



Research Paper

Afr. J. Traditional,
Complementary and
Alternative Medicines
www.africanethnomedicines.net

ISSN 0189-6016©2006

EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF THE FRUIT-RIND OF
PICRALIMA NITIDA (APOCYNACEAE)

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Abstract

The methanol (M) extract of the fruit-rind of *Picralima nitida* (PN) (Apocynaceae) was tested for its anti-diarrhoeal activity. Like loperamide (3 mg/kg body weight), a single oral dose of PN-M (375, 750 mg/kg body weight) produced a significant decrease in the frequency of defecation and severity of diarrhoea. To understand the mechanism of its anti-diarrhoeal activity, its effect was further evaluated on intestinal transit; castor oil-induced intestinal fluid accumulation (enteropooling) and electrolyte concentration in the small intestinal fluid. PN-M produced a decrease in intestinal transit (18.81-21.86%) as compared to castor oil treated animals. Unlike atropine, PN-M significantly inhibited castor oil-induced enteropooling. However it did not alter the electrolyte concentration in intestinal fluid as compared to castor oil treated rats.

Keywords: *Picralima nitida*, antidiarrhoeal activity, castor oil

Introduction

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhoea. There are a large number of epidemiological and experimental evidence pertaining to worldwide acute diarrhoea disease which is one of the principal causes of death in infants (Syder *et al.*, 1982, Lutterodt *et al.*, 1989). The incidence of diarrhoeal diseases still remains high despite the efforts by many governments and international organizations to curb it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs which are not always free from adverse effects (Harman *et al.*, 1992). A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these *traditional anti-diarrhoeal medicines* has not been scientifically evaluated and *Picralima nitida* is one of these.

Picralima nitida (PN) (Apocynaceae) known as limeme (Congo), Eban, Obero (Gabon), Erin (Nigeria), Bamborutuk, Eban (Cameroon) is an entirely glabrous shrub of 3-10 m high. The leaves are opposite, oblong or elliptic, 6-20 cm long and 3-10 cm broad, with tough 14 to 20 pairs of thin lateral nerves. The flowers are white, about 3 cm long. The fruits are ovoid and yellowish at maturity (Adjanohoun et al., 1996). PN is a species occurring in African forest region, spread through Ivory Coast to Zaire and Uganda (Adjanohoun et al., 1996). It has been shown to possess antiplasmodial, antimicrobial, anti-inflammatory, antipyretic, as well as anti-trypanosomiasis properties (François et al., 1996; Fakeye et al., 2000; Ezeamuzie et al., 1994; Iwu et al., 1994; Wosu et al., 1989). Medicinally, the bark is used to prepare remedies to treat malaria and male sexual impotence, while the fruits are used for dysmenorrhoea and gastrointestinal disorder (Adjanohoun et al., 1996).

The present study investigates the anti-diarrhoeal activity of PN against experimentally induced diarrhoea.

Materials and Methods

Animals

Wistar albinos rats (150-200g) of both sexes were obtained from the animal house of the Centre for research in Food and Nutrition (CRAN) of the Institute of Medical Research and Medicinal Plant Studies (IMPM), Yaoundé, Cameroon. The rats were given food and water *ad libitum*. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. All studies were carried out in group of 6 rats each. Each rat was housed separately in a metabolic cage.

Collection of plant materials.

The fruit of PN were collected in the morning in Zo-étélé, Yaoundé. Identification of the plant was confirmed in the National Herbarium Yaoundé (reference number of the plant: 2136/SRFK). The seeds were removed and the fruit-rinds were then air-dried at room temperature. The dry fruit-rinds were ground into a fine powder.

Preparation of plant extract

This was carried out by soaking the dry powdered plant (200g) in a bottle with 1.5l of methanol (M) and kept for 72 hours. The plant methanol mixture was then sieved. The filtrate (extract) was concentrated by evaporating methanol under reduce pressure using a rotary evaporator. The extract (PN-M) was further concentrated by allowing it to stand overnight in an oven at 30° C.

Drugs or Chemicals

Atropine sulphate and loperamide (standard reference anti-diarrhoeal drugs), castor oil (laxative agent), normal saline solution (NaCl 9‰), charcoal meal (10% activated charcoal in 5% gum acacia) and vehicle (0.5% v/v Tweens 80 in distilled water) were used.

