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Ameliorative Potential of Bambara Nuts against Acute Ethanol-Induced Oxidative Stress in Wistar Rats

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ABSTRACT

The aim of this study is to examine the ameliorative potential of Bambara nuts against acute ethanol-induced oxidative stress in Wistar rats. Bambara nut, the Songkhla 1 variety were purchased at Obimze area of Owerri, Imo State Nigeria and ground into fine powder using a coffee grinder. Extraction was done using soxhlet apparatus and methanol as the solvent. Twenty-four adult male Wistar rats were acclimatized for seven days during which they were fed ad libitum with standard feed and drinking water. They were randomly divided into four groups of six rats each. Animals in groups A and B were administered distilled water while those in groups C and D were administered Bambara nutextract for twenty-one days at a dose of 100 mg/kg body weight 12 hourly via oral route. At the end of the treatment, they were fasted overnight and animals in groups B and D were exposed to a single dose of 70% ethanol at 12 ml/kg body weight to induce oxidative stress. After 12 hours of ethanol administration, the animals were sacrificed and blood samples were collected via cardiac puncture. Oxidative stress parameters were determined using standard methods. Ethanol-induced oxidative stress significantly increased the activities of LPO, CAT, SOD and GPx but decreased GSH. Bambara nut was able to remedy these effects by regulating the oxidative stress biomarkers, thus possesses ameliorative potential against ethanol-induced oxidative stress and can protect the body against free radicals arising from oxidative stress. This also implies that it could boost the immune system. Thus, regular consumption of this nut is recommended.

Keywords: Ameliorative potential, Bambara nut, Ethanol, Oxidative stressbiomarkers

1. INTRODUCTION

Bambara nut (*Vignasubterranea*(L.) Verdc), a legume whose origin is Africa, is a rich source of protein and minerals [1]. The high nutritional composition promotes its utilization in many food applications. Traditional processing of the seed includes dehulling, roasting or fermentation, and milling to obtain flour. The use of milk and flour obtained from Bambara nut is widely studied [2,3]. Other studies also addressed the potential of Bambara nut as composite in home-made weaning foods for infants and young [4,5]. Bambara nut grows near or below the surface of the soil which serves as fungal inoculum [6]. Bambara nut has been ranked as the third most important grain legume, after groundnut *(Arachishypogaea L.)* and cowpea *(Vignaunguiculata)* in semi-arid Africa, but has not been accorded due attention in research [7].

Figure 1: Bambara Nut

It might be surprising to say that most people in Nigeria may not be conversant with the name

Bambara nut as the local name is commonly used but it forms most parts of some families' daily meal. Locally, it is called 'Okpa' in Igbo, 'Epa-Roro' in Yoruba, 'Kwaruru' or 'Gurjiya' in Hausa.

Excessive acute or chronic alcohol consumption poses a serious health hazard and can result into several metabolic disorders in organisms [8]. Alcohol is a commonly used hepatotoxin in experimental hepatopathy. Although the pathogenesis of alcohol-induced liver disease is not clearly defined, there is evidence that ethanol-induced liver injury is due to oxidative stress that leads to fibrosis and impaired liver functions [9,10]. Alcohol overuse is also characterized by central nervous system (CNS) intoxication symptoms, impaired brain activity, poor motor coordination, and behavioral changes [11]. Excessive alcohol consumption commonly causes hepatic, gastrointestinal, nervous and cardiovascular injuries leading to physiological dysfunctions [12]. Cellular disturbances resulting from excessive alcohol consumption results in increased formation of oxidative stress biomarkers such as malondialdehyde (MDA); reduction in the level of reduced glutathione level and a decrease in the activities of antioxidant enzymes [13,14]. Free radicals and reactive oxygen species (ROS) have been implicated in the oxidative damage of biomolecules and various organs of the body. Studies have shown the crucial role free radicals play in the pathogenesis of many human diseases namely, cardiovascular and pulmonary diseases, some types of cancer, immune/autoimmune diseases, inflammation, diabetes, cataracts and brain dysfunction such as Parkinson and Alzheimer [15]. However, the deleterious effect of free radicals can be corrected by antioxidants – both enzymatic and nonenzymatic. Oxidative stress is known to arise when there is an imbalance between free radical production (especially reactive oxygen species; ROS) and endogenous antioxidant defense system. This shift in balance is associated with oxidative damage to a wide range of biomolecules including lipids, proteins, and nucleic acids, which may eventually impair normal functions of various tissues and organs [16].

