



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2015; 4(1): 183-187

Received: 15-01-2015

Accepted: 19-02-2015

Joseph O.T. EmudainohwoDepartment of Pharmacology
and Therapeutics, Delta State
University, Abraka, Nigeria.**Goodies E. Moke**Department of Pharmacology
and Therapeutics, Delta State
University, Abraka, Nigeria.**Daniel E. Ejebe**Department of Pharmacology
and Therapeutics, Delta State
University, Abraka, Nigeria.**Earnest O. Erhirhie**Department of Pharmacology
and Therapeutics, Delta State
University, Abraka, Nigeria.

An investigation into the anti-diarrhoeal effects of aqueous and ethanol stem bark extracts of *Alchornea cordifolia* in wistar rats

Joseph O.T. Emudainohwo, Goodies E. Moke, Daniel E. Ejebe, Earnest O. Erhirhie

Abstract

This study investigated the anti-diarrhoeal effect of aqueous and ethanol bark extract of *Alchornea cordifolia* on castor oil-induced diarrhoeal; gastrointestinal motility; and enteropooling. The results revealed that, compared with the positive control (loperamide-treated) group which showed the most reduction in mean weight of stool, the 400 mg/kg and 200 mg/kg of both extracts also elicited dose dependent reduction in the mean weight of stool, with the 400 mg/kg body weight of the aqueous extract causing greater reduction than the other extracts doses. The anti-motility effect and percentage inhibitive effect was also shown to be greater in the loperamide-treated group, compared to the ethanol and aqueous extract-treated groups, which demonstrated a dose-dependent effect. The intestinal volume (enteropooling) was equally found to be most markedly increased in the positive control (atropine-treated) groups followed by a dose-dependent fashion in the 200 mg/kg and 400 mg/kg doses of the ethanol and aqueous stem bark extracts treated groups. Thus, the study showed that the aqueous and ethanol stem bark extracts of *Alchornea cordifolia* possess anti-diarrhoeal properties.

Keywords: Anti-diarrhoeal, loperamide, *Alchornea cordifolia*, Castor oil-induced Diarrhoea

1. Introduction

Diarrhoeal disease has been identified as the second leading cause of death, especially in the low-income regions of most developing countries, and has also been identified as a major basis of malnutrition in the under 5 years ^[1]. The diseases presents with an abnormal increase in the frequency, volume or liquidity of stools which precipitate dehydration and loss of body fluid and salt ^[2]. Contaminated food and water are the main sources of diarrhoea ^[3], and this is due to unhygienic mannerisms before eating or sharing food, or after using toilet facilities ^[4]. This is even with the institution of preventive measures by the World Health Organization ^[1] which focuses on providing safe drinking-water; improving sanitation; practice of good personal and food hygiene; promoting health education, and encouraging rotavirus vaccination, amongst people in many developing countries ^[1].

Herbal medicines derived from various plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, ^[5, 6]. This practice is very common amongst those in low income regions of developing countries who still prefer traditional herbal practices for their regular health care needs ^[7]. This is in spite of the huge technological advancement in modern medical practices that has been employed by the World Health Organization (WHO) in the move against diseases such as diarrhoeal in the low income regions of developing countries.

This preference for traditional/herbal medicine in the low income regions of developing countries has also made the WHO to encourage studies that relates to the advancement of traditional medical practices for the treatment and prevention of common diseases such as diarrhoeal, which has been shown to greatly contribute to mortality in developing countries and has been investigated with different traditional medical herbs ^[8, 9, 10, 11].