The Experimental procedure

Castor oil induced diarrhoea

24 rats were allowed to fast for 18 hours and divided into 4 groups of 6 animals each. All groups received castor oil in the dose of 1 ml /animal orally (p.o.) (Doherty et al., 1981). Thirty minutes after castor oil administration, the control group received vehicle (0.5% Tween 80 in distilled water), the second and third groups PN-M 375 mg/kg and 750 mg/kg body weight, respectively. The fourth group received the reference drug loperamide (3 mg/kg p.o.). After this administration, the animals were kept in separate metabolic cages with filter paper placed beneath the cage to collect the faeces. The filter paper was changed every hour. The severity of diarrhoea was assessed each hour for six hours. The total number of faeces (diarrhoea and non-diarrhoea) and diarrhoea faeces excreted, the weight of total number of faeces were recorded during a 24 hour period and compared with the control group. The total score of diarrhoea faeces of the control group was considered 100%. The weight of the total number of faeces of the control group was considered 100%. The results were expressed as a percentage of inhibition of the diarrhoeal frequency and as a percentage of inhibition of the weight of the faeces passed (Zaval et al., 1988).

Small intestinal transit of charcoal meal

This was done according to the method proposed by Mujumdar et al., (1998) using charcoal meal as a diet marker. The Rats were divided into four groups of 6 animals each. First group (the control group) was orally administered the vehicle (0.5% Tween 80). The second and third groups were given orally PN-M, 375 mg/kg and 750 mg/kg body weight respectively. The fourth group the standard drug, atropine sulphate (5 mg/kg body weight). Half an hour later, each animal was given 1 ml of charcoal meal orally (10% activated charcoal in 5% gum acacia). Also thirty minutes after this treatment, each animal was sacrificed; the intestine and stomach were removed. The pylorus was attached to a glass rod and the intestine was suspended for 20 seconds with a weight of 3 g attached to ileocaecal junction to straighten it out. The distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as a percentage of the total length of the intestine. The results were compared with control group. The distance travelled by the charcoal meal of the control group was considered 100%. The results were expressed as a percentage of inhibition of the propulsive effect.

Castor oil induced enteropooling and electrolyte secretion

Intraluminal fluid accumulation was determined by the method of Robert et al. (1976). Rats were divided into 5 groups of 6 animals each. Group one was given 2 ml of normal saline solution and group 2 received 2 ml of castor oil. Groups 3, 4 and 5 received atropine sulphate (0.1 mg/kg) through the intraperitoneal route (i.p.), PN-M (375 mg/kg p.o.), and PN-M (750 mg/kg p.o.), respectively, 1 hour before the oral administration of castor oil. Two hours later the rats were anaesthetised using ether and sacrificed. The small of each intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected into a graduated tube and the volume was recorded. The intestine was reweighed and the difference between full and empty intestines was calculated. The intraluminal fluid was centrifuged at 3000 rpm for 15 minutes and the Na⁺ and K⁺ concentrations in the supernatant were measured by flame photometry.

Phytochemical Screening

The freshly prepared extract was chemically tested qualitatively for the presence of chemical constituents such as tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars. They were identified by characteristic colour changes using standard procedures (Odebedy and Sofowora, 1978).

Statistical analysis

The values were expressed as means \pm standard deviation. The statistical analysis of data was by Analysis of Variance (ANOVA) using 5% level of significance. The statistical package used was SPSS 10.1. A One way ANOVA enabled us to see the significant differences between the values. The Duncan test was used to identify these differences.

Results

Effect of PN-M on castor-oil induced diarrhoea

In the castor oil-induced diarrhoea experiment, the rats that did not receive the plant extract, showed typical diarrhoea signs such as watery and frequent defecation. The extract of PN fruit-rinds produced a marked anti-diarrhoeal effect in the rats. Both doses of PN-M significantly decreased ($p < 0.05$) the total number of faeces produced by administration of castor oil (7.17 at the dose of 375 mg/kg and 6.17 at the dose of 750 mg/kg) as compared to the castor oil-treated control group (20.83). The percentage of inhibition of castor oil-induced diarrhoea in PN-M treated rats was 65.58 and 70.38 %, respectively, at 375 mg/kg and 750 mg/kg dose.

