaf extract of *P. hirsuta* (LEP). The percentage yield (w/w) of the extract was calculated using the formula below:

Weight of extracted material ÷ weight of starting material ×100. The LEP was separated into fractions using column chromatography (Harbourne, 1991). Briefly Silica gel 60 G for column chromatography (Vicker, West York England) was used as the stationary phase and 10 g of LEP was adsorbed to it. The column was eluted with Petroleum-ether, chloroform, ethyl acetate and methanol in ascending order of polarity as shown in table 1. Two hundred and thirty five aliquots 10 ml column fractions were collected and spotted on pre-coated silica gel GF254 aluminum plate for thin layer chromatography (TLC) (Merck, Germany) and eluted with chloroform-methanol-ethyl acetate (1:3:1) in a small chromatographic tank to separate the various fractions based on their relative mobility on TLC plates and color reactions with UV light (Buchi Labotechnik, Switzerland). This procedure yielded a total of 7 fractions which were to read which were used for the experiments. The fractions were concentrated to dryness using rotary evaporator at 200 milibar and 40°c and were referred to as Leaf Extract of *P. hirsuta* fractions (LEPfr).

Castor oil induced Diarrhea in Mice

Twenty five mice were divided into five groups of five. Group 1 received 10 ml/kg distilled water, group 2 received 5 mg/kg diphenoxylate, and groups 3 - 5 received 50, 100 and 150 mg/kg respectively of LEP. Thirty minutes post treatment 0.5ml of castor oil (Bell sons and co. Southport England) was administered to all the animals. The animals were kept in individual cages and floor of which were lined with blotting paper and the number of both normal and watery droppings counted for each mouse every hour over a period of 4 h. Mean of the stools passed by the treated groups were compared with that of the control (Vander *et al*., 2007).

Small Intestinal Transit (SIT) and Fluid Accumulation (FA)

Twenty five mice were divided into five groups of five and were fasted for 16hr before the experiment. Group 1 received 10 ml/kg distilled water, group 2 received 5 mg/kg diphenoxylate, while groups 3 - 5 received 50, 100 and 150 mg/kg LEP. For the LEPfr, 27 mice of both sexes were grouped into 9 groups of 3 mice each. While the controls received either 10 ml/kg distilled water or 5 mg/kg diphenoxylate, groups 3- 9 received 50 mg/kg of LEPfr 1- 7 respectively. One hour post treatment, standard charcoal meal (0.5ml of 5% activated charcoal suspension in 5% gum acacia) was administered to all the animals. The animals were sacrificed 30 min post-administration of charcoal meal under mild ether anesthesia and the intestine immediately isolated and ligated at pyloric sphincter and at the ileo-cecal junction. The SIT (the peristaltic index) of each mouse was expressed as percentage of distance travelled by the charcoal meal relative to the total length of the small intestine from the pyloric sphincter to the ileo-cecal junction of each mouse. The FA was determined by weighing the intestine and its contents, then milking out the intestinal contents, and finally reweighing the empty intestine to determine the final weight. The difference between full and empty intestine was determined. Percentage inhibition of FA was calculated (Rao *et al*., 1997). The mean fecal output and the mean SIT were analyzed using one way analysis of variance and Variant means were separated post-hoc using the least significant difference (LSD). Results were expressed as means ± standard error of means (SEM) and significance was accepted at the probability level $p<0.05$. Ratio of normal to watery droppings, percentage inhibition of diarrhea and percentage inhibition of intestinal fluid accumulation were expressed as percentages.

Spot Phytochemical Analysis of LEPfr

Phytochemical analysis of LEPfr was carried out using standard procedure (Trease and Evans, 1999). LEPfr was tested for the presence of alkaloids, flavonoids, tannins, glycosides, starch and carbohydrates.

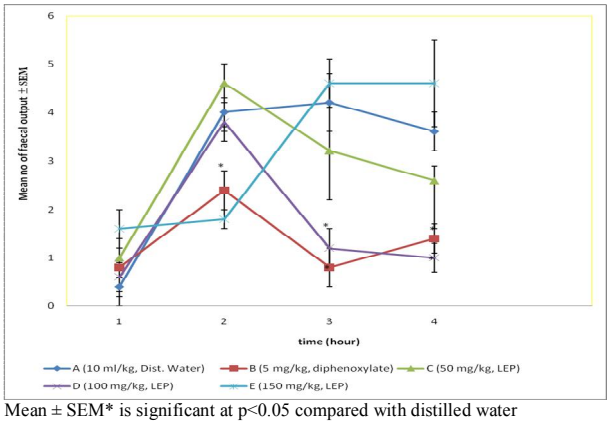
RESULTS

Yield of the extract

The methanolic leaf extract of *P.hirsuta* yielded 8.77%w/w material which was dark green in color, pasty in consistency and had a sharp pungent smell. Bioassay-guided chromatographic Separation of LEP yielded seven fractions. Spot phytochemical analysis of LEPfr2, which was the most active fraction showed the presence of tannins.

