Original Research Article

Bambara Groundnut (*Vignasubterranea*) Condiment Extract Reversed Diarrhoeal Condition in Male Wistar Rats

ABSTRACT

Aims: The goal of the investigation is to verify the anti-diarrhoeal properties of bambara groundnut seed condiment.

Study design: The study involved thirty male Wistar rats divided into five groups. Rats in groups 2,3, and 4 received different dosages of the methanol extract of bambara groundnut condiment (BGNCE). Group 1 served as a control and received loperamide, a standard medication for treating diarrhoea, while group 5 received distilled water. The rats were subjected to castor oil to induce diarrhoea, and various parameters were evaluated over a four-hour period.

Place and Duration of Study: The study was conductedat the Department of Veterinary Biochemistry and Physiology, Faculty of Veterinary Medicine, University of Ibadan, and theDepartment of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta; between July, 2017 and November, 2017.

Methodology:.The methodology involved administering castor oil to induce diarrhoea, evaluating various diarrhoea parameters over four hours, and analyzing serum concentrations of albumin, creatinine, blood urea nitrogen (BUN), and electrolytes (K+, Na+, Cl-) using standard methods.

Results: The rats in group 1 (loperamide) showed the highest level of inhibition of diarrhoea (100%), while rats in group 5 (distilled water) had the least inhibition (21.85%). The treatment withbambara groundnut condiment extract improved kidney and liver functions in rats, as indicated by changes in blood urea nitrogen(18.80 mg/dlvs 10.67 mg/dl);creatinine(0.83mg/dlvs 0.74 mg/dl); and albumin (0.89 g/dlvs 0.88 g/dl), for rat groups 4 and 1 respectively. BGNCE also up-regulated serum concentrations (mmol/L) of (K^+, Na^+, Cl^-) ions close to control.

Conclusion: The study concludes that bambara groundnut condiment have anti-diarrhoeal potential, and untreated acute diarrhoea can impair liver and kidney functions in rats.

Keywords: Diarrhoea, Bambara groundnut seeds, Albumin, Blood Urea Nitrogen, Creatinine,

1. INTRODUCTION

Diarrhoea is a deviation in an animal's or human's regular bowel movement, it is distinguished by an increase in the frequency, volume, and water content of the stool, and abdominal pain. Diarrhoea is a serious public health challenge[6]. The intestinal lumen fills with water, ions, or electrolytes during diarrhoea; inadequate reabsorption of these materials and other nutrients causes the frequent passing of watery stools [14]. Diarrhoea is the most common cause of morbidity and mortality in many developing countries [17]. Diarrhea can be caused by various factors, such as infections, food intolerances, intestinal disorders and so on.Entero-toxins, which are generated by bacteria such as *Salmonella typhi, Escherichia coli, and Vibrio cholera;* viruses such as *rota virus*; and parasites such as *Giardia intestinalis*, are frequently fingered in diarrhoeal infections [13]. Children, especially infants are mostly affected by diarrhoea because their immune system is not well developed. The micro-organisms causing diarrhoea are usually food borne, thus when food providers like mothers or house minders prepare food without proper washing of hands, diarrhoea may be acquired from food or water that has been contaminated byfaeces. Diarrhoea usually lasts for a few days, but results in fluid loss. About 2 billion cases of diarrhoea disease are reported globally every year; out of this number, 1.9 million children (less than 5 years of age), die annually, especially in developing countries [10]. In Nigeria, prevalence of diarrhoea is estimated at 18.8 % [17]. Currently, the treatment of diarrhoea is not yet specific and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movements. Chemical drugs are often used to treat diarrhoea, but these anti-diarrhoeal medications can occasionally have negative side effects on users [8] Moreover, microbes also develop resistance towards them, making such medications to be ineffective [11].One significant area of ongoing research is the pursuitof safer and more affordable anti-diarrhoeal medications derived from plants, or "phyto-medicine." Phytotherapy, or the use of plants to treat illnesses, has long been a significant aspect of Nigerian indigenous culture, regardless of a person's tribe.Fermentation is a food processing technique that has been embraced in traditional medicine.