The effect of PN-M was comparable to that of the standard drug, loperamide (3 mg/kg) which produced an inhibition of 70.38% (Table 1). The average weight of faeces in the control group was 7.38g. Treatment with both doses of PN-M significantly reduced ($p < 0.05$) the weight of faeces to 4.24-4.05g (Table 1)

Table 1: Effect of PN-M on castor oil induced diarrhoea

Group	Total number of faeces	Number of diarrhoeal faeces	Percentage of inhibition of diarrhoeal faeces frequency (%)	Total weight of faeces (g)	Percentage of inhibition of the weight of faeces passed (%)
Castor oil (1ml) + vehicle (0.5% Tween 80)	24.83 \pm 4.53 ^d	20.83 \pm 5.115 ^b	0	7.383 \pm 2.20 ^c	0
Loperamide (3mg/Kg) + castor oil (1ml)	9.83 \pm 0.93 ^a	6.17 \pm 1.60 ^a	70.38	1.76 \pm 0.83 ^a	76.83
PN-M (375 mg/Kg) + castor oil (1ml)	15.67 \pm 3.20 ^b	7.17 \pm 1.32 ^a	65.58	4.24 \pm 0.93 ^b	42.54
PN-M (750 mg/Kg) + castor oil (1ml)	14.33 \pm 1.03 ^b	6.17 \pm 2.22 ^a	70.38	4.05 \pm 1.20 ^b	45.07

Values in the same column with different superscript letters are significantly different ($p < 0.05$)

Effect of PN-M on small intestinal transit of charcoal meal

The administration of PN-M also slowed down the propulsion of charcoal meal through gastro-intestinal tract when compared to the castor oil-treated rats. The percentage of intestinal length traversed by charcoal meal in PN-M pre-treated (375 and 750 *mg/kg*) and castor oil-treated rats was 58.27, 60.54 and 74.57, respectively. Atropine on its part, produced a marked decrease in the propulsive movement and the intestinal length travelled by charcoal meal was 40.33 (Table 2).

Table 2: Effect of PN-M on charcoal-induced gut transit changes

Group	Percentage of distance travelled by charcoal meal	Percentage of inhibition of the propulsive effect (%)
Vehicle (0.5% Tween 80)	74.57 ± 9.90 ^c	0
Atropine sulphate (5 mg/kg)	40.32 ± 4.25 ^a	45.93
PN-M (375 mg/kg)	58.27 ± 11.20 ^b	21.86
PN-M (750 mg/kg)	60.54 ± 8.17 ^b	18.81

Values in the same column with different superscript letters are significantly different ($p < 0.05$)

Effect of PN-M on castor oil-induced enteropooling and electrolyte secretion

PN-M was also found to possess anti-enteropooling activity. Oral administration of castor oil produced a significant increase ($p < 0.05$) in the intestinal fluid (3.12 *ml*) compared to normal rats (1.28 *ml*). PN-M when given orally 1 hour before castor oil, significantly inhibited ($p < 0.05$) the enteropooling, 1.08 *ml* (375 *mg/kg*) and 1.38 *ml* (750 *mg/kg*). The volume of intestinal fluid was similar to that obtained in normal group (1.28 *ml*) (Table 3).

The weights of intestinal contents were also significantly decreased ($p < 0.05$) following treatment with castor oil (from 3.54 to 1.02 *g* in normal rats). However, PN-M produced a marginal decrease in the weight of intestinal content and the secretions were more viscous. Treatment of rats with castor oil significantly increased ($p < 0.05$) the Na⁺ concentration to 11.00 mEq/l as compared to the control group (8.75 mEq/l). PN-M at both doses as well as atropine sulphate pre-treatment did not alter the Na⁺ concentration in intestinal fluid as compared to the castor oil treated group.

Discussion

In this study, the methanol extract of PN fruit-rinds exhibited a significant inhibition of castor oil-induced diarrhoea though not in a dose-dependent manner. The results were similar to that of the standard drug loperamide (3 *mg/kg*). The extract slowed down the propulsion of charcoal meal through gastro-intestinal tract and also led to a marked reduction in the weight and the volume of the intestinal contents.

