Antioxidant Effects of Bambara Nut (*Vigna subterranea*) Extract in Improperly Processed Bitter Cassava (*Manihot esculenta*) Flour Konzo-Induced Male Wistar Rats

ABSTRACT

Konzo is a neurological disorder characterized by clinical spastic paresis and increased reactive oxygen species. The study aim was to investigate the antioxidant effects of Bambara nut extract in improperly processed Bitter Cassava flour Konzo-induced Male Wistar rats. Twenty-five (25) Adult Male Wistar rats weighing between 180–200g were randomly divided into five (5) groups of five rats. After two weeks of acclimatization of the rats, Konzo was induced following improperly processed bitter cassava flour feeding for a period of three weeks. The rats were subsequently fed as follows for 28 days: Group 1: Control group - fed with water and rat feed; Group 2: Konzo-Induced Group - fed with bitter cassava flour; and Groups 3-5 were fed with different doses of Bambara nut (BN) extract: 100mg/kg, 200mg/kg and 300mg/kg respectively. 24 hours after the last feeding, rats were placed under chloroform anaesthesia and blood samples collected through cardiac puncture for the estimation of serum concentration of oxidative stress markers: Malondialdehyde (MDA), Glutathione reductase (GSH), Catalase (CAT) and Superoxide dismutase (SOD). The results showed that compared to Group 1 rats, Konzo-induced rats exhibited a significant decrease in GSH ($p<0.05$) and a significant increase in CAT, SOD, and MDA levels ($p<0.05$). These changes indicate possible oxidative stress in Group 2 rats. The groups 3-5 administered 100mg/kg, 200mg/kg and 300mg/kg BN showed a significant increase in GSH, CAT and SOD levels ($p<0.05$), and a significant decrease in MDA level ($p<0.05$) compared to Group 2 rats. These findings suggest that BN extract has antioxidant effect against oxidative stress induced by Konzo. Conclusively, BN extract decreased MDA and increased GSH, CAT and SOD in a dose dependent manner, resulting in a possible prevention of cell death due to lipid peroxidation, by inhibiting the lipid peroxidation process.

Keywords: Bambara nut, Konzo, Bitter Cassava flour, Oxidative stress

1. INTRODUCTION

Konzo as a neurological disorder, affects selective upper motor neurons. It is characterized by a clinical spastic paresis and increased reactive oxygen species (ROS) [1]. The name “Konzo” means “tied legs”, as derived from the local Congolese dialect, which physically describes the resulting spastic gait. Giovanni Trolli, an Italian doctor, in the 1930’s, in the former Belgian Congo, now Democratic Republic of Congo, was the first to describe the Konzo disease. The

disease is linked with poverty and agricultural crises that are provoked by drought or war. It affects mostly those living in remote rural areas, and it's globally increasing. It is believed that the consumption of improperly processed cassava root causes the disease, especially the bitter cassava species which are known to contain high dietary cyanogen [2]. As such, it remains a health problem in Sub-Saharan Africa.

Bambara nut (BN), as a native crop, is widely cultivated in most sub-Sahara African countries. As an indigenous African legume it belongs to the family of *Fabaceae* and sub-family of *Faboidea*. The common names of BN vary across the continent. It is called Okpa in Igbo-speaking parts of Nigeria, Epa-roro in Yoruba-speaking parts of Nigeria, and Gurujia in Hausa-speaking parts of Nigeria. The South Africans call it Njugo, Malawians call it Nzama, while Zambians call it Katoyo [3]. Bambara nut (BN) is cultivated, in most rural areas in sub-Saharan Africa, mainly for its seed [4]. It was reported that BN has been less researched on despite its anecdotal applications in traditional medicine [5]. It's been anecdotally reported to possess nutraceutical properties including antioxidant, antidiabetic, and antimicrobial potentials [6].

In the light of the above-described beneficial properties of Bambara nut, the current study was set out to investigate the antioxidant effects of Bambara nut (*Vigna subterranea*) extract in bitter cassava (*Manihot esculenta*)flour konzo-induced in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Procurement of Animals

Twenty-five (25) adult male Wistar rats weighing 180–200g were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. The rats were housed in different sawdust bedding, clean and disinfected wooden

cages. The animal house provided a controlled environment with a 12-hour light/dark cycle, 50–60% humidity, and a temperature of roughly 30°C. These conditions were maintained throughout the acclimatization and experimental periods. The rats had free access to clean water and standard animal feed.