There is an increasing global interest concerning the use of medicinal plants in the prevention and treatment of different pathologies [17, 18]. The beneficial effects of plants are attributed to the presence of secondary metabolites such as polyphenols, tannins, terpenoids, alkaloids, flavonoids [19]. Considering the central role played by free radicals in the initiation and progression of many diseases, the use of natural products with antioxidant constituents has been proposed as an effective therapeutic and/or preventive strategy against diseases and the search for potent and cost-effective antioxidants of plant origin has since increased [20]. Many plants have been shown to possess antioxidant potentials [21,22]. This has thus raised interest in the investigation of commonly consumed plants for their phytochemicals with nutritional and chemotherapeutic potentials. Therefore, the need to argument synthetic chemotherapeutic compounds with natural products is the drive for the exploitation of natural products from plants; as they may have little or no side effects yet meeting the nutritional, chemotherapeutic and economic needs [23,24]. Moreover, despite the efforts of pharmaceutical companies in the production of synthetic antibiotics, there yet exists a marked increase in pathogen population exacerbated by multi drug resistant microorganisms. Consequently, there is increased research into phytochemicals for the effective therapeutics combat of this menace. The therapeutic effects of plant-based drugs have been documented to be due to the phytochemicals that constitute the plants [25,26]. These constituents selectively target toxins and pathogens without significant detrimental effect on the human host. This present study is therefore aimedat examining the ameliorative potential of Bambara nut against acute ethanol-induced oxidative stress in Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of PlantMaterial

Bambara nut, the Songkhla 1 variety (red seed coat) were purchased at Obimze area of Owerri, Imo State Nigeria and were identified by a botanist. Immature and damaged seeds were removed. The seeds were peeled and ground to a fine powder using a coffee grinder and stored in screw-cap bottle at -20°C.The extraction was done using soxhlet apparatus and methanol as the solvent according to the methods described by Airaodion et al. [27,28]. About 25 gof the powder was packed into thethimbleof the soxhlet extractor. 250 mL of methanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to

the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the methanol was evaporated in a rotary evaporate at 35 ^{o}C with a yield of 2.17 g which represents a percentage yield of 8.68%. The extract was preserved in the refrigerator until when needed.

2.2. Animal Treatment

Twenty-four (24) adult male Wistar rats with body weight between 140 and 160 g were used for the experiment. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into four (4) groups of six (6) rats each. Animals in groups A and B were administered distilled water while those in groups C and D were administered Bambara nutextract for twenty-one days at a dose of 100 mg/kg body weight 12 hourly via oral route of administration. At the end of the treatment, they were fasted overnight and animals in groups B and D were exposed to a single dose of 70% ethanol at 12 ml/kg body weight to induce oxidative stress. The dosage of ethanol used in this study has been documented to induce tissue toxicity and oxidative damage in rats [29]. After 12 hours of ethanol administration, the animals were anaesthetized using diethyl ether and were sacrificed and blood samples were collected *via* cardiac puncture.

2.3 Determination of Oxidative Stress Biomarkers

Determinationof Lipid Peroxidation (LPO), Reduced Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione peroxidase (GPx) were carried out according to the methods previously described by Airaodion et al. [30].

2.4 Statistical Analysis

Results are expressed as mean ± standard error of the mean (S.E.M). The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values < 0.05 were considered statistically significant

3. RESULTS

Observation from the result of this study revealed that alcohol induced oxidative stress and was ameliorated by Bambara nut extract as presented in figures 2-6.

4. DISCUSSION

Alcohol metabolism results in oxidative and nitrosative stress via elevation of NADH/NAD^{+} redox ratios, induction of nitric oxide synthase (NOS) and NADPH/xanthine oxidase [31,32]. Lipid peroxidation, a primary mechanism of cell membrane destruction and cell damage is a common feature of both acute and chronic alcohol consumption [33,34]. The presence of a high concentration of oxidisable fatty acids and iron significantly contributes to ROS production. A rise in lipid peroxidation level is only identified if there is oxidative damage due to the increase in free radical generation. Generally under normal conditions, the animals tend to maintain a balance between generation and neutralization of ROS in the tissues [35]. But, when the organisms are subjected to xenobiotic stress, the rate of production of ROS including O_{2}□,H_2O_2, OH^{-}, ROO^{-},exceeds their scavenging capacities [35,36]. All the organisms have their own cellular antioxidant defense system composed of both enzymatic and non-enzymatic components. Enzymatic antioxidant pathway consists of SOD, CAT and GPx. Superoxide anion O_{2}□is dismutated by SOD to H_2O_2, which is reduced to water and molecular oxygen by CAT or is neutralized by GPX, which catalyzes the reduction of H_2O_2 to water and organic peroxide to alcohols using GSH as a source of reducing equivalent. Glutathione reductase (GR) regenerates GSH from oxidized glutathione (GSSG), which is a scavenger of ROS as well as a substrate for other enzymes. Glutathione S-transferase (GST) conjugates xenobiotics with GSH for exclusion.

