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Anti-oxidant and Anti-inflammatory Properties of Bambara groundnut (V*igna subterranean verde L.*) Condiment Extract (BGNCE) in Castor oil-induced Diarrhoeal Rats

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Abstract

Past works have established that bambara groundnut seeds are used in the treatment and prevention of diarrhoea. The mechanism by which bambara-groundnut-condiment extract (BGNCE) prevents diarrhoea was studied by assessing the anti-inflammatory as well as anti-oxidant properties of BGNCE (*in- vivo*). Two trials were carried out and each trial had 5 groups of rats (n=6). Diarrhoea was induced in rats using castor-oil. Rats with diarrhoea received the following treatment for seven days: the initial trial involved group 1 rats which received 2 mg loperamide (Lpr) per KGBW; while the second trial comprised group 1 rats which received 100 mg vitamin C per KGBW. In both trials, group 2 rats received 100 mg BGNCE per KGBW, group 3 rats received 250 mg BGNCE per KGBW, group 4 rats received 500 mg BGNCE per KGBW and rats in group 5 received distilled water. Group 1 was the control-group for each trial. After the trials, rats were bled into plain bottles, and serum was obtained from whole blood. From the first trial, inflammatory-indicators like TNF-alpha, NFK-beta and LDH (Lactate dehydrogenase) were assessed from serum. The activity of four anti-oxidants: superoxide-dismutase (SOD), catalase (CAT), reduced-glutathione (GSH), and glutathione-peroxidase (GPx) were assessed in the liver and serum for the second trial. The data was analysed using SPSS (version 20. BGNCE treatment lowered the levels of TNF-alpha, NFK-beta, and LDH in the serum of diarrhoeal-rats by 25.82–45.37%, 8.54–60.48%, and 8.63–21.06%, respectively. Also, treatment of diarrhoeal-rats with BGNCE up-regulated the activities of the monitored antioxidants in a dose dependent-manner. This work established that BGNCE plays its anti-diarrhoeal role through its anti-inflammatory and anti-oxidant properties.

Introduction

In Nigeria, diarrhoea constitutes a major public-health-problem, due to its effects on children under five years and thereby leading to high mortality. The incidence of diarrhoea among children, who are less than five-year-old in Nigeria was reported to be 18.8 percent according to Peter and Umar, (2018). The symptoms of diarrhoea include frequent passing of watery-stool at close intervals which is often associated with pains at the gastro-intestinal-tract. When diarrhoea is not treated, it can cause death especially in children within the age of five- year-old. Annually, about two-billion cases of diarrhoea-disease is reported globally; out of which, about 1.9 million children of less than five- years are dead in developing-countries (Getachew *et al*., 2018). It is worrisome to note however that children who survived from diarrhoea are likely to have defective intelligence-quotient. This was

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proven by the reported-data published by Pikerton *et al*. (2016) which stated that children who suffered severe-diarrhoea in early-stage of life were observed to have scored lower-grades in a series of tests measuring intelligence-quotient. When diarrhoea occurs, the effectiveness of oral-drugs is reduced due to the increased-rate of peristalsis through the gastro-intestinal-tract (G.I.T), and this prevents active-assimilation of the drug by the villi into the blood-stream. Pertinently, several attempts have been made in the treatment of diarrhoea and these include but not limited to: the use of anti-diarrhoeals like oral-rehydration-therapy (for young-children), giving of probiotics and continuous-feeding, as well as administration of drugs (Yakubu and Salimon, 2015). Conversely, some allergic-reactions such as vomiting, abdominal-cramps and light-headedness were associated with the use of anti-diarrhoeal-drugs (Faure, 2013). Prolonged-abuses of these drugs can also cause –resistance of the micro-organisms, and thereby rendering the use of anti-diarrhoeal-drugs in-effective (Gupta and Birdi, 2017). From the time immemorial, the use of phyto-therapy in the treatment of human-ailments and diseases has been an integral part of Nigeria's local-culture, irrespective of tribes. In essence, Nduche and Omosun (2016) listed some plants that have been used to treat diarrhoea, these included but not limited to the Roselle -calyces in form of zobo (*Hibiscus sabdariffa)*, stem of ginger (*Zingiber officinale*) and seeds of sweet basil (*Ocimum basilium*) among others. Bamishaye *et al.* (2011) emphasized that bambara-groundnut was used to treat diarrhoea, and this was corroborated by Jideani and Diedericks (2014). One of the food-processing techniques that have been documented to increase poly-phenol content of legumes, and improve their anti-oxidant properties is fermentation (Ademiluyi *et al*., 2010). In this research, fermented bambara-groundnut condiment was used in the treatment of diarrhoea induced-rats. The overall aim was to determine the anti-inflammatory and anti-oxidant properties of the bamabara-groundnut condiment extract using loperamide and vitamin C as controls for trial-1 and trial-2 respectively, *vis-a-viz* diarrhoea prevention.

Materials and Methods

Sample preparations: Bambara-groundnut-condiment extraction (BGNCE)

The modified technique of Farinde *et al.* (2007) was previously used to make Bambara-groundnut-condiment flour. By macerating 250 g of Bambara-groundnut-flour in 2,500 ml of 70% pure methanol for three days (72 hours), the flour was subjected to methanolic-extraction. Filtrate was produced by running aqueous-extract through Whatman No1 filter-paper. A vacuum rotary evaporator (Buchi Rota-vapor R210/215, Switzerland) was used to concentrate the aqueous-extract at a temperature and pressure of 40°C and 204 millibar, respectively. The solvent was then allowed to evaporate by placing the rotary evaporator on a water bath set at 40°C. The dry and crude-extract was weighed into a clean-bottle.

Acclimatisation of animals

Thirty male, healthy Wistar-rats weighing 200–220 grams were used for each study. The Covenant Farm, Ibadan, Oyo State provided the experimental-animals, which were housed in the Biochemistry Department's facility at the Federal University of Agriculture in Abeokuta. Proper-handling, care-and-use of rats followed the guidelines outlined in the National Institutes of Health's guidance for experimental-animals such as: 27°C, 55% humidity, and a 12-hour cycle of light and darkness were maintained in the room (NRC, 2011). Six rats were placed in each of the ten cages after the animals were sorted by weight and divided into five cages for each trial. Every day, wood shaving bedding was changed to keep the area tidy. Each trial's cages were clearly labeled, and the rats were identified by tail-tags. Rats were given unlimited amounts of rat food and water while they adjusted to their new habitat for seven days.