Table 1 Solvent System for Chromatographic Separation of LEP

Pet ether %	Chloroform %	Ethyl acetate %	Methanol %
100			
70	30		
50	50		
20	70	10	
	80	20	
	60	40	
	40	50	10
	20	60	20
		70	30
		50	50
		30	70
		10	90
			100



Mean ± SEM* is significant at $p<0.05$ compared with distilled water

Figure 1 Effect of LEP on castor oil-induced diarrhea in mice

Table 2 Effect of LEP on fecal consistency in castor oil-induced diarrhea in mice

Groups	% solid droppings	%watery droppings	% inhibition of diarrhea
A	18	82	0
B	41	59	56
C	42	58	7
D	61	39	46
E	19	81	0

A: 10 ml/kg distilled water, B: 5 mg/kg diphenoxylate, C – E (50, 100 and 150 mg/kg) LEP respectively

Effect of LEP on castor oil-induced diarrhea

The result of the castor oil induced diarrhea in mice as shown in fig. 1 showed that, there was no significant difference in the mean number of fecal output among the groups one hour post-administration of castor oil. Fecal output at this stage was only the solid type. At two hours post-administration of castor oil, there was a general increase in the mean number of fecal output among the groups, but diphenoxylate (5mg/kg b.w.) significantly ($p<0.05$) decreased the mean number of fecal output when compared with the LEP treated groups. There was also significant ($p<0.05$) decrease in the mean number of fecal output in the LEP (100 mg/kg b.w) treated groups 3h and 4h post-administration of castor oil compared with distilled water-treated group, though there was no significant ($P>0.05$) compared with diphenoxylate (5mg/kg). Comparison of the ratio of solid to watery stool (table 2.)

Table 3 Effect of LEP on small intestinal transit in mice

Groups	% SIT
A (10 ml/kg b.w. dist. Water)	80.29 ± 7.88
B (5 mg/kg b.w. diphenoxylate)	55.10 ± 9.53*
C (50 mg/kg b.w. LEP)	45.71 ± 6.02*
D (100 mg/kg b.w. LEP)	49.50 ± 2.45*
E (150 mg/kg b.w. LEP)	45.84 ± 5.31*

Mean ± SEM* is significant at $p<0.05$ compared with distilled water

Table 4 Effect of LEP on intestinal fluid accumulation in mice

Groups	% inhibition of intestinal fluid accumulation
A (10ml/kg, distilled water)	0
B (5 mg/kg, diphenoxylate)	0
C (50 mg/kg, MLEPH)	4
D (100 mg/kg, MLEPH)	0
E (150 mg/kg, MLEPH)	0

Table 5 Effect of LEPfr on small intestinal transit in mice

Groups	% SIT
A (10 ml/kg b.w. dist water)	76.28±3.3
B (5 mg/kg b.w. diphenoxylate)	54.44±3.8*
C (50 mg/kg b.w. LEPfr1)	76.24±9.3
D (50 mg/kg b.w. LEPfr2)	54.37±4.2*
E (50 mg/kg b.w. LEPfr3)	60.29±3.5
F (50 mg/kg b.w. LEPfr4)	68.81±10.7
G (50 mg/kg b.w. LEPfr5)	82.58±4.2
H (50 mg/kg b.w. LEPfr6)	68.50±4.3
I (50 mg/kg b.w. LEPfr7)	58.42±7.5

Mean ± SEM* is significant at $p<0.05$ compared with distilled water

showed that LEP 100 mg/kg b.w. produced 39% watery stools against 59% by diphenoxylate (5 mg/kg b.w.) while

Table 6 Effect of LEPfr on intestinal fluid accumulation in mice

Groups	% inhibition of EP
A (10 ml/kg, distilled water)	0
B (5 mg/kg, diphenoxylate)	0
C (50 mg/kg, LEPfr1)	0
D (50 mg/kg, LEPfr2)	2
E (50 mg/kg, LEPfr3)	7
F (50 mg/kg, LEPfr4)	0
G (50 mg/kg, LEPfr5)	0
H (50 mg/kg, LEPfr6)	0
I (50 mg/kg, LEPfr7)	0

Comparison of the percentage inhibition of diarrhea among the groups showed that diphenoxylate (5 mg/kg b.w.) caused 56% inhibition as against 46% and 7% by 100 mg/kg and 50 mg/kg LEP respectively.