In this study, acute ethanol exposure significantly elevated the malondialdehyde (MDA) levels indicating enhanced peroxidation and breakdown of

the antioxidant defense mechanisms. Decomposition products of lipid hydroperoxide such as malanoldehyde and 4-hydroxynonenal, can cause chaotic cross-linkage with proteins and nucleic acids, which plays an important role in the process of carcinogenesis [29]. In this investigation, lipid

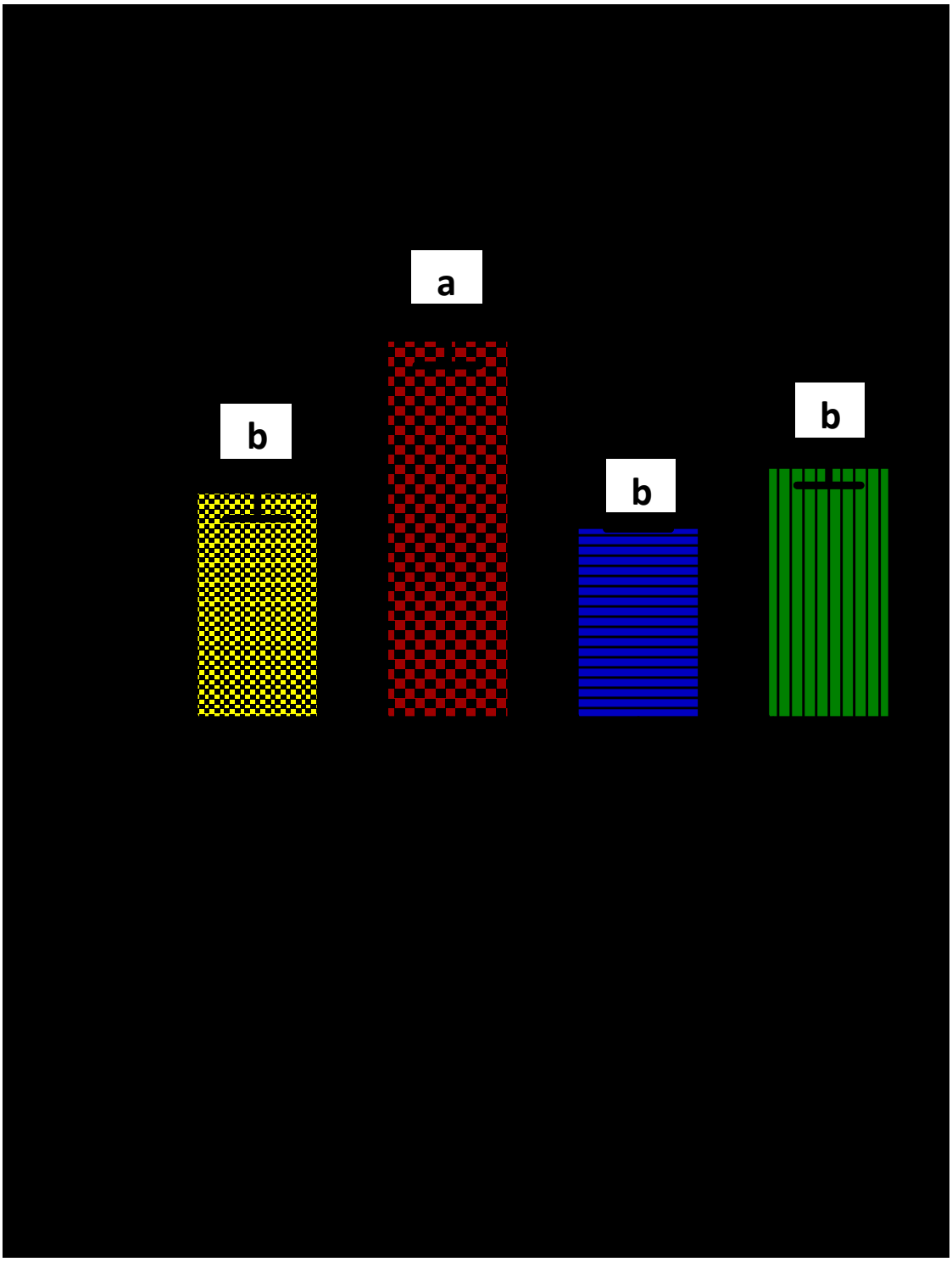

Figure 2: Effect of Bambaranut on Lipid Peroxidation (LPO) of Animals after 21 Days of Administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at $P<0.05$