Induction of diarrhoea using castor-oil

After the seven-day acclimatization phase, rats were denied food overnight. The protocol for castor-oil-induction for diarrhea as prescribed in Yakubu and Salimon (2015) was adopted in this study. By oral gavage, 1.00 ml of castor-oil was given to each rat. The rats started having diarrhoea four hours after receiving castor-oil.

Trial one-determining concentration of inflame-matory-biomarkers in castor-oil-induced diarr-hoeal rats

The following medications were administered to diarrhoeal rats over the course of seven days: First group received 2 mg of loperamide (Lpr) per KGBW, second group received 100 mg BGNCE per KGBW, third group received 250 mg BGNCE per KGBW, fourth group received 500 mg BGNCE per KGBW, and fifth group received distilled water. Rats from first group were used as a control. Rats were sacrificed at the conclusion of the experiment, and blood was drawn from the jugular vein and placed in sample bottles. To get serum, the whole blood was centrifuged at 4,500 revolution per minute for 10 minutes, after standing at for 30 minutes at ambient temperature. Serum was carefully put into pristine eppendoff tubes and kept in storage at -4 °C for later use. Lactate dehydrogenase (LDH), TNF-alpha, and NF-k beta concentrations were measured in rat serum samples as markers for inflammation.

TNF-alpha concentration in rat serum

TNF-alpha concentration was measured using an ELISA kit from Elabscience with the model number CK-E30635. The ELISA plate was placed in the Biotek ELISA reader ELx 800 with Gen 5 software for ELISA reading once the reactions were finished as described in Table 1. At a wavelength of 450 nm, the absorbances of the wells were produced simultaneously. The quantity of TNF- in the samples was determined by comparing their absorbance to that of earlier prepared standard solutions (S0 to S5).

NF-k beta concentration in rat serum

Using the Elabscience ELISA kit with the model number CK-E92153, the concentration of Nuclear Factor Kappa beta (NF-k beta) was measured. The ELISA plate was placed in the Biotek ELISA reader ELx 800 with Gen 5 software for ELISA reading, once the reactions were finished as described in Table 2. The values of each well's absorbance were generated at 450 nm wavelength. The concentrations of the NF-k beta in the samples were extrapolated from the graph of absorbance against concentration earlier generated for the standard solutions (S0 to S5).

Determination of LDH concentration in sera of rats

The LDH kit from Cypress Diagnostics, Belgium, CEHBEL041 was used. Spectrophotometric kinetic assay was performed to evaluate the activities of LDH in the serum of diarrhoeal rats. The entire reagent set was taken out of the freezer and allowed to come to room temperature. 100 µl of the sample were combined with 3 ml of the working reagent which comprise imidazole-65 millimolar per litre, pyruvate-0.6 millimolar per litre, and NADH-0.18 millimolar per litre. The resultant mixture was stirred for one minute. At start point, (taken as 0 seconds), absorbance of the mixture was read; readings were also taken once per minute for the next three minutes i.e. at 60, 120 and 180 seconds. Variation in absorbance readings per minute was calculated and used to evaluate activity of LDH in the samples.

$$\text{LDH (U / L)} = \Delta\text{Abs/min x } 4{,}925$$

4,925 is the concentration of reference solution

Trial 2-Determination of *in- vivo* anti-oxidant activities of BGNCE in castor- oil-induced diarrhoeal rats

The following treatments were administered to diarrhoeal-rats over the course of seven days: first group received 100 mg of vitamin C per KGBW, second group received 100 mg of BGNCE per KGBW, third group received 250 mg of BGNCE per KGBW, fourth group 4 rats received 500 mg of BGNCE per KGBW, and fifth group received distilled water. Rats were then sacrificed after seven days, and blood was taken into clear bottles via the jugular vein. To get serum, the whole blood was centrifuged at 4,500 revolution per minute for ten minutes, after standing at ambient temperature for 30 minutes. A clean Eppendof-tube was used to gently collect the serum, which was then kept in storage at -4 °C for later use. Rat liver was excised after blood was collected, cleaned in standard saline -solution, and dried before being kept in appropriately labeled packaging and kept at -4 °C pending usage.

Preparation of liver samples for evaluation of in-vivo anti-oxidant activities in diarrhoeal--rats

A portion (0.3g) of the liver of each animal was homogenized in 2.7 ml of 50 mM phosphate buffer to create a 10% liver homogenate. The mixture was placed in a centrifuge, where it was spun at 3,500 revolutions per minute for 10 minutes. Supernatant

Table 1: Protocol for determination of TNF-apha concentrations in sera of rats

	Sample (µl)	Standard (µl)	TNF-alpha AB (µl)	Streptavidin HRP (µl)	After washing		
					Chromogen A	Chromogen B	Stop solution
Sample	40 µl	-	10 µl	50 µl	50 µl	50 µl	50 µl
Standard	-	50 µl	-	50 µl	50 µl	50 µl	50 µl
Blank	-	-	-	-	50 µl	50 µl	50 µl

Table 2: Protocol for determination of NF-k beta concentrations in sera of rats

	Sample (µl)	Standard (µl)	NF-k beta AB (µl)	Streptavidin HRP (µl)	After washing		
					Chromogen A	Chromogen B	Stop solution
Sample	40 µl	-	10 µl	50 µl	50 µl	50 µl	50 µl
Standard	-	50 µl	-	50 µl	50 µl	50 µl	50 µl
Blank	-	-	-	-	50 µl	50 µl	50 µl

obtained was used for evaluating the *in-vivo* anti-oxidant activities in the liver.

Superoxide-dismutase-SOD activities in serum and liver homogenate of castor-oil-induced diarrhoeal rats

Using a modified version of Marklund and Marklund (1974) methodology, superoxide dismutase (SOD) activity was evaluated. Sample-20 microlitre, tris-HCl-buffer (concentration 50 millimolar and pH 8.2)-180 microlitre, and distilled water-250 microlitre were mixed together. Afterward, 50 microlitre of pyrogallol was added. By flipping the Eppendof-tube in which the mixture was placed, the mixture was thoroughly shaken. Every 30 seconds, values of absorbance at 420 nm were read until time reached 180 seconds. The unit of enzyme-activity was expressed as: unit/mg protein.

Absorbance increase per minute (Δ Abs / min) = $(A_3-A_0)/T$

where A_0 is30-seconds absorbance.

A_3 is 180-seconds absorbance

T is the absorbance total time in seconds (180 seconds).

