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## Protective Effects of Bambara Nut (*Vigna subterranea*) Extract on Paracetamol-Induced Oxidative Stress in Wistar Rats: An In Vivo Evaluation

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### Abstract

Paracetamol (acetaminophen) is one of the most widely used antipyretic and analgesic agents globally. Despite its therapeutic benefits at recommended doses, overdose or prolonged use is associated with hepatotoxicity primarily mediated by the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). NAPQI depletes intracellular glutathione (GSH) reserves and covalently binds to cellular macromolecules, thereby disrupting membrane integrity, impairing mitochondrial function, and promoting oxidative stress. This cascade ultimately compromises hepatic structural and functional integrity. The present study investigated the protective potential of Bambara nut (*Vigna subterranea*) extract against paracetamol-induced oxidative stress in Wistar rats using an in vivo model. Preliminary phytochemical screening of the Bambara nut extract revealed the presence of bioactive constituents including saponins, tannins, flavonoids, glycosides, steroids, amino acids, phenolic compounds, and alkaloids—classes of compounds known for antioxidant and cytoprotective properties. Twenty adult male Wistar rats were randomly assigned into four groups (A-D), with five animals per group. Groups A and B received distilled water orally for 14 days, while Groups C and D were administered Bambara nut extract at a dose of 100 mg/kg body weight every 12 hours for the same duration. Following an overnight fast, oxidative stress was induced in Groups B and D using a single oral dose of paracetamol (3000 mg/kg body weight). After 48 hours, animals were sacrificed under mild anesthesia, and blood samples were collected for biochemical evaluation of oxidative stress markers using standard analytical methods. Results showed that rats administered 3000 mg/kg paracetamol without Bambara nut pre-treatment exhibited significant oxidative stress, characterized by elevated activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and increased lipid peroxidation (LPO) levels. A marked depletion of reduced glutathione (GSH) was also observed, indicating compromised antioxidant defense. Conversely, pre-treatment with Bambara nut extract attenuated these alterations, suggesting stabilization of antioxidant enzyme activity and reduction of lipid peroxidative damage. These findings demonstrate that *Vigna subterranea* possesses significant antioxidant and protective properties against paracetamol-induced oxidative stress. Regular dietary inclusion of Bambara nut may therefore confer protective benefits against oxidative damage mediated by free radicals.

**Keywords**  
Ameliorate,  
Bambara Nut,  
Paracetamol,  
NAPQI,  
Oxidative stress  
biomarkers



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## Introduction

In Nigeria, a huge number of people, including infants and adults, use paracetamol, commonly known as acetaminophen, as a first aid medication for pain, fever, and colds. It is an analgesic, antipyretic, and pain reliever that can be taken with or without a prescription. Although it is safe when used in therapeutic dosages (Ryder & Beckingham, 2011), abusing it might cause liver damage (Ahmed & Khater, 2001). The development of NAPQI (N-acetyl-p-benzoquinone imine), an oxidant that diminishes the liver antioxidant system and erodes components of healthy cells and membranes, ultimately resulting in loss of structural integrity and function, is a manifestation of paracetamol toxicity.

Excessive production of the harmful NAPQI metabolite, which is exacerbated by characteristics of oxidative stress, glutathione (GSH) depletion, and mitochondrial dysfunction that cause ATP stores to be depleted (Jaeschke & McGill, 2015). Research supports the hypothesis that NAPQI, which binds to various cellular proteins, especially mitochondrial proteins, is produced by the metabolic activation of paracetamol. Adherence to mitochondrial proteins is important, especially when GSH diminishes, because mitochondrial protein binding lowers natural antioxidant activities and alters the mitochondrial ATP-synthase  $\alpha$ -subunit, leading to inefficient ATP production (Jaeschke *et al.*, 2012).

