Nigerian Journal of Pharmaceutical Research
Vol. 8 No 1 pp. 138 - 144 (September 2010)

ANTI-DIARRHOEAL AND ANTISPASMODIC EFFECTS OF LEAF EXTRACT OF *PTEROCARPUS SANTALINOIDES*

***T. C. Okoye[1], P. A. Akah[1], C. O. Okoli[1], O. O. Ndu[1], A. C. Ezike[1], M. O. Okoye[2], F. N. Mbaoji[1] and E. U. Agba[1]**

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka

[2]Department of Clinical Pharmacy and Pharmacy Management; Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.

E-mail: theokuba@yahoo.com.,Tel.: +234 803 668 4506

Abstract

Diarrhoea is one of the popular gastrointestinal disorders with high mortality especially in children. Certain medicinal plants are being used in traditional medicine practice to treat and control diarrhoea. One of such plants is the leaves of *Pterocarpus santalinoides.* The need to scientifically ascertain this folkloric claim and the possible identification of the active constituents therein motivated this study. The methanol leaf extract of *P. santalinoides* was prepared and screened for activity using castor-oil induced diarrhoea, gastrointestinal motility tests (charcoal transit test) and inhibition of contraction induced by histamine (H) and acetylcholine (Ach) on isolated rabbit jejunum. The results indicated that the extract at 200 mg/kg dose exhibited a significant inhibition ($P < 0.05$) of castor-oil induced diarrhoea when compared with a standard antidiarrhoeal agent, diphenoxylate hydrochloride (5 mg/kg). The extract also at 200 mg/kg dose level showed a significant inhibition ($P<0.05$) on gastrointestinal motility when compared with the vehicle (3 % Tween 80). The extract also exhibited a dose dependent inhibition on the contraction induced by acetylcholine and histamine on rabbit jejunum as well as the abolition of spontaneous pendular movements of the smooth muscles of the rabbit jejunum. The acute toxicity study exhibited an LD_{50} greater than 5000 mg/kg. The phytochemical screening revealed the presence of carbohydrates, resins, terpenoids, steroids, saponins. The extract exhibited both antidiarrhoeal and antispasmodic effects which could be through inhibition of histaminergic and cholinergic mechanisms via their respective receptors abundant in the GIT. The antidiarrhoeal effect of this plant can be explored for therapeutic advantages as an alternative for treatment of diarrhoea and further research is expected to confirm the active phytochemical constituent(s) responsible for the activity.

Key words: *P. santalinoides*, antidiarrhoeal, antispasmodic and rabbit jejunum

INTRODUCTION

Diarrhoea is a leading cause of childhood mortality accounting for an estimated 4 million deaths annually of children below the age of 5 years especially in developing countries (Fauci *et al*., 1998). High mortality rate of diarrhoea has made it necessary for adequate measures to be put in place with respect to the disease control, prevention and treatment of dehydration. Proper rehydration judicious use of antibiotics for cholera and dysentery could reduce the disease fatality (Kosec *et al*., 2003). Each episode of diarrhoea contributes to under nutrition and when episodes are prolonged their impact on growth is increased (Fauci et al., 1998). Diarrhoea is classified into four types depending on the severity, duration of occurrence and presentation as acute diarrhoea (which begins quickly and becomes severe and of short duration, less than 14 days); persistent diarrhoea

*Corresponding author
ISSN 0189-8434

(diarrhoea of long duration at least 14 days); chronic diarrhoea (recurrent diarrhoea, that is long-lasting); and dysentery with visible blood in the faeces with sudden onset of explosive water stool along with abdominal cramps (Wilson, 2005). Also abuse of laxative may cause chronic diarrhoea which may lead to serious electrolyte imbalance, steatorrhoea, protein loss and weight loss (Cummings *et al.*, 1979) with visible blood in the faeces. A lot of microorganisms like protozoa and viruses have been implicated in many diarrhoea cases and these include *Giardia lamblia, E. coli, Shigella spp, Salmonella Campylobacter* and *Staphylococcus species* (Dupont, 1971; Wilson, 2005). Also viruses include retroviruses have been implicated in some cases of diarrhoea (Wilson, 2005). Clinical features of diarrhoea include vomiting, abdominal cramp and pain, passage of watery stools, and low-grade fever (Thorn, 1998). Management of diarrhoea include oral rehydration therapy (Murphy, 1998), dietary management (Garrin *et al*., 1996) and therapeutic management (Janssen *et al*., 1959; Kachel *et al.*, 1986; Karim and Adaikan, 1977). Equally herbal preparations are used in the management of diarrhoea in developing countries of Africa such as Nigeria. One of such plants employed in the management of diarrhoea is the decoction from the leaves of *Pterocarpus santalinoides* (Fabaceae) in south eastern Nigeria (Igoli *et al*., 2005: Nworu *et al*., 2009). *P. santalinoides* is popularly called Uturukpa by the Ibo speaking tribe of south eastern Nigeria. *P. santalinoides* has also been reported for its antispasmodic and digestive activities (Quarshi, 1985). Therefore, the aim of this study is to verify the ethnomedicinal claim of *P. santalinoides* and to ascertain the active phytochemical (s) responsible for the effects.