To determine the activity of SOD, the following formula was used:

$$\text{Activity of SOD} = \frac{\frac{\Delta\text{Abs}}{\text{min}}(\text{blank}) - \frac{\Delta\text{Abs}}{\text{min}}(\text{sample})\ x\ 100}{\frac{\Delta\text{Abs}}{\text{min}}(\text{blank})}$$

where:

$\frac{\Delta\text{Abs}}{\text{min}}(\text{blank})$ = Increase in absorbance per minute of blank

$\frac{\Delta\text{Abs}}{\text{min}}(\text{samle})$ = Increase in absorbance per minute of sample

Specific activity of SOD (U/mg protein) = $\frac{\text{SOD activity}}{\text{Total protein (mg)}}$

Catalase-CAT activities in serum and the liver of castor-oil-induced diarrhoeal rats

To quantify activity of catalase enzyme in the serum and the liver of diarrhoeal rats, Goth (1991) method was used, following modification as done by Hadwan and Abed (2016). Two sets of reaction mixtures were used; the reaction mixture (for test) consists of 100 microlitre of sample, 1,000 microlitre of 20 millimolar hydrogen peroxide in a buffer of potassium phosphate, concentration 50 millimolar. Reaction mixture (for test-control) consists of 100 microlitre of sample and 1,000 microlitre of distilled water. At 37 °C, the mixture was incubated for three minutes. To halt the reaction, 4,000 µl of ammonium molybdate (32.4 mM) was introduced into the mixture. The standard used was hydrogen- peroxide. Changes in absorbance against a reagent blank were measured at 374 nm.

Activity of catalase was estimated with the formula:

$$\text{Activity of Catalase (kU)} = \frac{2.303}{T} X\ log\frac{(S1)}{(S2-M)} X\ Vt/Vs$$

S1 = Optical density reading of standard

Vt = Total volume of reagent
S_2 = Optical density reading of sample
Vs = volume of serum or liver homogenate
M = Optical density reading of control
T = Time in seconds

Concentration of reduced-glutathione (GSH) in serum and liver homogenate of castor- oil-induced diarrhoeal rats

The modified procedure by Beutler *et al.* (1963) prescribed by Kozer *et al.* (2003) was used to determine reduced-glutathione (GSH) concentration in the samples. The procedure involved adding 50 microlitre of sample and 50 microlitre of TCA (10%) to an Eppendof-tube. The mixture was put in a centrifuge and spun for ten minutes at a speed of 4,000 revolution per minute. Supernatant obtained thereafter was used. 250 microlitre of phosphate buffer (concentration 0.1 molar, pH 7.8) and 5 microlitre of DTNB (0.01 molar) were combined with 25 microlitre of supernatant. Following that, the mixture was left for ten minutes to incubate and develop yellow colour. Absorbance of the yellow product was taken, with the spectrophotometer set to 412 nanometer wavelength. Additionally, GSH working standard of different concentrations were made. 250 microlitre phosphate-buffer (concentration 0.1 molar, pH 7.8) and 5 microlitre of 0.01 molar DTNB were added to each of the GSH standard solutions, spectrophotometer reading was taken at wavelength of 412 nanometer. A graph of absorbance vs concentration was drawn for the GSH standard solutions, to produce the GSH standard curve. From the curve generated, GSH concentrations of serum and liver samples were estimated.

Activity of glutathione-peroxidase (GPx) activities in serum and liver homogenate of castor oil-induced diarrhoeal rats

Using a modified version of Mohandas *et al.* (1984) method, glutathione peroxidase (GPx) activities were assessed. GPx working reagent was produced by adding 2 ml of phosphate-buffer (concentration 0.4 molar; pH 7.0); 1 ml of sodium azide, 2 ml of GSH (4 millimolar), and 1 ml of hydrogen peroxide (25 millimolar). After mixing, 75 microlitre of the GPx working reagent was added to the 25 microlitre of sample, mixture was left for ten minutes for incubation to take place. Twenty five microliter of TCA solution (15%) was added to stop the process. Mixture was put in the centrifuge and spun for ten minutes at speed of four thousand revolutions per minute. To 25 microlitre of the supernatant obtained, 250 microlitre phosphate buffer (concentration 0.1 molar, pH 7.8) and 5 microlitre of 0.01 molar DTNB were combined with 25 microlitre of supernatant. Following that, the mixture was kept on the stand for 10 minutes at ambient temperature. This was followed by spectrophotometer reading, taken at wavelength 412 nanometer. Activity of GPx was estimated by the formula:

$$\text{GPx (U/mg protein)} = \frac{2.3}{T\ (min)}\ X\ \log GSH$$

Where:
2.3 = constant
T = Time used for incubation (10 minutes)

Statistical analyses

The data was examined using SPSS (version 20). The mean values and Standard Error of Means were evaluated using one-way-analysis of variance (ANOVA). The Duncan Multiple-Range- Test (DMRT) was used to distinguish the means. The level of significance was adjudged at $p < 0.05$

Research ethical approval

Permission for this study was given by the Research Ethical Committee, Biochemistry Department, Federal University of Agriculture Abeokuta, Nigeria.

Results

The Bambara-groundnut extract's yield as a percentage was 11.60% and was used for this study.

Antioxidant properties (*in-vivo*) of BGNCE in diarrhoeal-rats

Figs, 1 to 8 showed the results of the impact of BGNCE on *in-vivo* anti-oxidant activities i.e. serum superoxide dismutase-SOD, liver SOD, serum catalase-CAT, liver CAT, serum reduced-glutathione-GSH, liver GSH, serum glutathione peroxidase-GPx and liver GPx respectively; in castor oil- induced diarrheoal rats. The rats given distilled water had the lowest levels of SOD activities in the serum and liver, while the highest activities were observed in the rat groups that were given 100 mg Vitamin C per KGBW representing control group. BGNCE treatment of

diarrhoeal-rats boosted SOD activity in the serum and liver of the animals (Figs. 1 and 2).

In Figs. 3 and 4, are the results of the effects of BGNCE on catalase activities in castor-oil induced diarrhoeal-rats, for serum and liver respectively. The results revealed that the rat group on 100 mg Vitamin C per KGBW recorded the highest activities of catalase both in the serum and liver. The least catalase activities were in rats that got distilled water. Furthermore, administration of BGNCE increased the catalase activities in serum and liver of diarrhoeal-rats.

