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Evaluation of the Anti-Diarrhoeal Activity of the Aqueous Extract from Leaves Of *Pterocarpus santalinoides*

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ABSTRACT

The effect of the aqueous extract from leaves of *Pterocarpus santalinoides* on gastrointestinal tract was evaluated using the intestinal transit, castor oil-induced diarrhoea and intestinal fluid accumulation in mice and rats. The extract of *Pterocarpus santalinoides* (25mg/kg) significantly reduced the intestinal movement of charcoal meal in fasted mice. The extract (10-50mg/kg) also caused a significant ($p<0.05$) decrease in the frequency of defaecation and severity of diarrhoea and afforded protection from diarrhoea in castor oil-treated mice. The greatest effects of the extract on the tested parameters were achieved at a dose of 25mg/kg while the higher dose of extract gave a relatively lower effect. However, the extract at all the doses used, did not show any appreciable activity on the weight and volume of the intestinal content and did not significantly modify the concentration of electrolytes (Na^+ and K^+) in the intraluminal fluid. The results obtained indicate that the aqueous extract of *Pterocarpus santalinoides* possesses significant anti-diarrhoeal activity due to its effect on motility and experimentally induced diarrhea.

Keywords: *Pterocarpus santalinoides*. Intestinal transit. Diarrhoea. Castor oil.

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INTRODUCTION

Local herbalists have depended on medicinal plants as a reliable means of treating diarrhoea. In the past two decades, there has been a search for drugs that might inhibit the process of diarrhoea development especially the secretory process. Although a number of drugs have emerged, none has found a place in the routine management of diarrhoea [1]. Hence, the use of medicinal plants that possess antidiarrhoeal activities has been explored as one measure that could be of benefit in combating widespread diarrhoeal infections especially in third world countries [2]. Medicinal plants use has been welcomed in the treatment of diarrhoea mainly due to the presence of a variety of ingredients in each herb which broadens and enhances the anti-diarrhoeal effect and also creates a good platform for combating the diverse underlying factors which trigger off diarrhoea. In many traditional medicine settings, plant or herbs are claimed to have anti-diarrhoeal efficacy without any scientific backing [3].
Pterocarpus santalinoides L'Herit ex DC (Family: *Fabaceae-papilnoideae*) is a shade-tolerant tree 9-12m tall, with low straggling branches, commonly found along riverine forests in Africa and tropical South America. It is native to Brazil, Cameroon, Ghana, Nigeria and Senegal [4].

Various morphological parts of *P. santalinoides* are used in traditional medicine, in many African countries, to treat an array of human ailments. Locally known as nturukpa, the fresh leaves of *P. santalinoides* are consumed locally, in soups, by the Igbos of South East Nigeria and is reputed to be useful in the treatment of diarrhoea and other gastrointestinal disorders [5, 6]. Also, the fresh-leaf extract combined with leaves of *Solanum macrocarpum* is used in the management of high blood pressure. Similar uses are known among the Igede tribe in Benue State, Nigeria [7]. The bark of the plant plus the leaf is used in the management of sleeping sickness in Ivory Coast. It is also used as an anti-abortive agent and in the treatment of malaria and other infections such as *Staphylococcus aureus* and *Escherichia coli*.
Barring a few studies on the anti-trypanosomal and antimicrobial activities, no literature exists on the anti-diarrhoeal and other gastrointestinal effects of this plant, either in humans or animals.

Using experimentally-induced diarrhoeal models, the present study is aimed at evaluating the acclaimed anti-diarrhoeal effects of *Pterocarpus santalinoides* in an attempt to provide scientific justification for its folkloric medicinal use.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Pterocarpus santalinoides* were obtained from the forest around Amaokwe Item, Bende Local Government Area, Abia State, Nigeria. Botanical authentication was done by Mr. T.K Odewo of the Forestry Research Institute of Nigeria (FRIN), Ibadan where a voucher specimen (Number FHI. 106640) was deposited for future reference.

