https://dx.doi.org/10.4314/jpb.v17i2.12
Vol. 17 no. 2, pp. 180-188 (September 2020)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Activity of methanol root extract of *Parquetina nigrescens* (Afzel.) Bullock on castor oil-induced diarrhoea in mice

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Received 20th July 2020; Accepted 24th August 2020

Abstract

This study aimed at providing pharmacological rationale for the ethnomedicinal use of *Parquetina nigrescens* root in the treatment of diarrhoea. The antidiarrhoeal activity of methanol root extract of *P. nigrescens* (MPN) was determined using castor oil-induced diarrhoea (COD), castor oil-induced enteropooling (COE) and gastrointestinal motility (GIT) tests. In COD, MPN at all doses significantly ($p \leq 0.01$) delayed the onset of diarrhoea and significantly ($p \leq 0.05$) decreased the number of wet faeces (with percentage inhibition of 52.2; 53.33 and 71.13 at 25, 50 and 100 mg/kg) respectively. The frequency of defecation was significantly ($p \leq 0.01$) decreased at 50 and 100 mg/kg. In COE, MPN at all doses significantly ($p \leq 0.01$) reduced the volume of intestinal content with percentage inhibition of intestinal fluid accumulation of 79.36; 47.62; 68.25 at doses of 25, 50 and 100 mg/kg respectively against control. In the GIT test, MPN significantly ($p \leq 0.01$) decreased the distance travelled by charcoal with a percentage reduction of peristalsis index of 41.19; 47.26; 43.46 at doses of 25, 50 and 100 mg/kg respectively against control. The percentage inhibition of GIT at all doses of MPN was 50.48, 39.00 and 45.62 respectively. MPN possesses antidiarrhoeal activity thus, the credence for its ethnomedicinal use in the treatment of diarrhoea.

Keywords: Diarrhoea, castor oil, enteropooling, gastrointestinal motility, *Parquetina nigrescens*

INTRODUCTION

Diarrhoea is the passage of three or more watery, loose, soft stools within 24 hours. It may be accompanied with abdominal bloating, loss of appetite, cramp, flatulence etc. [1, 2]. Diarrhoea is characterized by increased gastrointestinal motility and secretion with a decrease in the absorption of fluid and electrolytes [3, 4]. Diarrhoea can be acute (lasts less than 14 days without visible blood stain in stool); dysentery (with visible blood stain); and persistent (last at least 14 days) [5] The most important causes of diarrhoea are micro-organisms such as rotavirus, enterotoxigenic *Escherichia coli*, Shigella, *Campylobacter jejuni*, *Giardia lamblia*, *Cryptosporidium spp*, *Vibrio cholerae*, Salmonella *Entamoeba histolytica*, *Blastocytis hominis etc*. Other causes are side effects of medications, psychological stress and anxiety [6, 7, 8]. Diarrhoea is responsible for about 3.2 million deaths each year in children less than 5 years of age in developing countries [5]. Complications such as dehydration, dysentery,

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malnutrition (due to increased nutrient requirements and reduced appetite and nutrient absorption) and serious infections, such as pneumonia account for diarrhoea related death [9]. Diarrhoea affects a country's economy by reducing the health of its workforce [10].

Diarrhoea is self-limiting. In severe cases, oral rehydration therapy, zinc supplementation; and antimotility or antisecretory drugs is necessary [11]. Diphenoxylate, loperamide and bismuth subsalicylate have been widely used in the treatment of diarrhoea [12,13]. Antibiotics such as the fluroquinolones and cephalosporins are effective against many enteropathogens thus reducing the severity of infectious diarrhoea [14-16]. However, side effects of these drugs such as respiratory depression, lethargy, constipation, dry mouth, inability to prevent dehydration (due to hidden fluid loss in the ileum) have limited the use of opioids [17-19]. Renal toxicity and Reye's syndrome due to the high absorption of subsalicylate are peculiar to bismuth subsalicylate, while antibiotics resistance present a challenge in infectious diarrhoea [19-21].

