JMBR: A Peer-review Journal of Biomedical Sciences
December 2016, Vol. 15 No. 2 pp 12-20

THE ANTI-DIARRHOEAL ACTIVITY OF THE AQUEOUS STEM BARK EXTRACT OF PARKIA FILICOIDEA (FABACEAE).

Owolabi Omonkhelin Josephine[1]*, Ukoima George Silvanus[1], Inninh Silvanus Olu[2], Otokiti Ibrahim Osaremen[1]

ABSTRACT

Parkia filicoidea, Welw (Fabaceae) has been used in herbal medicine for the treatment of diarrhoea. This claim however need to be validated, hence this study. The study aimed at evaluating the anti-diarrhoeal activity of the stem bark of the plant. The plant was extracted using distilled water (AEPF) and tested at 100 and 200 mg/kg doses on castor oil induced diarrhoea, castor oil induced enteropooling, small intestinal transit and magnesium sulphate induced diarrhoea in both rats and mice. Positive control (loperamide 3 mg/kg) and negative control (distilled water) were used in each of this model. The results showed a significant reduction ($p < 0.05$) by the extract at both doses in the onset and frequency of stooling in both the castor oil and magnesium sulphate induced diarrhoea in comparison with the negative control. A significant reduction ($p < 0.05$) was also observed in transit time and enteropooling in the extract treated groups in comparison with the control. In conclusion, AEPF does have some anti-diarrhoea activity and compares well with loperamide.

INTRODUCTION

Diarrhoea occurs from an imbalance between the absorptive and secretory mechanism in the intestinal tract, resulting in an excess loss of fluid in the faeces. Diarrhoea has two basic pathological mechanisms: increased active secretion and decreased absorption of water and electrolyte. These two mechanisms lead to increase in stool volume and weight. Decreased absorption of water and electrolyte results from decreased transit time and abnormal motor activity invariably seen in patients with diarrhoea[1]. According to World Health Organization, it is one of the most common causes of mobility and mortality in many developing countries affecting mainly infants and children [2].

In many developed countries, death from diarrhea have been prevented by WHO recommended homemade remedies, oral rehydration solutions (ORS) which contains modest amount of salt and zinc tablets, use of anti-infective agents and non- antimicrobial anti diarrhea agents [3]. It has also been documented that medicinal plants have an advantage in toxicity considerations based on their long term use and it is expected that bioactive compounds obtained from such

KEYWORDS: Castor oil, Activated charcoal, Magnesium sulphate, Enteropooling

Owolabi Omonkhelin Josephine[1], Ukoima George Silvanus[1], Inninh Silvanus Olu[2], Otokiti Ibrahim Osaremen[1]*
[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria
[2]Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin.

**Correspondence:*

Owolabi OJ
Senior Lecturer, University of Benin
E-mail: owolabi@uniben.edu, omonowolabi@yahoo.com
+2348034120318
Conflict of interest: Authors declare they have no competing interest.

plants have low animal and human toxicity[4]. Based on this report individuals have embarked on the use of indigenous plants as a remedy against diarrhea diseases[5].

Parkia filicoidea is one of such plants with numerous ethnomedicinal uses; noticeable amongst these is the anti-diarrhea activity of the aqueous crude extract of the stem bank of the plant[6].

Parkia filicoidea is a large briefly deciduous tree with an open widely spreading crown, it can grow up to 30 m tall, popularly known as the African locust bean, called Ogiri amongst the Ibos, Dawadawa (Hausa) and Iru (Yoruba) in Nigeria. Other uses of the bark of Parkia filicoidae in traditional medicine are: as a decoction taken to stimulate lactation (galactogogue), treatment for malaria and to ease rheumatism and toothache.

This study is thus aimed at justifying the use of Parkia filicoidea in the treatment of diarrhoea based on its ethnomedicinal use.

MATERIALS AND METHODS

Plant material

Fresh stem barks of Parkia filicoidea were obtained from a farm area in Ajaokuta area of Kogi state, Nigeria in May, 2015. Botanical identification was done by Prof. B.A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin and a herbarium specimen exists in the Department.

Aqueous Extract of Parkia filicoidea (AEPF)

Fresh stem bark of Parkia filicoidae was dried in open air for about seven (7) days, after which the dried stem bark was pulverized. The powdered dried stem bark was boiled in 2 L of distilled water in a conical flask for about 15 minutes on a heating mantle, after which the mixture was allowed to cool. It was then filtered using cotton wool and the filtrate was passed through Whatmann No. 4 filter paper for further filtration, the filtrate was then concentrated and then allowed to dry in an oven at a temperature of 40^0 C. The desired stock concentrations to be administered were made from the extract. Fresh solutions were prepared each day of the study.