Oxidative stress is a consequence of an imbalance between the body's natural antioxidant defense system and the production of free radicals, especially reactive oxygen species (ROS). According to Lobo *et al.* (2010), this imbalance is linked to oxidative damage to a variety of biomolecules, including as lipids,

proteins, and nucleic acids, which may eventually affect normal operations of many tissues and organs. Free radicals and reactive oxygen species (ROS) have been connected to oxidative damage to several human organs and biomolecules. Numerous human diseases, such as lung and cardiovascular conditions, some types of cancer, immune and autoimmune disorders, inflammation, diabetes, cataracts, and brain disorders like Parkinson's and Alzheimer's, have been shown to be significantly impacted by free radicals (Rahman *et al.*, 2012).

The health advantages of plants are believed to be caused by secondary metabolites such as polyphenols, tannins, terpenoids, alkaloids, and flavonoids (Chikezie *et al.*, 2015). Since the usage of natural products with antioxidant components has been proposed as an efficient therapeutic and/or preventive strategy against diseases, there has been a growing quest for potent and fairly priced antioxidants derived from plants (Morales-González, 2013).

The grain legume crop known as bambara nut (*Vigna subterranea* (L.) Verdc) is a staple food in several semi-arid regions of Africa. Bambara nut (BN) is a natural crop grown in most sub-Saharan African countries. This native African legume belongs to both the *Faboidea* subfamily and the *Fabaceae* family. It is a crop with great potential to satisfy the dietary requirements of people living in both rural and urban areas. BN, as it is commonly called in the area, makes up the majority of daily meals in several households. Locals refer to it as "Okpa" in Igbo, "Epa-Roro" in Yoruba, and "Kwaruru" or "Gurjiya" in Hausa (Megwas *et al.*, 2021).

Regardless of climate change, poor sandy soils and drought-prone areas, bambara nuts are still relatively

neglected despite being an agronomically significant crop that is cheap to grow and requires little inputs. The bambara nut (*Vigna subterranea* (L.) Verdc) is a drought-tolerant annual legume that is native to sub-Saharan Africa. With 40–63% carbs, 15–25% protein, 4.5–7.4% fat, 5.2–6.4% fiber, 3.2–4.4% ash, and 2% minerals, this hardy crop is a vital and healthful dietary source (Bamisaiye et al., 2011).

The seeds can be roasted and then ground into flour to make porridge. As an alternative, they can be boiled, soaked, and crushed into a paste for use in fried or steamed meals. "Okpa" is the Nigerian term for this paste. Bambara groundnut flour is widely used in Zambia to produce soup, stew, and bread. Additionally, the seeds can be used to make milk, which can ferment to produce tempeh and dawadaw. The use of milk and wheat made from Bambara nuts has been the subject of numerous investigations (James et al., 2018).

Bambara nut 1 is the third most important grain legume in semi-arid Africa, behind groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata*), according to Megwas et al. (2021). According to Mayes et al. (2013), BN has not gotten much study attention despite its anecdotal benefits in traditional medicine. According to anecdotal reports, it has nutraceutical qualities such as antibacterial, antidiabetic, and antioxidant potential (Al Shareef et al., 2014). According to earlier studies, Bambara nut includes flavonoids, tannins, and phenolic acids, among other phenolic components. These phenolic compounds contain numerous hydroxyl groups that effectively eliminate free radicals, O<sub>2</sub><sup>-</sup>, and singlet oxygen. Additionally, polyphenols have been shown to possess advantageous characteristics and exhibit biological activities, including a protective effect against the development of cancer, diabetes, cardiovascular diseases, osteoporosis, and neurodegenerative diseases (Arts & Hollman, 2005).

They have the capacity to scavenge free radicals, control cell cycle arrest, apoptosis, antioxidant, anti-inflammatory, anti-allergic, antibacterial, antiviral, anti-proliferative, and anti-mutagenic activities, among other health-promoting qualities. This study aims to assess the ameliorative ability of

bambara nut (*Vigna subterranea* (L.) extract) against paracetamol-induced oxidative stress in wistar rats in light of the aforementioned advantageous features of the nut.

