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Antidiarrheal activity of *Pterocarpus erinaceus* methanol leaf extract in experimentally-induced diarrhea

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ABSTRACT

Objective: To investigate the antidiarrheal activity of the methanol leaf extract of *Pterocarpus erinaceus in vivo*. **Methods:** The methanol leaf extract of *Pterocarpus erinaceus* was evaluated using different doses (100, 200 and 400 mg/kg body weight) orally for antidiarrheal activity using castor oil-induced diarrhea, charcoal meal transit time and castor oil-induced enteropooling in different groups of albino Wistar mice. The activity of the extract at different doses were compared to diphenoxylate (5 mg/kg) and atropine sulphate (3 mg/kg) which were used as standard reference drugs and also to the distilled water administered negative control group of mice. **Results:** The extract at the doses used caused a significant ($P< 0.01$) reduction in the wet faeces passed by the mice in the castor oil-induced diarrhea, decreased the distance travelled by the charcoal meal by up to 54.8% and also caused a dose dependent and significant ($P< 0.001$) reduction in the intraluminal fluid accumulation in the castor oil-induced enteropooling. **Conclusions:** Our results indicate that *Pterocarpus erinaceus* extract produced significant antidiarrheal activity and the action may attribute to inhibition of gastrointestinal movement and fluid secretion.

1. Introduction

Diarrhea is the frequent passage of liquid faeces[1] and it is characterized by increased gastrointestinal motility and secretions[2].

In the tropical and subtropical countries of the world, diarrhea is still one of the major health threats to the population[3] and the causes include infectious agents, plant toxins, gastrointestinal disorders such as inflammatory or dysmolity problems and substances that increase gastrointestinal tract secretions[4].

In Nigeria, it remains the number one killer disease among children under 5 years with the most susceptible being children between the ages of 7-12 months[5]. By WHO estimates, about 3-5 billion cases occur annually with about 1 billion cases and 5% of deaths occurring in children[6].

Based on these, WHO have included a programme for control of diarrhea which involves the use of traditional herbal medicine and several plants including *Pterocarpus erinaceus* (*P. erinaceus*) have been reportedly used in the treatment and management of diarrhea[7].

P. erinaceus Poir belongs to the family Fabaceae and is a small to medium sized tree, 12-15 cm tall and 1.2-1.8 m in diameter. It is a perennial deciduous legume tree of African savannah and dry forests and is commonly called African Rose tree, African gum or African kino in English. In Nigeria, it is known as "Modobiya" or "Sha jini" in Northern Nigeria, "Apepe" or "Osun dudu" in Western Nigeria and "Oha Ofia" (wild variety of Oha) in Eastern Nigeria[8]. Some parts of the plant have shown medicinal properties. The stem bark extracts have demonstrated anti-microbial property, antioxidant, anti-inflammatory and analgesic activities. Also the stem bark has been shown to posses anti malarial and haemostatic activities[9,10].

The present study was designed to evaluate the anti diarrhea activity of *P. erinaceus* in experimentally-induced diarrhea.

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2. Materials and methods

2.1. Collection and identification of plant materials

Fresh leaves of *P. erinaceus* were collected from natural habitat in Amaeke- Afaranta, umuahia, Abia State in the month of June 2010 and identitified by Dr MC Dike of Forestry Department, College of Natural Resources and Environmental Management, Micheal Okpara University of Agriculture Umudike, Nigeria. A voucher specimen was deposited in the university herbarium.

2.2. Preparation of plant extract

The leaves were dried at room temperature and pulverized into a coarse powder of about 1 mm in diameter. Extraction was done by cold maceration in 80% methanol for 48 h with intermittent shaking every 2 h and later filtered with Whatman filter papers (NO 1) and the filtrate evaporated to dryness in an electric oven at 40℃. The obtained crude extract was stored in a refrigerator at 4℃ until time of use. The percentage yield of the extract was calculated using the formula below:

$$\% \text{ yield} = \frac{\text{Weight of the extract}}{\text{Weight of plant material}} \times 100\%$$

2.3. Experimental animals

Adult male Wistar albino mice weighing between 29-35 g were used for the experiments. The animals were procured from the laboratory animal unit of faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were allowed 7 days for acclimatization. The animals were kept in stainless steel cages and clean drinking water provided *ad libitum* while they were fed with standard commercial pelleted feed (Vital Feed® Nigeria). The temperatures varied between 28-30℃ and relative humidity of about 56%-60% with 12-hour light-dark cycle with adequate ventilation maintained in the animal house. The animals were handled in accordance with international principles guiding the use and handling of experimental animals[11].

2.4. Acute toxicity test

The method employed by George and Kapinga[12] was used for this study.

Twenty mice of both sexes were randomly divided into four groups (A-D) of five mice per group and were administered with 100, 500, 1 000 and 3 000 mg/kg of the extract orally by gastric gavage. The animals were given feed and water *ad libitum*. They were observed over a period of 48 hours for signs of toxicity and mortality.

