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ANTIDIARRHOEAL ACTIVITY OF *Pterocarpus santalinoides* LEAF EXTRACT IN MICE

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ABSTRACT
The antidiarrhoeal activity of the methanolic leaf extract of *Pterocarpus santalinoides* was evaluated in mice using different experimental models: castor oil – induced diarrhoea, gastrointestinal motility test and castor oil – induced enteropooling. The extract (200,400 and 800 mg/kg, p.o.) significantly ($p < 0.05$) reduced the number of wet faeces in the castor oil – induced diarrhoea, as well as inhibited the movement of charcoal meal plug through the gastrointestinal tract. Both actions were dose – dependent and comparable to those of Loperamide, used as the positive control. The extract, however had no effect on the intra luminal fluid content in the castor oil – induced gastric enteropooling. The extract did not demonstrate any acutely toxic effect in mice within the dose range used; hence it was well tolerated by the animals. The results point out the possible antidiarrhoeal effects of *P. santalinoides* and substantiate its use as a non – specific treatment for diarrhoea in folk medicine.

KEYWORDS: *Pterocarpus santalinoides*, diarrhoea, castor oil, Loperamide.

INTRODUCTION

Diarrhoea is the frequent passage of liquid faeces, a condition generally accompanied by abdominal cramps and sometimes nausea and vomiting (Rang *et al*, 2007). In Veterinary Medicine, diarrhoea is very common in neonates and occurs as a symptom or complication of most viral, bacterial, parasitic and nutritional diseases (Susan and Mays, 2005). In humans, diarrhoea has been recognised as one of the most important health problems in developing countries (Snyder Merson, 1982). In Nigeria, diarrhoea remains the number one killer among children aged 1-5 years and worldwide, the disease accounts for 4-5 million deaths among humans annually (Ezeigbo *et al*, 2010). The acute disease is characterised by progressive dehydration and death while in the sub acute form, diarrhoea may persist for several days resulting in malnutrition and emaciation (Susan and Mays, 1998).

Increased motility of the gastrointestinal tract and decreased absorption of fluid are major factors in diarrhoea, hence antidiarrhoeal drugs include antimotility agents, adsorbents and drugs that modify fluid and electrolyte transport (Harvey and Champe, 2009). Most of the existing antidiarrhoeal drugs such as: Diphenoxylate, Loperamide, Atropine etc exhibit side effects like: severe ileus ,xerostomia, urine retention, cycloplegia, tachycardia and CNS excitement (Rang *et al*, 2007). Perhaps owing to these adverse effects of the synthetic products, the World Health Organisation (WHO) in designing a programme for the control of diarrhoea included the use of herbal alternatives (Snyder and Merson, 1982).

Pterocarpus santalinoides belongs to the family *Fabaceae*. It grows 9-12m tall, with a trunk up to 1m in diameter and flanky bark. The leaves are pinnate 10-20cm long with 5-9 leaflets. The flowers are orange - yellow, produced in panicles. The fruit is a pod 3.5-6cm long, with a wind extending three-quarters around the margin. The plant is native to tropical West Africa and also to South America.

In South - East Nigerian folklore medicine, infusion of the leaves of *P. santalinoides* is used to control diarrhoea. This study is therefore designed to investigate the antidiarrhoeal activity of *P. santalinoides* using standard experimental models.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Pterocarpus santalinoides* were collected from Ugwu Nkpa in Bende Local Government Area of Abia State Nigeria. The Plant was identified by the Botany Department, Michael Okpara University of Agriculture, Umudike, Nigeria. A voucher specimen is deposited in our herbarium for further reference.

Preparation of Extract

The fresh leaves were dried under mild sunlight, then pulverised into coarse powder. Three hundred (300) grams of the sample were extracted in 96% methanol for 48 hours with intermittent shaking. Filtration was done using filter papers and funnel into an already weighed beaker. The solvent was allowed to evaporate in an electric oven at 40^0C. The percentage yield (w/w) of the extract was determined.

Animals

Swiss Albino mice (25 – 30 g) of both sexes were used. The animals were kept in stainless steel cages at room temperature and relative humidity of about 45 - 65% and fed *ad libitum* with standard commercial pelleted feed (Vital Feed, Nigeria). Clean drinking water was provided *ad libitum* and the animals were allowed 2 weeks for acclimatization before the experiments. Ethical rules guiding the use of laboratory animals for experiments according to Zimmerman (1983) were strictly followed.

Preliminary Phytochemical Analysis of the extract

The tests were carried out according to the procedures outlined by Harbourne (1991), Trease and Evans (1996).

Acute Toxicity Test

Twenty mice of both sexes weighing between 23 g and 36 g were randomly divided into 4 groups of 5 mice each. Group A served as control with each mouse receiving 10 ml/kg of distilled water. Groups B, C and D were treated orally with varying doses of the extract (500 mg/kg, 1000 mg/kg and 2000 mg/kg) respectively. The animals were allowed free access to feed and water for 48 h during which they were observed for signs of toxicity and death.