## MATERIALS AND METHODS

### Seed collection and seed extract

Bambara seeds (red seed coat) were locally sourced from a local market of Ilorin, Kwara state, Nigeria and were collected and properly identified by a botanist at the university of Ilorin. Damaged and immature seeds were eliminated. The seeds were peeled, ground into a powder using a coffee grinder, and kept at -20°C in a screw-cap bottle. Using a soxhlet device and ethanol as the solvent, seed extract was produced (Airaodion, 2021). For each 18-hour distillation, about 25 g of seed powder and 250 mL of ethanol were employed. An extract weight of 2.11 g, or roughly 8.44% of each sample distilled, was obtained by evaporating ethanol in a rotary evaporator at 35 °C. The extract was then refrigerated.

### Treatments and Experimental Design

Twenty (20) mature male Wistar rats weighing between 160 and 180 g were used in the experiment. Throughout the course of the study, they were housed in hygienic cages with adequate ventilation in humid tropical environments. They were given unlimited access to regular feed and drinking water and were acclimated for seven days. Every animal received humane treatment in compliance with the guidelines outlined in the National Academy of Science's 1 "Guide for the Care and Use of Laboratory Animals," which was released by the National Institute of Health (NRC, 2011).

They were randomly split into four groups of five rats each. For 14 days, animals in groups A and B received distilled water, while animals in groups C and D received Bambara nut extract orally at a rate of 100 mg/kg body weight every 12 hours. The control group consisted of the animals in group A. Following an overnight fast, animals in groups B and D were given a single dosage of paracetamol (3000 mg/kg body weight) to cause oxidative stress. The dosage of paracetamol used in this study has been documented to induce tissue toxicity and oxidative damage in rats

(Henneh et al., 2022). Following paracetamol administration, all of the animals were fasted for 48 hours. The animals were then given a mild anesthetic (pentobarbitone 50 mg/kg, i.p.) and their hearts were punctured to draw blood, which was then placed in gel-

separator vacutainer tubes for biochemical analysis. The animals' livers were removed for additional research after they were mercifully slaughtered by cervical dislocation. Table 1 displays the therapy schedule.

Table 1: Experimental study design

Group	Treatment	Dosage
A	Control	Distilled water
B	Paracetamol Only	3000mg/kg body weight
C	Bambara Nut only	100mg/kg body weight
D	Bambara nut + Paracetamol	100mg/kg + 3000mg/kg body weight

### Determination of Qualitative phytochemical constituents

The ethanolic extract of Bambara Nut was tested for phytochemicals using standard protocols. The methods (Harborne, 1973; Sofowora, 1993; Trease & Evans, 1989) were used to determine the saponins, tannins, flavonoids, terpenoids, glycosides, steroids, phlobatannins, amino acids, phenolics, and alkaloids.

### Determination of Oxidative Stress Biomarkers

Using the method of Airaodion et al. (2019i), blood concentrations of Lipid Peroxidation (LPO), Reduced Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione peroxidase (GPx) were measured.

### Statistical Analysis

The mean  $\pm$  standard error of mean (S.E.M.) is used to express the results. Turkey's test and One-way Analysis of Variance (ANOVA) were used to determine the degree of homogeneity between the groups. Graph Pad Prism 8 Software Version (8.0.1) was used for all analyses, and p values were deemed statistically significant.

## RESULTS

A preliminary phytochemical screening for the various constituents was performed on freshly prepared bambara Nut (*Vigna subterranea*) ethanolic extract. Saponins, tannins, flavonoids, glycosides,

steroids, amino acids, phenolics, and alkaloids were among the active ingredients found (table 1).

Table 1: Phytochemical Screening

Phytochemicals	<i>Vigna subterranea</i>
Saponins	+
Tannins	+
Flavonoids	+
Terpenoids	-
Glycosides	+
Steroids	+
Phlobatannins	-
Amino acid	+
Phenolics	+
Alkaloids	+

Key: + Moderately present  
- Absent

### Determination of Oxidative Stress Biomarkers

These results demonstrated that, in comparison to the control animals, the GSH concentration of the group given Bambara nut and pretreatment with Bambara nut rose, while the GSH concentration of the group given paracetamol only considerably dropped ( $p < 0.05$ ) (Figure 1).