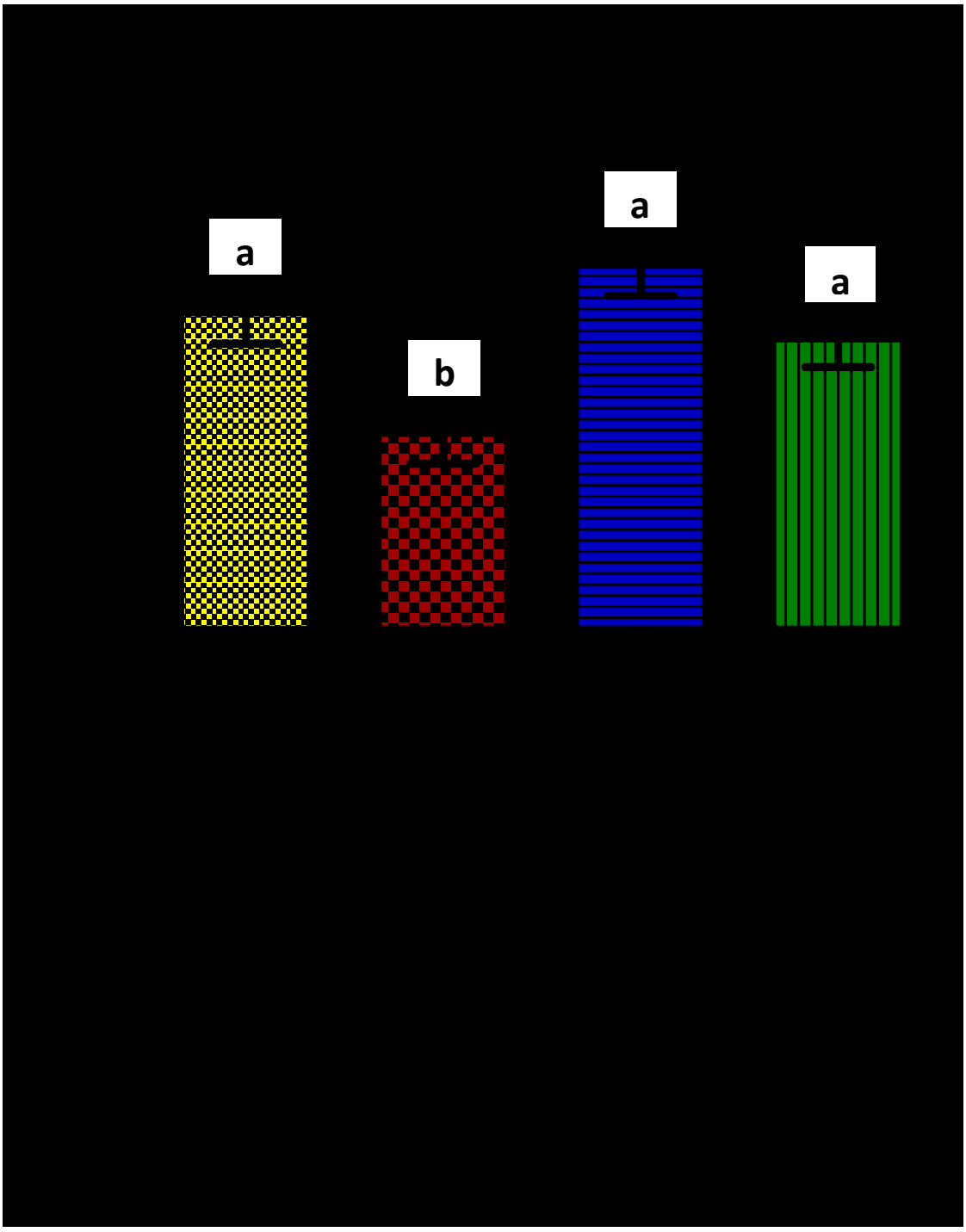

Figure 3: Effect of Bambara nut on the Concentration of Reduced Glutathione (GSH) of Animals after 21 Days of Administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