When legumes are fermented, the product formed is called condiment. A condiment is a flavor-enhancing ingredient that can be added to food in the form of sauce, powder, spread, or anything similar. [2]. Fermented locust bean (iru), fermented melon seed (ogiri), fermented soybean (dawadawa), and fermented cotton seed (ogiriowu) are among the common legume condiments in Nigeria. Fermentation of legumes leads to the liberation of polyphenol aglycones, by the catalytic action of microbial β-glucosidase enzyme [1].Studies conducted by Jideani and Diedericks [12] demonstrated the protective effects of plant-based diets on the control of diarrhoea and its related complications. African traditional medicine also asserted that in certain regions of South Africa and Kenya, a steep made by soaking bambara groundnut seeds overnight is used to alleviate diarrhoea [4]. Undocumented reports claim that travelers in Nigeria consume okpa, a food product made from bambara groundnut seeds, to avoid stooling while on a long journey. Notably, the authors are not aware of any scientific evidence that supports this assertion, which is why this investigation was necessary.

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2. MATERIAL AND METHODS

2.1 Kits for diagnostics

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The ELISA kits for determination of albumin, blood urea nitrogen (BUN) and bilirubin were Labkit products from Chemelex, South Africa. Diagnostic kits for the determination of sodium (Na^+) and potassium (K^+) ions as well as creatinine were from Inteco Diagnostics, U.K Kit for Chloride ion (Cl^-) determination was Labkit obtained from Chemelex, South Africa.

2.2 Preparation of bambara groundnut condiment extract (BGNCE)

Previously made bambara groundnut condiment flour(250 g) was macerated in 2500 ml of 70% purity methanol and let to soak for 72 hours. After that, the mixture was filtered through Whatman No. 1 filter paper. Using a vacuum rotary evaporator (Buchi Rotavapor R210/215, Switzerland), the filtrate was concentrated under reduced pressure (204 mbar) at a controlled temperature (40 °C). After that, it was placedin a water bath at 40 °C to allow the solvent to evaporate. The extract yield as a percentage was assessed.

2.3 Oral acute toxicity study of bambara groundnut condiment extract (BGNCE) in Wistar rats

This was done by the modified fixed dose method of OECD [16]. Nine rats used for the experiment were allowed to acclimatise for 7 days. On the 7th day, the animals were fasted overnight. On the 8th day, each rat received single dose of 5000 mg of the BGNCE by oral gavage. Rats were monitored over twenty four hours for toxicity signs (convulsion, hypo-activity, hyper-activity, salivation, ataxia, weakness, micturition, and respiratory disorders).

2.4 Evaluation of the anti-diarrhoeal activities of bambara groundnut condiment extract (BGNCE)

According to Lakshimanarayana et al. [14] the anti-diarrhoeal properties of bambara groundnut condiment extract were assessed. Thirty male wistar rats were bought from Covenant Farm Nigeria Enterprises, Ibadan. The rats were sorted and divided into 5 groups (of six rats each). Each rat was housed in a cage within the Allentown-M0-3-4-D-34-stainless steel metabolic cages made specifically to collect urine and faeces separately. Rats were kept in the animal house of the Department of Veterinary Biochemistry and Physiology, Faculty of Veterinary Medicine, University of Ibadan. The conditions there included a 12 hour/12 hour light/dark cycle, a temperature of 27°C, and a humidity of 55%. The rats were provided free access to a commercial pelleted meal and unlimited water during their one-week acclimatisation period. Treatment of the animals followed; and group 1 rats were given 2 mg/kg bwloperamide (Loperamide hydrochloride 2 mg, Diatex, Eurolife Healthcare PVT, India). Group 1 rats served as control. Group 5 rats were given distilled water, while groups

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2, 3, and 4 rats were given 100, 250, and 500 mg/kg bw methanol extract of bambara groundnut condiment (BGNCE), respectively. Two hours after dosing, each rat received 1 ml castor oil orally to induce diarrhoea and was monitored for the next four hours on some diarrhoeal parameters; specifically, onset time of diarrhoeafaeces, number of wet faeces and total number of faeces were assessed.The faeces were collected in plastic dishes placed at the base of the cages. Blood samples were taken from the jugular vein into plain sample bottles to obtain serum. Concentrations of albumin, blood urea nitrogen, creatinine, potassium (K^+), sodium (Na^+), chloride (Cl^-) ions were assessed in the serum.

2.5 Determination of electrolyte concentrations (Na^+, K^+, Cl^- ions) in serum of Rats

Colrimetric kits (MPRN401, MPRKTB2), manufactured by INTECO Diagnostics Ltd. (UK) were used to assay for sodium ion (Na^+) and potassium ion (K^+) concentrations respectively. The concentration of chloride ions (Cl^-) was measured using a spectrophotometric kit (LKBsDTT10) made available by CHEMELEX, S.A. The process followed the instructions included in the kit manuals.