Figs. 5 and 6 are the respective serum and liver concentrations of GSH. The findings showed that in the serum and liver of experimental rats, GSH concentration was highest in the group administered with 100 mg of vitamin C per KGBW and least in the group given distilled water. Administration of BGNCE at all the experimental doses (100 mg per KGBW, 250 mg per KGBW and 500 mg per KGBW increased concentration of GSH, and brought it close to control. This same trend was observed in Figs. 7 and 8 for serum GPx and liver GPx, respectively.

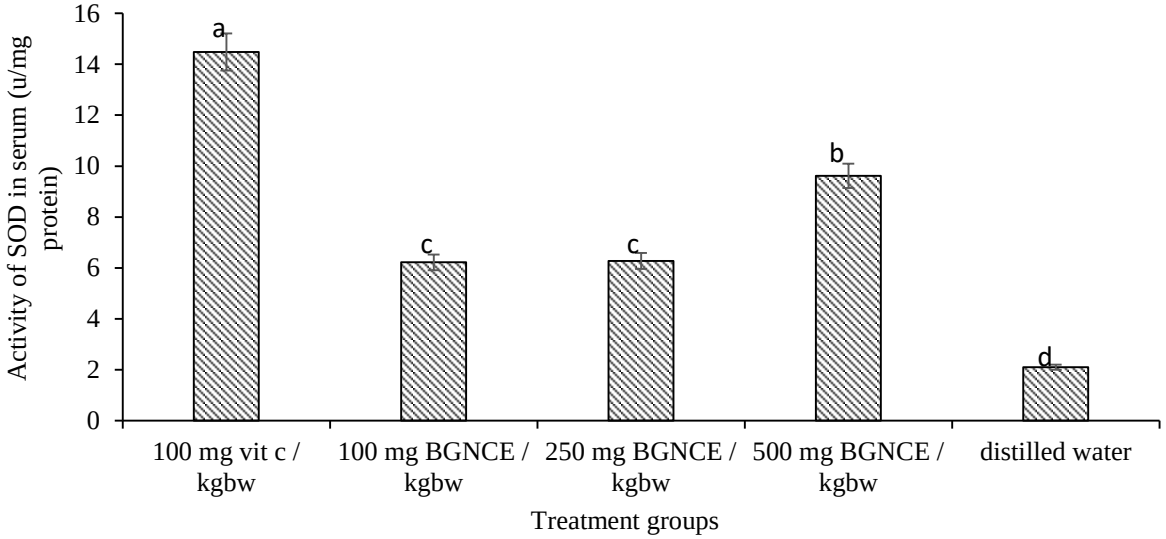

Fig. 1: Effect of BGNCE and Vit. C on the activity of superoxide-dismutase-(SOD) in serum of Castor-oil-induced diarrhoeal- rats.

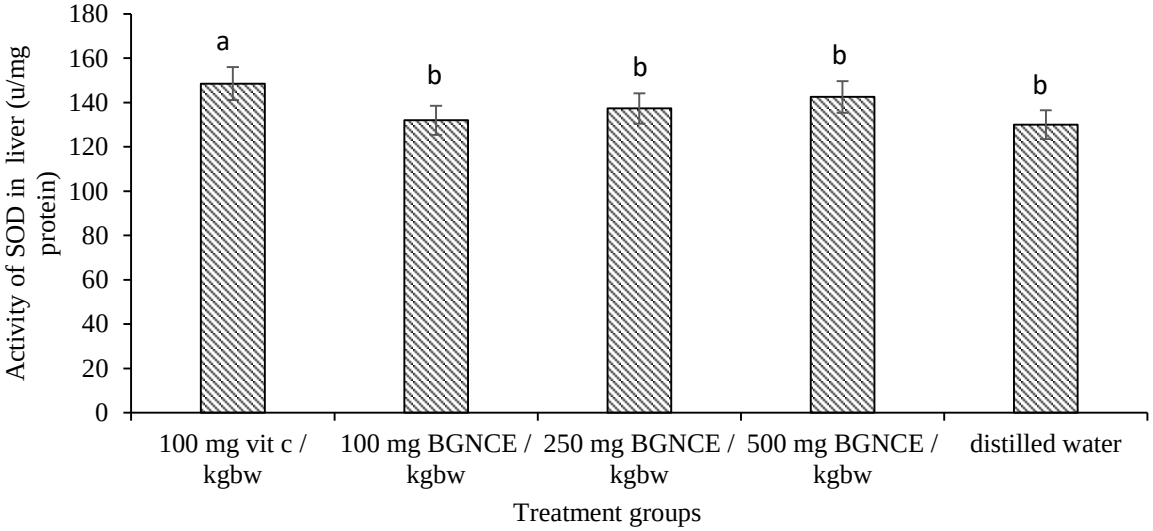

Fig. 2: Effect of BGNCE and Vit. C on the activity of superoxide- dismutase-SOD in liver of Castor-oil-induced diarrhoeal- rats.

*Values are means of six determinations ± SEM); Bars with different alphabets are significantly different ($p < 0.05$); B*GNCE = Bambara-groundnut condiment extract

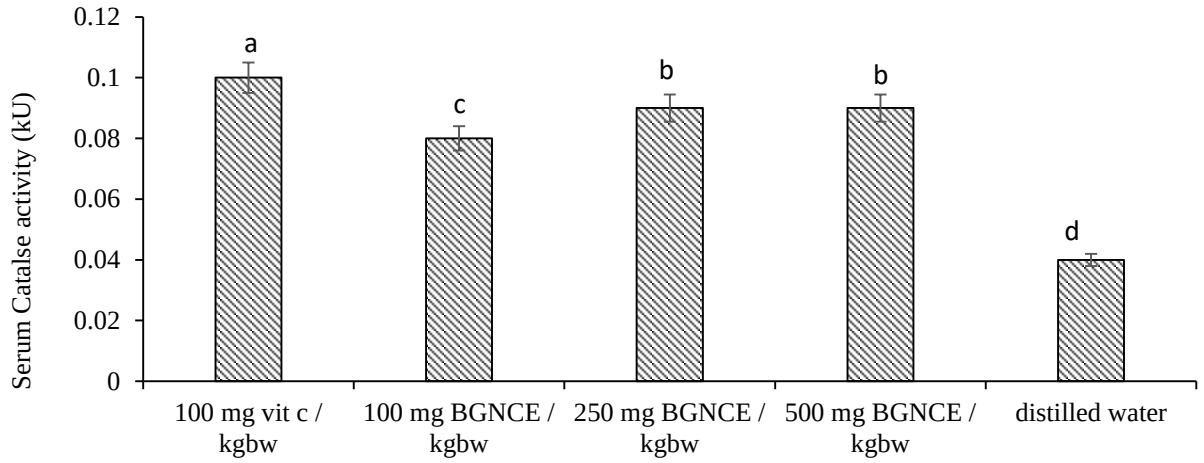

Fig. 3: Effect of BGNCE and vitamin C on catalase activity in serum of castor-oil-induced Diarrhoeal-rats.

