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Full Length Research Paper

Antidiarrhoeal activity of cashew (*Anacardium occidentale*) leaf extract enriched with zinc in wistar albino rats

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This study investigated the antidiarrhoeal activity of zinc, aqueous extract of cashew leaf and zinc enriched leaf extract. Diarrhoea was induced in wistar albino rats by the administration of 2 ml of castor oil. The negative control group received normal saline while the positive control group received loperamide- a standard antidiarrhoeal drug. Zinc (15, 30 and 45 mg/kg), aqueous extracts of cashew leaf (100, 250 and 500 mg/kg) and Zinc plus extract (500 mg/kg extract with 15 mg/kg zinc), were administered orally to different groups of rats. Diarrhoealfaeces and colonal nitric oxide concentration were determined. The lowest dose of 15 mg/kg zinc showed the highest significant reduction in both the diarrhoealfaeces and colonal nitric oxide concentration relative to the castor oil control group. Aqueous extract (500 mg/kg) had the highest reduction in diarrhoealfaeces and colonal nitric oxide concentration relative to the castor oil control group.There was a higher reduction in diarrhoealfaeces and colonal nitric oxide concentration in extract with zinc group than that obtained for the cashew leaf alone. An excellent antidiarrhoeal activity was obtained on the co-administration of loperamide and zinc. The result showed that zinc, cashew leaf and cashew leaf extract enriched with zinc are good antidiarrhoeal agents but the later gave the best antidiarrhoeal activity.

Key words: *Anarcadium occidentale,* antidiarrheal activity, castor oil, nitric oxide, nitric oxide synthase.

INTRODUCTION

Diarrhoea is the passing out of increased amount of loose stool as a result of an irritation of the mucous membrane of the intestine, resulting in imbalance between secretion and absorption of water (Kazi and Henry, 2006). Nitric oxide, an endogenous signaling molecule has been considered a mediator of the pathological conditions of diarrhoea and an effector substance in both laxatives and antidiarrhoeal agents (Mourad, 1999). Nitric oxide (NO) is synthesised from L-arginine by the enzyme nitric oxide synthase (NOS). This complex reaction involves the transfer of electrons from NADPH, via the flavins FAD and FMN in the carboxy-terminal reductase domain,tothehaem in the amino-terminal oxygenase domain, where the substrate L-arginine is oxidised to L-citrulline and NO (Andrew and

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Mayer,1999). The enzyme is a homodimeric protein with 125- to 160-kDa per monomer and the haem is responsible for the dimerization (Andrew and Mayer, 1999). The dimer is structurally stabilized by a zinc ion which is situated at the oxygenase domain interface of the dimer (Fischmann et al., 1999). The zinc ion is tetrahedrally coordinated by four cysteine (two from each monomer - Cys109 and Cys104). In mammals, NOS appears as 3 isozymes: neuronal NOS (nNOS), cytokine-inducible NOS (iNOS) and endothelial NOS (eNOS). The zinc ion is found at a region which connects the N-terminal hook and the subunit core. The coordination of zinc arranges the N-terminal hooks so that they interact with their own subunit. However, when there is no zinc ion present, two of the thiolate ligands (cysteines) form a disulfide bond connecting the two subunits (Craneet al., 1999). Little wonder both nitric oxide and zinc is implicated in diarrhoea. It is a public health problem, generally caused by poor sanitation and hygiene and kills some 194,000 Nigerian children under five every year (UNICEF, 2012). As diarrhoeal diseases are oral in nature, one of the simplest and most inexpensive barriers to infection is hand washing with soap or ash at critical times, such as before handling food and after defecation or changing a diaper. Hygiene promotion and proper hand washing is the most cost effective of public health intervention against diarrhoeal diseases. However, poverty is inextricably linked to poor sanitation and hygiene in developing countries. It therefore appears that poor sanitation and hygiene persist as poverty persists in developing countries and the use of antidiarrhoeal drugs are indispensable. Antidiarrhoeal drugs are expensive and are not easily accessible by majority of the rural poor people who still rely on traditional healing practices and medicinal plants for their daily health care needs (Jebunnessa et al., 2009), despite immense technological advancement in modern medicine. Cashew is a multipurpose tree of the Amazon that grows up to 15 m high. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground. Cashew trees are often found growing wild on the drier sandy soils in the central plains of Brazil and are cultivated in many parts of the Amazon rainforest (Abulude et al*.,* 2010). Cashew leaf or bark tea is still widely used throughout the tropics as an effective diarrhoea and colic remedy, considered gentle enough for children (Aiswariya et al., 2011). Zinc deficiency often develops with gastrointestinal manifestation including diarrhoea, abdominal pain, vomiting and fever (Li et al., 1999) indicating that the intestine is one of the tissues most sensitive to zinc deficiency. Intestinal zinc loss has been reported in children with dehydrating diarrhoea (Bhutta et al*.,* 2000). The objective of this work therefore, is to establish a scientific basis for the use of the cashew leaf as an antidiarrhoeal agent and to establish its further efficacy when enriched with zinc.