The percentage yield was determined using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract} \times 100}{\text{Weight of the plant material}}$$

The dried extract was stored in the refrigerator prior to use.

Animals

Adult albino rats weighing 160-280 g and albino mice (20-35g) of both sexes were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The animals were kept under standard environmental conditions on a regular feed (standard growers mash, Top feeds, Nigeria) and had access to water ad libitum.

All animals were handled according to the standard protocols for the use of laboratory animals (National Institute of Health, USA: Public Health Service Policy on Humane care and use of Laboratory animals, 2002).

Drugs/Chemicals

Castor oil (Bell Sons& company, Southport, England), Magnesium sulphate (BDH Chemicals UK), Activated charcoal, Loperamide (Neros Pharmaceuticals Ltd, Lagos).

Castor oil induced diarrhoea

The method as reported by [7,8] were used in assessing the anti-diarrhoeal effect of the AEPF in rats using the castor oil. Rats of either sex (160 – 280g) were fasted for 18 hours prior to experiment but had access to water. The animals were divided into four groups (n = 5). Group (I) received distilled water orally (2ml/kg) which served as negative control. Animals in group (II) received loperamide (3mg/kg) orally and served as the positive control while groups (III) and (IV) orally received 100 and 200 mg/kg AEPF respectively. 60 minutes after oral administration of drug/extracts, the animals received castor oil (1ml) orally and were individually placed in a cage, the bottom of which was lined with a white plain sheet of paper for observation of the number and consistency of faecal matter. The onset of stooling, number and weight of wet feacal droppings were recorded every hour for 4 hrs. The means of the stool passed by the treated groups were compared with that of the control. The mean number of wet droppings pulled by the control group was considered as 100%.

Castor oil induced enteropooling

The effect of AEPF on pooling of enteric contents was assessed using the castor oil induced enteropooling method [9]. Rats of either sex were again fasted for 18 hours prior to experiment but had access to water. The animals were divided into four groups (n=5). Animals in group (I) received distilled water (2ml/kg) orally. Group (II) loperamide (3mg/kg) orally, while groups (III) and (IV) received doses of 100 and 200 mg/kg AEPF orally. An hour after the above treatment, castor oil (1ml) was administered to all the animals. One hour after oral administration of treatments, animals were sacrificed by cervical dislocation and the small intestine removed after tying both ends with ligature at the pyloric end and illeoceacal junction respectively. Intestinal contents were collected by milking into a graduated tube and their volumes were measured using a syringe. The level of reduction in volume intestinal content was calculated relative to the control. The mean volume of intestinal content of control rats was considered as 100%.

Magnesium sulphate induced diarrhoea

Mice of either sex (20 – 35g) were fasted for 18 hours prior to procedure but had access to water. The animals were divided into four groups (n = 5). Group (I) received distilled water orally (2mg/kg), animals in group (II) received loperamide (3mg/kg) orally, while groups (III) and (IV) received 100 and 200 mg/kg AEPF respectively orally. 60 minutes following oral drug/extract administration, each animal in all groups received magnesium sulphate (2g/kg) orally. Each mouse was placed in a metal cage, the floor of which was lined with plain white paper and observed for 4 hours. The onset of stooling, number and weight of wet stools were noted every hour for 4 hours and recorded.

Small intestinal transit

The effect of AEPF on gastrointestinal motility was accessed using intestinal motility of mice [10]. Mice of either sex were fasted for 18 hours prior to experiment but had access to water. The animals were divided into four groups, (n=5). Animals in group (I) received distilled water (2ml/kg) orally, group (II) received

loperamide (3mg/kg) orally, groups (III) and (IV) orally received AEPF at doses of 100 and 200 mg/kg body weight respectively. One hour after oral administration of treatment, each animal received 0.2 ml of charcoal meal orally. Thirty minutes later, the animals were sacrificed by cervical dislocation and the small intestine was carefully separated from the mesenterum avoiding being stretched. The length of the intestine from pyloric sphincter to the ileoceacal junction (Ceacum) and the distance traveled by the charcoal meal were measured. For each animal, gastrointestinal transit was calculated as the percentage distance travelled by the charcoal meal, relative to the total length of the intestines. The inhibitory effect of AEPF on gastrointestinal transit was calculated relative to the control.

Statistical analysis

All data are expressed as mean ± SD. The significance of the differences among the group were assessed using two way ANOVA. The test followed Dunnet's test. P values < 0.05 were considered as significant.