The high incidence of diarrhoea in developing countries coupled with poor healthcare coverage make medicinal plants a good alternative for the treatment of the disease. Various parts of plants in forms off infusion, decoction and enema are used in the treatment of diarrhoea [22]. Despite lack of adequate dosing and scarce report of toxicities of medicinal plants, availability and anecdotal report of efficacy have made them important alternative treatment of diarrhoea (23,24).

Parquetina nigrescens possesses important therapeutic properties and its various parts are used in the treatment of gastro-intestinal disorders, rickets, diarrhoea, skin lesions, menstrual disorders, erectile dysfunction, gonorrhoea and sickle cell disease in the south western part of Nigeria [25-27]. It is commonly found in West Africa especially in Ghana and Nigeria and belongs to the family Aasclepiadaceae [28,29]. Various parts of the plant have been validated to possess anti-inflammatory and analgesic [30], anti-ulcer, antianaemic, antidiabetic [31,32], antimicrobial [33,34], antimalaria [35] anticancer effect [36] and cognitive enhancing potentials [37,38]. This study therefore aimed at investigating the antidiarrhoeal activity of the methanol stem extract of *P. nigrescens*.

EXPERIMENTAL

Drugs. Methanol (Sigma-Aldrich, Germany), atropine, loperamide (Jiangsu Ruinian Qianjin Pharm. Co. Ltd, China), castor oil, activated charcoal (Rambaxy Laboratories, India), distilled water (Juhel Pharm. Ltd, Nigeria).

Animals. Swiss Albino mice of both sexes weighing 20-25 g were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Animals were maintained on standard laboratory animal feed and allowed water *ad libitum*. They were acclimatised for one week before experimentation.

Ethical clearance. Institutional Animal Ethics Committee's approval was obtained with an approval number: ABUCAUC/2017/023. Experiment was carried out between 9:00 and 18:00 hours of the day.

Plant collection and extract preparation. The whole plant was collected in a bush within Samaru, Sabon Gari Local Government Area, Kaduna State, Nigeria in the month of June, 2016. The freshly harvested plant material was identified by Mallam Umar Gallah of the National Institute for Chemical Research Technology (NARICT) Zaria, Kaduna State. A voucher specimen was prepared (voucher number 01624) and deposited in the herbarium unit of NARICT. The roots were removed, washed and air-dried under the shade for three weeks until constant weight was obtained after which it was grounded to coarse powder using mortar and pestle. The powdered sample (665

g) was extracted with 6 litres of 70 % methanol by cold maceration with occasional shaking for one week. The macerated mixture was filtered using a filter paper and the filtrate concentrated using a rotary evaporator. The concentrate was further dried over a water bath at a temperature of 40 ^{0}C to obtain a dried dark-brown solid mass subsequently referred to as methanol root extract of *P. nigrescens* (MPN). This was stored in a desiccator until required for use. Fresh solution of the extract was prepared for each study.

Phytochemical screening. Phytochemical screening was carried out on the methanol root extract of *P. nigrescens* according to the methods described by Evan [39] to determine the class of phytochemicals present in the extract.

Acute toxicity test. Oral median lethal dose was determined according to the method described by Lorke [40]. Briefly, the method is divided into phase 1 and 2 using a total of 12 mice. Animals were administered different doses of the extract in both phases and observed for mortality. The highest non-lethal and lowest lethal doses of 600 and 1000 mg/kg respectively were obtained from which the LD_{50} was calculated as shown below:

$LD_{50} = \sqrt{}(Do \times D_1)$

Do = Highest non-lethal dose; D_1 = Lowest lethal dose.