MATERIALS AND METHODS

Animals and diet

Wistar albino rats obtained from the animal house of Veterinary Department of University of Nigeria, weighing between 64 – 72 g were used for the study. The animals were acclimatized at room temperature in a standard wire cages for 7 days prior to commencement of the experiment. During the period of study, the animals were fed with standard diet and water. In this study, all the animals experimentation were carried out according to the standard ethics and guidelines.

Collection of plant materials

Fresh leaves of cashew tree were collected. Botanic identification was performed at the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria and voucher number assigned was NAU/291.

Preparation of aqueous cashew leaf extract

Fresh samples of cashew leaves were washed, chopped into smaller pieces, air dried in the laboratory and then ground to powder. 300 g of the powder was mixed with 250 ml distilled water for 24 h. The mixture was filtered using a glass funnel plugged with a white sieve cloth. The resulting filtrate was evaporated using gentle heating at 20°C using water bath. A deep brownish coloured (10 g) concentrated extract was obtained which was kept in refrigerator at 4°C.

Chemicals

Loperamide (reference antidiarrhoeal drug), castor oil (laxative) were purchased from Golden pharmaceutical, Ltd, Enugu, Nigeria. Zinc gluconate, citrullin solution, diacetylmonoxime, potassium persulphate, trichloroacetic acid, saline, acetate buffer and urease solution were of pharmacological and analytical grade and were purchased from Sigma Chemical Company Ltd, St. Louis, U.S.A.

Induction of diarrhea

The method proposed by Meite et al. *(*2009) was used to induce diarrhoea. Adult rats were fasted for 18 h and

Table 1. The effect of zinc on castor oil induced diarrhoea in wistar albino rats.

Treatment	Total amount of faeces	Total amount of wet faeces	% reduction of diarrhea	Concentration of nitric oxide (mg/100ml)
Distilled water (2ml)	7.3 ±3.06	0.00± 0.00		0.72± 0.04
Castor oil (2ml)	13.7 ±1.53[a]	12.0±1.73	0.00	1.62 ±1.730[a]
Zinc (15mg/kg)	11.66±2.08[ab]	6.67±1.53[b]	44%	1.29±0.070[ab]
Zinc (30mg/kg)	12.7±1.53[ab]	9.30±1.53[b]	22.5%	1.31±0.050[ab]
Zinc (45mg/kg)	12.60±0.58[ab]	9.00±2.65[b]	25%	1.34±0.035[ab]
Loperamide(5mg/kg)	9.67±1.52[ab]	4.00±2.65[b]	66%	0.94±0.040[ab]
Zinc(15mg/kg) + loperamide	6.60±3.22[bc]	3.00±0.70[b]	75%	0.83±0.066[ab]

Result = mean S.E.M. [a]significantly higher compared to normal control. [b]Significantly lower than the castor oil control group. [c]Significantly lower than normal group. P < 0.01.

Table 2. Effect of aqueous cashew leaf extract on castor oil induced diarrhea.

Treatment	Total amount offaeces	Total amount of wet faeces	% reduction of diarrhoea	Concentration of nitric oxide (mg/100 ml)
Distilled water (2ml)	7.3±3.06	0.00±0.00		0.72±0.04
Castor oil (2ml)	13.7±1.53[a]	12.0±1.73	0.00	1.62±1.73[a]
Leaves extracts (100mg/kg)	13.33±2.08[a]	11.67±1.53	3%	1.56±0.07[ab]
Leaves extracts (250mg/kg)	10.67±1.53[ab]	8.67±1.15[b]	28%	1.47±0.10[ab]
Leaves extracts (500mg/kg)	11.70±1.16[ab]	7.00±1.00[b]	42%	1.40±0.05[ab]
Loperamide (5mg/kg)	9.67±1.52[ab]	4.00±2.65[b]	66%	0.94±0.04[ab]

Values are expressed as Mean ± S.E.M. [a]significantly higher compared to normal control. [b]significantly lower than the castor oil control group. [c]significantly lower than normal group. P < 0.01.

divided into groups of four animals each. Following the oral administration of 2 ml castor oil to induce diarrhea, the animals were treated and placed in separate wired cages for observation. The cages were covered with filter paper to identify both solid and diarrhoealfaeces. The total faeces and wet diarrhoealfeaces were recorded over a period of three hours. The percentage diarrhoea inhibition was calculated as function of the castor oil control:

% inhibition = (control-test) × 100/control.