Extraction

The fresh leaves of *Pterocarpus santalinoides* (44g) were squeezed with distilled water (150ml). The resulting extract was filtered, decanted into glass beakers of known weights and oven dried at 40^{o}C. The dried extract (yield = 21%) was dissolved in distilled water to form a concentration of 100mg/ml before use.

Chemicals

The drugs and chemicals used were Castor oil (Bell, England), Gum acacia (BDH, England), Indomethacin (Sigma Chemical Co., USA), Loperamide hydrochloride (Xepa-Soul Pattinson, Malaysia), Ultracarbon® (Merck, Germany).

Animals

Sprague-Dawley rats (172±7.5g) and albino mice (25±-3g) of both sexes were obtained from the Nigeria Institute for Medical Research (NIMR), Yaba, Lagos and Laboratory Animal Center, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The animals maintained under standard laboratory conditions (temperature of 25±1.5°C, relative humidity of 35-78 %, and 12hr light /dark cycle) had access to standard chow (Bendel Feeds, Plc Ewu, Nigeria) and water *ad libitum*.

Animals were acclimatized for, at least, 2 weeks before use and fasted overnight with free access to water prior to experiments. Approval for the use of animals in the experiments was obtained from the Ethical Committee on the Use of Laboratory Animals, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Castor oil-induced diarrhoea

Groups of male and female mice (at least 5 per group) were administered orally, the extract (10-50mg/kg) and Loperamide (5 mg/kg), thirty minutes before the administration of castor oil (0.2ml per mouse) [8]. The control group was given distilled (10ml/kg). Each mouse was placed under a glass funnel, the floor of which was lined with a filter paper and observed for 4 hours. The parameters observed were; the onset of diarrhoeal stool (first wet stool that leaves a halo on the filter paper), number of wet stools, weight of wet stools and total weight of fecal output numerical score based on stool consistency was assigned as follows; Hard or normal stool = 1, mild or semi solid stool = 2 and copious or watery stool = 3.

Calculations were made for the delay in diarrhoeal onset and purging index by comparison with the control group. The *in vivo* anti-diarhhoeal index (ADI) was then expressed according to the formular [9].

$$ADI_{in\ vivo} = \sqrt[3]{D_{freq} \times G_{meq} \times Pfreq}$$

Where: D_{freq}- delay in defaecation time or anti-diarrhoeal onset (in % of control)
G_{meq} – gut meal travel reduction (in % of control)
P_{freq} - purging frequency, as number of stool reduction (in % of control).

Small intestinal transit

Male and female mice were allotted to groups of, at least, five animals each. Animals were treated orally with the extract (25mg/kg), Distilled water (10ml/kg) as control and loperamide (5mg/kg). Thirty minutes after treatment with the extract, control or standard drug, the mice were administered, intragastrically, (0.2ml per mouse) a freshly prepared standard charcoal meal (10% charcoal suspension in 5% gum acacia). All the animals in each treatment group were sacrificed 30minutes after the administration of the charcoal meal, and the small intestine immediately isolated. The distance traveled by the charcoal meal from the pylorus to the ileocaecal junction was measured and expressed as the percentage of the total length of the intestine (peristaltic index) [9].

Intestinal fluid accumulation and electrolyte secretion

Rats of both sexes were randomly allotted into different treatment groups. Group 1 received distilled water (10ml/kg), groups 2 and 3 received the extract 25mg/kg and 50mg/kg, respectively, while group 4 received indomethacin (10mg/kg). All administrations were by oral intubation.

One hour after the above treatments, rats received castor oil (2ml/rat) intragastrically [10]. The animals were sacrificed one hour later, the entire small intestine removed, after ligation at the pyloric end, and ileocaecal junction, respectively, and weighed. The intestinal content was expelled into a graduated tube and the volume measured. The intestine was reweighed and the difference between full and empty intestine was calculated.
The Na^+ and K^+ concentrations in the intraluminal fluid were analyzed using a flame photometer.

Statistical analysis

Results are presented as mean $\pm$ SEM and n represents the number of animals per group. Data comparisons were made using the Student's t-test or one way ANOVA with Tukey-Kramer post hoc test. Chi square test was employed where appropriate. Values were considered statistically significant at $p<0.05$.