Castor oil-induced diarrhoea

The method of Biswas *et al* (2002) as modified by Ezekwesili *et al* (2004) was used. Mice fasted for 18 h were randomly divided into 5 groups of 6 mice each. Groups A, B and C were treated orally with 200 mg/kg, 400 mg/kg and 800 mg/kg respectively of the methanol extract of *P. santalinoides*. Group D received loperamide (5 mg/kg, p.o.) while group E received distilled water (10 mg/kg, p.o.) and served as the negative control. After 1 h, each animal was administered with 0.5 ml of castor oil and placed in a separate cage. Transparent blotting papers were placed beneath each cage and the animals were observed for a period of 4 h for the presence of wet faeces.

Gastrointestinal motility test

The effect of *P. santalinoides* on charcoal meal transit time was evaluated using the method of Mascola *et al* (1994), modified by Chidume *et al* (2001). Thirty mice were fasted for 18 h but allowed free access to clean water. They were randomly divided into 5 groups of 6 mice each. Groups A, B and C were treated orally with 200 mg/kg, 400 mg/kg and 800 mg/kg respectively of the methanol extract of *P. santalinoides*. Group D received the standard drug loperamide (5 mg/kg, p.o.) while group E received distilled water (10 mg/kg, p.o.). five minutes after drug administration, 0.5 ml charcoal meal (10% charcoal suspension in 5% acacia gum) was administered to each mouse by gastric intubation. Thirty minutes later all the animals were sacrificed by cervical dislocation and the gastrointestinal tract removed. The distance travelled by the charcoal meal from the pylorus was measured and expressed as a percentage of the total length of the small intestine, extending from the gastropyloric to the ileocecal junction. The percentage motility was derived from the equation:

$$\% \text{ Motility} = \frac{\text{Distance travelled by the meal}}{\text{Total length of the small intestine}} \times \frac{100}{1}$$

Castor oil – induced Enteropooling

Intraluminal fluid accumulation was determined by the method of Robert et al.,(1976). Thirty mice fasted for 18 h were randomly divided into 5 groups of 6 animals each. Group A served as control and received distilled water (10 ml/kg, p.o.) while groups B, C and D received 200, 400 and 800 mg/kg of the extract respectively, by gastric intubation, 1 h before oral administration of 0.5 ml castor oil. Group E received 3 mg/kg, i.p. of atropine (reference drug) 20 minutes before administration of castor oil. Two hours later the mice were sacrificed, the

small intestine was removed after tying the ends with thread. The content of each intestine was milked into a graduated tube and the volume recorded.

Statistical Analysis

The experimental results are presented as mean ± SEM (Standard error of the mean) and analysed using one way analysis of variance (ANOVA). The difference between the means were tested using the Post Hoc LSD and values of $p < 0.05$ were considered significant in the study.

RESULT

Extraction

Extraction of *P. santalinoides* with methanol gave a yield of 1.3% w/w dry matter. The extract was greenish brown in colour and had a sweet smell.

Preliminary Phytochemical Analysis

A preliminary phytochemical screening of the methanolic extract of *P.santalinoides* showed the presence of carbohydrates, alkaloids, resins, flavonoides, tannins, saponins and reducing sugar. Whereas flavonoids were present in small concentrations, alkaloids, saponins and resins were present in moderately high concentrations. Carbohydrates, reducing sugar and tannins were present in high concentrations, while glycosides, proteins fats and oil were absent (Table 1) .

Acute Toxicity

No death was recorded in all doses of the extract, hence the LD_{50} could not be determined. Also, the animals did not exhibit any acute toxicity sign within the 48h observation.

Effect of the extract on castor oil – induced diarrhoea

Thirty minutes after administration of castor oil diarrhoea was clinically apparent in all the animals of the negative control group and persisted throughout the 4h observation period. Diarrhoea in the test groups commenced about 45 minutes following castor oil administration. The extract at doses of 200, 400 and 800 mg/kg showed in a dose – dependent manner, significant ($p < 0.05$) reduction in the number of wet defecations over four hours when compared with the untreated control mice. The activity was similar to that of loperamide (5 mg/kg), the standard antidiarrhoeal agent (Table 2).

Effect of the extract on small intestinal transit

The result of *P. santlinoides* on small intestinal transit is presented in Table 3. The result showed that all the doses of the extract (200, 400 and 800 mg/kg) significantly decreased the propulsion of the charcoal meal through the gastrointestinal tract. The activity was dose - related and comparable to that of loperamide (5 mg/kg).

Effect of the extract on castor oil – induced enteropooling

Studies on the castor oil – induced enteropooling showed that the extract, at all doses did not decrease the intra-luminal fluid accumulation in the test animals, when compared with the negative control (Table 4).