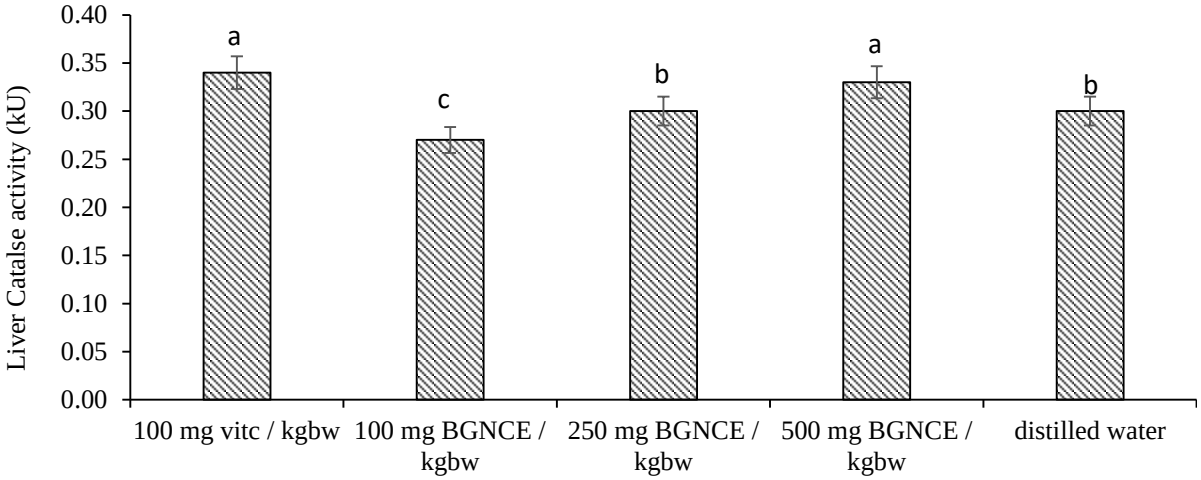

Fig. 4: Effect of BGNCE and Vit.C on activity of catalase in liver of castor- oil-induced Diarrhoeal-rats.

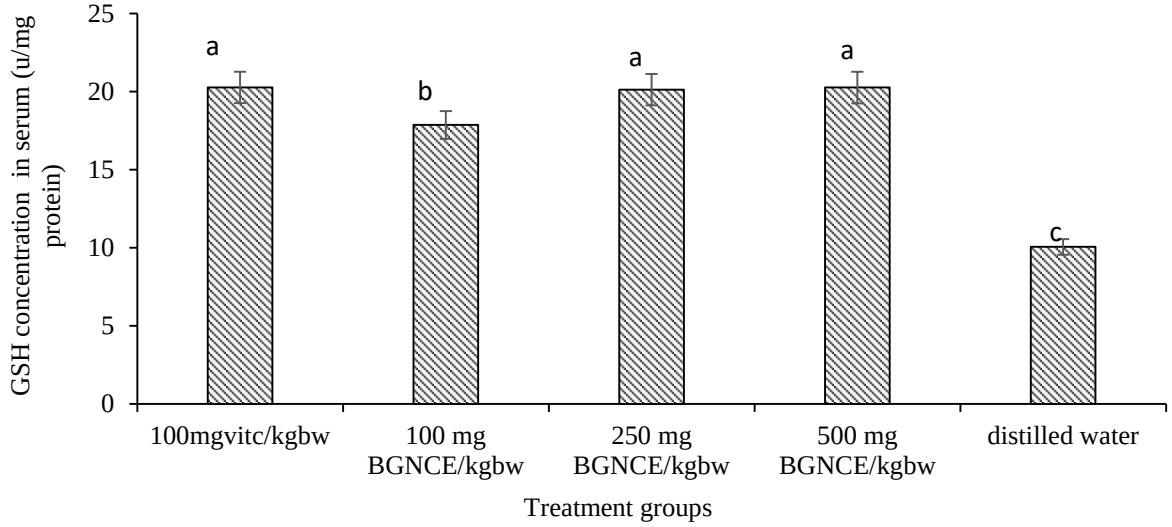

Fig. 5: Effect of BGNCE and Vitamin C on reduced-glutathione (GSH) concentration in serum of diarrhoeal-rats.

Values are means of six determinations ± SEM; Bars with different alphabets are significantly different ($p < 0.05$); BGNCE = Bambara-groundnut condiment extract

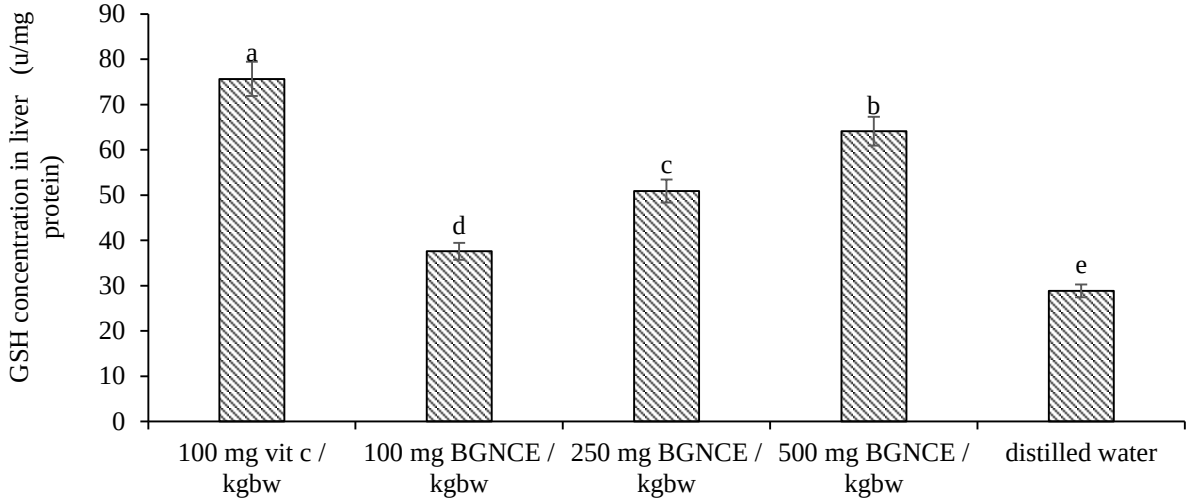

Fig. 6: Effect of BGNCE and vitamin C on reduced-glutathione (GSH) concentration in liver of diarrhoeal-rats.