2.5.1 Determination of Na^+ ion concentration in serum of diarrhoeal rats

The labels 'blank', 'standard' and 'samples' were put on respective Eppendof tubes. A 1000 µl dose of sodium reagent (R1) was pipetted into every tube. Then, 10 µl of sample and 10 µl standard were pipetted into their respective eppendoff tubes. The mixture was allowed to sit at room temperature for five minutes. At 630 nm, the absorbance of the samples and standard was measured in relation to a reagent blank.

Concentration of Na^+ ion in the sample was calculated by the formula:

$$Concentration\ of\ Na^+ = \frac{Absorbance\ \ of\ sample}{Absorbance\ \ of\ standard} x\ sodium\ standard\ concentration \text{--------1}$$

Where sodium standard concentration = 150 mmol/ Litre, Na = Sodium; Na^+ = sodium ion

2.5.2 Determination of potassium ion (K^+) concentration in serum of diarrhoeal rats

Eppendof tubes were labelled with tags of blank, standard and samples. Tetraphenyl boron (TPB) reagent (1000 µl) was put in all the eppendof tubes; this was followed by the addition of 20 µl of sample into the sample tubes, while 20 µl of standard was put in the standard tube. The mixture was incubated for 10 minutes at room temperature. Absorbances of standard and samples were taken against reagent blank at 630 nm. Concentration of K^+ ion in the sample was calculated by the formula:

$$Concentration\ of\ K^+ = \frac{Absorbance\ \ of\ sample}{Absorbance\ \ of\ standard} x\ Concentation\ of\ Potassium\ standard \text{ -----2}$$

Where concentration of potassium standard = 5.0 mmol/ Litre,

K^+ = potassium ion

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TPB reagent comprise sodium tetraphenyl boron (0.2 mol / Litre), sodium hydroxide (2.2 mol / Litre), preservative (0.1 %)

2.5.3Determination of chloride ion (Cl^-) concentration in serum of diarrhoeal rats

Eppendorf tubes were labelled as blank, standard and samples. Reagent 1 i.e. thiocyanate mercury (1000 µl) was put into each tube by micro pipette. 10 µl of sample was also added into the sample tubes, while 10 µl of standard was put in the standard tube. The mixture was incubated for 5 minutes at room temperature. Absorbance of standard and samples were measured against reagent blank at 480 nm. Concentration of Cl^- ion in the sample was calculated by the formula:

$$Concentration\ of\ Cl^- = \frac{Absorbance\ \ of\ sample}{Absorbance\ \ of\ standard}\ x\ Concentration\ of\ chloride\ standard$$ -------3

Where concentration of Chloride standard = 125 mmol /Litre

Cl = Chlorine; Cl^- = Chloride ion

2.6 Assay of albumin, blood urea nitrogen and creatinineconcentrations in rat sera

Albumin, Blood Urea Nitrogen (BUN) and Creatinine were assayed using standard procedure as described in the manual of their respective spectrophotometric kits (Labkit-LkBsDTT07, Labkit-LkBsDTT35, INTECO kit-MPRCRE3).

2.6.1 Determination of albumin concentration in serum of diarrhoeal rats

Eppendof tubes were labelled respectively as blank, standard and samples. Each sample tube had 5 µl of sample added to 1000 µl of R. The standard tube had 5 µl of albumin standard added to 1000 µl of R. The blank tube had only 1000 µl of R. The mixtures were incubated at room temperature for 10 minutes after which absorbance of samples and albumin standard were read against blank in a spectrophotometer at 630 nm. Albumin concentration in the sample was determined using the formula:

$$Concentration\ of\ albumin = \frac{Absorbance\ \ of\ sample}{Absorbance\ \ of\ standard}\ x\ Concentration\ of\ standard$$------ 4

Concentration of albumin standard = 5 g/dl; R = bromocresol green

2.6.2Determination of creatinine concentration in serum of castor oil-induced diarrhoeal rats

Reagent (R) of 50 ml was created by combining 25 ml of R1 and R2 together. Eppendorf tubes were labelled as blank, standard and samples. In every tube, we pipetted 1000 µl of reagent R. Then, 100 µl of sample was added into each sample tube, while 100 µl of creatinine standard was put into the standard tube. The blank tube had only 1000 µl of reagent R. For aduration of thirty seconds, the mixtures were left to incubate. After that, the absorbance of samples and standard was measured against a blank at 492 nm (this was taken as A1). After turning on the

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stopwatch, another absorbance measurement was made precisely ninety seconds after the initial reading (this was taken as A2).