MATERIALS AND METHODS

Reagents: Methanol (Fluka), Tween 80, Diphenoxylate hydrochloride, Atropine, Castor oil, chloroform, and ethanol solution.

Collection and preparation of plant material

The fresh leaves of *P. santalinoides* were collected in the month of May, 2008 from Ideato South Local Government Area of Imo State. The botanical identification was done by Mr. Anthony Ozioko, of Bioresources Development and Conservation Programme centre, Nsukka Enugu state. The leaves were air-dried at laboratory temperature (30-37^{0}C) and then grounded to powder. The powdered material (257 g) was extracted with methanol (Analar grade) by a 48 h cold maceration with intermittent shaking and filtered afterwards. The filtrate was evaporated using rotary evaporator at reduced pressure to obtain the methanol extract (ME) which weighed 11.9 g. The methanol extract was subjected to phytochemical screening using standard procedures (Trease and Evans, 1989). Also acute toxicity studies were carried out using Lock's method (1983).

Animals

Adult albino mice (18-19 g) and rabbit (450 g) of both sexes obtained from the animal unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The animals were housed under standard conditions. The mice were maintained on growers mash (Altran International Ltd® Nsukka, Nigeria) while the rabbit was fed with conventional rabbit grass. The animals were allowed free access to drinking water. All animal experiments were conducted in compliance with the National Institute of Health Guidelines for Care and Use

of Laboratory Animals (Pub No. 85 – 23, revised 1985) and approval of the University of Nigeria Ethical Committee on the use of laboratory animals.

Castor-oil-induced diarrhoea in mice

A modified method of Awounters *et al.,* (1978) was used in the experiment. Mice were fasted for 18 hours prior to the experiment and were randomly divided into 5 groups (n=5). The first group received 0.2 ml/kg of the vehicle (10% Tween 80 p.o.). The second group received a suspension of standard antidiarrhoeal agent, diphenoxylate hydrochloride (5 mg/kg, p.o.). The last three groups were treated with *P. santalinoides* extract (100, 200 and 400 mg/kg, p.o.). Thirty minutes after treatment with extract, castor oil (1.0 ml/kg, p.o.) was administered to each mouse and thereafter the mice were separated into single cages for observation for defecation up to 4^{th} hour, on a white filter paper placed beneath the cages. The antidiarrhoeal effect of the test drug, the standard agent and the control were calculated based on the frequency of defecation when compared to untreated mice.

Gastrointestinal motility tests (Charcoal Transit Test)

Adult albino mice (17-19g) of both sexes were randomly divided into five groups (n = 5) and were fasted for 18 h prior to the experiment. The first group (control group) received 0.2 ml/kg, (p.o.) of 10% Tween 80, while the second group received atropine sulphate (0.1 mg/kg, p.o.) as standard treatment group. The last three groups received (100, 200 and 400 mg/kg, p.o.) extract of *P. santalinoides* respectively. Twenty minutes later, 0.5ml of a 5% activated charcoal suspension in 10% aqueous slurry were administered to all the animals. The mice were sacrificed 30 minutes later, dissected and the small intestine removed and straightened on a dissecting board. The distance travelled by the charcoal meal was measured from the pylorus to the caecum and expressed as a percentage of the total length of the intestine (Akah *et al.*, 2007).