The plant *Alchornea cordifolia*, also known as 'Christmas bush', is a medicinal plant with extensive use as remedy in African folk traditional medicine ^[12]. The leaves are mostly used in traditional medicine, but the stem bark, the root, and the fruits have been mentioned in local traditional medicine ^[13]. They have been used for the treatment of bacteria, fungi, parasitic and inflammatory disorder, and have also been indicated in the treatment of ulcers, wounds, and for cicatrization ^[14]. The effect of the two extracts from the stem bark of *Alchornea cordifolia* (ethanol and aqueous) shall be investigated for their anti-diarrhoeal properties in a castor

Correspondence:**Goodies E. Moke**Department of Pharmacology
and Therapeutics, Delta State
University, Abraka Delta State,
Nigeria.

oil-induced diarrhoea model in Wistar rats. Research information on the anti-diarrhoea property of ethanol and aqueous bark extract of *Alchornea cordifolia* (if any), will not only improve ways of managing and treating diarrhoea, but could well create a lead for drug development.

2. Materials and Methods

2.1 Plant collection and identification of the plant

Alchornea cordifolia plant was collected in June 2013, from the bush behind the Staff Quarters in site III of the Delta State University, Abraka, Nigeria. The *Alchornea cordifolia* plant was identified in the Department of Botany, Faculty of Science, Delta State University, Abraka, Nigeria.

2.2 Preparation of extract

The bark of *Alchornea cordifolia* plant was peeled off the stem and was sun-dried for 1 week. The dried bark was blended into powdered form with the aid of a clean and sterile blending machine. The resultant powder was weighed, and was divided into two equal parts of 250 grams each and kept in a plastic can. The blended powder in the first can was soaked with 1.5 litre of ethanol, while the blended powder in the second can was soaked with 1.5 litre of distilled water for 72 hours. A clear filtrate of the soaked extract was obtained using an electrical evaporator extraction apparatus (rotary evaporator) set at 40 °C. The solvent were extracted under heat, pressure and a paste-like extract were obtained and oven-dried to a complete solid, which was grinded to powder. The powder were stored in air-tight glass ware and kept in the refrigerator at 4 °C pending when they were used experimentally.

2.3 Animal

Thirty (30) mature Wistar rats, weighing between 130 - 180 grams was obtained from the Animal House of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were moved in plastic cages to the Animal House of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria, where the study took place. Rats of either sex were housed in separate cages and kept at room temperature of 24± 2 °C in an approximate 12 hour light/dark cycle. The animals were fed with standard rodent pellet diet (Savannah Feeds, Nigeria) and provided with drinking water *ad libitum*. The animals were acclimatized for one week and received humane care in compliance with the ethical guide approved by the College of Health Sciences, Delta State, University, Abraka, Delta State, Nigeria, similar to the NIH guidelines for the care and use of laboratory animals.

2.4 Experimental Design

The experimental administration of extracts and standard drug took place between 8:00 am and 11:15 am and lasted for just one (1) day.

2.4.1 Effect of aqueous and ethanol bark extract of *Alchornea cordifolia* on castor oil-induced diarrhoea.

Thirty (30) rats were randomly allotted to 6 groups of 5 rats each. Group 1 – the control group, received 10 ml/kg of distilled water. Group 2 received 2 mg/kg loperamide as the standard anti-diarrhoeal drug, while groups 3 and 4 received 200 and 400 mg/kg of body weight of the ethanol extract of

Alchornea cordifolia orally. Group 5 and 6 also received 200 and 400 mg/kg body weight of the aqueous extract of *Alchornea cordifolia* orally. After 1 hour of the treatments, diarrhoea was induced by administration of 1 ml of castor oil orally to each rat which was then observed for 4 hours. The characteristic diarrhoeal droppings were noted in the absorbent paper placed beneath the individual rat perforated cages ^[15].