RESULTS

Castor oil induced diarrhoea

Onset of stooling/Number of stools

The Results are presented in table 1 and figure 1. At 100 mg/kg, the onset of stooling was 52 mins which was noted to be higher than that of the control (45 mins). The group that received 200 mg/kg AEPF did not stool for the entire 4 hour observation period.

Loperamide, the positive standard, produced an effect similar to that of 200 mg/kg AEPF, as there was no stooling during the 4 hour observation period.

A significant reduction ($p < 0.0001$) in the number of stools following treatment with both doses of the extract was observed in comparison with the control group. At 100 mg/kg, the number was reduced from 13.4 ± 2.19 (control) to 2.8 ± 1.64, while no stool was seen at 200 mg/kg.

The loperamide and 200 mg/kg AEPF groups had similar results and both showed statistically significant difference ($P < 0.0001$) in comparison with the control. This is presented in Figure 1.

Table 1: The effect of AEPF on castor oil-induced diarrhoea

Group (mg/kg)	Time of first stool (mins)	Total number of stools	Ratio of animals that stooled
CONTROL (2 ml/kg)	45.0 ± 1.00	13.4 ± 2.19	5/5
LOPERAMIDE (3)	-	0.0 ± 0.0*	0/5
AEPF (100)	52 .0± 1.05	2.8 ± 1.64*	5/5
AEPF (200)	-	0.0 ± 0.0*	0/5

Values are Mean±SD, n = 5 per group.

*P<0.0001 significantly different from the control group.

Control animals received distilled water

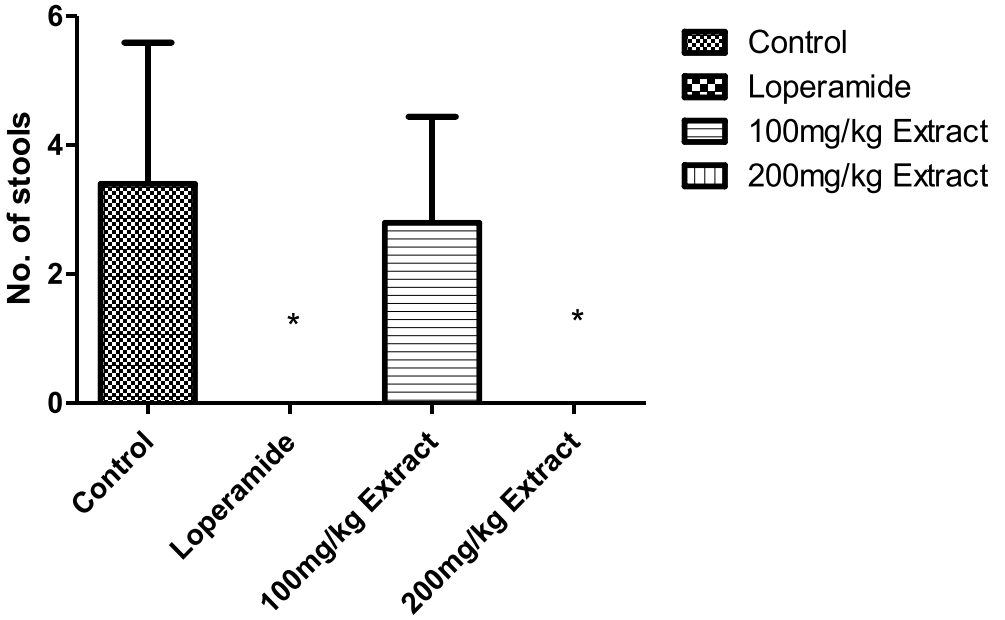

Fig 1: Effect of AEPF on castor oil-induced diarrhea (number of stools)

Values are Mean±SD, n = 5 per group.

*P<0.0001 significantly different from the control group.

Control animals received distilled water

Castor oil induced enteropooling

All the groups treated with the extract/loperamide had reduction in their volume of intestinal contents when compared to the control. However the 200 mg/kg AEPF group had the least volume of 2.6 ± 0.37 and was significantly different from the control; 3.8 ± 0.56 ($p < 0.05$). The intestinal content volume for loperamide and 100 mg/kg AEPF groups were 2.62 ± 1.1 and 3.1 ± 0.55 respectively (table 2).

Table 2: Effect of AEPF on Castor oil-induced enteropooling

Group (mg/kg)	Volume of intestinal content	% inhibition of intestinal volume
CONTROL (2ml/kg)	3.80 ± 0.56	---
LOPERAMIDE (3)	2.62 ± 1.11*	31.05
AEPF (100)	3.10 ± 0.55	18.42
AEPF (200)	2.60 ± 0.37*	31.57

Values are Mean±SD, n = 5 per group.