2.2 Collection and Identification of Plant Materials

Bitter cassava root and Bambara nut were procured from the Ministry of Agriculture, Agricultural Development Programme Retail Outlet, Rumuodumaya, Rivers State, Nigeria. The plant materials were identified by Dr. Edwin Nwosu of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

2.3 Preparation of bitter cassava root

The cassava roots were washed in water to eliminate any strenuous materials. Peeled roots were cut into little chunks (2-5cm thickness) and minimally air dried to retain its cyanide content. It was subsequently grinded to powder for easy digestion and fed the animals.

2.4 Extraction of Barbara nut

Procured Bambara nuts were washed to eliminate dust and other impurities. The nuts were air-dried at room temperature for a minimum of 7 days to remove moisture. Nuts were weighed and grounded into powder using an electric grinding machine [7]. Specifically, 2 kilograms of the powdered material was macerated in 80% methanol for 72 hours. After three-days, the mixture was filtered through a double-layered muslin cloth to remove debris, and the liquid portion was

further filtered through Whatman filter paper. The collected filtrate was concentrated at a temperature of 45°C using a rotary evaporator. It was subsequently transferred to an evaporating dish and dried over a water bath. Once dried, the extract was safely stored in a desiccator [8] [9] [10].

2.5 Induction of Konzo Disease

After an initial period of acclimatization, the experimental animals were fed unprocessed bitter cassava flour for a period of three weeks. The quantity of powdered cassava flour administered was equivalent to 86 grams per kilogram of the rats' body weight [1].

2.6 Ethical Approval and Acute Toxicity Study

Ethical approval was obtained from the University of Port Harcourt Ethical Committee through a communication referenced: UPH/CEREMAD/REC/MM80. The acute toxicity of Bambara nut (*Vigna subterranea*) extract was determined and found to be consistent [11]. The study was conducted in accordance with the guidelines for the care and use of laboratory animals [12].

2.7 Experimental Design

Twenty-five (25) adult male Wistar rats weighing between 180–200g were randomly divided into five (5) groups of five (n=5) rats per group. After an initial period of acclimatization, Konzo was induced following unprocessed bitter cassava flour feeding for a period of three weeks. The rats were subsequently fed as follows for 28 days:

Group 1: Control group - rats in this group received normal distilled water and rat chow

Group 2: Konzo-Induced group – rats in this group received improperly processed bitter cassava flour

Group 3: Low Dose Extract group - rats in this group received 100mg/kg BN

Group 4: Medium Dose Extract group - rats in this group received 200mg/kg BN

Group 5: High Dose Extract group - rats in this group received 300mg/kg BN

24 hours after the last feeding, the rats were placed under chloroform anaesthesia and blood samples collected through cardiac puncture for the estimation of serum concentration of oxidative stress markers including: Malondialdehyde (MDA), Glutathione reductase (GSH), Catalase (CAT) and Superoxide dismutase (SOD).

2.8 Biochemical Assay

Using wavelengths of 525 nm for excitation and 547nm for emission, the malondialdehyde (MDA) produced, reacts with the chromogenic reagent and 2-thiobarbituric acid (TBA) to yield a pink coloured complex. The concentration of MDA was calculated using the molar extinction coefficient of the chromospheres [13].

The determination of catalase activity was based on the fact that catalase in the sample preparation split hydrogen peroxide (H_2O_2) which can then be measured spectrophotometrically at 240nm. One unit of catalase enzyme activity equals the amount of protein that converts 1µmol H_2O_2 utilized per minute [14].

The measurement of superoxide enzyme activity was done spectrophotometrically. The superoxide anion reduction of nitrobluetetrazolium (NBT) to blue formazan was determined at 560nm.The ability of superoxide enzyme to cause a 50% inhibition in Nitrobluetetrazolium (NBT) reduction refers to one unit of enzyme activity [15].