The colonal nitric oxide concentration was determined by measuring colonalcitrullin concentration using a method described by Gornall and Hunter (1941) since both products were produced in equimolar concentration. Briefly, the colon was excised after 3 h of treatment, instilled isotonic saline and incubated with acetate buffer pH 5.0. Urease solution and trichloroacetic acid were added to break down urea and for deproteinisation respectively. After a brief heating, potassium per sulphate was added to the filtrate to develop citrullin color. The absorbance was read at 440 nm and citrullin concentration obtained using a citrullin standard calibration curve.

Data analysis

Data were analyzed using the students't' test distribution. The results were expressed as a mean ± S.E.M. At 99% confidence interval, $p < 0.01$ was considered statistically significant.

RESULTS

The effect of zinc (15, 30, 45 mg/kg) on castor oil induced diarrhoea in wistar albino rats are shown in Table 1. At 15 mg/kg, zinc significantly decreased ($p < 0.01$), the total number of wet faeces by 44% (6.67 ± 1.53) upon administration of castor oil (12.0 ± 1.73). Colonal nitric oxide concentration level also decreased significantly from (1.62 ± 1.73) mg/100 ml castor oil control group to (1.29 ± 0.07) mg/100 ml.

The effects of aqueous cashew leaf extract on castor oil induced diarrhea are shown in Table 2. The aqueous extract of cashew leaf showed significant antidiarrheal activity ($p < 0.01$) against castor oil induced diarrhoea in rats. It reduced the number of diarrhoealfeaces produced by castor oil administration from (12.0 ± 1.73) 100% control group to (11.6 ± 1.53) 3%, (8.67 ± 1.15)28%,

Table 3. The effect of zinc enriched cashew leaf extract on castor oil induced diarrhea.

Treatment	Total amount of faeces	Total amount of wet faeces	% inhibition of nitric oxide synthase	Concentraation of nitric oxide(mg/100ml)
Distilled water(2ml)	7.30± 3.06	0.00±0.00		0.72± 0.041
Castor oil(2ml)	13.70 ±1.53[a]	12.00±1.73	0.00	1.62 ±1.73[a]
Zinc(15mg/kg)+leaves (500mg/kg)	9.30± 3.06[ab]	4.33±1.53[bc]	64%	1.16 ±0.072[ab]

Results are expressed as mean S.E.M. [a]significantly higher compared to normal control. [b]Significantly lower than the castor oil control group. [c]Significantly lower than normal group. $P < 0.01$.

(7.00 ± 1.00) 42% when experimental animals were respectively administered 100, 250 and 500 mg/kg of leaf extracts.

The effects of zinc enriched cashew leaf extract on castor oil induced diarrhea are shown in Table 3. Zinc-enriched leaf extractsgave 9.30 ± 3.06and 4.33 ± 1.53 as the total amount of faeces and total amount of wet faeces respectively while the % inhibition of nitric oxide synthase and the concentration of nitric oxide are 64% and 1.16 ± 0.072 mg/100 ml respectively.

Discussion

As shown in Table 1, zinc-enriched loperamide at 75% reduction (3.00 ± 0.70) has a better antidiarrheal activity than loperamide alone with diarrhoea reduction of 66% (4.00 ± 2.65). Colonal nitric oxide concentration showed the same pattern. Although the pathophysiological mechanism that either link zinc deficiency with severe diarrhoea or explain the efficacy of zinc in reducing diarrhoea is not yet well understood, successful clinical studies concluded that the possible mechanism for the beneficial effect of zinc on the duration of diarrhea involves improved absorption of water and electrolyte by the intestine and faster regeneration of gut epithelium (Mourad, 1999). Another publication established that zinc inhibits cAMP-induced calcium-secretion by inhibiting basolateral potassium channel in *in-vitro* studies with rat ileum (Kazi and Henry, 2006). The leaf extract (Table 2) is believed to stimulate the re-absorption of water from the intestinal lumen resulting to the normalization of the deranged water transported across the mucosal cells which are seen in the type of faeces produced. Cashew leaf extracts also reduced the colonal concentration of nitric oxide, the endogenous mediator of diarrhoea from castor oil control group of 1.62 ± 1.73 mg/100 ml to 1.56 ± 0.07, 1.47 ± 0.10, 1.40 ± 0.05mg/100ml when 100, 250, 500 mg/kg of leaf extracts where administered. Though the reduction was not significant, the result suggests that the extracts might have an inhibitory effect on colonal nitric oxide synthase. As shown in Table 3, zinc-enriched leaf extracts showed a better antidiarrheal activity by significantly reducing both diarrhoealfaeces and colonal nitric oxide from castor oil control group 12.00 ± 1.73 and 1.62 ± 1.73 mg/100 ml to (4.33 ± 1.53) 64% and (1.16 ± 0.072) mg/100 ml respectively.

Conclusion

The results strongly established that zinc, cashew leaf extract and zinc-enriched cashew leaf extracts are good antidiarrhoeal agents of plant origin.

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