2.5. Chemicals/Drugs

Methanol (Sigma-Aldrich), castor oil (Bell and Son, South Port), atopine sulphate(Nigbo Chemicals), activated charcoal and gum acasia (Rambaxy).

2.6. Effect of P. erinaceus on castor oil-induced diarrhea in mice

The method previously described by Mbagwu and Adeyemi[13] was used for the experiment. 25 adult mice were fasted for 18 h and were randomly divided into five groups of five mice each. Mice in the first group received 10 mL/kg distilled water, the second group received loperamide (5 mg/kg) while third, fourth and the fifth groups received 100 mg/kg, 200 mg/kg and 400 mg/kg of extract, respectively all by gastric gavage. After 1 h of administration of extract and drugs, castor oil (0.5 mL) was given to each of the mouse also by gastric gavage. Following the administration of castor oil, the animals were placed in separate cages containing transparent blotting papers for observation and the total number of faeces and the number of wet faeces passed were recorded 4 h after the administration of castor oil. The percentage diarrhea inhibition was calculated with wet faeces using the formula below.

% Inhibition = (control − test)/control × 100%.

2.7. Effect of P. erinaceus on gastrointestinal transit time

This was evaluated using the charcoal meal marker diet test[14].

Twenty five mice randomly divided into five groups (1-5) of five mice each, fasted for 12 hours but allowed free access to drinking water was treated as follows: The mice in group one received 10 mL/kg of distilled water. Group two received diphenoxylate (5 mg /kg) and those in groups three, four, and five received *P. erinaceus* extract, 100, 200 and 400 mg/kg, respectively, all by gastric gavage. Five minutes after drug administration, 0.5 mL of 10% charcoal suspension in 5% acacia gum was administered to each mouse by gastric gavage. All the mice were sacrificed by cervical dislocation 30 min later, the abdomen opened and the total length of the small intestine was measured with a calibrated ruler. The distance traveled by the charcoal plug from the pylorus to caecum was determined and expressed as a percentage of the total length of the small intestine from where the percent inhibition of movement was calculated by subtracting the percentage traveled from 100%.

2.8. Effect of P. erinaceus on castor oil-induced enteropooling

The effect of the extract on inhibition of intraluminal fluid accumulation was determined by measuring the volume of fluid accumulated in intestine of mice over time[15]. One hour before administration of castor oil (0.5 mL/mouse), 18 h fasted mice were divided into five groups of five mice per group and were given 10 mL/kg distilled water to group one, 3 mg/kg of atropine sulphate to group two and 100, 200 and 400 mg/kg of *P. erinaceus* extract to groups three, four and five, respectively. One hour after administration of castor oil, the mice were sacrificed by cervical dislocation, laparatomized, the pyloric and caecal ends of the small intestine were tied and the intestines were removed. The contents of each intestine were milked into a graduated test tube. The volume was recorded and percent inhibition of secretion was calculated.

2.9. Data analysis

The results were presented as mean±SEM and were analyzed using one way analysis of variance. The differences between the means were tested using post Hoc LSD and values of $P< 0.05$ were considered statistically significant.

3. Results

3.1. Extraction

The yield of the extract was 12.1% w/w dry matter and the acute toxicity test of the extract produced no death or signs of toxicity after 48 h.

3.2. The effect of P. erinaceus on castor oil-induced diarrhea.

The methanolic leaf extract of *P.erinaceus* and diphenoxylate significantly ($P<0.01$) inhibited diarrhea induced by castor oil. The extract reduced the wet faeces from 25.5±0.13 in the distilled water treated group to 17.5 ±0.11, 14.4±0.15 and 20.2±0.18 at the doses of 100, 200 and 400 mg/kg, respectively representing 31.4%, 43.5% and 20.8% inhibition of diarrhea. The highest activity was observed at the dose of 200 mg/kg (Table 1).

3.3. Effect of P. erinaceus on charcoal meal transit time

The extract in all the doses used caused a dose dependent inhibition of the charcoal plug reducing the distance travelled by 37.2% , 44.9% and 54.8% at the doses of 100, 200 and 400 mg/kg, respectively while diphenoxylate (5 mg/kg) caused 44.5% inhibition.

3.4. Effect of P. erinaceus on castor oil-induced enteropooling

Studies on the effect of *P. erinaceus* on castor oil-induced enteropooling showed that both the extract and the reference drug atropine (3 mg/kg) caused a significant ($P< 0.001$) and dose dependent decrease in intraluminal fluid accumulation in the mice when compared to the distilled water treated group. The extract at the dose of 400 mg/kg had a better effect than the reference drug reducing the volume of intestinal content from (0.39±0.03) mL in the negative control group to (0.19±0.01) mL which represents 51.3% reduction as against (0.23±0.02) mL (41.0%) by atropine (Figure 1).