The glutathione (GSH) was measured based on its ability to react with 5,5'-dithio-bis (2-nitrobenzoic acid) (DNTB, Ellman's reagent) to produce the conjugate GS-TNB as well as the yellow TNB (5'-thio- 2- nitrobenzoic acid) detected at 412nm. The rate of TNB production was proportional to the GSH concentration in the extract [16] [17].

2.9 Data Analysis

GraphPad Prism and Microsoft Excel 2019 were used to analyze the data. Values are presented as mean ± standard error of mean. One-way analysis of variance (ANOVA) was used to compare the groups. The confidence interval was set at 95%, and a p-value less than 0.05 was considered statistically significant.

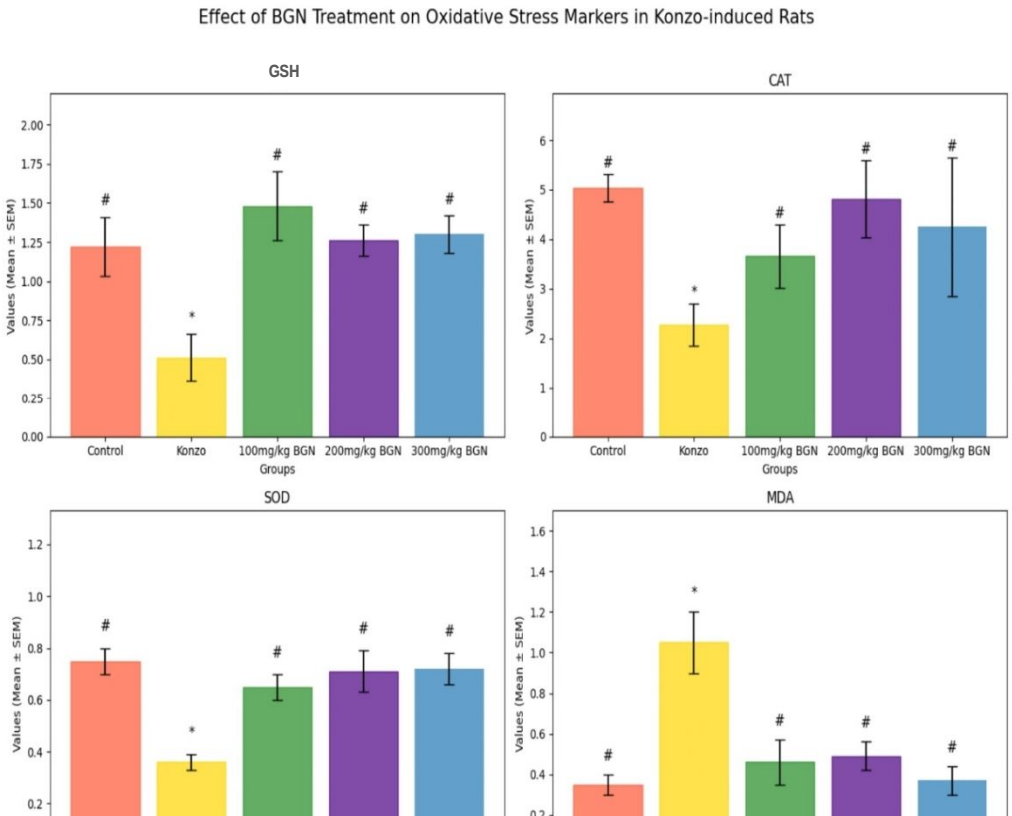
3 RESULTS

3.2 Effect of BN Extract on Oxidative Stress Markers in Konzo-induced Rats

Table 1 presented the result of the effect of BN extract on oxidative stress markers in konzo-induced rats. Compared to Group 1 (Control) rats, Konzo-induced rats exhibited a significant decrease in GSH ($p<0.05$) and a substantial increase in CAT, SOD, and MDA levels ($p<0.05$). These changes indicate a possible oxidative stress amongst Konzo group (Group 2) rats. Upon administration of BN extract, a dose-dependent response was observed. The groups 3-5 administered 100mg/kg, 200mg/kg and 300mg/kg BN showed a significant increase in GSH, CAT and SOD levels ($p<0.05$) compared to Konzo induced group (Group 2) rats, however, MDA level was significantly lowered by graded doses of the extract ($p<0.05$). These findings

collectively suggest that BN extract has a protective effect against oxidative stress induced by Konzo.