Antispasmodic effect on isolated rabbit jejunum

A modified method described by Mukherjee (2007) was adopted for the antispasmodic effect. Segment of the jejunum (3 cm piece) isolated from freshly sacrificed rabbit was suspended in 50 ml organ bath containing Tyrode solution at 37^0C aerated with oxygen. The tissue was allowed to acclimatize for 30 minutes under resting tension of 0.5 g before exposure to drugs and extract. During this equilibration period, the tissue was washed with fresh Tyrode solution every 10 minutes to prevent the accumulation of metabolic end products. Maximal responses were obtained for Acetylcholine (Ach) and Histamine (H) from the graded responses. The various doses for both Ach and H that evoked the maximal contraction were then recorded. Increasing concentrations in microgram of the ME was administered together with the respective doses of ACH and H that evoke the maximal response on the rabbit jejunum to obtain inhibitory effects on the maximal contractions they exhibited. This was repeated three times and the mean as well as the percentage inhibition calculated. Abolition of the intrinsic pendular movement of the jejunum was also assessed.

Statistical analysis

The statistical analysis was done using SPSS Analysis of Variance (ANOVA) version 16 and post hoc was Dunnet for multiple comparisons with the

control. $P < 0.05$ is regarded as significant.

RESULTS

Yield of extract

The extraction yield was 4.63 % w/w of ME from the starting material.

Acute toxicity test

Acute toxicity and lethality studies indicated an LD_{50} greater than 5000 mg/kg. This implied that the extract of *P. santalinoides* is relatively safe without potentials of causing obvious acute toxic reactions in man and animals.

Phytochemical studies

Preliminary phytochemical tests revealed the presence of resins, terpenoids, steroids, saponins, glycosides and carbohydrates phytochemicals whereas flavonoids, tannins, alkaloids, reducing sugars and acidic compounds were absent (Table 1).

Effect on castor oil induced diarrhoea

The ME at the doses tested showed decrease in the frequency of defecation. At 200 mg/ kg dose the effect was significant ($P < 0.05$) compared to the control (Table 2).

TABLE 1: Phytochemical constituents of *P. santalinoides* extract

Constituent	ME
Carbohydrates	+
Alkaloids	-
Reducing sugars	-
Glycosides	+
Saponins	+
Tannins	-
Flavonoids	-
Resins	+
Fats and oils	-
Steroids	+
Terpenoids	+
Acidic compounds	-

Treatment	Dose (mg/kg)	Frequency of Defecation
Control	-	11.33 ± 0.33
Diphenoxylate	5	3.33 ± 0.33*
ME	100	7.25 ± 0.85
ME	200	6.50 ± 0.87*
ME	400	7.50 ± 0.33

+ = present; - = absent

TABLE 2: Effect on castor oil induced diarrhoea

Figures are in Mean ± SEM, *Significant $P < 0.05$, ANOVA compared with the control; n = 5;

Effects on gastrointestinal motility

The ME significantly ($P < 0.05$) exhibited dose – dependent inhibition on gastrointestinal motility when

TABLE 3: Effects of the ME on the percentage inhibition of intestinal movement

Treatment	Dose (mg/kg)	% inhibition of charcoal meal
control	-	66.00 ± 4.57
Atropine	0.1	47.12 ± 7.27*
ME	200	43.67 ± 6.22*
ME	400	54.34 ± 5.00

Figures are in Mean ± SEM; *Significant $P < 0.05$, ANOVA compared with the control; n = 5;

compared with the control. The percentage inhibition of intestinal motility obtained at 200 mg/kg dose was comparable to that of the effect of

atropine (Table 3).

Antispasmodic effect

The results showed that the ME did not evoke contraction of the rabbit jejunum at concentrations tested, but exhibited dose dependent inhibition of contraction evoked by acetylcholine and histamine on isolated rabbit jejunum. At 160 µg/ml concentration the ME gave 100% and 62.5% attenuation of response exhibited by H and Ach respectively indicating a more potent activity at the histaminergic receptors than cholinergic receptors. The ME also exhibited a reasonable attenuation of the inherent pendular movement of the jejunum (Fig. 1).