Effect of LEP and LEPFr on SIT

In the small intestinal transit (table.3.), LEP at doses (50, 100 and 150 mg/kg b.w.) significantly ($p<0.05$) decreased the SIT when compared with the distilled water control group. The was no significant ($P>0.05$) difference between the extract-treated groups and diphenoxylate (5 mg/kg) the effect of LEP on FA is shown in table.4. LEP (50 mg/kg b.w.) caused 4% inhibition of FA compared with diphenoxylate. Table.5. presents the effects of LEPfr on the SIT and it showed that LEPfr2 (50 mg/kg b.w.) significantly ($p<0.05$) decreased the SIT when compared with the distilled water group. There was no significant ($P>0.05$) difference between the effect of LEPfr2 and diphenoxylate (5 mg/kg). The effect of LEPfr on FA (Table.6.) showed that LEPfr2 and LEPfr3 caused 2 and 7% inhibition of intestinal fluid accumulation when compared with diphenoxylate.

DISCUSSION

Diarrhea is as a result of imbalance between absorptive and secretory mechanisms in the intestinal tract involving two components; motility and secretory (Chitme *et al*., 2004). A good anti diarrheic agent must have significant effect on any or both of these components. Castor oil causes irritation of the intestinal mucosa via liberation of recinoleic acid and the irritation leads to release of prostaglandin which causes excessive intestinal fluid secretion and motility (Chitme *et al*., 2004). Normal intestinal fluid absorption is also impaired by castor oil through inhibition of intestinal $Na^{+}K^{+}$ ATPase activity (Gaginella *et al*., 1978).

Anti-diarrheic effect of LEP became apparent 3 h post administration of castor oil and sustained till the end of the experiment. Pretreatment of mice with LEP possibly resulted

in amelioration of irritation and inflammation of intestinal mucosa induced by recinoleic acid liberated from castor oil, thus leading to decrease in intestinal motility and secretion, for an overall reduction in the rate of passage of watery stool.

It is also possible that LEP activated the intestinal Na^+K^+-Atpase activity, to enhance normal fluid absorption, thereby reducing diarrhea.

The motility and secretory activity of the GIT are controlled by various neurotransmitters secreted by the enteric nervous system. Some are excitatory like acetylcholine and will lead to increased gut motility and secretion while some are inhibitory like noradrenaline and will lead to decreased gut motility and secretion (Guyton and Hall, 2001). LEP at all doses and its fraction (LEPfr2, 50 mg/kg, b.w.) significantly slowed down charcoal meal transit in the gastrointestinal tract, showing that LEP could have inhibitory effects on the excitatory neurotransmitters in the gastrointestinal tract thus leading to relaxation of the gut muscles and slowing down motility.

There was also a 4% decrease in intestinal fluid accumulation at 50mg/kg b.w. LEP and 2% by LEPfr2, pointing to mild antisecretory effect. The effects of LEP on the small intestinal transit and fluid accumulation tend to decrease with purification, signifying that other phytochemical constituents of LEP are important for effective anti-motility and antisecretory effects. It also seems that beyond 100 mg/kg, effectiveness of LEP as gastrointestinal protectant decreases.

It has been found that anti-diarrheic properties of medicinal plants are due to tannins, flavonoids alkaloids, saponins, sterols, triterpenes and reducing sugars (Longanga-Otshudi *et al*., 2000; Teke *et al*., 2010) . The major phytochemical constituents of LEP were found to be tannins, flavonoids glycosides and proteins (Anaga *et al*., 2009). The flavonoids, tannins or glycosides may have mediated the anti-diarrheic effects of LEP.

Phytochemical analysis of LEPfr2 showed that the major constituents were tannins, therefore suggesting that the anti-diarrheic effects of *P.hirsuta* leaves was mediated mainly by the tannin content. Tannin containing drugs will precipitate proteins and have been used internally for the protection of inflamed surfaces of mucous membranes (Trease and Evans, 1999). The astringent actions of tannins function to precipitate microproteins on inflamed mucous membranes, thereby forming a protective layer over the mucosal lining and protect the underlying mucosa from irritants and toxins (Clinton, 2009).

CONCLUSION

Our results suggest that *P.hirsuta* possesses promising anti-diarrheic effects which were comparable with diphenoxylate; a standard anti-diarrheic drug. The effects were probably mediated mainly by the tannin component. The effects on the small intestinal transit and fluid accumulation suggest that the anti-diarrheic mechanism was through relaxation and retardation gastrointestinal smooth muscles and motility, with only a mild effect on intestinal fluid secretion. These effects of *P.hirsuta* validate its traditional use as an anti-diarrheic agent. We recommend further pharmacological and toxicological investigations into the anti-diarrheic properties of *P. hirsuta* leaves in other to exploit the full potential of this medicinal plant as an anti-diarrheic agent.

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