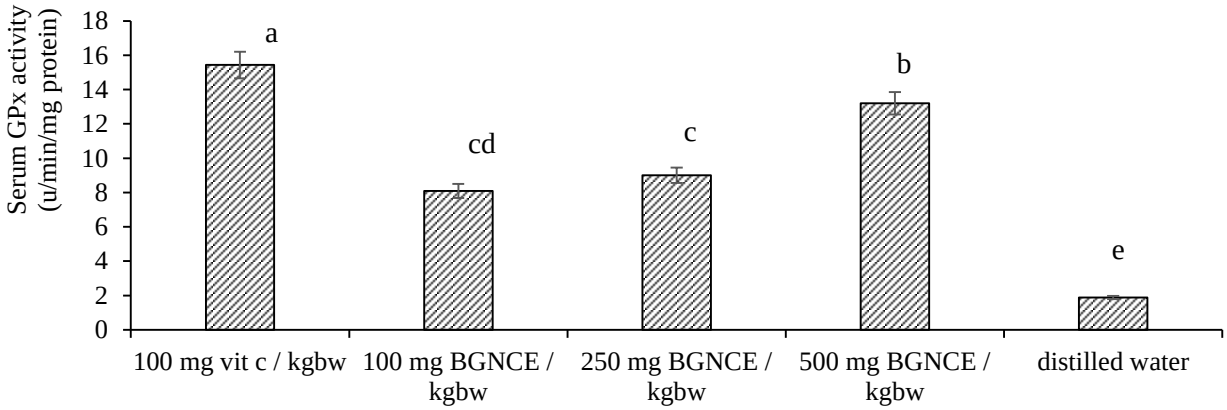

Fig 7: Effect of BGNCE and vitamin C on glutathione-peroxidase (GPx) activity in serum of Castor-oil-induced diarrhoeal- rats.

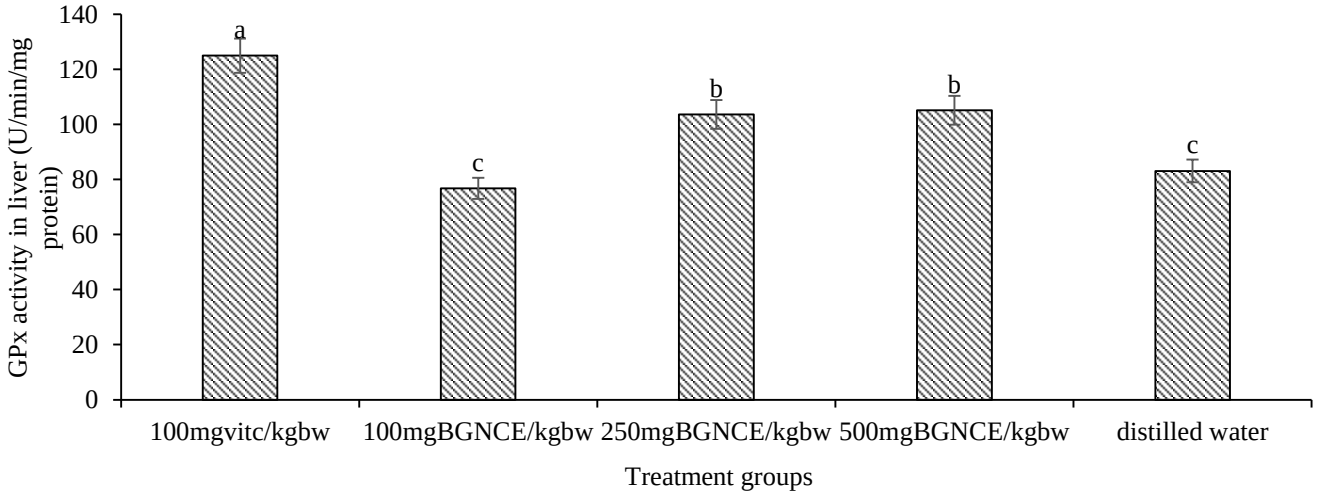

Fig. 8: Effect of BGNCE and vit. c on activity of glutathione peroxidase-GPx in liver of castor-oil-induced diarrhoeal- rats.

Values are means of six determinations ± SEM; Bars with different alphabets are significantly different ($p < 0.05$); BGNCE = Bambara-groundnut condiment extract

Effect of BGNCE on inflammatory-biomarkers (TNF-alpha, NF-k beta and LDH) in castor oil-induced diarrhoeal-rats

The variations in TNF-alpha concentrations in rats with diarrhoea are shown in Fig. 9. The rats that received distilled water had the greatest levels of TNF-alpha concentration; whereas the rat groups that received loperamide (the control group) had the lowest levels. Administration of BGNCE caused a decrease in serum concentration of TNF-alpha, close to the value recorded in the control group. The observed-decrease in TNF-alpha concentration increases with BGNCE concentration. The trend for NFk-beta was slightly different (Fig. 10); it was found that treating diarrhoeal-rats with BGNCE at dosages of 250 mg per KGBW and 500 mg per KGBW was able to reduce the concentration of NFk-beta in the rats sera, but that treating diarrheal rats with 100 mg per KGBW of BGNCE did not sufficiently reduce the concentration of NFk-beta. Fig. 11 demonstrates how the LDH activity changed in diarrhoeal-rats when treated with BGNCE. Treatment at all doses caused reduction in LDH activity in rats' sera, though not to a level that was close to the control.