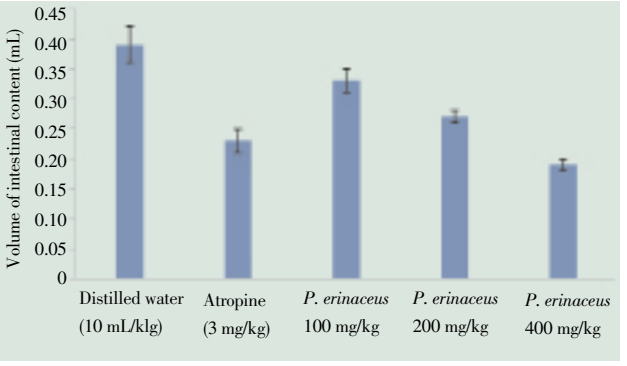

Figure 1. Effect of *P. erinaceus on* castor oil-induced enteropooling.

Table 1
Effect of *P. erinaceus* leaf extract on castor oil-induced diarrhea in mice.

Group	Treatment	Total no of faeces	No of wet faeces
A	Distilled water(10 mL/kg) + castor oil	30.50±0.16	25.50±0.13
B	Diphenoxylate(5 mg/kg)+castor oil	33.00±0.23	12 .00±0.07**
C	*P. erinaceus* (100 mg/kg)+ castor oil	22.00±0.20	17.50±0.11**
D	*P. erinaceus* (200 mg/kg)+ castor oil	21.80±0.19	14.40±0.15**
E	*P. erinaceus* (400 mg/kg)+ castor oil	32.60±1.15	20.20±0.18*

*$P<0.01$ ** $P < 0.001$ *vs*. group A.

4. Discussion

The effect of the methanolic leaf extract of *P. erinaceus* on experimentally induced diarrhea was evaluated using castor oil-induced diarrhea, charcoal meal transit time and castor oil induced enteropooling in mice. This is in recognition of the fact that in some diarrheas, the secretory component predominates while other diarrheas are characterized by hyper motility of the gastrointestinal tract[15].

Castor oil increases volume of intestinal content by prevention of the re- absorption of water and the liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa leading to release of prostaglandins which results in stimulation of motility and secretion and the prevention of re-absorption of NaCl and water[16]. This is characterized by an increase in the secretion of water and electrolytes, an increase in intestinal transit time and an increase in wet faeces[14].

The extract of *P. erinaceus* showed significant antidiarrheal activity just like the reference drug by significantly reducing the castor oil-induced diarrhea in mice. The highest effect was observed at the dose of 200 mg/kg of the extract which suggests that the antidiarrhoic activity revolves around this dose. The plant extract may have brought about this activity by stimulation of the re-absorption of water from the intestinal lumen or by anti prostaglandin activities that contribute to the patho-physiological functions in the gastro intestinal tract.

Administration of castor oil to experimental animals stimulated small intestinal transit as shown by the over 90% of the small intestine travelled by the charcoal plug in the distilled water treated mice. Oral administration of *P. erinaceus* brought about a dose dependent and significant

reduction in the percentage of the intestinal transit with the extract at the dose of 400 mg/kg being more effective than the reference drug and lower doses of the extract. This activity is probably due to the ability of the extract to inhibit intestinal motility. This reduction in percentage distance travelled can be used to establish the intestinal smooth muscle relaxation. The property of reducing intestinal contractions (and consequently, intestinal transit) is demonstrated by most antidiarrhoeal drugs[17] and this property was shown by *P. erinaceus* further demonstrating its antidiaarheal activity. This activity is probably due to the ability of the extract to inhibit intestinal motility. Reduction of intestinal transit time may possibly be due to its anticholinergic effect[18].

Intraluminal fluid accumulation was determined by castor oil-induced enteropooling. Castor-oil and its active principle, ricinoleic acid, induces changes in mucosal fluid and electrolyte transport that results in hypersecretory response and fluid accumulation in the intestine[19]. Also liberation of ricinoleic acid from castor oil leads to release of prostaglandins which stimulate motility and secretion[20]. The result of our study showed that the reference drug atropine (3 mg/kg) and the extract in all the doses used significantly (P<0.001) reduced the volume of intestinal fluid accumulation in mice in a dose dependent manner with the extract at 400 mg/kg having a better activity than the reference drug. The prevention of intraluminal fluid secretion by *P. erinaceus* in this study may be due to inhibition of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or may be due to promotion of absorption of water and electrolytes in the gut.

Phytochemical screening of the crude extract of *P. erinaceus* revealed the presence of tannins, flavonoids, triterpenes and steroids[12]. Tannins, flavonoids and other plant metabolites possess antidiarrheal activity in different experimental animal models and flavonoids also inhibit diarrhea induced by castor oil[21-23]. It is therefore possible that tannins and flavonoids content of the plant among others may be responsible for the antidiarrheal activity of *P. erinaceus*.

In conclusion, the results of this study have shown that *P. erinaceus* demonstrates significant antidiarrheal activity and may be working through anti secretory and anti motility mechanisms or through inhibition of prostaglandin activities and or synthesis.

Conflict of interest statement

We declare that we have no conflict of interest.

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