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Effect of Chloroform-Ethanol Extracts of Cashew (*Anacardium occidentale*) Kernel on Electrolyte Imbalance in Castor Oil-induced Diarrhea Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAO and OFCN designed the study, wrote the protocol and supervised the work. Authors DAO and OFCN carried out all laboratories work. Author PEJ performed the statistical analysis. Authors DAO and PEJ managed the analyses of the study. Authors DAO, OFCN and PEJ wrote the first draft of the manuscript. Authors DAO, CXA and OFCN managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Diarrhea results from an imbalance between the absorptive and secretary mechanisms in the intestinal tract, accompanied by watery bowel movement resulting in excess fluid and electrolytes in faeces. This study was undertaken to evaluate the effect of chloroform-ethanol extracts of Cashew (*Anacardium occidentale*) kernel at the dose of 21 mg/kg and 84 mg/kg body weight on electrolyte imbalance in castor oil induced diarrheal rats. Acute toxicity and lethality (LD_{50}) and phytochemical constituents of the extracts where also evaluated. The results showed the extract significantly ($P<0.05$) reduced the concentration of sodium and potassium ion in the intestinal solution compared to the control animals induced with castor oil only. The results of the qualitative

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phytochemical analysis showed that the chloroform-ethanol extract (ethanol, chloroform and middle layers) tested positively to flavonoids, alkaloids saponin, reducing sugars, glycosides and steroids while, chloroform layer and middle layer tested positive to fat and oil. Acute toxicity and lethality studies on chloroform-ethanol extracts revealed an oral LD_{50} equal to or more than 5000mg/kg body weight in mice. These results showed that kernels of *A. occidentale* possess anti-diarrheal properties through inhibition reduction of the intestinal electrolyte secretion which can substantiate its use in the treatment of diarrhea in traditional medicine.

Keywords: Diarrhea; electrolytes; castor oil; phytochemicals.

1. INTRODUCTION

Plants and their derivatives play key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs are derived from plants [1]. According to the World Health Organization, about 80% of the world's population relies essentially on plants for primary health care [2]. Diarrhea is the condition of having three or more loose or liquid bowel movements per day; it results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hyper-motility, resulting in excess loss of fluid in the faeces [3]. It is a common cause of death in developing countries and the second most common cause of infant deaths (16%) after pneumonia (17%) worldwide. In 2009 diarrhea was estimated to have caused 1.1 million deaths in people aged 5 and over and 1.5 million deaths in children under the age of 5 [4]. Oral rehydration salts and zinc tablets are the treatment of choice and have been estimated to have saved 50 million children in the past 25 years. The loss of fluids through diarrhea can cause dehydration and electrolyte imbalances [5]. The incident of diarrheal diseases still remains high despite the intervention of government agencies and international organization to halt the trend [6]. The use of herbal drugs in the treatment of diarrhea is common in many African countries [7]. Despite immense technology and advances in medicine, many people in developing countries still rely on traditional healing practices and medicinal plants for their health care need [8]. The World Health Organization (WHO) encourage studies into traditional medicinal prevention of diarrheal diseases [9].Therefore, there is urgent need for intensification of research into claim of the use of medicinal plants in the management of diarrheal diseases [10]. Cashew (*Anacardium occidentale*) is a well known member of the Anacardiaceae family and is commonly found in Northeast Brazil. The cashew nut has been commercially exploited since colonization. Brazil, India and Mozambique are the leading cashew nuts producers in the world [11]. The kernels of cashew (*A. occidentale)* plant have been reported by Orwa et al. [12] to be used for curing diarrhea in some parts of the world. This research attempts at a possible investigation on the anti-diarrheal properties of ethanol and chloroform extracts of *Anacardium occidentale* kernel in experimentally induced acute diarrheal rats.

2. MATERIALS AND METHODS

All reagents used were of analytical grade. Drug (Lomotil) used were supplied by Emzor Pharm Ind. Ltd., Nigeria.