2.4.2 Effect of aqueous and ethanol bark extract of *Alchornea cordifolia* on gastrointestinal motility

Thirty (30) rats were randomly allotted to 6 groups of 5 rats each. Group 1 received 10 ml/kg of distilled water. Group 2 received 2 mg/kg loperamide, while groups 3 and 4 received 200 and 400 mg/kg of body weight of the ethanol extract of *Alchornea cordifolia* by stomach intubations. Group 5 and 6 also received 200 and 400 mg/kg body weight of the aqueous extract of *Alchornea cordifolia* by stomach intubations. After 5 minutes of treatment with extracts, distilled water or standard drug (loperamide), 0.5 ml of 10% charcoal suspension in 5% acacia gum was administered to each mouse by stomach intubations. Thirty minutes later, all the rats were sacrificed by inhalation anaesthesia using chloroform. The abdomen was opened and the total length of the small intestine was measured with a calibrated ruler. The distance travelled by the charcoal plug from the pylorus to caecum was determined and expressed as a percentage of the total length of the small intestine. The percent inhibition of movement was also calculated by deducting the percentage travelled from 100% ^[16, 17].

2.4.3 Effect of aqueous and ethanol bark extract of *Alchornea cordifolia* on Castor oil - induced enteropooling

Thirty (30) rats were randomly allotted to 6 groups of 5 rats each and fasted for 12 hours with free access to distilled water. Rats in group 1 received 10 ml/kg of distilled water. Group 2 received 3 mg/kg Atropine, while groups 3 and 4 received 200 and 400 mg/kg of body weight of the aqueous extract of *Alchornea cordifolia* by stomach intubations. Group 5 and 6 also received 200 and 400 mg/kg body weight of the ethanol extract of *Alchornea cordifolia* by stomach intubations. One hour after treatment, each rat was given 0.5 ml castor oil by oral gavage. Two hours after administration of castor oil, the rats were sacrificed by inhalation anaesthesia using chloroform. The abdomen was opened and the small intestine identified and tied at the pyloric and ceecal junction and dissected out. The small intestine was weighed with analytical weighing balance (Mettler H30). The content of each intestine was squeezed into a graduated test tube, and its content measured ^[18].

2.5 Data Analysis

Results are expressed as mean ± SEM (Standard error of mean). The results were subjected to statistical analysis by independent students' t-test using statistical package for social science (SPSS) version 16 for windows. The significance differences between groups were considered significant when $P < 0.05$.

3. Result

The result of the study is presented in Table 1-3.

Table 1: Effect of aqueous and ethanol stem bark extracts of *Alchornea cordifolia* on castor oil-induced diarrhoea in wistar rats

Group	Mean Weight of Stool \pm SEM (g)	Percentage Inhibition
Distilled water (10 ml/kg)	3.26 \pm 0.68	0
Loperamide (2 mg/kg)	0.39 \pm 0.02*	88.0
200 mg/kg (EE)	1.83 \pm 0.02 ^a	43.7
200 mg/kg (AE)	1.85 \pm 0.04 ^{ac}	43.3
400 mg/kg (EE)	1.20 \pm 0.189 ^{ab}	63.2
400 mg/kg (AE)	0.96 \pm 0.06 ^{abd}	70.6

EE = Ethanol Extract; AE = Aqueous Extract

Values are expressed as mean \pm standard error of mean (SEM), n=5. *P<0.05: Significant value when compared with control. ^aP<0.05: Significant value when compared with group standard drug. ^bP<0.05: Significant value when compared with 200 mg/kg ethanol extract. ^cP<0.05: Significant value when compared with group 400 mg/kg ethanol extract. ^dP<0.05: Significant value when compared with group 200 mg/kg aqueous extract.

Table 2: Effect of aqueous and ethanol stem bark extracts of *Alchornea cordifolia* on charcoal transit time in wistar rats.