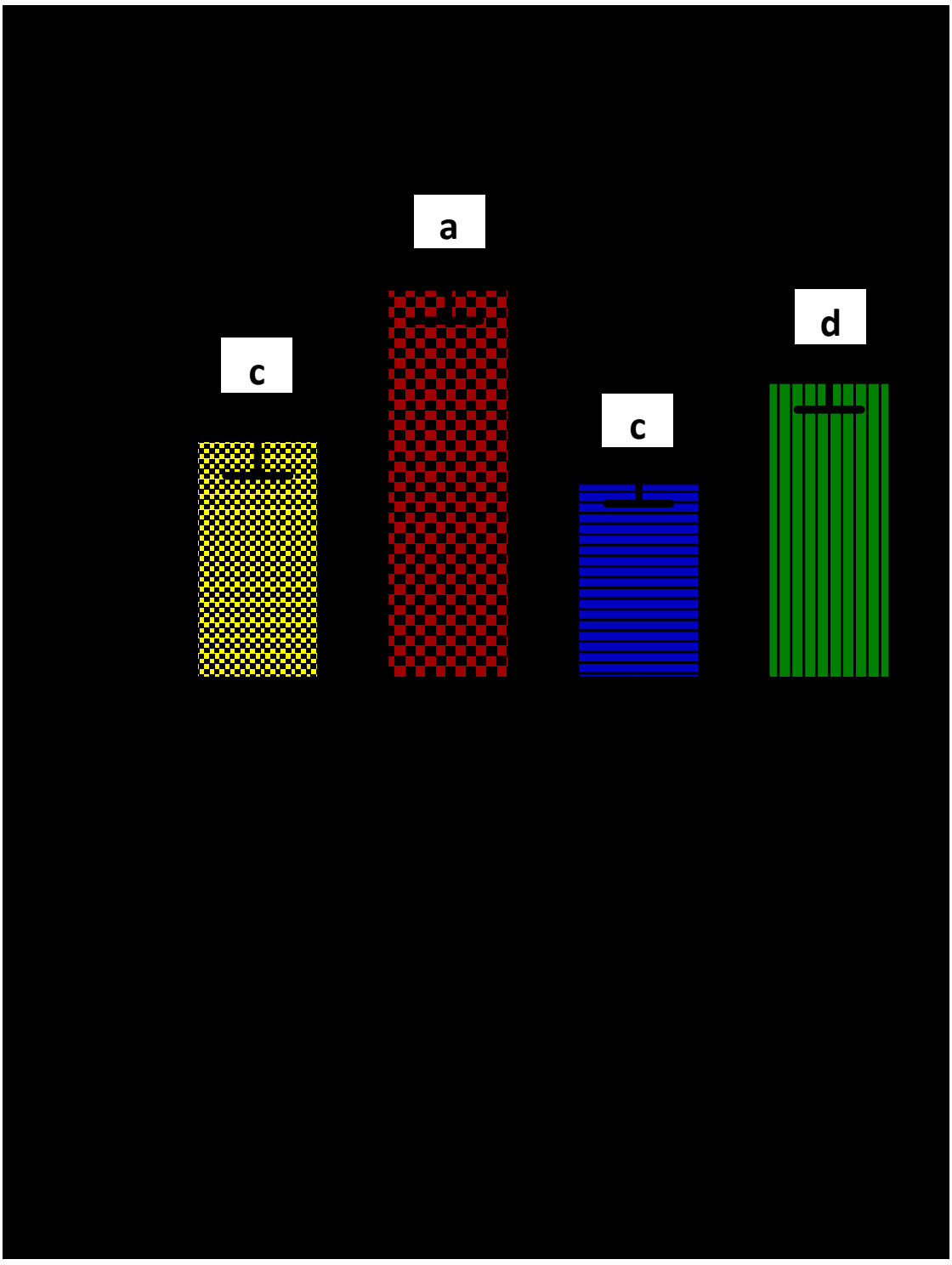

Figure 4: Effect of Bambara nut on the Activity of Catalase (CAT) of Animals after 21 Days of Administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