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RESULTS

Castor oil-induced diarrhea

Table 1. Effect of *P. santalinoides* on castor oil-induced diarrhea.

Group	Dose (mg/kg)	Onset of diarrhoea (min)	No. of wet stool	Total no. of stools	Weight of wet stools (g)	Total weight of stools (g)	Diarrhea score[†]	Protection (%)
Control	-	100.56±9.23	8.22±1.34	9.00±1.22	0.44±0.05	0.44±0.05	25.22±3.05	-
P. santalinoides	10	166.33±15.98***[a]	4.33±1.05*[a]	4.50±1.06*	0.30±0.09	0.32±0.09	12.50±2.93[‡]	52.52
	25	175.71±30.62***[a]	3.00±1.48**[a]	3.14±1.48**[a]	0.24±0.12	0.25±0.11	8.29±3.89[‡]	68.51
	50	164.67±17.56***[a]	3.66±0.84**[a]	4.16±0.91*[a]	0.36±0.12	0.38±0.13	11.17±2.54[‡]	57.58
Loperamide	5	114.00±12.46*	7.33±1.11	7.50±1.06	0.49±0.09	0.50±0.09	21.33±3.01	18.99

Values are mean±SEM of at least 6 animals, *p<0.05, **p<0.01, ***p<0.001 compared with control values; [a]p<0.05 compared with Loperamide (ANOVA; Tuckey-Kramer post hoc test). [†]Diarrhoea score was analyzed by Chi square test, [‡]p<0.05 compared to control value.

Table 2. *In vivo* antidiarrhoeal index of *P. santalinoides*.

Group	Dose (mg/kg)	Delay in defaecation (time of onset, D_{freq})	Gut meal travel distance (G_{meq})	Purging frequency in no. of stools	In vivo antidiarrhoeal index (%)
P. santalinoides	25	53.89	33.21	65.11	48.02
Loperamide	5	9.60	37.22	16.67	18.15

All the mice in the control group produced copious diarrhea four hours after castor oil administration. The aqueous leaf extract of *Pterocarpus santalinoides* (10-50mg/kg) produced a significant ($p<0.05$) delay in onset of diarrhoea, frequency of stooling (reduction in number of wet stools and total number of stools), and the general diarrhoea score without any appreciable effect on the other parameters. These effects were produced in a non-dose dependent manner (Table 1). The greatest *in vivo* anti-diarrhoeal index was achieved at the dose of 25mg/kg body weight of the extract. This was much higher than that of the standard drug - loperamide (5mg/kg) (Table 2). In addition, since the protection (as measured by the diarrhoea score) by 25mg/kg body weight of the extract was higher than that obtained with the

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highest dose of the extract, this dose (of 25mg/kg) was selected as a standard for subsequent experimental models.

Intestinal transit

Table 3. Effect of *P. santalinoides* on small intestinal transit

Group	Dose (mg/kg)	Peristaltic Index (%)	Inhibition (%)
Control	-	78.75 ± 4.95	-
P. santalinoides	25	52.60 ± 7.91*	33.21
Loperamide	5	49.44 ± 6.90*	37.22

Values are mean ± SEM of 4-6 animals. *p<0.001 compared to control (ANOVA).

In control animals, the charcoal meal transversed 78.75 ± 4.95% of the total length of the small intestine. The aqueous extract (25mg/kg) produced a significant decrease in the propulsive movement of the charcoal meal through the small intestine comparable to the effect produced by the standard drug-loperamide (5mg/kg) (Table 3).

Intestinal fluid accumulation

Table 4. Effect of *P. santalinoides* on intestinal fluid accumulation.