Group	Mean Length of Intestine (cm)	Mean Distance Travelled by Charcoal (cm)	Mean % Distance Travelled (cm)	% Inhibition
Distilled water (10 ml/kg)	93.4 \pm 95.1	81.96 \pm 1.1	87.6	12.2
Loperamide (2 mg/kg)	95.1 \pm 1.61 ^{ns}	36.84 \pm 0.6*	38.7	61.3
200 mg/kg (EE)	96.3 \pm 1.67 ^{ns}	55.8 \pm 0.5 ^a	57.9	42.1
200 mg/kg (AE)	98.9 \pm 0.44 ^{ns}	56.3 \pm 0.8 ^{acd}	56.9	43.1
400 mg/kg (EE)	98.7 \pm 0.64 ^{ns}	43.9 \pm 1.3 ^{ab}	44.5	55.5
400 mg/kg (AE)	97.1 \pm 0.6 ^{ns}	45.9 \pm 0.9 ^{ab}	47.3	52.7

EE = Ethanol Extract; AE = Aqueous Extract

Values are expressed as mean \pm standard error of mean (SEM), n=5. *P<0.05: Significant value when compared with control. ^aP<0.05: Significant value when compared with group standard drug. ^bP<0.05: Significant value when compared with 200 mg/kg ethanol extract. ^cP<0.05: Significant value when compared with group 400 mg/kg ethanol extract. ^dP<0.05: Significant value when compared with group 200 mg/kg aqueous extract. ^{ns}P<0.05: Value not significant when compared with control group.

Table 3: Showing the results of the effect of aqueous and ethanol bark extract of *Alchornea cordifolia* on mean volume of intestine content in wistar rats.

Group	Mean Volume of Intestine (ml)	% protection
Distilled water (10 ml/kg)	4.25 \pm 0.02	0
Atropine (3 mg/kg)	6.61 \pm 0.08*	-55.5%
200 mg/kg (EE)	5.3 \pm 0.11 ^a	-24.7%
200 mg/kg (AE)	5.44 \pm 0.05 ^{ac}	-28.0%
400 mg/kg (EE)	5.82 \pm 0.05 ^{ab}	-36.9%
400 mg/kg (AE)	5.82 \pm 0.07 ^{abd}	-36.9%

EE = Ethanol Extract; AE = Aqueous Extract

Values are expressed as mean \pm standard error of mean (SEM), n=5. *P<0.05: Significant value when compared with control. ^aP<0.05: Significant value when compared with group standard drug. ^bP<0.05: Significant value when compared with 200mg/kg ethanol extract. ^cP<0.05: Significant value when compared with group 400mg/kg ethanol extract. ^dP<0.05: Significant value when compared with group 200mg/kg aqueous extract.

4. Discussion

In this study, the anti-diarrhoea properties of the two preparations from *Alchornea cordifolia* (ethanol and aqueous stem bark extracts) were investigated in castor oil-induced diarrhoea in animal model.

As shown in Table 1 above, treatment with loperamide and two doses of aqueous and ethanol bark extract significantly (P<0.05) reduced the mean weight of stool when compared with the control group. Reduction in stool volume was marked in loperamide group while 200 mg/kg and 400 mg/kg of the two extracts elicited dose dependent effects. Percentage inhibition values in stool weight was highest in loperamide treated group (88%) followed by the 400 mg/kg of aqueous (70.6%) and ethanol (63.2%) bark extract and was least in the 200 mg/kg doses of ethanol (43.7%) and aqueous (43.3%) bark extract of *Alchornea cordifolia*. The mechanism of action of loperamide is based on its ability to reduce motility of the circular and longitudinal muscles of the intestine, consequent

upon its action at the myenteric plexus [19]. The effect is that it increases the amount of time that the faecal matter stays in the intestine, allowing time for more water to be absorbed from of fecal matter. The high percentage of inhibition recorded for loperamide as with the extract of *Alchornea cordifolia* in this study justifies its being ascribed with anti-diarrhoeal property. Table 2 above, illustrates the effect of aqueous and ethanol bark extract of *Alchornea cordifolia* on charcoal transit time in Wistar rats. The result shows that the administration of loperamide (36.84 \pm 0.6* cm); 200 mg/kg ethanol (55.8 \pm 0.5^a cm); 400 mg/kg ethanol (43.9 \pm 1.3^{ab}cm); 200 mg/kg aqueous (56.3 \pm 0.8^{acd} cm); and 400 mg/kg aqueous (45.9 \pm 0.9^{ab} cm) bark extract of *Alchornea cordifolia* significantly (p<0.05) reduced the distance travelled by charcoal meal when compared with control group (81.96 \pm 1.1cm). Decrease in distance travelled by charcoal meal was dose dependent in ethanol and aqueous extract-treated groups. Thus 400 mg/kg doses of both extract had more anti-motility effects while

