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Antidiarrhoeal Effects of Ethanol Seed Extract of *Picralima nitida* in Rodents

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ABSTRACT
Picralima nitida Stapf (Apocynaceae) seed is one of the traditional remedies of the Ibibios of AkwaIbom state of Nigeria in the management of traveler's diarrhoea. This study was undertaken to evaluate the antidiarrhoeal activities of ethanol seed extract of P. nitida. The extacts (90 – 270mg/kg) were tested on wistar albino rats and Swiss mice using castor oil – induced diarrhoeal model, small intestinal propulsion model and castor oil – induced fluid accumulation experimental model. Result showed that the seed extract inhibited diarrhoea significantly (p< 0.05) in all the tested experimental models in a dose - related manner (90 – 270mg/kg) relative to control and comparable to prototype drugs used in the study. The ability of the ethanol seed extract to inhibit diarrhoea in these models supports its folkloric use in AkwaIbom State ethnomedicine.
Keywords: *Antidiarrhoeal, Picralimanitida, castor oil*

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INTRODUCTION

Globally, diarrhoea remains one of the commonest diseases of children and one of the major causes of death in infants in developing world. An estimated annual death of about 3.3million has been reported amongst children under 5 years.*Picralimanitida*(Apocynacece) is a tree or shrub of 4 – 35m high that is widely distributed in Dahomy, Ghana, Ivory Coast, Nigeria, Sierra Leone, South of Congo and Angola [1]. There are about eight species in the *Picralima*genusbut amongst them, *Picramila nitida* have been widely embraced for its numerous medicinal uses. Throughout its distribution area, the seeds, bark and roots have a reputation as febrifuge and remedy for malaria, pain disorders and pneumonia. The seeds and bark are usuallycrushed or chewed for these purposes but the roots are prepared by decoction [2].
In Nigeria, a decoction of the bark is administered orally against measles while crushed seeds are applied to abscecces. The seeds, roots or fruit pulp are also ingredient for arrow poison. The seeds are distributed around human habitats to scare away snakes [1]. Many compounds have been isolated from the matured seed butakuammidine, a principal alkaloid displayed a strong sympathomimetic and local analgesic activities [3]. The ethanolic seed extract of *P. nitida* exhibited in *vivo*antiplasmodial activity in mice which correlated earlier *invitro* report [4]. The seed extract also studied for hypoglycaemic activity did not reduce blood glucose in rats rather the extract increased blood glucose concentration in the sub acute groups of rats in the study [5].
In AkwaIbom State ethnomedicine, the matured seeds are eaten by travelers while on transit to control diarrhoea. This research is aimed at providing scientific basis for the use of the seeds as anti-diarrhoeal agent.

MATERIALS AND METHODS

Plant Collection and Extraction

Ripe fruits of *P. nitida* were collected from Ubulu, a town in Orlu West Local Government Area of Imo State and identified at the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo and a voucher specimen deposited.

Preparation of Seed Extract

The ripe fruits were cut opened to reveal the seeds. The seeds were removed from the pulp, washed with water and sun – dried. After 24 hours, the seed-coats were removed and the seeds air dried for another two weeks. These seeds were pulverized and 300g of the crushed seed extracted using 50% ethanol for 72 hours after which the mixture was filtered to obtain the ethanol extract. The ethanol extract was evaporated *in vacuo* using a rotary evaporator and then dried in an oven at 40^{o}C. The dried extract was stored in a refrigerator at -4^{o}C from where it was used for the experiments.

Animal Stock

Adult albinoWistar rats (151 – 195g) and adult Swiss mice (25-39g) of both sexes were obtained from the Animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria and they were handled with humane care in accordance with internationally accepted guide for care and use of laboratory animals as adopted by the Ethics Regulation Commitee of the Faculty of Pharmacy, University of Uyo, Uyo, AkwaIbom State, Nigeria.

Acute Toxicity Study

According to the method used, mice were randomized and divided into groups of three mice per group and fasted for 24 hours prior to the experiment [6]. The extract was administered intraperitoneally in a dose range of 600 – 2000 mg/kg body weights.