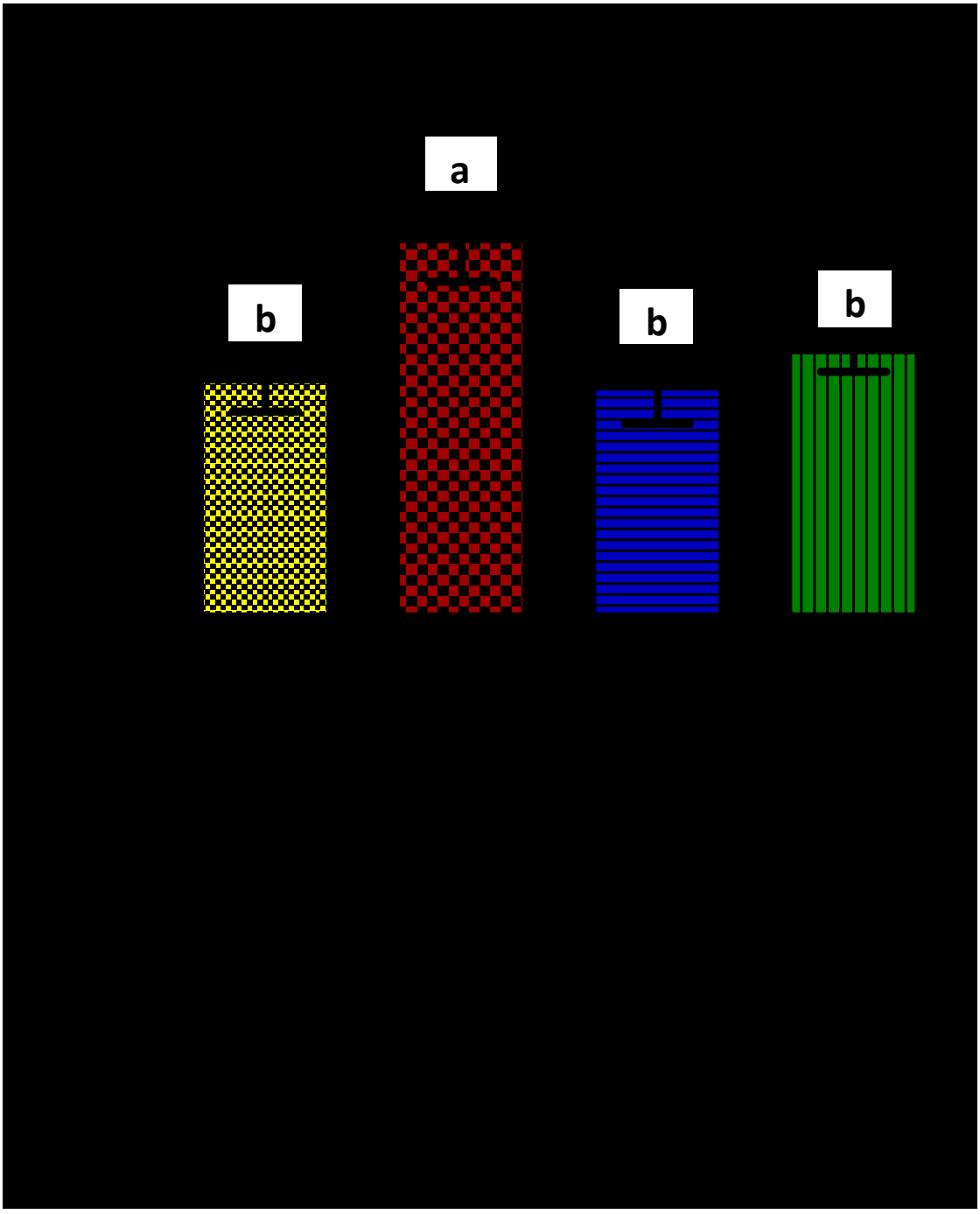

Figure 5: Effect of Bambara nut on the Activity of Superoxide Dismutase (SOD) of Animals after 21 Days of Administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at $P<0.05$