Table 3: Effect of PN-M on castor oil enteropooling and electrolyte secretion

Group	Volume of intestinal content (ml)	Weight of intestinal content (g)	Concentration of Na ⁺ (mEq/l)	Concentration of K ⁺ (mEq/l)
Castor oil (2 ml)	3.12 ± 0.37 ^d	3.54 ± 0.61 ^c	11.00 ± 1.50 ^a	5.64 ± 1.41 ^a
Normal saline (2 ml)	1.28 ± 0.24 ^{ab}	1.02 ± 0.25 ^{ab}	8.75 ± 1.49 ^c	6.34 ± 0.98 ^{ab}
Atropine sulphate + castor oil (2ml)	1.80 ± 0.31 ^c	2.46 ± 0.36 ^b	15.83 ± 3.46 ^{ab}	7.78 ± 0.20 ^{ab}
PN-M (350 mg/Kg) + castor oil (2ml)	1.08 ± 0.84 ^a	1.88 ± 0.16 ^a	16.71 ± 2.20 ^{ab}	7.65 ± 1.80 ^{ab}
PN-M (750 mg/Kg) + castor oil (2ml)	1.38 ± 0.19 ^{ab}	2.30 ± 0.19 ^{ab}	15.23 ± 5.73 ^{ab}	8.07 ± 1.44 ^b

Values in the same column with different superscript letters are significantly different ($p < 0.05$)

Castor oil is reported to induce diarrhoea by increasing the volume of intestinal content by prevention of the reabsorption of water. This property of castor oil is due to its active metabolite ricinolic acid (Ammon, 1974; Watson, 1962). The liberation of ricinolic acid results in irritation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (Pierce et al., 1971). Thereby prevents the reabsorption of NaCl and H₂O (Galvez et al., 1993). Probably extract increased the reabsorption of water by decreasing intestinal motility as observed by the decrease in intestinal transit of charcoal meal. The delay in faecal emptying by the extract allows more time for fluid absorption and subsequently reduces fluid losses in the stool.

Weights and volumes of intestinal contents decrease when electrolyte concentrations are slightly increasing. Logically, osmotic pull by the electrolytes should cause intraluminal fluid retention. May be strong *in vivo* inhibition of tone by rat duodenum could provide an explanation. But this was not tested.

Earlier studies have shown that anti-dysenteric and anti-diarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars (Anonymous, 1962; Galvez et al., 1991, 1993; Longanga et al., 2000). The phytochemical screening of the extract revealed the presence of alkaloids, saponins and flavonoids. Thus, alkaloids, saponins and flavonoids may be responsible for the mechanism of action of PN-M anti-diarrhoeal activity.

Loperamide is a synthetic opiate analogue developed specifically for use in diarrhoea. All opiate agonists have effects on intestinal smooth muscle. Loperamide regulate the gastrointestinal tract by inhibiting the propulsive motor activity, predominantly in the jejunum, and this effect is partially inhibited by opiate antagonists. Other effects on intestinal motility may be mediated through inhibition of prostaglandin stimulation of gut motility and/or through calcium antagonist actions (W.H.O., 1990). Apart from regulating the gastrointestinal tract, loperamide is also reported to reduce colonic rate of flow, and

consequently increase colonic water absorption, but it does not have any effect on colonic motility (Theoderau et al., 1991).

Atropine produced a significant reduction in intestinal transit time. This is possible due to its anticholinergic effect (Brown and Taylor, 1996). However, it did not inhibit castor oil induced enteropooling suggesting thereby that mediators other than acetylcholine are involved in castor oil induced enteropooling. Further, an increase in intestinal transit time with atropine could also be due to a reduction in gastric emptying (Izzo et al., 1999).

Conclusion

The result of this investigation revealed that PN-M possesses significant antidiarrhoeal activity due to its inhibitory effect on gastrointestinal propulsion. This property establishes the use of PN as a traditional anti-diarrhoeal medicine.

Further research is to be carried out to fraction and purify the extract, in order to find out the fractions and molecules responsible for the anti-diarrhoeal activity observed.

Acknowledgement

We are Grateful to the Centre for the Research in food and Nutrition (CRAN) of the Institute of Medical Research and Medicinal Plant Studies (IMPM), Yaoundé, for their material support.

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