Concentration of creatinine was calculated thus:

$$Concentration\ of\ creatinine = \frac{A2\ sample\ -A1\ sample}{A2\ standard\ -A1stand\ ard}\ x\ Concentration\ of\ standard$$-------- 5

A2= Absorbance after 120s of incubation

A1= Absorbance after 30s of incubation

Concentration of creatinine standard = 2 mg/dl; R_1 = picric acid, R_2 = sodium hydroxide

2.6.3 Determination of blood urea nitrogen (BUN) concentration in serum of castor oil-induced diarrhoeal rats

Eppendof tubes were labelled as standard, blank, and samples respectively. Five microliter of urea standard was pipetted into the tube labeled standard, then 200 µl of R_1 was also added. The sample tubes had 5 µl of sample pipetted into each tube followed with the addition of 200 µl of R_1. The blank tube had only 200 µl of R_1 in it. The content in the tubes were mixed gently and allowed to stay for 1 minute. Then 200 µl of R_2 was added to each tube (i.e) samples, standard and blank tubes and incubated for 15 minutes at 37°C. Absorbance was read at 510 nm. Urea concentration was calculated as:

$$Urea\ concentration = \frac{Absorbance\ \ od\ sample}{Absorbance\ \ of\ standard}\ x\ concentration\ of\ \ standard$$ -------- 6

Concentration of standard Urea = 50 mg /dl, R1 = O-phthalaldehyde, R2 = Borate solution

2.7 Statistical analyses

SPSS (version 20) was used to analyze the data, using one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to segregate means. Data was presented as Mean ± S.E.M. At p = 0.05, differences were deemed significant.

3. RESULTS AND DISCUSSION

3.1Oral acute toxicity of bambara groundnut condiment extract (BGNCE) in Wistar rats

There was no mortality recorded at thedose level of 5000 mg/kg bwof bambara groundnut condiment extract (BGNCE) over the 24 hour period of examination. Furthermore, the rats did not show signs of restlessness, vomiting, micturition, salivation, drowsiness and aggression.

3.2Effects of bambara groundnut condiment extract (BGNCE) and loperamide On diarrhoeal rats

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The results of BGNCE on castor oil-induced diarrhoea in rats are shown in (Table 1). According to the findings, BGNCE, in a dose-dependent way, improved the diarrhoeal state in the rats. There was 100 % inhibition of diarrhoea in the rat group that received 2 mg/kg bwloperamide (a standard anti-diarrhoeal drug), while the percentage inhibition against diarrhoea for the rat groups that received 100, 250 and 500 mg per kg body weight of BGNCE was 28.57 %, 61.53 % and 80.00 % respectively. At all experimental dosage, bambara groundnut condiment extract (BGNCE) was able to improve the diarrhoeal state in castor oil-induced diarrhoeal rats; however, the amelioration was dose dependent. The rat groups that received BGNCE had aprolonged onset time of diarrhoea, in comparison with the group that had distilled water. There was adecrease in the number of wet faeces as well as anincrease in the percentage inhibition of defaecation, in contrast to the untreated rat group that received distilled water. Generally, anti-diarrhoeal medications act by decreasing the gastrointestinal and / or secretions; hence bambara groundnut condiment extract is an effective anti-diarrhoeal agent.

Table 1. Some diarrhoeal parameters in castor oil-induced diarrhoeal rats treated with bambara groundnut condiment extract (BGNCE) / lopramide

Parameters	Group 1 2mg/Kg bw Loperamide	Group 2 100 mg / Kg bw BGNCE	Group 3 250mg / Kg bw BGNCE	Group 4 500 mg / Kg bw BGNCE	Group 5 Distilled water
Onset-time for diarrhoealfaeces (min)	Not established	52.00± 2.3^{c}	75.00 ± 0.1^{b}	90.00 ± 5.6^{a}	30.00 ± 2.1^{d}
Number of wet faeces	0.00 ± 0.0^{e}	5.00±0.1^{b}	2.00±0.1^{c}	1.00 ± 0.0^{d}	10.16± 2.3^{a}
Total number of faeces	0.00±0.0^{d}	7.00±0.1^{b}	5.20±0.2^{c}	5.00 ±1.0^{c}	13. 00 ± 0.0^{a}
Inhibition of defaecation (%)	100±0.0^{a}	28.57±4.4^{d}	61.53±2.4^{c}	80.00±0.0^{b}	21.85 ± 0.1^{e}