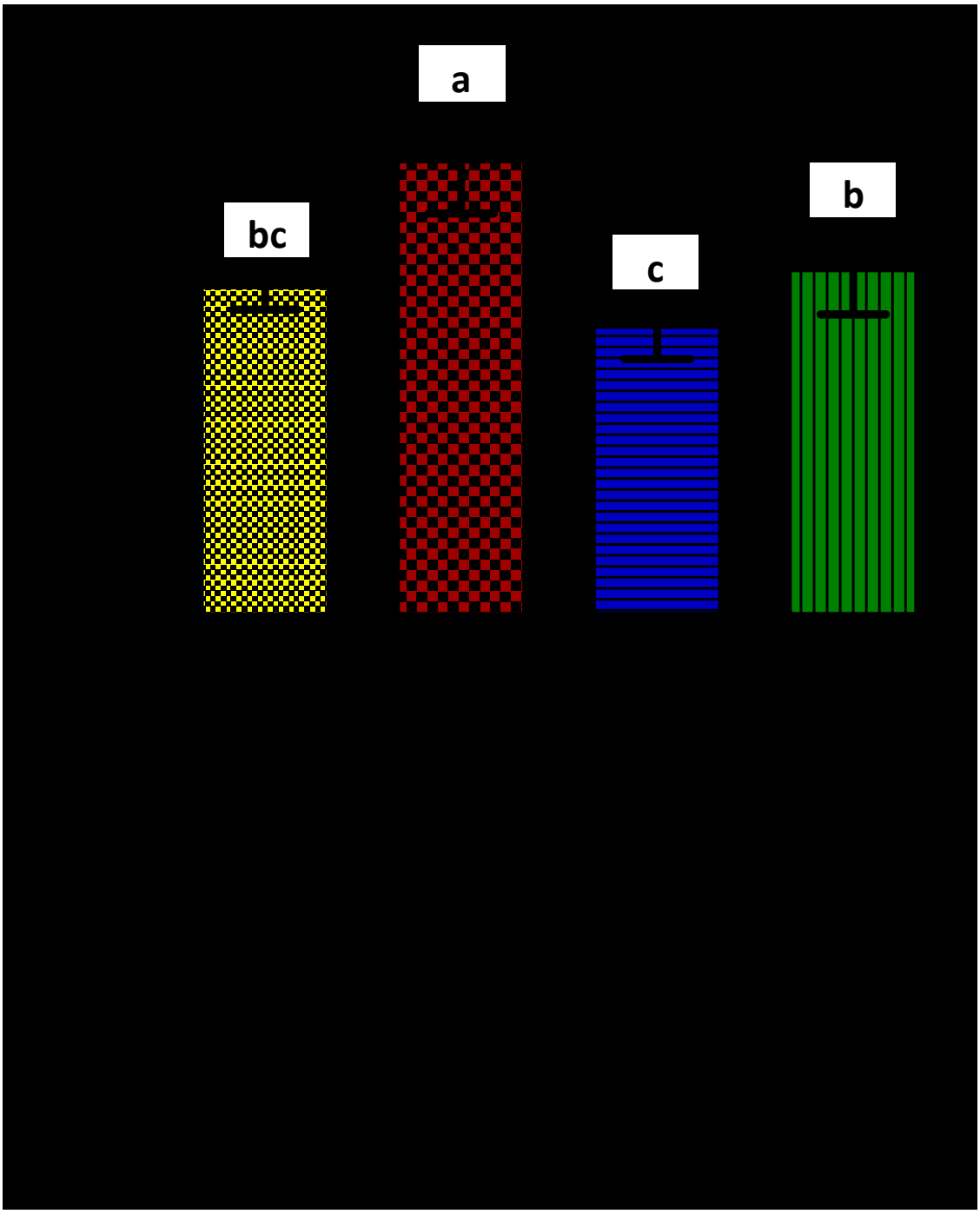

Figure 6: Effect of Bambara nut on the Activity of Glutathione Peroxidase (GPx) of Animals after 21 Days of Administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at $P<0.05$

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peroxidation (LPO) activities show significant increase due to ethanol intoxication. Furthermore, extensive damage to tissues in a free radical mediated LPO results in membrane damage and subsequently decreases the membrane fluid content [30]. Bambara nutpretreatment significantly reversed these alterations causing a significant decrease in MDA levels, suggesting its protective effects against ethanol-induced oxidative damage. This is consistent with the study of Airaodion et al. [30] who reported the hepatoprotective effect of *Parkiabiglobosa*on acute ethanol-induced oxidative stress in Wistar rats.

Glutathione (GSH) is a tripeptide (L-α-glutamylcysteinol glycine) which is highly abundant in all cell compartments and it is the major soluble antioxidant. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism [34]. Glutathione detoxifies hydrogen peroxide and lipid peroxide by donating electron to hydrogen peroxide to reduce it to water and oxygen protecting macromolecules such as lipids from oxidation. In this study, the decrease in the reduced glutathione level in animals treated with ethanol only is connected with ethanol-induced oxidative stress and direct conjugation of GSH with acetaldehyde and other reactive intermediates of alcohol oxidation. This result is in agreement with the findings of Airaodion et al. [29] who reported that acute ethanol treatment caused reduction in the glutathione levels in different tissues. The significant increase ($P < 0.05$) in the glutathione levels of Bambara nut-treated rats prior to ethanol-administration may be due to the direct ROS—scavenging effect of Bambara nut or an increase in GSH synthesis. This is consistent with the report of Airaodion et al. [37] who reported the ameliorativeefficacy of methanolic extract of *Corchorusolitorius* leaves against acute ethanol-induced oxidative stress in Wistar rats.