loperamide (the standard drug) had the most anti-motility effect. Table 2 above also showed the percentage inhibition in distance travelled by charcoal meal. The pattern of percentage inhibition in distance travelled by charcoal meal took the following pattern: Loperamide (61.3%) > 400 mg/kg ethanol extract (55.5%) > 400 mg/kg aqueous extract (52.7%) > 200mg/kg aqueous extract (43.1%) > 200mg/kg ethanol extract (42.1%). The percentage inhibition was thus shown to be dose dependent in the ethanol and aqueous extract-treated groups.

Table 3 shows the results of the effect of aqueous and ethanol bark extract of *Alchornea cordifolia* on mean volume of intestine content of the different groups of animals. The result revealed that the volume of intestine content was significantly ($P < 0.05$) decreased in the Atropine ($6.61 \pm 0.08^*$ ml); 200 mg/kg ethanol ($5.3 \pm 0.11^{*a}$ ml); 400 mg/kg ethanol ($5.82 \pm 0.05^{*ab}$ ml); 200 mg/kg aqueous ($5.44 \pm 0.05^{*ac}$ ml); and 400 mg/kg aqueous ($5.82 \pm 0.07^{*abd}$ ml) bark extract of *Alchornea cordifolia*, when compared with control group (4.25 ± 0.02 ml). However, the intestinal volume was most markedly increased in the Atropine-treated groups followed by a dose dependent fashion in the 200 mg/kg and 400 mg/kg doses of ethanol and aqueous bark extracts of *Alchornea cordifolia*.

Castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport, which result in a hyper-secretory response and diarrhea^[20]. Ricinoleic acid causes a release of prostaglandins, which promotes motility and secretion within the gut^[21]. The significant ($p < 0.05$) increase in the volume of intestine contents of rats treated with extracts suggests the presence of compounds that could inhibit the action of ricinoleic acid, and thus, causes inhibition of the release of prostaglandins. Before now, phytochemical studies on *Alchornea cordifolia* have revealed the presence of tannins, reducing sugars, sterols, flavonoids and triterpenes which had been reported for their anti-diarrhoeal activity^[22, 23, 24], as well as inhibition of intestinal motility^[25]. This goes on to support our suggestion in this study that, indeed, the aqueous and ethanol bark extracts of *Alchornea cordifolia* does possess some anti-diarrhoeal properties.

5. Conclusion

The present study showed that *Alchornea cordifolia* does possess some anti-diarrhoeal properties, however, the anti-diarrhoea properties of the aqueous and ethanol stem bark extract of *Alchornea cordifolia* is lower than those of the standard drugs (loperamide and atropine). Our suggestion is that more research be carried out on the plant - *Alchornea cordifolia*, to determine the exact mechanism(s) of action of the extract and to isolate and characterize the active biochemical substances.

6. Acknowledgements

The authors would like to thank the Laboratory Technologist – Mr. G.C. Ahatty, of the Department of Pharmacology and Therapeutics, Delta State University, Abraka, Nigeria, for his moral support during the course of this study. We also thank Mrs. Joy Eyakpegba for helping to type the manuscripts.

7. Authors' Contributions

All authors significantly contributed to this research work. Emudainohwo, J.O.T.; Moke, E.G., and Ejebe D.E. conducted the experiments, while Erhirhie, E.O. and Emudainohwo, J.O.T. carried out the statistical analysis.

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