Values expressed are Means of six determinations ± SEM. Means within the same row with different alphabets are significantly different (P = .05) BGNCE = bambara groundnut condiment extract

3.3Electrolyte concentrations (Na^{+}, K^{+}, Cl^{-} ions) in serum of diarrhoeal rats

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The electrolytes, i.e. potassium ion (K+), sodium ion (Na+), and chloride ion (Cl-) concentrations in the serum of diarrhoeal rats are summarized in (Figures 1, 2, and 3 respectively). The concentrations of the electrolytes were significantly lowest ($p<0.05$) in the rat group that was given distilled water. However, treatment with BGNCE increased the concentrations of the electrolytes in a dose dependent manner. The results of concentrations of the electrolytes (potassium ion (K^+), sodium ion (Na^+) and chloride ion (Cl^-) monitored in the experimental rats corroborated earlier reports byDo *et al*. [7] that diarrhoeal condition leads to loss of body electrolytes. Treatment of castor oil-induced diarrhoeal rats with bambara groundnut condiment extract at all experimental doses improved the diarrhoeal condition of the rats. This study revealed that bambara groundnut condiment extract has anti-electrolyte permeability action, and that is one of the mechanisms by which bambara groundnut condiment extract exhibits its anti-diarrhoeal action. Castor oil is hydrolyzed by lipases in the small intestine to glycerol and ricinoleic acid [18]. Through enhanced fluid and electrolyte secretion, ricinoleic acid predominantly operates in the small intestine to expedite the intestinal transition [14]. The release of ricinoleic acid in the small intestine, cause inflammation and irritation; thereby leading to the release of prostaglandins. High concentration of prostaglandins increases intestinal motility and discharges, resulting into diarrhoea [19]. The anti-diarrhoeaal action of bambara groundnut condiment against castor oil-induced diarrhoea is due to its anti-electrolyte permeability action [18].

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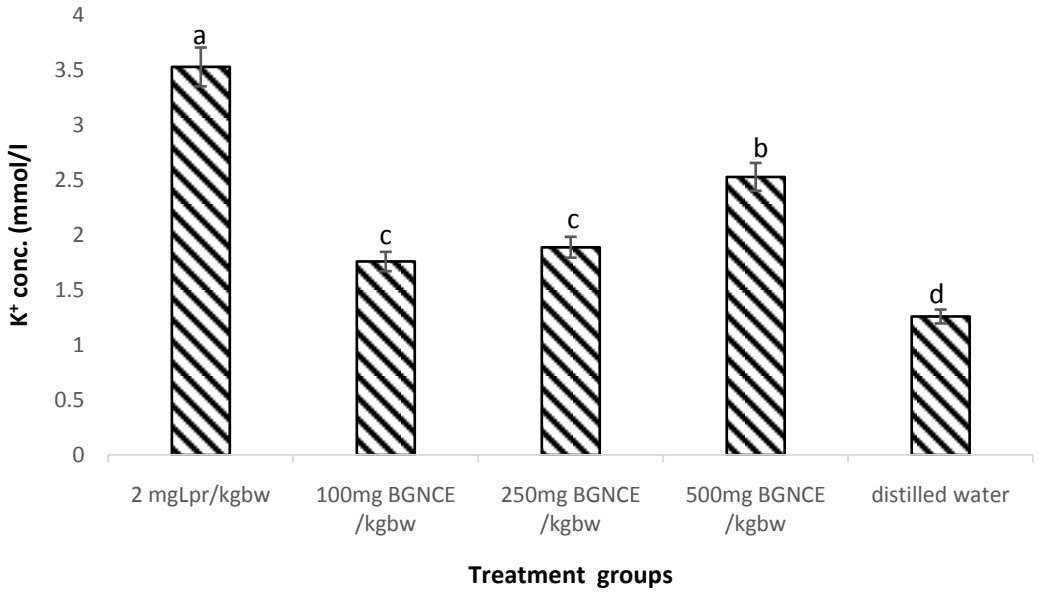

Fig.1.Potassium ion (K^+) concentration in serum of castor oil-induced diarrhoeal rats treated with BGNCE / Loperamide

Values expressed as Means of six determinations ± SEM. Means within the same row with different alphabets are significantly different (*P* = .05)