Catalase (CAT) contributes to ethanol oxidation, by oxidizing a small amount of ethanol in the presence of a hydrogen peroxide (H_2O_2) generating system to form acetaldehyde [38]. In this study, a significant increase was observed in the activity of catalase in control animals and those treated with Bambara nutextract without ethanol induction when compared with ethanol-induced animals with Bambara nutextract pretreatment. This contradicts the findings of Airaodion et al. [30] who reported a nonsignificant difference when animals were treated with *Parkiabiglobosa*. The activity of catalase in animals pretreated with Bambara nutprior to ethanol induction was significantly reduced when compared with those without Bambara nut pretreatment. This might be an indication that ethanol-induced oxidative stress generated elevated ROS which CAT tend to combat, thereby increasing its activity. Bambara nutwas able to reduce the ROS generation with subsequent decrease in CAT activity due to its high phytochemical content [39]as well as its antioxidant potential reported by Chinnapun[40]. Increased CAT activity in this study following acute ethanol exposure suggests elevated ethanol oxidation and formation of oxidising product-acetaldehyde.

Superoxide dismutase (SOD) plays an important role in reducing the effect of free radicals attack, and SOD is the only enzymatic system quenching O_2- to oxygen and H_2O_2 and plays a significant role against oxidative stress [41]. These radicals have been reported to be deleterious to polyunsaturated fatty acids and proteins [42]. In this study, no significant difference was observed in the activity of SOD in control animals and those treated with Bambara nutextract only when compared with ethanol-induced animals with Bambara nut extract pretreatment. The activity of SOD in animals pretreated with Bambara nutprior to ethanol induction was significantly reduced when compared with those without Bambara nut pretreatment. This might be that ethanol-induced oxidative stress generated elevated ROS which SOD tend to combat thereby increasing its activity. *Bambara nut*was able to reduce the ROS generation with subsequent decrease in SOD activity due to its high phytochemical content [39] as well as its antioxidant potential reported by Chinnapun [40]. The increased activity of SOD observed in ethanol-induced animals contradicts the study of Halliwell and Gutterberidge [43] who reported that SOD activity was considerably reduced during ethanol intoxication.

Glutathione peroxidase (GPx) is another enzymatic antioxidant that acts as a defense against oxidative stress [35,36]. In this study, no significant difference was observed in the activity of GPx in control animals and those treated with Bambara nutextract only when compared with ethanol-induced animals with Bambara nutextract pretreatment. The activity of GPx in animals pretreated with Bambara nutprior to ethanol induction was significantly reduced when compared with those without Bambara nut pretreatment. This might be suggestive that ethanol-induced oxidative stress generated elevated ROS in animals which GPx tend to combat thereby increasing its activity. Bambara nutwas able to

reduce the ROS generation with subsequent decrease in GPx activity due to its high phytochemical content [39] as well as its antioxidant potential reported by Chinnapun [40]. The increased activity of GPxobserved in ethanol induced animals contradicts the studies of Airaodion et al. [30] who observed no significant difference in the activity of GPx in the study of hepatoprotective effect of *Parkiabiglobosa*against acute ethanol-induced oxidative stress in Wistar rats and that of Yang et al. [44] who also observed a non-significant difference in GPx activities in rats hepatocyte exposed to varying concentrations of ethanol at an incubation time of 12 hours. The toxicity of ethanol is related to the product of its metabolic oxidation. Acetaldehyde and acetate, produced from the oxidative metabolism of alcohol are capable of forming adducts with cellular macromolecules, causing oxidative damage and affecting metabolic processes [38,45]. Catalase and glutathione peroxidase further detoxify H_2O_2 into H_2O and O_2 [42]. Thus, SOD, catalase and GPx function mutually as enzymatic antioxidative defense mechanism to counter the deleterious effect of ROS. The effect of Bambara nut against alcohol-induced oxidative stress observed in this study is consistent with the findings of Chinnapun [40] who reported that Bambara nut has the capacity to protect plasmid DNA against 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative damage.

4. CONCLUSION

Observations from this present study indicated that ethanol induced oxidative stress as shown in the perturbation of the biomarkers. Bambara nut was able to remedy this effect by regulating the oxidative stress biomarkers, thus possesses ameliorative potential against ethanol-induced oxidative stress and can protect the body against free radicals arising from oxidative stress. This also implies that Bambara nut could boost the immune system. Thus, regular consumption of this nut is recommended.

CONSENT

It is not applicable.

ETHICAL DISCLAIMER

Animal ethic Committee approval has been collected and preserved by the authors.

CONFLICT OF INTERESTS

Authors wish to declare that no conflict of interests exist in this study and publication.

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