Figure 1: Effect of BN on Oxidative Stress Markers in Konzo-induced Rats



Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at $p<0.05$ when compared to the control values; # =means values are statistically significant at $p<0.05$ when compared to group 2 (konzo) values.

4 DISCUSSION

Konzo is a neurological disease characterized by increase in reactive oxygen species primarily affecting populations in sub-Saharan Africa who rely mainly on cassava as a staple food. The disease manifests as sudden onset paraparesis, leading to severe muscular weakness and cell membrane peroxidation. Bitter cassava, which contains high level cyanide, is believed to be the causative agent in the development of Konzo.

Oxidative stress represents an imbalance between reactive oxygen species (ROS) production and the body's ability to neutralize them with antioxidants [13]. ROS development has been linked with various diseases including neurodegenerative diseases, cancer, and cardiovascular diseases [5] [6]. Oxidative stress markers serve as indicators of the level of oxidative damage in cells and tissues. Malondialdehyde (MDA), a pro-oxidant marker, is used to assess increased oxidative damage in cells [16].

Furthermore, antioxidant markers like Glutathione reductase (GSH), Catalase (CAT), and Superoxide dismutase (SOD) are pivotal in protecting against oxidative damage. The Konzo-

induced rats in this study demonstrated an elevated level of MDA due to the debilitating effects of the disease and its association with oxidative stress. This finding is in consonance with a previous study which established a strong connection between oxidative stress and diseases including Konzo [18]. Additionally, the activity level of GSH, CAT, and SOD were reduced in the tissues of these rats, which further confirms the relationship between Konzo and oxidative stress [18].

Though, administration of BN at different doses specifically: 100mg/kg, 200mg/kg, and 300mg/kg demonstrated a dose-dependent improvement in oxidative stress markers. These findings show that BN treatment effectively increased the levels of GSH, CAT, and SOD, while simultaneously reducing the levels of MDA. These results suggest that BN possesses potent antioxidant properties, thereby offering protection against oxidative stress-induced damage in the rats. Our findings indicate that Bambara nut extract (BN) administration at the respective doses of 100mg/kg, 200mg/kg, and 300mg/kg elicited a dose-dependent improvement in oxidative stress markers following Konzo induction in male Wistar rats. Findings from this study are consistent with [5] [6] who prospectively highlighted possible antioxidant properties of Bambara nut and its potentials in mitigating oxidative stress-related damage in various pathological conditions.

The observed increase in the levels of Glutathione reductase (GSH) following BN administration suggests a reinforcement of the cellular antioxidant defense system. GSH is a crucial endogenous antioxidant that plays a pivotal role in neutralizing reactive oxygen species (ROS) and protecting cells from oxidative damage. The upregulation of GSH levels aligns with previous studies demonstrating the ability of Bambara nut to enhance antioxidant defenses.

Elevation in Catalase (CAT) levels with BN administration further supports the antioxidative potential of Bambara nut. Catalase is an enzyme responsible for breaking down hydrogen peroxide into water and oxygen, thereby mitigating oxidative stress. The increased CAT levels indicate a potential enhancement of the enzymatic antioxidant defense system, contributing to the overall reduction of oxidative stress in Konzo-induced rats administered BN. Moreover, the observed rise in Superoxide dismutase (SOD) levels following BN administration underscores its role in enhancing the cellular antioxidant capacity. SOD is a key enzyme involved in the dismutation of superoxide radicals, preventing the formation of more harmful reactive oxygen species (ROS). The upregulation of SOD aligns with other studies demonstrating the antioxidant potential of Bambara nut extracts.

Furthermore, the significant decrease in Malondialdehyde (MDA) levels, a marker of lipid peroxidation and oxidative damage, provides additional evidence of the protective effect of BN extract against oxidative stress. This is consistent with previous research indicating the ability of Bambara nut in reducing lipid peroxidation and oxidative stress in various experimental models.

5 CONCLUSION

In conclusion, Bambara nut extract dose dependently decreased MDA and increased GSH, CAT and SOD, resulting in a possible prevention of cell death due to lipid peroxidation, by inhibiting the lipid peroxidation process. Bioactive inherent compounds present in the extract may be responsible for the observed physiologic and pharmacologic effects of the extract.

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