Lpr = Loperamide

BGNCE = Bambara groundnut condiment extract

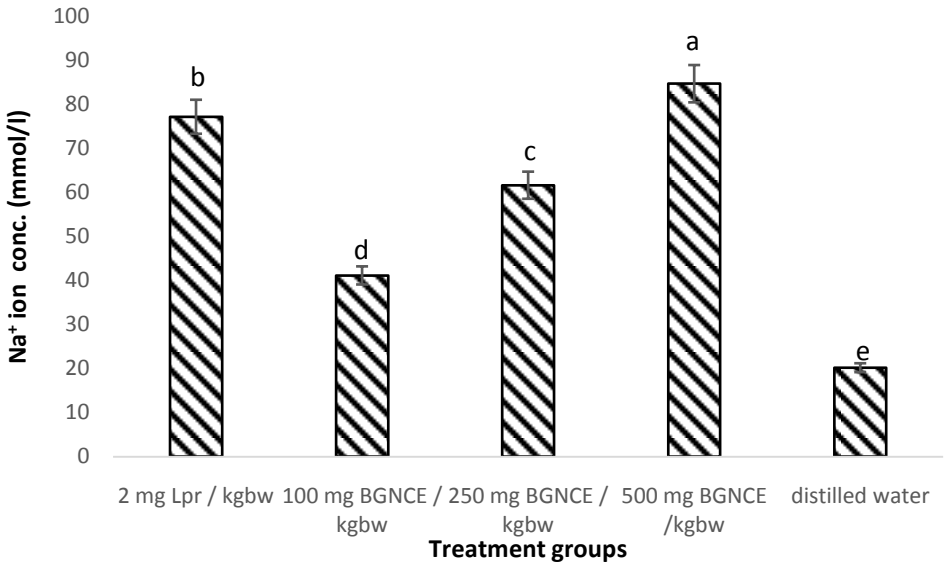

Fig 2: Sodium ion (Na^+) concentration in serum of castoroil-induced diarrhoeal rats treated with BGNCE / Loperamide.

Values expressed as Means of six determinations ± SEM. Means within the same row with different alphabets are significantly different (*P* = .05)

Lpr = Loperamide

BGNCE = Bambara groundnut condiment extract

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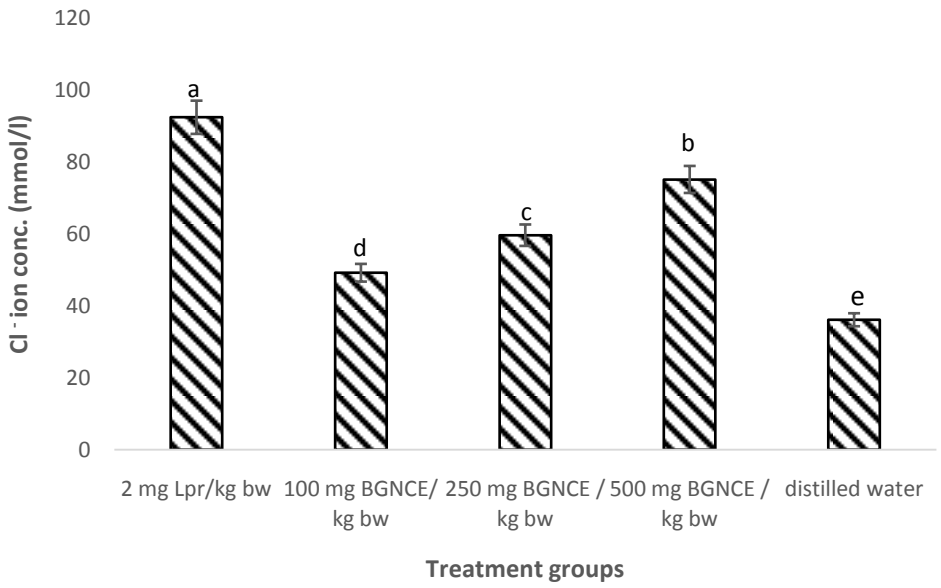

Fig. 3. Chloride ion (Cl^-) concentration in serum of castoroil-induced diarrhoeal rats treated with BGNCE and Loperamide.