When 1 animals in group D received prophylactic therapy with Bambara nut extract before paracetamol induction, the rise in CAT, SOD, and GPx activities brought on by paracetamol induction was minimized.

However, compared to the control mice, treatment with paracetamol only increased the activities of CAT, SOD, and GPx, whereas Bambara nut only lowered them (Figure 2-4).

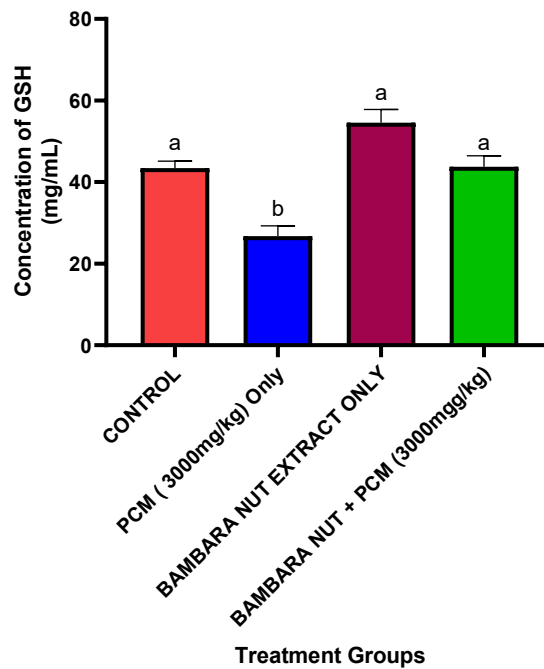


Figure 1: Bambara nut's effect on concentrations of reduced glutathione (GSH)  
The findings are displayed with  $n = 5$  as mean  $\pm$  standard deviation.  
 $P < 0.05$  indicates a significant difference between bars with different letters.

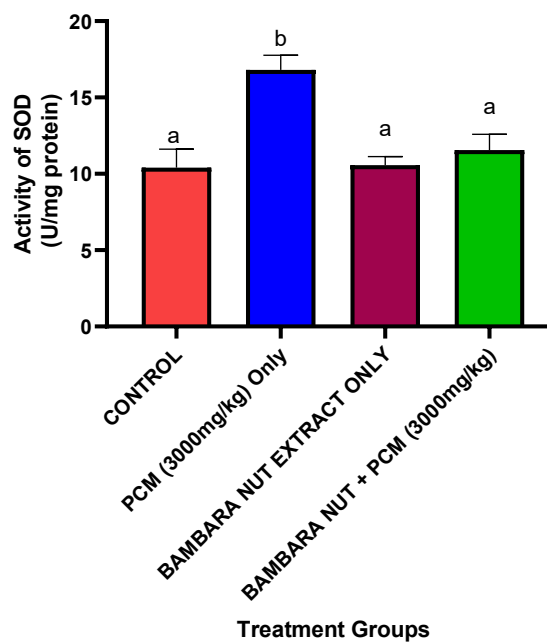


Figure 2: Bambara nut's effect on superoxide dismutase activity (SOD)  
The findings are displayed with  $n = 5$  as mean  $\pm$  standard deviation.  
 $P < 0.05$  indicates a significant difference between bars with different letters.

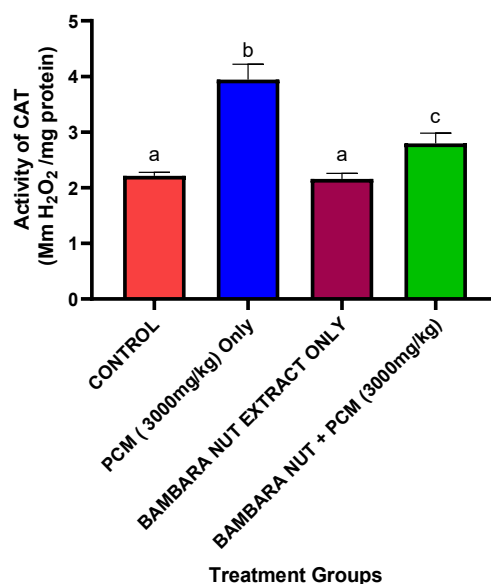


Figure 3: Bambara nut's effect on catalase activity (CAT)  
The findings are displayed with  $n = 5$  as mean  $\pm$  standard deviation.  
 $P < 0.05$  indicates a significant difference between bars with different letters