*P<0.05 significantly different from the control group.

Control animals received distilled water

Magnesium sulphate induced diarrhoea

Onset of stooling

Treatment with AEPF significantly ($p < 0.0001$) prolonged the onset of stooling in comparison with the control. A similar prolongation was observed with loperamide treated group though not significant. This was 3 minutes for the control group, increased to 5 minutes for loperamide group, while the 100 and 200 mg/kg AEPF groups had their onset of stooling as 15 minutes and 20 minutes respectively. This is presented in Table 3.

Number of stools

A significant reduction ($p < 0.0001$) in the number of stools was seen following treatment with 100 and 200 mg/kg AEPF compared to the control group at the end of 4 hours. Control produced 25.5 ± 5.57 stools compared to 3.75 ± 3.10 and 4.6 ± 1.15 for 100 and 200mg/kg AEPF group respectively. The positive control, loperamide, also produced a significant reduction ($p < 0.05$) in the number of stools (8.25 ± 9.11), as compared to 25.5 ± 5.57 stools for the control. This is also shown in table 3.

Table 3: Effect of AEPF on Magnesium sulphate-induced diarrhea

Group (mg/kg)	Time of first stool (mins)	Total number of stools	Ratio of animals that stooled
CONTROL (2 ml/kg)	3.0± 1.05	25.5 ± 5.57	4/4
LOPERAMIDE (3)	5.0± 1.05	8.25 ± 9.11[a]	3/4
AEPF (100)	15.0 ± 1.00*	3.75 ± 3.10*	4/4
AEPF (200)	20.0± 1.00*	4.6 ± 1.15*	4/4

Values are Mean±SD, n = 4 per group.

[a]P<0.05 and *P<0.0001 significantly different from the control group.

Control animals received distilled water

Small intestinal transit

There was a significant ($p < 0.05$) decrease in the distance travelled by the charcoal meal on treatment with both doses of AEPF when compared to the control, a similar significant reduction in the distance traversed by charcoal meal on treatment with loperamide was also noted ($p < 0.05$).

On conversion to percentage distance, the distance transversed by the charcoal meal for the control was 79.59%, significantly decreased ($p < 0.05$) on treatment with loperamide, 100 and 200mg/kg AEPF (47.15%, 48.37% and 54.69%) respectively.

The percentage inhibition for loperamide, 100 and 200 mg/kg AEPF were 52.8, 51.63 and 45.3 % respectively. This is presented in table 4.

Table 4: Effect of AEPF on small intestinal transit

Group (mg/kg)	Total length of intestine	Distance travelled by charcoal	Percentage travelled (%)	Percentage inhibition (%)
CONTROL (2ml/kg)	45.7± 2.44	35.5± 4.5	79.59	-
LOPERAMIDE (3)	45.0± 2.16	21.5± 11.03	47.15*	52.85
AEPF (100)	44.25± 1.5	21.5± 5.31*	48.37*	51.63
AEPF (200)	45.63± 4.03	25.25± 8.80*	54.69*	45.30

Values are Mean±SD, n = 5 per group.

*P<0.05 significantly different from the control group.

Control animals received distilled water

DISCUSSION

Castor oil is a triglyceride in which approximately 90% of the fatty acid chains are ricinoleic acid. Oleic and linoleic acids are the other significant components. Castor oil induces diarrhea via ricinoleic acid [11,12]. Ricinoleic acid, a monosaturated 18-carbon fatty acid is unusual in that it has a hydroxyl functional group on the 12th carbon. This functional group causes ricinoleic acid to be polar [11].

Castor oil stimulates peristaltic activity in the small intestine, leading to changes in the intestinal mucosa. Its action also stimulates the release of prostaglandins.

Our results show that the AEPF possesses anti-diarrhoea activity. Several mechanisms have been proposed to be involved in the diarrhoea effect of castor oil [13]. These include decrease in fluid absorption, increase secretion in the small intestine and colon, and it affects smooth muscle contractility in the intestine. The inhibition of intestinal Na+, K+ -ATPase activity to reduce normal fluid absorption, activation of adenylyl cyclase, stimulation of prostaglandin formation, platelet activator factor and recently nitric oxide was observed to contribute to the diarrhoea effect of castor oil. Despite the fact that a number of mechanisms have been involved in the diarrhoea effect of castor oil, it has not been possible to define its precise mechanism of action. AEPF may act on any of the mechanisms mentioned above.