Values expressed as Means of six determinations ± SEM. Means within the same row with different alphabets are significantly different (*P* = .05)
Lpr = Loperamide
BGNCE = Bambara groundnut condiment extract

3.4Serum concentrations of albumin, blood urea nitrogen and creatininein diarrhoealrats

The rat group that got standard medication (lopeamide) had the highest serum albumin concentration, whereas the rat group that received distilled water had the lowest concentration, as shown in (Figure4). Treatment of diarrhoeal rats with BGNCE increased the concentration of serum albumin and brought it close to control. The effect of BGNCE on the blood urea nitrogen (BUN) content of diarrhoeal rats is given in (Figure5). The rat group that was given distilled water only (not treated) had the highest concentration of BUN in the serum while the rat group that was given standard drug (loperamide) had the least BUN concentration. Treatment with BGNCE decreased the serum concentration of BUN close to the control; this same trend was observed in the result of creatinine concentration as displayed in (Figure 6). Elevated serum levels of urea and creatinine, which are normally eliminated by urine, are indicative of kidney problems. When kidney function is impaired, these chemicals are reabsorbed into the circulation, resulting in elevated serum levels. [9]. The result of this research revealed that untreated diarrhoea could lead to compromise in the function of the kidney as shown by elevated levels of urea and creatinine concentrations in

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the serum. The relative decrease in the concentration of albumin in the untreated diarrhoeal rats indicated that the liver is compromised. Albumin is a globular protein produced by the liver; it is responsible for thetransport of materials and theproper distribution of body fluids. The normal range of albumin is between 3.4 to 5.4 g/dl. Low concentration of serum albumin, as seen in the untreated diarrhoeal rats showed that the liver is compromised. Treatment of diarrhoeal rats with BGNCE ameliorated this condition. This result agrees with the report that acute diarrhoea resulted in impairment of renal function [5].

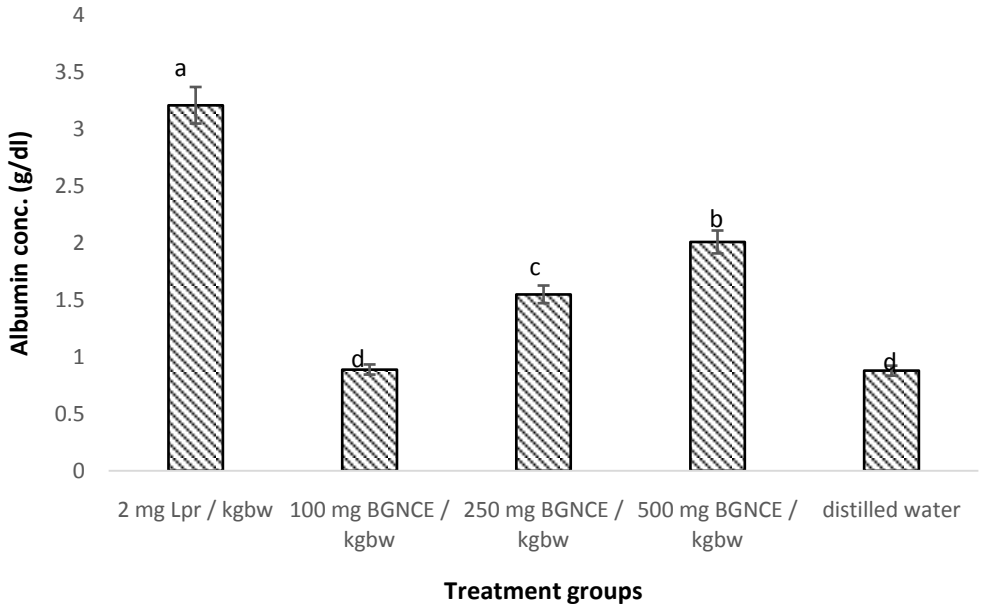

Fig. 4. Albumin concentration in serum of castoroil-induced diarrhoeal rats treated with BGNCE and Loperamide.

Values expressed as Means of six determinations ± SEM. Means within the same row with different alphabets are significantly different (*P* = .05)
Lpr = Loperamide
BGNCE = Bambara groundnut condiment extract