2.1 Plant Material

Cashew (*Anacardium occidentale*) nuts used were collected in Obolloafor, Enugu State, Nigeria. They were authenticated by Mr. A. Ozioko of the Bioresource Development and Conservation Programme (BDCP) Research Centre in Nsukka.

2.2 Extraction Procedure

The cashew kernels were isolated using a simple cutter knife. This was used to slit each nut open and a pointed knife employed to remove the kernel immediately from the shell to minimize contamination with the cashew nut shell liquid (CNSL). The kernel was then subjected to roasting at 80ºC for one hour to remove the testa as described by Onilude, et al. (2010). The roasted kernel was ground into coarse form.

The pulverized kernel (1071 g) was macerated in 3 L mixture of chloroform and ethanol (2:1) for 48 hours. The macerate was passed through Whatman No 4 filter paper. The filtrate was shaken with 20% of distilled water to obtain three (3) layers. The upper layer (ethanol layer) was drawn out; the middle layer was also separated

from the lower layer (chloroform layer). The three layers were concentrated with a rotatory evaporator and dried in a boiling water bath. The weight of the sample was taken after drying. The extract yields were 0.53%, 1.48% and 0.74% for upper, middle and lower layers respectively.

3. EXPERIMENTAL ANIMAL

Adult female Wistar albino rats of 5-6 weeks old with average weight of 160±14 g and female mice weighing 30±0.75 g were obtained from the Animal House of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were acclimatized for 7 days under standard environmental conditions, with a 12-hour light and dark cycle maintained on a regular feed and water *ad libitum*. The animals were used according to the NIH animal care guidelines with approval of the Departmental Animal Committee (DAC/IW-OT/1-07).

3.1 Phytochemical Test

Basic qualitative phytochemical screening of the ethanol, chloroform and middle layers of the water-treated extract of the kernel sample was carried out by testing for the presence or absence of the following plant constituents: flavonoids, tannins, saponins, glycosides, fat and oil, sterol, alkaloid, reducing sugar and carbohydrate. The phytochemical analysis of the sample was carried out using procedures outlined by Harborne, [13] and Trease and Evans [14].

3.2 Acute Toxicity and Lethality (LD_{50}) Test

The acute toxicity and lethality of chloroform-ethanol extract of the cashew kernel was determined using the modified method of Lorke, [15]. The test was divided into two stages. In stage one, twenty-seven (27) randomly selected adult mice were divided into nine groups, three per group (n=3) and received 10, 100 and 1000 mg/kg body weights of the ethanol, middle layer and chloroform extracts respectively and the signs of toxicity and number of death for a period of 24 hours. After 24 hours observation, the doses for the second phase were determined based on the outcome of the results of the first phase. Since there was zero death, a fresh batch of animals was used following the same procedure in phase 1 but with higher dose ranges of 1900, 2600 and 5000 mg/kg body weights of the extract. The animals were also observed for 24 hours for signs of toxicity and possible number of death. The LD_{50} was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose [15].

3.3 Preparation of Sample for Electrolyte Test

Female albino Wistar rats were fasted for 18hours and divided into groups with four animals (n=4) per group. Each rat was administered 1ml of castor oil. After a duration of one hour, rats in group A was administered (oral) tween 20 (vehicle) for dissolving the extract; group ii rats were administered (oral) 2.5 mg/kg body weight of lomotil as a standard anti-diarrhea drug; group C, D, E and F rats were administered (oral) 21 mg/kg and 84 mg/kg body weight of ethanol and chloroform layers of the extract. After one hour of the treatment, the rats were sacrificed by cervical dislocation, the small intestine were located and tied at the pyloric sphincter and ileo caecal junction and cut out; the small intestine. The content of each intestine were milked out into a graduated test tube and the volume were recorded. The effluent from the intestinal loops (serosal solution) was centrifuge at 1500G for 30 minutes. The supernatant was obtained and analyzed for Na^+ and K^+.