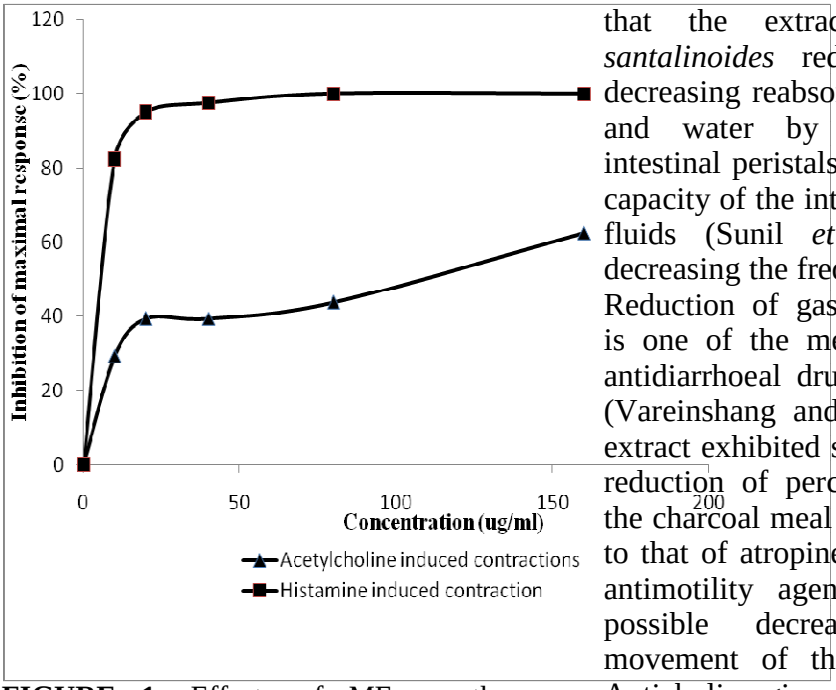

FIGURE 1: Effects of ME on the Acetylcholine and Histamine induced concentrations on rabbit jejunum

DISCUSSION

The methanol extract of *P. santalinoides* exhibited antidiarrhoeal and antispasmodic effects from the experimental models investigated. The ME exhibited a significant inhibition of castor oil induced diarrhoea. Castor oil contains ricinoleic acid which is responsible for its purgative effects through irritation and inflammation of the gastro intestinal tract (GIT) leading to change in the electrolyte transport, increased peristaltic activity and diarrhoea (Awouter *et al*, 1978; Evans, 2009). This effect was however, inhibited by the extract at the doses tested. This is an indication that the mechanism of the activity of ME might have been due to the inhibition of prostglandin biosynthesis and decrease in the reabsorption of electrolytes and water in the GIT (Awouter *et al*, 1978; Nworu *et al.*, 2009). The antidiarrhoeal effect of ME was comparable to that of diphenoxylate an opioid antimotility and antisecretory agent (Jafri and Pasricha, 2001) used as a positive control in the study. This also suggest that the extract of *Pterocarpus santalinoides* reduced diarrhoea by decreasing reabsorption of electrolytes and water by inhibiting induced intestinal peristalsis and increasing the capacity of the intestines to retain their fluids (Sunil *et al.*, 2001) hence decreasing the frequency of defecation. Reduction of gastrointestinal motility is one of the mechanisms by which antidiarrhoeal drugs elicit their effect (Vareinshang and Yadar, 2004). The extract exhibited significant ($P < 0.05$) reduction of percentage inhibition of the charcoal meal which is comparable to that of atropine sulphate a standard antimotility agent. This suggests a possible decrease in peristaltic movement of the gut by the ME. Anticholinergics such as atropine possess good antispasmodic effects by the blockade of cholinoceptors in the smooth muscles of the gut causing decreased GIT motility, prolonged gastric emptying time and relaxation of the smooth muscles of GIT wall (Katzung, 2004). Since abdominal cramp and pain is one of the clinical

symptoms of diarrhoea (Thorn, 1998) a good antidiarrhoea agent should as a matter of pharmacological importance possess a significant antispasmodic effect. Inhibition by the ME of smooth muscles contractions of the rabbit jejunum induced by the spasmogens Ach and H as well as the abolition of the spontaneous pendular movement of the jejunum are indications of its antispasmodic activity. The extract did not contract the jejunum confirming its antagonistic effects on the gut. The extract dose dependently inhibited the contractions induced by Ach and H. Histamine and acetylcholine are important endogenous spasmogens and agents that inhibit their contractions may have a good antispasmodic potential. There is an indication of a non-specific antagonism by the ME. This antagonism could either be through the receptor site such as the muscarinic and histaminic receptors or through other musculotropic route such as influx or outflux of calcium ions (Okoye *et al*., 2010). Phytochemical tests showed the presence of glycosides, saponins, terpenoids, and steroids which may be attributable to the antidiarrhoea and antispasmodic effects of the *P. santalinoides* extract though none of these phytochemicals could be confirmed at this level of the study.