Antidiarrhoeal studies

Castor oil-induced diarrhoea. The method previously described by Shoba and Thomas [41] was adopted with slight modification. Thirty mice were fasted for 18 hours and randomly divided into 5 groups of 6 animals each. Group 1, 2, 3, 4 and 5 received 10 mL/kg distilled water, 5 mg/kg loperamide, 25, 50 and 100 mg/kg of methanolic root extract of *P. nigrescens* (*p.o*) respectively. One hour after administration, all mice received 1 mL of castor oil (*p.o*) and individually placed in a cage whose floor was covered with plain paper and observed for 4 hours. The floor lining was changed every 1 hour for the period of observation. The onset of diarrhoea (time taken to produce diarrhoea stool), the frequency of defecation (total number of faecal output), consistency of faeces (total number of wet and dry diarrhoeal drops for the 4 hours duration) were recorded and compared with the control group. The results were expressed as a percentage inhibition of diarrhoea:

$$\frac{\text{Mean NWF of (negative}-\text{treated)}}{\text{Mean NWF negative}} \text{ X } 100$$

Where NWF = number of wet faeces.

Castor oil-induced enteropooling. The method employed by Ezeja *et al.*, [42] was adopted with slight modification. Thirty mice were deprived of food for 18 hours and allowed water *ad libitum*. The animals were randomly divided into 5 groups of 6 mice each. Group 1, 2, 3, 4 and 5 received 10 mL/kg distilled water, 5 mg/kg loperamide, 25, 50 and 100 mg/kg methanol root extract of *P. nigrescens* per oral respectively. One hour later, 1 mL of castor oil was administered to all the mice per oral. One hour after castor oil administration, the mice were sacrificed and the small intestine of each from the pylorus to the caecum was isolated, tied at both ends and weighed. Then, the intestinal content of all individual animals were collected by milking into a graduated tube and the volume of each was measured and recorded. After milking the intestinal content, the intestine of each mouse was reweighed. Then, intestinal weight reduction (IWR) and percentage inhibition of intestinal fluid accumulation (IFA) were calculated as shown below: $IFA = \frac{\text{Mean VIC of (negative}-\text{treated)}}{\text{VIC negative}} \text{ X } 100$

$$Intestinal\ weight\ reduction\ (IWR) = weight\ before\ milking - weight\ after\ milking$$

Where VIC = volume of intestinal content.

Gastrointestinal motility test. The method described by Ezeja and Anaga [43] was adopted. Thirty mice were fasted for 18 h and allowed water *ad libitum.* The animals were randomly divided into 5 groups of 6 mice each. Group 1, 2, 3, 4 and 5 received distilled water 10 mL/kg (*p.o*), 1 mg/kg atropine (*i.p*), 25, 50

and 100 mg/kg methanol root extract of *P. nigrescens (p.o*) respectively. One hour after drug administration, 1 mL of castor oil was administered to each animal orally in all groups. One hour later, 0.5 mL of 5% charcoal suspension in distilled water was administered to all the mice orally. Then, all animals were humanely sacrificed by cervical dislocation after 30 minutes and the small intestine of each mouse was isolated. The total length of the intestine and the distance moved by the charcoal meal from the pylorus towards the caecum were measured using a transparent meter rule. The peristalsis index (PI) and the percentage inhibition of motility were calculated as shown below:

$$PI = \frac{\text{Mean DTC)}}{\text{Mean TLI}} \text{ X } 100$$

Where DTC = Distance travelled by charcoal; TLI = Total length of intestine.

$$\% \text{ Inhibition of motility} = \frac{\text{Mean DTC Of (negative}-\text{treated)}}{\text{Mean DTC negative}} \text{ X } 100$$

Statistical analysis. Data were presented as mean ± standard error of mean (SEM) analyzed using SPSS statistical software, version 20. Comparisons between groups were made using One-way ANOVA followed by Dunette's post hoc test for multiple comparisons. The difference between the compared groups was considered statistically significant at $p \leq 0.05$.

RESULTS

Phytochemical screening. The methanol root extract contains alkaloids, phenolics, tannins, saponins, cardiac glycosides, unsaturated sterols and carbohydrates. Anthraquinones was absent.

Acute toxicity test. The oral median lethal dose (LD_{50}) of the methanol root extract was estimated to be 775 mg/kg.