3.4 Determination of Sodium Ion (Na^+) Concentration (Teco Diagnostic Kit)

The method is based on modification of method described by Tietz, [16] using Teco diagnostic kit in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate with the excess uranium then being reacted with ferrocyanide producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

3.5 Determination of Potassium Ion (K^+) Concentration (Teco Diagnostic Kit)

The method is based on modification of method described by Tietz [16] using Teco diagnostic kit. The amount of potassium ion is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension.

3.6 Statistical Analysis

The data obtained from the laboratory result of the tests were subjected to both one way and two way analysis of variance (ANOVA).

Significant differences were observed at p≤0.05. The results were expressed as mean and standard error of mean (SEM). These analyses were done using computer software known as Statistical Package for Social Sciences (SPSS), Version 16.

4. RESULTS

4.1 Acute Toxicity and Lethality (LD_{50}) Test

Oral administration of up to 5000 mg/kg body weight of chloroform-ethanol extract to mice caused no death in the two stages of the test. Thus, oral LD_{50} of the extract in mice was estimated to be greater than 5000 mg/kg body weight.

4.2 Phytochemical Test

The qualitative phytochemical compositions as observed in Table 1 showed relatively moderate presence of bioactive compounds such as flavonoid and reducing sugar in the three layers. The chloroform and middle layers showed relatively moderate presence of alkaloid, saponin, fat and oil. Glycoside and steroid were relatively present in low concentration while ethanol layer showed moderate presence of steroid only and low concentration of glycoside, saponin and alkaloid were apparently present. The bioactive compounds found to be relatively absent in the extracts were tannins and carbohydrate as shown in Table 1.

4.3 Effect of Ethanol and Chloroform Layers of *A. occidentale* Kernel Extract on the Serosal Fluid Sodium Ion Concentration

Result shown in Table 2 indicates that ethanol and chloroform layers of cashew (*A. occidentale*) kernel extract at different doses caused significant (P<0.05) reduction of serosal fluid sodium ion concentration compared with the rats induced with castor oil only. The rats dosed with ethanol layer of the extract (84 mg/kg body weight) showed significant (P<0.05) increase in sodium ion concentration to the rats in the standard drug group, while the animals administered ethanol layer (21 mg/kg b.w) and chloroform layer (21 mg/kg and 84 mg/kg b.w) of the extract showed increase in the serosal fluid sodium concentration compared with the rats administered the standard drug (lomotil).

4.4 Effect of Ethanol and Chloroform Layers of *A. occidentale* Kernel Extract on the Serosal Fluid Potassium Ion Concentration

Result shown in Table 3 indicates that ethanol and chloroform layers of Cashew (*A. occidentale*) kernel extract at different doses significantly (P<0.05) reduced serosal fluid potassium ion concentration compared with the control group. The rats administered chloroform layer (84 mg/kg b.w) of the extract showed significant (P<0.05) reduction in the potassium ion (K^+) to the rats administered the standard drug. Although all the rats treated with the extract showed significant (P<0.05) reduction in the serosal fluid potassium ion concentration, but the rats administered ethanol layer (21 mg/kg and 84 mg/kg b.w) and chloroform layer (21 mg/kg b.w) of the extract showed significant (P<0.05) reduction compared with the standard drug group (Table 3).

5. DISCUSSION

Usually the chloroform-ethanol extract yields two layers after partitioning with water. Surprisingly, in this study partitioning of the chloroform-ethanol extract yielded three layers consistently (n=3). Even in the presence of potassium chloride (KCl) or sodium chloride (NaCl) there were still three layers.