Table 1: Phytochemical Screening of *P. santalinoides*

Test	Result
Carbohydrates	+++
Alkaloids	++
Reducing sugar	+++
Glycosides	-
Saponins	++
Tannins	+++
Flavonoids	+
Resins	++
Proteins	-
Fats and oil	-

+++ present in high concentration; ++ present in moderately high concentration; + present in low concentration; - absent.

Table 2: Effect of *P. santalinoides* extract on castor oil – induced diarrhoea in mice

Group	Treatment	Mean number of wet faeces
A	Extract (200 mg/kg)	5.67±0.33*
B	Extract (400 mg/kg)	4.33±0.33*
C	Extract (800 mg/kg)	3.50±0.34*
D	Loperamide (5 mg/kg)	1.33±0.21*
E	Distilled water (10 ml/kg)	6.67±0.42

Values are expressed as mean ± SEM; * $p < 0.05$

Table 3: Effect of *P. santalinoides* extract on small intestinal transit in mice

Group	Treatment	Length of small intestine (cm)	Dist. travelled by charcoal meal (cm)	% Motility
A	Extract (200 mg/kg)	46.1±1.96	27.2±1.33	60.3±2.90*
B	Extract (400 mg/kg)	42.9±0.76	25.2±1.93	58.7±4.81*
C	Extract (800 mg/kg)	45.3±1.48	25.7±0.70	56.8±2.30*
D	Loperamide (5 mg/kg)	44.6±1.00	18.1±1.99	41.0±5.50*
E	Distilled water (10ml/kg)	40.8±1.26	26.7±1.71	70.2±3.64

Values are expressed as mean ± SEM; * $p < 0.05$

Table 4: Effect of *P. santalinoides* extract on castor oil – induced enteropooling in mice

Group	Treatment	Volume of intestinal content (ml)
A	Distilled water (10 ml/kg)	0.15±0.009
B	Extract (200 mg/kg)	0.28±0.009
C	Extract (400 mg/kg)	0.25±0.008
D	Extract (800 mg/kg)	0.37±0.011
E	Atropine (3 mg/kg)	0.10±0.006*

Values are expressed as mean ± SEM; * $p < 0.05$

DISCUSSION AND CONCLUSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, resulting in an excessive loss of fluid in the faeces. In some diarrhoeas, the secretory component dominates while others are characterised by hypermotility (Harvagiray *et al*, 2004). The castor oil used to induce diarrhoea in the experiment is broken down in the small intestine to ricinoleic acid, which causes irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins which stimulate motility and secretion (Harvagira *et al*, 2004, Harvay *et al*, 2009). Loperamide, an analog of meperide have opioid – like actions on the gut, activating presynaptic opioid receptors in the enteric nervous system to inhibit acetylcholine release and decrease peristalsis (Rang *et* al, 2007). Loperamide and atropine also exhibit antisecretory actions in addition to their antimotility effect (Rang *et al*, 2007). These actions thus justify their use as standard antidiarrhoeal agents in the experiment.

The results of the present study shows that the methanolic extract of *P. santaniloides* produced a statistically significant reduction in the severity and frequency of diarrhoea produced by castor oil. It is also noted that the extract significantly reduced the intestinal propulsive movement in the charcoal meal treated model. Inhibition of the intestinal motility and reduction in the severity and frequency of wet defecation could be useful actions in the treatment of diarrhoea (Tavs *et al*, 2010). In these two experimental models, the extract showed activity similar to that of loperamide.

It should be observed that the extract, at the doses tested could not reduce the castor oil induced intestinal fluid accumulation (enteropooling). This may have resulted from the crude nature of the extract, which helped to increase the intestinal content in the animals within the test groups.

The purpose of an acute toxicity test is to determine the nature and the extent of the adverse reactions to a single dose or an overdose of the drug (Tavs *et al*, 2010). Incremental doses of the methanolic extract of *P.*

santalinoides up to 2 g/kg administered to mice *per os* were not lethal, so the LD_{50} could not be determined. This is an indication of the low toxicity of the extract, therefore P. santalinoides can be said to be relatively safe and may be used for the treatment of diarrhoea.

Previous reports have demonstrated the antidiarrhoeal activity of tannins, flavonoids, alkaloids, saponins, reducing sugars, steroids and/ or terpenes - containing plant extracts (Venkatesan, *et al*, 2005).A preliminary phytochemical analysis of the extract revealed the presence of alkaloids, saponins, tannins, reducing sugars and flavonoids. These constituents may be responsible for the antidiarrhoeal activity of the methanolic extract of *P. santalinoides*.

The results could indicate that the methanolic extract of *Ptericarpus santalinoides* possesses significant antidiarrhoeal activity due to its inhibitory effect on gastrointestinal propulsion. This justified the use of the plant as an antidiarrhoeal agent in folk medicine. Further investigations are underway to determine the extract's phytoconstituents which are responsible for the antidiarrhoeal activity.

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