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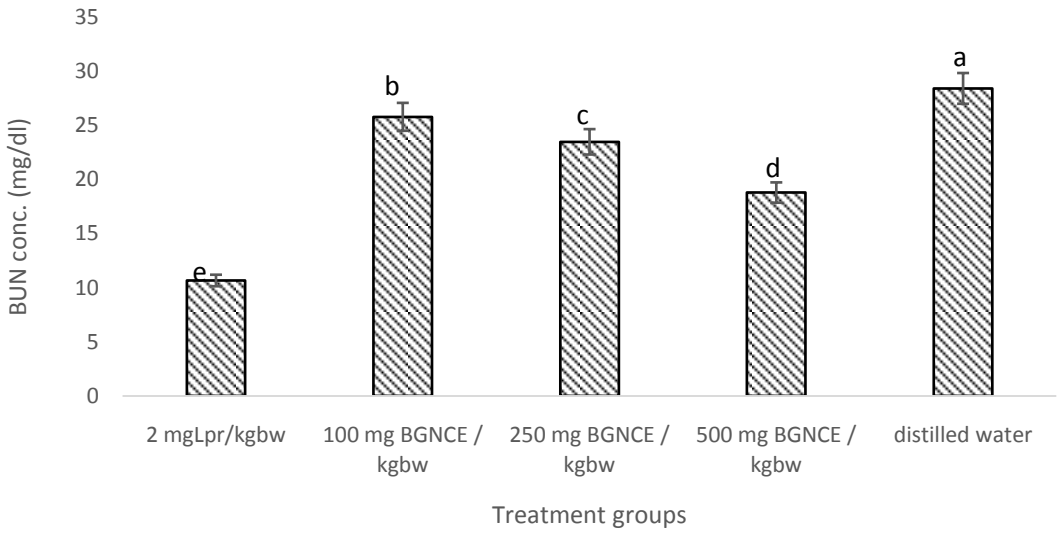

Fig. 5.Concentration of blood urea nitrogen (BUN) in serum of castoroil-induced diarrhoeal rats treated with BGNCE and Loperamide

Values expressed as Means of six determinations ± SEM. Means within the same row with different alphabets are significantly different (*P* = .05)
Lpr = Loperamide
BGNCE = Bambara groundnut condiment extract
BUN= Blood urea nitrogen

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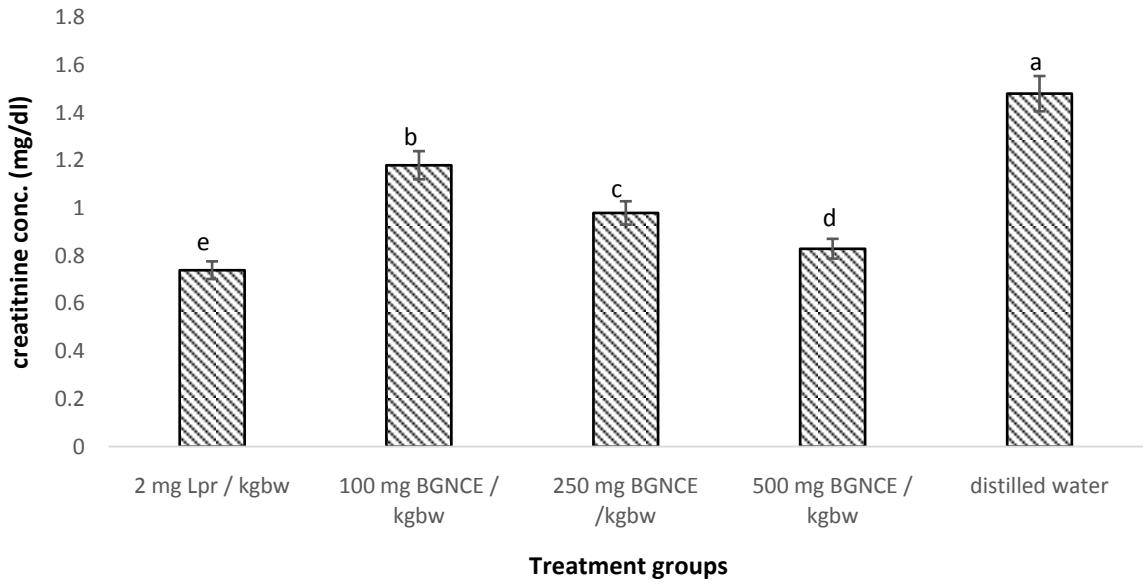

Fig. 6. Concentration of creatinine in serum of castoroil-induced diarrhoeal rats treated with BGNCE / Loperamide.

Values expressed as Means of six determinations ± SEM. Bars with different alphabets are significantly different (*P* = .05)
Lpr = Loperamide
BGNCE = Bambara groundnut condiment extract

4. CONCLUSION

This work revealed that BGNCE has antidiarrhoeal properties, which is due to its anti-electrolyte permeability action. Untreated acute diarrhoea impacts negatively on the ability of kidney and liver to function properly. The use of bambara groundnut seeds for the treatment of diarrhoea in folklore medicine was validated in this study**.**

ETHICAL APPROVAL

This study with the number PG 14/0101 was approved by the Post-graduate Review / Ethics Panel of the Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria. Authors hereby declare that animals were handled as stated in the “Principles of laboratory animal care” (NIH publication No 85-23, revised 1985) regarding the use of animals.

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