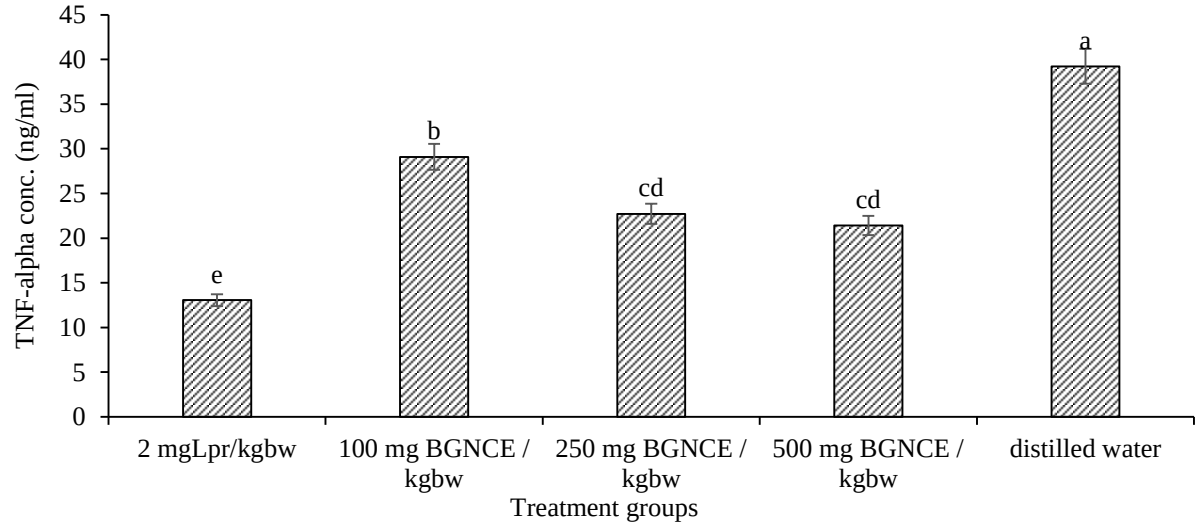

Fig. 9: Effect of BGNCE and Loperamide on TNF-apha concentration in castor- oil-induced diarrhoeal-rats

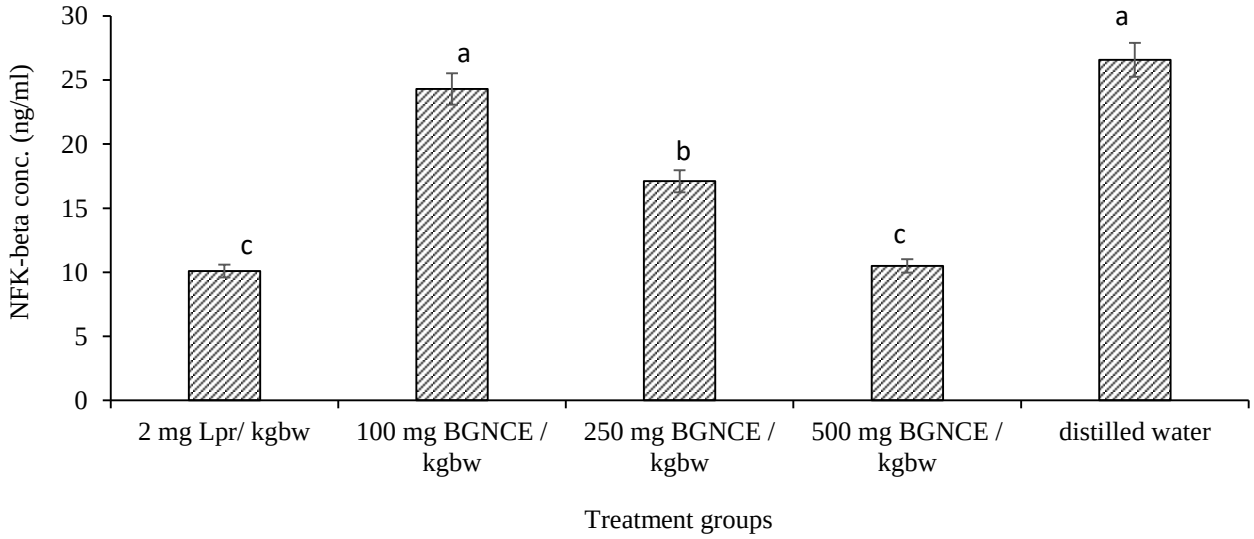

Fig. 10: Effect of BGNCE and Loperamide on Nuclear factor- kappa beta (NFK-beta) concentration in castor- oil-induced diarrhoeal-rats

Values are means of six determinations ± SEM; Bars with different alphabets are significantly different ($p < 0.05$); Lpr = Loperamide BGNCE = Bambara-groundnut condiment extract.

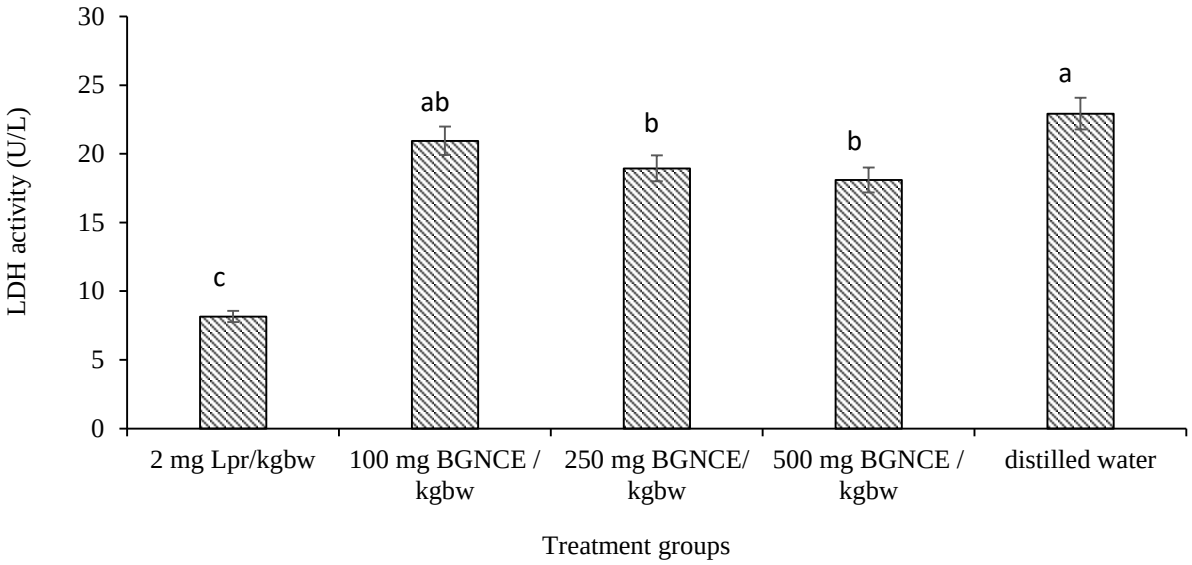

Fig. 11: Effect of BGNCE and Loperamide on LDH activities in serum of castor- oil-induced diarrhoeal-rats

Values are means of six determinations ± SEM; Bars with different alphabets are significantly different ($p < 0.05$); Lpr = Loperamide BGNCE = Bambara-groundnut condiment extract.