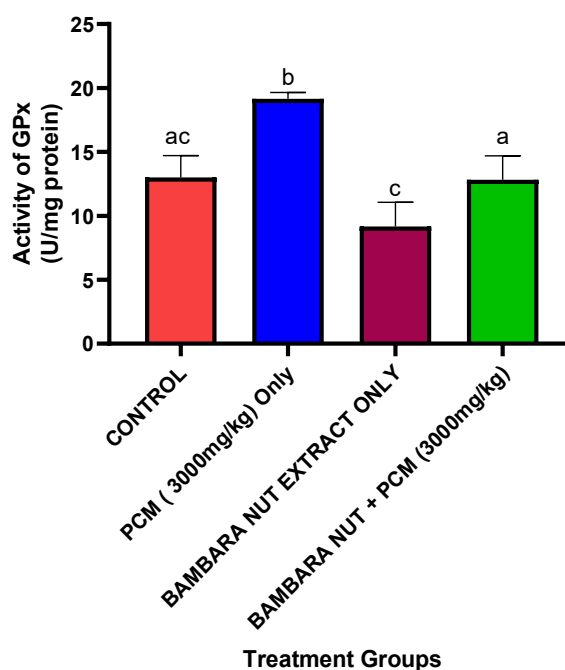


Figure 4: Bambara nut's effect on glutathione peroxidase (GPx) activity  
The findings are displayed with  $n = 5$  as mean  $\pm$  standard deviation.  
 $P < 0.05$  indicates a significant difference between bars with different letters

One of the final byproducts of lipid peroxidation is malondialdehyde (MDA). In the current study, the concentration of MDA in the group given paracetamol alone was significantly higher ( $P < 0.05$ ) than in the control group. Lipid peroxidation levels were lower in

animals treated with Bambara nut prior to paracetamol administration and in rats given solely Bambara nut, which is always indicative of lower MDA concentrations (Figure 5).

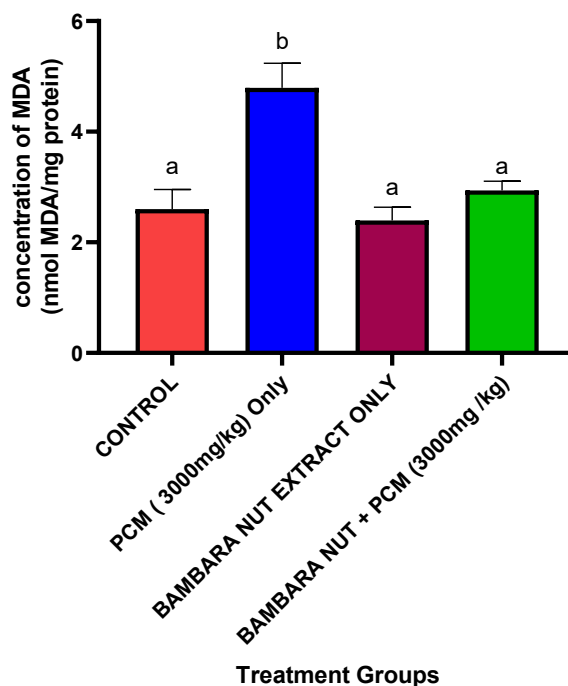


Figure 5: Bambara nut's effect on Malondialdehyde concentration  
 The findings are displayed with  $n = 5$  as mean  $\pm$  standard deviation.  
 $P < 0.05$  indicates a significant difference between bars with different letters