CONCLUSION

The leaf extract of *Pterocarpus santalinoides* possess anti-diarrhoeal and antispasmodic effects which could be through the non specific inhibition of the histaminergic and cholinergic mechanisms. There is the need to study the anti ulcer effects as agents with anticholinergic as well as antispasmodic effects possess potent anti ulcer effects**.**

REFERENCES

Akah PA, Okoli CO and Ndu OO. (2007). Experimental Methods in Physiology and Pharmacology. ABIC Publishers, Enugu, Nigeria. Pp: 147.

Awouter F, Niemergeer CJE, Lenaerls FM, Janssen PAJ. (1978). Delay of castor-oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis *J. Pharm. Pharmacol*. *30*:41-45.

Cummings JH, Mullan NA, Barr M. (1979). The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. *British Journal of Nutrition, 41*:447.

Dupont HL. (1971). Pathogenesis of *E. coli* diarrhoea. New Eng. J. of Med. 285: 1-9.

Evans WC. (2009). Trease and Evans Pharmacognosy, 16th Edn. Saunders Elsevier, New York. Pp: 190-191.

Fauci, AS, Bravnwold, E and Wilson, ID. (1998). *Harrison's Principles of Internal Medicine*. New York. McGraw Hill Company pp.236-242.

Garrin N, Merrik N and Davidson B. (1996). Efficacy of glucose based oral rehydration therapy. Paediatrics, 98(1): 45-51.

Igoli JO, Ogaji OG, Tor-Anyim, Igoli NP (2005). Traditional medicine practice amongst the Igede people of Nigeria, Part II. Afr. J. Trad. CAM 2(2): 134-152.

Janssen PA, Powell DW and Lichstein J. (1959). Synthetic antidiarrhoeal agents: some pharmacological properties of R-1132 and related compounds. Journals of Medicinal and Pharmaceutical Chemistry, 1:299.

Kachel G, Beubler E and Lembeck F. (1986). Human intestinal motor activity and transport; Effects of a synthetic opiate. Gastroenterology, 90:85.

Karim SMM and Adaikan PG. (1977). The effect of loperamide on prostaglandin induced diarrhoea in childhood: Results of double blind, placebo controlled clinical trail. Annals of Tropical Paediatrics, 7:122.

Katzung BG. (2004). Basic and Clinical Pharmacology. 9th Edn., Mc Graw Hill, Boston. Pp : 114-115.

Kosec M, Bern C, Guerrant RL (2003). The magnitude of the global burden of diarrhoeal disease from studies published 1991-2000. Bull. World Health Organisation. 81: 197-204.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol., 53*:275-287.

Mukherjee PK. (2007). Quality Control of Herbal Drugs. (An Approach to Evaluation of Botanicals). Business Horizon Publishers, New Delhi, India. Pp: 570-575.

Murphy MS. (1998). Guidelines for Managing acute gastroenteritis based on a systemic review of published research. Archives of diseases in childhood, 79:279-284.

Nworu CS, Akah PA, Nwachukwu JO, Asogwa C. (2009) Antidiarrhoeal activity of *Perocarpus santalinoides* L'Herit ex DC leaf extract. Journal of Complementary and Integrative Medicine, 6(1): 1-9.

Okoye TC, Akah PA, Okoli CO, Ezike AC, Mbaoji FN (2010) Antimicrobial and antispasmodic activity of leaf extract and fractions of *Stachytarpheta cayennensis*. Asian Pacific J. of Trop. Med. 3(3): 189-192.

Quarshi MH (1985). *Tibbi Pharmacopia*. Lahore, Pakistan, Kareemi Press. 1:33.

Sunil B, Bedi K, Singla A, Johri R. (2001). Antidiarrhoeal activity of piperine in mice. Planta medica, 67:284-287.

Thorn GW. (1998). Harrison's Principles of Internal Medicine, 8th edn. McGraw-Hill, New York. Pp 210-214.

Trease GE, Evans WC. Text book of Pharmacognosy. 15th ed. London: Bailliere Tindall; 1989: 315-679.

Vareinshang, T. and Yadar, A.K. (2004). Antidiarrhoeal activity of *Rhus javenica* ripened fruit extract in albino mice. Fitoterapia, 75:39-44.

Wilson ME. (2005). Diarrhoea in nontravellers: risk and etiology. Clin. Infect. Dis. 41 suppl. 8: S 581-6.