Group	Dose (mg/kg)	Weight of intestinal content (g)	Volume of intestinal content (ml)	Na^+ Concentration (mM/L)	K^+ Concentration (mM/L)
Control	-	1.43±0.23	1.85±0.50	116.60±11.51	30.04±3.55
P. santalinoides	25	1.14±0.18	1.56±0.27	107.50±12.08	29.58±0.84
P. santalinoides	50	3.03±0.40	2.60±0.50	85.20±6.37*	31.28±2.41
Indomethacin	10	2.07±0.50	2.79±0.53	117.17±5.92	28.37±0.81

Values are mean ± SEM of 5 animals. *P<0.001 compared to control (ANOVA)

Oral administration of castor oil produced intestinal fluid volume and content weight of 1.85±0.50ml and 1.43±0.23g, respectively, in the control rats. The aqueous extract (25mg/kg) administered, orally, one hour before castor oil reduced both the intestinal fluid volume (1.56±0.27ml) and weight (1.14±0.18g) but these were not significantly different from the control (p>0.05)(Table 4). However, the secretions from animals pretreated with the extract or indomethacin were more viscous compared to the control. None of the treated groups produced any significant change in the sodium or potassium ion concentration in the luminal fluid relative to control.

DISCUSSION

The aqueous leaf extract of *Pterocarpus santalinoides* produced a significant decrease (p<0.001) in the propulsive movement of the standard charcoal meal in the small intestine suggesting an anti-spasmodic activity.

The extract produced a significant ($p<0.05$) but non dose-dependent reduction in frequency and severity of diarrhoea produced by castor oil. There was delay in the onset of diarrhoea with protection of 52.52%, 68.51% and 57.58%, of the animals, at doses of 10, 25 and 50mg/kg, respectively. The number of wet stools and total number of stools were also decreased in a non dose-dependent manner with the highest effect observed at 25mg/kg dose level. The effect of the extract on the aforementioned parameters were significantly greater than those produced by loperamide (5mg/kg) hence the general diarrhoeal score of the extract (25mg/kg) was 68.51% compared to loperamide (5mg/kg, 18.99%).

Drugs affecting motility and frequency/consistency of diarrhoea also affect secretion [11]. Castor oil, upon enzymatic action, releases ricinoleic acid and its stereoisomer, which induces changes in mucosal fluid and electrolytic transport that result in a hypersecretory response and diarrhoea [12, 13]. The aqueous extract (25mg/kg) inhibited the castor oil induced intestinal fluid accumulation (enteropooling) and reduced the weight of intestinal content, but this was not significantly different from control. These parameters were increased (higher than the control) at the dose of 50mg/kg of extract and indomethacin. We can not, at this time, adduce any reasons for this seeming discrepancy, especially with indomethacin which is known to decrease fluid accumulation. However, the intestinal content in the indomethacin- and extract-treated rats was more viscous than the control with the highest viscosity observed with the former. The increased viscosity of the intestinal content of the extract-treated animals may be due to the effect of the extract on intestinal transit (slowing the intestinal motility). This, however, may not hold true for indomethacin since it is not known to inhibit intestinal motility.

Diarrhoea reflects a disruption in the bi-directional transport of Na^+ and Cl^- ions through intestinal epithelial cells [14] and castor oil inhibits the enzyme sodium-potassium ATPase [15]. Clinically, diarrhoea may result from disturbed bowel function, presenting as impaired intestinal absorption, excessive intestinal secretion of water and electrolyte and a rapid bowel transit [3]. The Na^+ concentration was not significantly decreased in the intraluminal fluid of the rats treated with the extract (25mg/kg) compared to the control. This suggests a lack of effect of the extract on the electrolyte secretory property of castor oil on intestinal mucosa.

The anti-diarrhoeal index (ADI) is a measure of the combined effects of the different components of diarrhoea such as purging frequency, onset of diarrhoeal stools and intestinal motility. The extract showed a much higher anti-diarrhoeal index than loperamide (5mg/kg).
The inhibitory effect of the aqueous extract of *Pterocarpus santalinoides* on gastrointestinal functions such as gastrointestinal propulsion, castor oil-induced diarrhoea and fluid accumulation shows that it may possess a high capacity in decreasing propulsive gut motility and secretions. This may be the basis for its use in traditional medicine. However, it is difficult to clarify the precise mechanism(s) of action based on this study. Further studies will have to be conducted in this regard.

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