In all animals, oral administration of castor oil induced/produced diarrhea. The results of the study on electrolytes transport showed that the extract caused absorptive efflux of potassium and sodium ion from the serosal solution to the merosal solution to varying extents and antagonized the ion transport alteration effects of castor oil on K^+ and Na^+ fluxes. From these one may deduce that the extract induced hyperpolarization (i.e. the absorptive flux of potassium ion). Net movement of K^+ in jejunum and ileum occurs only down the electrochemical gradient i.e. largely by passive transport [17]. Potassium diffuses primarily through the lateral spaces and tight junction. It is apparent from the results that the ability of the extract to cause hyperpolarization may account for its inhibition of castor oil induced diarrhea. The extract may therefore exert its anti-diarrheal effect by promoting water and electrolyte absorption.

The specific constituent responsible for the anti-diarrheal properties of cashew kernel is yet to be identified. None of the several phytochemical

Table 1. Qualitative phytochemical constituents of the chloroform-ethanol extract of *Anacardium occidentale*

Phytochemical constituents	Ethanol layer	Chloroform layer	Middle layer
Flavonoid	++	++	++
Alkaloid	+	++	++
Carbohydrate	ND	ND	ND
Saponin	+	++	++
Tannins	ND	ND	ND
Fat and oil	ND	++	++
Reducing sugar	++	++	++
Glycoside	+	+	+
Sterol	++	+	+

Key: ND= Not detected, + = Low, ++ = Moderate

Table 2. Effect of ethanol and chloroform layers of *Anacardium occidentale* kernel extract on the serosal fluid sodium ion concentration

Group	Treatment	Dose	Sodium ion (Na^+) concentration (mEq/L)
A	Control	1ml	267.75±6.01
B	Lomotil	5 mg/kg b.w	190.4±5.80
C	Ethanol layer	21 mg/kg b.w	219.5±12.30*
D	Ethanol layer	84 mg/kg b.w	198.9±12.02*
E	Chloroform layer	21 mg/kg b.w	230.35±6.01*
F	Chloroform layer	84 mg/kg b.w	202.0±4.67*

*Values in mean ± SD: *represents significant difference at p< 0.05*

Table 3. Effect of ethanol and chloroform layers of *A. occidentale* kernel extract on the serosal fluid potassium ion concentration

Group	Treatment	Dose	Potassium ion (K^+) concentration (mEq/L)
A	Control	1 ml	13.50±4.82
B	Lomotil	5 mg/kg b.w	6.77±0.45
C	Ethanol layer	21 mg/kg b.w	10.72±1.67*
D	Ethanol layer	84 mg/kg b.w	10.23±0.83*
E	Chloroform layer	21 mg/kg b.w	9.00±0.91
F	Chloroform layer	84 mg/kg b.w	7.77±0.31*

*Values in mean ± SD: *represents significant difference at p< 0.05*

constituents identified from the extracts has been reported to possess anti-diarrheal properties. Although, the result of the qualitative phytochemical analysis observed in this study showed moderate presence of such bioactive compounds as flavonoids and reducing sugars in the three layers; alkaloids, saponins and fats and oil in the chloroform and middle layers. The bioactive compound that was not detected in the three layers of the extract was tannins. However, the experimental data from this study is insufficient to directly ascribe the anti-diarrheal activity to any of the three layers of the extract.

Acute toxicity test on the extracts in mice established a high LD_{50} value of less or equal to 5000 mg/kg body weight which suggests that the kernels may be generally regarded as safe with a remote risk of acute intoxication. The high degree of safety is also consistent with its popular use of the kernel as food [18].

6. CONCLUSION

The present study revealed some of the pharmacological basis for the ethnomedical use of Cashew (*A. occidentale*) kernel in the treatment of diarrhea. Results of this study showed that the constituents of Cashew (*A. occidentale*) kernel possess anti-diarrhoeal properties mediated through absorption of water and electrolytes from the serosal solution. The chloroform layer showed decreases in the serosal fluid electrolyte which indicate that it

contain more anti-diarrheal compounds which can be elucidated in further studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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