Discussion

The study's findings showed that castor-oil-induced diarrhoea led to a rapid inflammatory response, as evidenced by a rise in the concentrations of LDH, TNF-alpha and NFk-beta. The data obtained from this research supported the claim of Yakubu and Salimon, (2015) which stated that ricinoleic acid, a castor-oil metabolite when utilized had caused diarrhoea in the tested-animals, thereby causing inflammation-and irritation in the guts of the tested-animals. The increased-levels of inflammatory-biomarkers observed in the untreated diarrhoeal-rats may be due to the action of ricinoleic acid in the small intestine of the rats. The use of BGNCE in the treatment of rats that had come down with diarrhoea reduced serum-concentrations of TNF-α, NFk-β and LDH in all doses (100, 250, 500) mg per kilogram of body weight; this reduction in inflammatory-biomarkers was in dose-wise-manner; suggesting that BGNCE has anti-inflammatory properties, especially at acute levels. This result was at par with the findings of Olayinka *et al*. (2021), which emphasized that Bambara-groundnut-seed extract has anti-inflammatory properties. The result obtained strongly concurs with those of earlier studies on other legumes. Take for instance, Esmailzadeh and Azadbakht (2012) reported that there was inverse relationship between legume consumption and serum inflammatory- biomarkers. In the same vein, Zhu *et al*. (2017) also reported that consumption of food legumes brought about a decrease in some serum anti-inflammatory-biomarkers. Inflammation is defined as a complex-process which involves many chemical-signals working together. And these chemical-signals included but not limited to enzymes, co-factors, inflammatory-mediators e.t.c. Lactate- dehydrogenase is primarily a cardiac function biomarker and is a useful inflammatory- biomarker in diseased conditions (Lu *et al*., 2015; Pucino *et al*., 2016; Santotoribo and Jimenez-Romero, 2019). The primary inflammatory-mediators like TNF-alpha, cyclo-oxygenase 1 (COX-1), the transcription-factor-NFk-beta, and other interleukins are typically the targets of anti-inflammatory agents like medications and plant extracts. The transcription-factor known as NFk-beta is responsible for controlling the expression of the genes linked to inflammation (Barnig et al., 2019). The prevalence of inflammatory illnesses like diarrhoea is significantly influenced by these key inflammatory-mediators, particularly NFk-beta and TNF-alpha (Yamamoto and Gaymor, 2019). The ability of Bambara-groundnut-condiment extract to reduce the amount of TNF-alpha, NFk-beta, and LDH in the rats' serum thus points to its anti-inflammatory-characteristics.

Reports have been made on the reduction in risks of chronic diseases with regular consumption of foods rich in natural anti-oxidants like fruits and vegetables, as well as legumes (Nevarro-Hoyos *et al.,* 2017). Foods, dietary-supplements, and conventional medications all include natural anti-oxidants (He *et al*., 2017). Anti-oxidant-rich diets have reportedly been shown to improve consumer's overall health (Anwar *et al.,* 2018). The *in-vivo* assay results of various endogenous enzymatic- and non-enzymatic anti-oxidants in this work confirm previous findings that Bambara- groundnuts have potent antioxidant activities (Nyau et al., 2015; Salawu, 2016). Almost all of the anti-oxidants measured showed dose-dependent up-regulation of anti-oxidant activity in the rat groups treated with BGNCE. Superoxide-dismutase (SOD) is well distributed within the system, it is found in almost all cells within the body, where it protects against oxidative injury caused by oxidants and free- radicals (Salibury and Bronas, 2015). Within the cells, injury causing superoxide ion, O^{2-} is generated during oxidative-phosphorylation. SOD then reacts with O^{2-} radical, turning it into harmless hydrogen peroxide (H_2O_2). By using additional anti-oxidant enzymes such as glutathione-peroxidase (GPx) and catalase (CAT), the hydrogen peroxide is further broken down into water and oxygen (Ighodaro and Akinloye, 2018). The findings of this study demonstrated that diarrhoeal-rats not given any treatment had considerably lower superoxide-dismutase (SOD), catalase (CAT), reduced-glutathione (GSH), and glutathione-peroxidase (GPx) activity than did diarrhoeal rats given BGNCE. The increase in amount of superoxide ions (O^{2-}) and hydroxyl-radicals (OH*) within the cells may be the reason for the decreased superoxide-dismutase and catalase-activities in the sera and liver diarrhoeal-rats. The accumulation of O^{2-} and OH* radicals within cells, overwhelm anti-oxidants, thereby causing damage to cell membrane-function and integrity, and leading to incidences of many diseases (Kaushal *et al*., 2018). Removal of the offending superoxide ions and hydroxyl-radicals from the cells will therefore reduce the incidences of diseases. Reduced-glutathione (GSH), a substrate for glutathione-peroxidase (GPx), works to remove free-radicals within the body- system (Ighodaro and Akinloye, 2018). Therefore, reduction in the concentration of reduced- glutathione in animal-tissue causes injury which impairs cellular-defense against reactive- oxygen-species. This research work confirmed that reduced-glutathione (GSH) concentration is decreased in diseased state as earlier reported by Kangralkar *et al*. (2010). The decrease in the activity of GPx in the untreated diarrhoeal-rats may be due to unavailability of GSH within the cells. Generally, anti-oxidants scavenge the reactive-oxygen-species(ROS) by donating electrons and hydrogen, thereby decomposing the peroxide and quenching singlet-oxygen (Engwa *et al.,* 2016). In order to combat free-radicals, the anti-oxidants both enzymatic and non-enzymatic have to terminate the chain-reactions before vital molecules are harmed and thereby turning the dangerous free-radicals into harmless-compounds. The observed decrease in the activities of anti-oxidant-enzymes in castor-oil-induced diarrhoeal-rats that were not treated, lends credence to the idea that oxidative stress plays a role in diarrhoeal attack.

Conclusion

This research demonstrated that BGNCE has anti-inflammatory and anti-oxidant properties. These potentials may be responsible for the anti-diarrhoealic-function of BGNCE. This can be harnessed for optimum health-benefit of Bambara-groundnut consumers. The researchers hereby call for inclusion of Bambara-groundnut-condiment in the diet of Nigerian families, especially children.

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