Small Intestinal Propulsion

The effect of the ethanol extract of *P. nitida* was tested using charcoal meal [7, 8]. Twenty five adult mice were randomized and allotted into five groups of five animals per group. Group one animals received distilled water (10ml/kg), groups 2-4 animals received the aqueous extracts of *P. nitida* at doses 90mg/kg, 180mg/kg and 270mg/kg respectively, while animals in group five received atropine at 0.5mg/kg. Twenty minutes after the pretreatment of the animals, each mouse was administered with 0.2ml standard charcoal meal (10% activated charcoal suspended in 5% gum acacia).

The mice were sacrificed after 30 minutes by cervical dislocation and the small intestine rapidly opened and placed on a clean surface. The distance travelled by the charcoal meal from the pylorus was measured and expressed in percentage (%).

Castor Oil - Induced Diarrhoea in Mice

Induction of diarrhoea was carried out by the oral administration of castor oil at a dose of 0.2ml/kg [9, 10]. The animals were randomized into five groups of five animals per group. Group 1 (control) received distilled water (10ml/kg), groups 2-4 received the aqueous extracts of *P. nitida* at doses 90mg/kg, 180mg/kg and 270mg/kg respectively, while animals in group five received atropine at 0.5mg/kg. After 1hour, each mouse received 2ml of castor oil and was then observed for consistency of faecal matter and frequency of defecation for 4hr.

Castor Oil - Induced Intestinal Fluid Accumulation

Twenty five adult albino rats were randomly allotted into five (5) groups of five (5) rats each [7]. The control group (group 1) received distilled water (10ml/kg). Rats in groups 2 to 4 were pretreated with the aqueous extract of *P. nitida* at doses of 90mg/kg, 180mg/kg and 270mg/kg and rats in group five received atropine (10mg/kg). All the treatments were done orally except for group 5 animals that were pretreated subcutaneously.

Thirty (30) minutes later, the rats were sacrificed by cervical dislocation and exsanguinated, the small intestine was ligated at both the pyloric and the ileocaeccal junctions and weight taken. The intestinal contents were measured using measuring cylinder. The difference between the full and empty intestines was determined.

Statistical Analysis

The data obtained were subjected to ANOVA followed by Turkey-kramer multiple comparison test and expressed as mean $\pm$SEM and a probability level ($P< 0.05- 0.01$) considered significant.

RESULTS

Acute Toxicity

The extract was seen to produce signs of toxicity such as decreased motor activity, decreased respiratory rate, decreased body and limb tone with eventual death. The intensity of these signs were directly related to the various doses of the extract administered. The LD_{50} calculated as the geometrical mean of the minimal dose (1300mg/kg) that killed all the animals and the maximal dose (600mg/kg) that killed none of the animals was 883mg/kg and dosages used for the experiments were extrapolated as 0.1%, 0.2% and 0.3% of the LD_{50}.

Castor Oil - Induced Diarrohea

Table 1 shows the effect of ethanol fruit extract of *Picralima nitida* on castor - oil induced diarrhea. Administration of *P.nitida* fruit extract (90, 180 and 270mg/kg) significantly (P<0.05) reduced the castor - oil induced diarrhoea in a dose-dependent manner when compared to control. The effect at doses 180mg/kg and 270mg/kg of the extract shows increase activity of inhibition of diarrhoea more than the standard drug atropine.

Castor - Oil-Induced Intestinal Propulsion

The effect of castor oil- induced intestinal propulsion is as shown in table 2.The fruit extract of P. nitida (90 – 270mg/kg)inhibited intestinal propulsion in a dose dependent manner when compared to control. The dose of 180mg/kg produced the greatest percentage inhibition (71%) which is comparable to that of the standard drug atropine (72%).

Castor Oil - Induced Intestinal Fluid Accumulation

Table 3 presents the effect of Picralima nitida fruit extract in intestinal fluid accumulation due to castor oil administration relative to control. The highest dose of 270mg/kg produced the highest percentage inhibition (44) which is less than that of atropine (68%).