Secretory diarrhoea is associated with an activation of Cl^- channels, causing Cl^- efflux from the cell, the efflux results in the massive secretion of water into the intestinal lumen and profuse watery diarrhea.

The observed inhibition of castor oil induced enteropooling in rats suggests that the AEPF produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effect [9].

In castor oil induced diarrhoea, the liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to the production of prostaglandins. This results in stimulation of secretion by preventing the reabsorption of sodium chloride and water. Probably, AEPF increased the reabsorption of sodium chloride and water by decreasing intestinal motility as observed in intestinal transit of charcoal meal. AEPF may have inhibited the above mechanism.

Magnesium sulphate is an inorganic salt that acts as an osmotic laxative. Because it is poorly absorbed in the intestinal lumen, it increases bulk and exerts osmotic effect [14].

In addition to this local effect, Magnesium sulphate is believed to cause release of hormones such as cholecystokinin or activation of constitutive nitric oxide synthase which might contribute to this pharmacological effect [13].

The AEPF also inhibited magnesium sulphate induced diarrhea and thus showed anti- diarrhoea activity, as observed in the result for the magnesium sulphate induced diarrhoea in mice. This suggests that AEPF may have interfered with any of these mentioned mechanisms.

CONCLUSION

This study has shown that the AEPF possesses significant anti-diarrhoeal activity as it was able to reduce peristalsis and inhibit active secretion as evident from its inhibitory effects on castor oil and magnesium sulphate induced diarrhea, and reduction of the transit of the charcoal meal. Further studies will have to be carried out to fully investigate the chemical constituents responsible for this effect and the precise mechanisms involved in the anti-diarrhoeal activity of Parkia filicoidea.

Acknowledgement

Our profound gratitude goes to Mr Ibeh and Mr Stephen and Miss Chikodi Nweke for their contributions towards this research.

References

1 Hogenauer C, Hammer HF, Krejs GJ, Reisinger EC. Mechanisms and management of antibiotic-associated diarrhea. Clin Infect Dis 1998; 27: 702 -10.

2 Fernando C, Ramon AA, Halley P. Effect of Plants used in Mexico to treat gastromtestinal disorders on charcoal gum acacia induced hyperperistalsis in rats. J. Ethnopharmacol 2014; 128:49-51

3 World Health Organization “Guideline for Assessment of the Herbal Medicines” Programme on Traditional Medicine, WHO, Geneva, 2010, pp 56-91.

4 Fabricant DS, Farnsworth NR. The value of plants in traditional medicine for drug discovery. Environ. Health Perspect 2001; 109 (1): 69-75.

5 Etuk EU, Mohammed BJ. Informant consensus selection method: A reliability assessment on medicinal plants used in north western Nigeria for the treatment of diabetes mellitus. Afr J Pharm & Pharmacol 2009; 3(10): 496-500.

6 Milliogo-Kone H, Guissou IP, Nacoulma O, Traore AS. Comparative study of leaf and stem of bark extracts of Parkia biglobosa against Enterobacteria. Afr. J. CAM 2008; 5(3): 238 -243

7 Nwodo, O.F.C. and Alumanah, E.O. (1991). J Ethnopharmacol, 31: 395-398.

8 Nwafor, P.A., Jacks, T.W., Ekanem, A.U. and Ching, F.P. (2005). Nig J Natl Prod & Med, 9: 66-70.

9 Robert, A., Nazemis, J.E., Lancaster, C., Hanchar, A.J. and Kleeper M.S. (1976). Enteropooling assay; a test for diarrhea produced by Prostaglandins 11: 809 – 828.

10 Mascolo, N., 1zzo, A.A., Avtore, G; Barboto, F. and Capasso, F. (1994). J.Pharmacol & Exp Thera, 268: 291-295.

11 Ammon, H.V. and Philips, S.F. (1974). Inhibition of ileal water absorption by intraluminal fatty acids. Influence of chain length, hydroxylation, and conjugation of fatty acids. J. Clin. Invest 53:205-210.

12 Capasso, F., Mascolo, N., Izzo, A.A. and Gaginella, T.S. (1994). Br. J. Pharmacol, 113: 1127-1368

13 Izzo, A.A., Mascolo, N., Viola, P. and Capasso, F. (1993). Inhibitors of nitric oxide synthase enhance rat ileum contractions induced by ricinoleic acid in vitro. Eur J Pharmacol, 243: 67-90.

14 Laurence, D.R., Bennett, P.N. and Brown, M.J. (1997). Clinical Pharmacology (8th Edidtion), Churchill Living Stone Edinburg.