## DISCUSSION

The bambara groundnut's (*Vigna subterranean*) **phytochemical** investigation identified chemical components that have been demonstrated to have certain pharmacological properties that are helpful in ethnomedicine. Saponins, tannins, flavonoids, glycosides, steroids, amino acids, phenolics, and alkaloids are among the notable and identified phytochemicals. The pharmacological properties of these secondary metabolites, such as their ability to scavenge free radicals, regulate cell cycle arrest, apoptosis, and other health-promoting properties, as well as their antioxidant, anti-inflammatory, anti-allergic, antimicrobial, antiviral, anti-proliferative, and

anti-mutagenic effects, have been closely associated. Similar compounds, particularly polyphenols and alkaloids, were discovered to be prevalent in extracts prepared with the polar solvent ethanol; these results are consistent with those of Abdulrashid & Hassan's investigation (2021).

The biotransformation of chemicals into highly reactive metabolites that induce cellular toxicity has attracted a lot of attention in recent years. Numerous substances, even therapeutically beneficial medications, have the potential to harm cells by metabolically activating into very reactive substances such as nitrenes, carbenes, and free radicals (Gupta *et al.*, 2004). Many human neurologic and other illnesses

now seem to be fundamentally caused by free radicals or oxidative damage (Atawodi, 2005). Following the delivery of a paracetamol oral dose, as a result its poor acidity and lipid solubility, the gut absorbs it quickly. It is converted into its main, pharmacologically inert glucuronidated and sulfated conjugates, which are eliminated in the urine, in about 50-60% of cases. Cytochrome P450 isoforms (CYP2E1, CYP2A6) convert a small amount of paracetamol (5-10%) in the liver microsomes into the reactive metabolite N acetyl-para-benzo-quinone imine (NAPQI).

NAPQI is an oxidant that attacks healthy cell and membrane components, weakening the liver's antioxidant system and ultimately leading to a loss of structural integrity and function. There is a clear correlation between the amount of paracetamol consumed and the cellular damage that NAPQI causes. When NAPQI is consumed in a non-toxic manner, hepatic glutathione quickly conjugates it through glucuronidation and sulfonation processes to produce mercaptate and cysteine complexes, which are then excreted in the urine.

The primary soluble antioxidant, glutathione (GSH) is a tripeptide (L-aglutamylcysteinol glycine) that is prevalent in all cell compartments. By conjugating NAPQI (Manyike et al., 2000) to generate GSSG (oxidized form of GSH), glutathione maintains hepatocyte integrity and directly reduces ROS such lipid peroxides. It also plays a significant role in xenobiotic metabolism (Livingstone & Davies, 2007) as well as preserving the integrity of the hepatocyte by conjugating NAPQI, which results in the production of GSSG (oxidized form of GSH) (Manyike et al., 2000). GSH levels are depleted by this process (Jaeschke et al., 2012), as demonstrated by the group that received paracetamol alone, which had a lower concentration of GSH, and the group that received bambara nuts, which had a large increase ( $p < 0.05$ ). This suggests that the extracts could spare or aid GSH in detoxifying the metabolites by being oxidized itself. In this work, oxidative stress and direct conjugation of GSH with the metabolite NAPQI are linked to the decrease in reduced glutathione levels in animals given just paracetamol.

This suggests that the hepatotoxic dose causes most of the paracetamol to be metabolized by the CYP2E1 pathway, which depletes glutathione through the

activation of GST-S-Transferases, the accumulation of NAPQI at toxic concentrations, and ultimately an overabundance of GSH in the system (Jaeschke et al., 2012). The direct ROS scavenging effect of Bambara nut or an increase in GSH synthesis could be the cause of the considerable ( $P < 0.05$ ) rise in glutathione levels in rats fed with Bambara nut before paracetamol was administered. This outcome is consistent with the findings of Ojo et al. (2006), who studied how extracts of green tea (*Camellia sinensis*) and lemongrass (*Cymbopogon citratus*) inhibited oxidative stress caused by paracetamol in rats. Superoxide dismutase (SOD) and catalase are two significant endogenous antioxidant enzymes that are essential for mitigating the effects of free radicals. While superoxide dismutase is responsible for converting superoxide anion to hydrogen peroxide and oxygen (Zelko et al., 2002), catalase naturally converts hydrogen peroxide to water and oxygen (Chelikani et al., 2004). In the current investigation, there was no discernible difference in SOD activity between the control group and either the group that received Bambara nut alone or the group that received Bambara nut prior to PCM induction.