Antidiarrhoeal studies. MPN at all doses significantly ($p \leq 0.01$) delay the onset of diarrhoea and significantly ($p \leq 0.05$) decreased the number of wet faeces (with a percentage inhibition of diarrhoea of 52.2; 53.33 and 71.13) at 25, 50 and 100 mg/kg respectively compared with the distilled water group. Loperamide significantly ($p \leq 0.05$) delayed the onset of diarrhoea and significantly decreased ($p \leq 0.05$) the number of wet faeces with a percentage inhibition of diarrhoea of 48.6. The total number of faeces (frequency of defecation) was significantly ($p \leq 0.01$) decreased only at 50 and 100 mg/kg of the extract compared with distilled water group. There was non-significant increase in the number of dry faeces (Table 1).

MPN at all doses significantly ($p \leq 0.01$) reduced the volume of intestinal content with a corresponding percentage inhibition of intestinal fluid accumulation of 79.36; 47.62; 68.25 at doses of 25, 50 and 100 mg/kg respectively compared to the distilled water group. The extract at all doses significantly ($p \leq 0.01$) caused intestinal weight reduction. Loperamide significantly ($p \leq 0.05$) decreased volume of intestinal content with a percentage inhibition of intestinal fluid accumulation of 31.74 and significantly ($p \leq 0.05$) caused intestinal weight reduction compared to the distilled water group (Table 2).

MPN at all doses tested and atropine significantly ($p \leq 0.01$) decreased the distance travelled by charcoal meal with a percentage reduction of peristalsis index of 57.75; 41.19; 47.26; 43.46 with atropine, 25, 50 and 100 mg/kg of the extract respectively compared with the distilled water group. The percentage inhibition of gastrointestinal motility by atropine, 25, 50 and 100 mg/kg of the extract are 26.21; 50.48, 39.00 and 45.62 respectively (Table 3).

Table 1: Effect of methanol root extract of *Parquetina nigrescens* on castor oil-induced diarrheal activity in mice

Group (/kg)	Onset (min)	NWF	NDF	TNF	% Inhibition
D/W 10 mL	37.67±2.75	15.00±1.98	2.17±0.83	17.17±1.44	-
LOP 5 mg	58.00*±2.03	7.67*±2.84	2.67±0.88	10.33±2.70	48.60
MPN 25 mg	85.67**±3.68	7.17*±1.25	4.00±0.63	11.17±1.25	52.20
MPN 50 mg	104.67**±3.75	7.00*0.89	2.50±0.43	9.50*±1.06	53.33
MPN100 mg	114.5**±6.31	4.33**1.33	4.67±1.67	9.00*±2.72	71.13

Data presented as mean ± SEM; n=6; * and ** denote ($p \leq 0.01$ and 0.05*)* respectively compared to D/W group; NWF = Number of wet faeces; NDF = Number of dry faeces; TNF = Total number of faeces (frequency of defecation); D/W = Distilled water; LOP = Loperamide; MPN = Methanol root extract of *P. nigrescens*

Table 2: Effect of methanol root extract of *Parquetina nigrescens* on castor oil-induced enteropooling in mice

Group (/kg)	VIC (mL)	% IFA	IWR (g)
D/W 10 mL	0.63±0.04	-	0.78±0.05
LOP 5 mg	0.43*±0.03	31.74	0.56*±0.04
MPN 25 mg	0.13**±0.04	79.36	0.29**±0.05
MPN 50 mg	0.33**±0.07	47.62	0.47**±0.09
MPN 100 mg	0.20**±0.04	68.25	0.34**±0.05

Data presented as mean ± SEM; n=6; * and ** denote ($p \leq 0.01$ and 0.05*)* respectively compared to D/W group; VIC = Volume of intestinal content; IFA = Intestinal fluid accumulation; IWR = Intestinal weight reduction; D/W = Distilled water; ATP = Atropine; MPN = Methanol root extract of *P. nigrescens*

Table 3: Effect of methanol root extract of *Parquetina nigrescens* on gastrointestinal motility of mice

Group (/kg)	TLI (cm)	DTC (cm)	% PI	% IGM
D/W 10 Ml	45.75±1.44	37.7±3.42	82.4	-
ATP 5 mg	48.17±1.61	27.82**±1.61	57.75	26.21
MPN 25 mg	45.33±1.08	18.67**±1.00	41.19	50.48
MPN 50 mg	48.67±0.67	23.00**±3.12	47.26	39.00
MPN 100 mg	47.17±1.57	20.50**±2.00	43.46	45.62