Table 1: Effect of *P. nitida* ethanol extract on castor oil diarrhoea in rats

Drug/extract	Dose (mg/kg)	Mean fecal matter	% inhibition
Distilled water (10ml/kg)	-	5.40 ± 0.51	-
P. nitida extract	90	3.60 ± 1.17	33.33
	180	0.20 ± 0.20*	96.20
	270	0.20 ± 0.20*	96.20
Atropine	0.5	1.60 ± 0.92*	70.37

Data were expressed as mean ± SEM. Significance at *P< 0.05 and n = 5

Table 2: Effect of *P. nitida* ethanol extract on small intestinal propulsion in mice

Drug/extract	Dose (mg/kg)	Charcoal length	Intestinal length	% inhibition
Distilled water (10ml/kg)	-	23.19 ±1.56	40.10 ± 0.92	58
P. nitida extract	90	19.14± 2.91	40. 20 ±1.61	48
	180	30.90±2.79	43.74 ± 1.38	71
	270	29.52±2.21*	45.50 ±1.59	65
Atropine	0.5	30.32± 3.83*	42.22 ±1.52	72

Data were expressed as mean ± SEM. Significance at *P<0.05 andn=5

Table 3: Effect of *P. nitida*ethanol extract on castor oil induced fluid accumulation in rats

Drug/extract	Dose (mg/kg)	mean volume of fluid	% inhibition
Distilled water (10ml/kg)	-	2.25 ± 0.25	-
P. nitida extract	90	2.96± 0.33*	32
	180	2.98± 0.37*	32
	270	3.23±0.08*	44
Atropine	0.5	3.79 ±0.36**	68

Data were expressed as mean ± SEM. Significance at *P< 0.05,**P< 0.01 and n=5

DISCUSSION

Antidiarrhoeal and antidysentrice properties of medicinal plants are found to be due to the presence of tannins, alkaloids saponins, flavonoids, steroids and/or terpenoids as earlier reported to be present in the extract of P. nitida [4].In this study, ethanol fruit extract of *P.nitida* exhibited a significant antidiarrhoeal activity in the models tested. The ethanol extract of *P. nitida* demonstrated a dose-dependent anti-diarrhoeal effect in castor oil induced diarhoea which was statistically (P< 0.05- 0.01) significant when compared to control. This effect was higher than that of atropine, a standard drug at doses 180mg/kg and

270mg/kg. Castor oil induces diarrhoea due to the active ingredient ricinoleic acid liberated as a result of action of lipases on castor oil which leads to the stimulation of peristaltic activity in the small intestine, thus resulting in changes in the electrolyte permeability of the intestinal mucosa. In this process, endogenous prostaglandins are also released [7]. Castor oil elicits secretory and motility diarrhea [11]. The ability of *P. nitida* ethanol extract to inhibit diarrhoea induced by castor oil may be related to its ability to inhibit the synthesis of prostaglandin.

The ethanol extract (90–270mg/kg) of *P. nitida* also inhibited the small intestine propulsion movement with percentages of 48%, 65% and71% in a dose – related manner relative to control and the effect was comparable to that of the prototype drug, atropine with 72% inhibition of intestinal peristalsis. Conversely, the extract may also be inhibiting the small intestinal movement through some other mechanism such as antagonism of alpha-2 adrenoceptor stimulation.

In the castor oil-induced fluid accumulation, the ethanol extract inhibited fluid accumulation (enter pooling).This effect was statistically ($P<0.05$ - 0.01) significant when compared to control. The prototype drug was more potent with a percentage inhibition of 68% as against P. nitida extracts with 32% and 44% at the various doses used for the study.

CONCLUSION

The results obtained from this study support the ethnomedicinal use of P. nitida in diarrhoeatreament. Further studies should be carried out to elucidate the exact mechanism by which P. nitidainhibits diarrhoea.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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