The group that received just paracetamol showed a significant increase in SOD and CAT activities. This could be because paracetamol causes oxidative stress and increased ROS, which SOD and CAT typically fight off, increasing their activity. Because of its high phytochemical content (Nwadi et al., 2019) and antioxidant capacity (Chinnapun, 2018), bambara nut was able to lower the formation of ROS and consequently lower SOD activity. MDA is a persistent consequence of the cumulative oxidative destruction of lipids, it functions as an indirect biomarker for this process. Increases in malondialdehyde (MDA) concentrations indicates either a decline in the body's antioxidant capacity or an increase in the formation of reactive oxygen species (ROS).

On the other hand, a decline in MDA levels suggests that the body's antioxidative defense mechanisms are functioning well, which lowers lipid peroxidation (Ayala et al., 2014). Lipid peroxidation can only be observed when there is oxidative damage caused by an increase in free radical generation. Under normal conditions, animals frequently maintain a balance between the

generation and neutralizing of ROS in their tissues (Airaodion et al., 2020b).

However, under xenobiotic stress, the rate of formation of ROS, such as  $O_2^-$ ,  $H_2O_2$ ,  $OH^-$ , and  $ROO^-$ , exceeds the organisms' capacity to scavenge them (Airaodion et al., 2020c). The fact that rats given paracetamol without first consuming Bambara nut had the highest levels of malondialdehyde (MDA) and LPO activity, which indicate an enhanced peroxidation, supports the theory that paracetamol overdose is a precursor of pro-oxidants, which mediate oxidative stress and cell death. The MDA levels of the PCM group in this study were considerably greater than those of the control group, however the groups that were pretreated with Bambara nut extract were able to reduce their MDA levels.

Malondialdehyde (MDA) concentration which suggest increased peroxidation, were highest in rats given a paracetamol dose without having previously consumed Bambara nut. The results of this investigation are consistent with those published by Gujral et al. (2002).  $H_2O_2$  is further detoxified into  $H_2O$  and  $O_2$  by catalase and glutathione peroxidase (Airaodion et al., 2020c). In order to counteract the harmful effects of ROS, Superoxide dismutase, catalase, and Glutathione Peroxidase work together as an enzymatic antioxidative defense mechanism. Hydroperoxides are reduced by the cytosolic enzyme glutathione peroxidase (GSH-Px; E.C. 1.11.1.9). GSH-Px serves several crucial roles in phagocytic cells and is the most potent antioxidant in erythrocytes against oxidative stress.

The activity of GPx in the control and Bambara nut extract-treated animals in this investigation did not differ significantly from that of the paracetamol-induced mice with Bambara nut extract pretreatment. When comparing animals who received bambara nut pretreatment before paracetamol induction to those that did not, there was a substantial decrease in GPx activity. This may indicate that paracetamol-induced oxidative stress raised ROS in rat, which GPx seeks to offset by increasing its activity. Bambara nut was able to reduce ROS generation and, consequently, GPx activity due to its high phytochemical content (Nwadi, 2019) and antioxidant capability (Chinnapun, 2018).

The efficacy of bambara nut against paracetamol-induced oxidative stress demonstrated in this study is consistent with the findings of Chinnapun (2018), who discovered that Bambara nut can protect plasmid genome from 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative injury.

## CONCLUSION

The study's findings clearly show that Bambara Nut can mitigate the effects of oxidative stress and, as a result, shield the body from free radicals that occur from oxidative stress. It is therefore advised to eat this nut on a regular basis.

## ETHICAL APPROVAL

The Institutional Animal Ethics Committee authorized all animal-related experimental procedures, which were carried out in compliance with national and international standards for the use and care of lab animals.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR'S DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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