Data presented as mean ± SEM; n=6; * and ** denote ($p \leq 0.01$ and 0.05) respectively compared to D/W group; TLI = Total length of intestine; DTC = Distance travelled by charcoal; PI = Peristalsis index; IGM = Inhibition of gastrointestinal motility; D/W = Distilled water; ATP = Atropine; MPN = Methanol root extract of *P. nigrescens*

DISCUSSION

There is high incidence of diarrhoea especially in rural areas of developing countries such as Africa and South Asia [44, 45]. This coupled with limitations of access to effective antidiarrhoeal drugs and poor healthcare coverage make medicinal plants a good alternative for the treatment of diarrhoea for these folks. The antidiarrhoeal activity of the methanol stem extract of *P. nigrescens* was investigated using castor oil-induced enteropooling, gastrointestinal motility, and castor oil-induced diarrhoea tests in mice.

In the castor oil-induced diarrhoea test, the methanol root extract of *P. nigrescens* reduced the episodes of diarrhoea by prolonging the onset, decreasing the number of wet faeces, the frequency of diarrhoea and formation of dry faeces. Increased in the frequency of stooling, passage of wet faeces and reduction or absence of dried faeces is commonly experienced in diarrhoea. Castor oil induces diarrhoea by increasing contractility and motility of intestinal smooth muscles thus, reducing water and electrolyte absorption with consequent formation of wet stool and increase diarrhoea frequency [6, 46].

In the castor oil-induced enteropooling, the methanol root extract of *P. nigrescens* and loperamide showed antidiarrhoeal activity by reducing the volume of intestinal content with a corresponding decrease in intestinal weight of the animals and inhibition of intestinal fluid accumulation. Castor oil induces diarrhoea by the action of the enzyme lipase which coverts it to the active form i.e. ricinoleic acid [1, 47].

Ricinoleic acid eventually induces diarrhoea by affecting electrolyte transport and smooth muscle contractility [48, 49]. Castor oil causes injury to the epithelia cells of the intestinal mucosa, release of prostaglandins leading to increase intestinal activity and secretion of fluid thus reducing water and electrolyte absorption through reduction of sodium potassium ATPase activity [50,51]. The ability of methanol root extract of *P. nigrescens* to reduce intestinal fluid accumulation maybe through inhibition of electrolyte secretion into the lumen which consequently decrease pool of fluid into the lumen. Drugs that are capable of reducing electrolyte permeability and secretion prevent castor oil-induced diarrhoea [6]

In the gastrointestinal motility test the methanol root extract of *P. nigrescens* and atropine decrease gastrointestinal motility by reducing the distance travelled by charcoal and correspondingly decreasing peristalsis index. The ability of the extract to reduce peristalsis implies a spasmolytic effect on the intestinal smooth muscles thus providing more time for fluid and electrolyte absorption consequently reducing the frequency and improving the consistency of stool in diarrhoea. Peristalsis is enhanced and water and electrolyte absorption decreased during diarrhoea. Spasmolytic agents such as anticholinergics inhibit the propulsive movement of the gastrointestinal tract thereby reducing the frequency of stool associated with diarrhoea [50].

Phytochemical constituents are known to show a vast array of pharmacological activities. The antidiarrheal activity of methanol root extract of *Parquetina nigrescens* may be due to one or more of the following: alkaloids, phenolics, tannins, saponins, cardiac glycosides, unsaturated sterols and carbohydrates present in the plant. Tannins, phenolics, saponins, alkaloids have been reported to possess antidiarrheal activity [52, 53].

In conclusion, the methanol root extract of *P. nigrescens* possesses antidiarrhoeal activity which may be associated with possible anti-secretory and antimotility effect.

Acknowledgement

We thank the entire staff of the Department of Pharmacology and Therapeutics for providing the necessary facilities to carry out this work.

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