ANTIOXIDATIVE POTENTIALS OF COOKED BAMBARA GROUNDNUT BASED DIET (VIGNA SUBTERRANEAN) ON LIPID PEROXIDATION STATUS IN PRETREATED ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study examines the antioxidant potentials of Cooked Bambara groundnut (Vigna subterranean) based diet in pretreated alloxan induced diabetic rats. Thirty (30) male albino rats (Rattus norvegicus) were randomly divided into three experimental groups (A, B, C) i.e 10 rats in each group. Group A received standard formulated diet, Group B were fed with 50:50 ratio of corn starch and cooked Bambara nut based diet, Group C received whole cooked Bambara nut based diet for four weeks. After four weeks of feeding, the three groups were sub-divided into subgroups A1, A2, B1, B2 and C1, C2. Subgroups A2, B2 and C2 were induced with 150mg/kg body weight of alloxan by a single intraperitoneal injection. Each Subgroups were maintained on their respective diet for two weeks, after which the blood samples were collected via cardiac puncture, heart was removed and assayed for Malondialdehyde (MDA), Reduced glutathione (GSH) and antioxidant enzyme activities (Catalase, Superoxide dismutase). Significant (p < 0.05) elevation in MDA, and a reduction in antioxidant activities were observed in plasma and heart of group A2 compared with group A1. A significant decrease in plasma and heart MDA level was observed in group C2 compared with group C1. Plasma and heart antioxidant activities of group C2 significantly increased (P ≤ 0.05) when compared with group C1. There was no significant difference in plasma and heart MDA level and antioxidant activities in group B2 compared with group B1. In conclusion, Cooked Bambara groundnut exhibits its antioxidant potentials by alleviating oxidative stress. However, it appears that increased concentration of cooked Bambara groundnut in the diet
could be more effective in preventing oxidative stress in pretreated alloxan induced diabetic rats.

KEYWORDS: Antioxidant, alloxan, oxidative stress, Vigna subterranean.

INTRODUCTION
Diabetes mellitus is an endocrine disease characterized by chronic hyperglycemia associated with abnormalities in carbohydrate, fat and protein metabolism caused by complete or relative insufficiency of insulin secretion and/or insulin action. Diabetes is broad in terms of classification, requiring a clear understanding of pathologic presentation and genetic contribution. Till date, immune-mediated diabetes also known as non-insulin dependent diabetes mellitus (NIDDM) have been reported.\[1\] Although type 1 and 2 diabetes mellitus are the major classes, minor classes including chemical induced diabetes, gestational diabetes and infection induced diabetes have been documented.\[2\] Treatment regimen for diabetes has been aimed at ensuring weight control, providing nutritional requirements, allowing good glycemic control with blood glucose levels as close to normal as possible and correcting any associated blood lipid abnormalities. These have been achieved in part by exogenous insulin and some oral hypoglycemic drugs.\[3\] The earliest record of ethnobotanical treatment for diabetes mellitus involved the use of plants. Quite a multitude of plants or plant materials have been described for the treatment of diabetes worldwide.\[4\]

Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. They are produced either from normal cell metabolisms in situ or from external sources (pollution, cigarette smoke and radiation). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of diseases such as Diabetes, cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through food.\[5\] Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing.\[6\] The body is incapable of producing enough endogenous antioxidants to combat free radicals on their own, therefore increase in the intake of exogenous antioxidants balance their loss due to disease or aging.\[7\] Several studies have shown that the therapeutic effects of some medicinal plants, fruits and even vegetables which are commonly used in
folklore remedies against many diseases can be attributed to the antioxidant properties of their phytoconstituents.\textsuperscript{[8]} Thus antioxidant activity of plants might be due to their phenolic compounds.\textsuperscript{[9]} Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action.\textsuperscript{[10]} The role of flavonoids are quite important in diabetes due to their ability to terminate free radicals and reduce oxidative stress. Flavonoids present in the plants have also been shown to regenerate the damaged β-cells of pancreas in some studies.\textsuperscript{[11]}

Bambara groundnut (\textit{Vigna subterranean}) is an African species, the cultivation of which predates that of groundnut. It represents the third most important grain legume in semi-arid Africa. Bambara groundnut is a member of the family Fabaceae. It is resistant to high temperature and is suitable for marginal soils where other leguminous crops cannot be grown.\textsuperscript{[12]} Bambara groundnut remains one of the crops less investigated but one with a great nutritional potential, it has a high nutritive value with 65% carbohydrate and 18% protein content.\textsuperscript{[13]} Due to its high protein value it is a very important crop for people in Africa. Bambara groundnut are used locally for preparing moi-moi (Igbo, Nigeria). It can be boiled and eaten as nut and can also be grounded into flour for preparing fufu for (Middle belt, Nigeria). Bambara groundnut is used to fortify maize for pap (Anambra State, Nigeria). The dry seeds can last for very long time and serves as famine food boosting food availability.

The aim of this study therefore is to examine the antioxidant potentials of Cooked Bambara groundnut in pretreated alloxan induced diabetic rats.

**MATERIALS AND METHODS**

Bambara groundnut was bought in Oja-oba market in Ado-Ekiti. The nut was washed, cooked and sundried until the mass was constant and ground into powdered form with a blender. The flour was then kept in air tight container until required for diet composition. All chemicals used for the study were of analytical grade (ANALAR).

**Animal Groupings**

Thirty (30) male albino rats (\textit{Rattus novergicus}) with average weight of about 90-100g were used for the study. They were obtained from College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria. The rats were kept in good conditions and were given normal rat feed and water \textit{ad libitum}. They were randomly divided into three experimental groups (A, B, C) i.e 10 rats in each group. GroupA received standard formulated diet, GroupB were fed with
50:50 ratio of corn starch and cooked Bambara nut based diet, Group C received whole cooked Bambara nut based diet (Table 1) for four weeks. After four weeks of feeding, the three groups were sub-divided into subgroups A1, A2, B1, B2 and C1, C2. Subgroups A2, B2 and C2 were induced with 150mg/kg body weight of alloxan by a single intraperitoneal injection. Each Subgroups were maintained on their respective diet for two weeks.

**Preparation of plasma and tissue homogenate**

Blood sample collected into Lithium heparin bottle was centrifuged at 3000rpm (revolution per minutes) for 10 minutes. After centrifugation, the supernatant which was the plasma was collected using a Pasteur’s pipette. The plasma, thus obtained were appropriately labeled and stored in a freezer at -10°C until required for further analysis such as assessment of lipid peroxidation (MDA), assay for Reduced Glutathione and Antioxidant enzymes (Superoxide dismutase and Catalase).

After sacrifice, the rats were dissected and tissue (heart) was isolated. The isolated tissue was blotted on a filter paper, weighed and immediately kept in a universal bottle containing ice-cold sucrose buffer solution (1:4 w/v). The heart cut with a clean scalpel was then subjected to homogenization using Teflon homogenizer in ice-cold sucrose buffer solution (1:4 w/v). The homogenate was centrifuged at 2500rpm for 10 minutes and the supernatant separated was stored in the freezer at -10°C until required for further analysis such as assessment of lipid peroxidation (MDA), assay for Reduced Glutathione and Antioxidant enzymes (Superoxide dismutase and Catalase).

**Table 1: Diet Composition (g/kg)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Starch</td>
<td>560</td>
<td>280</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin/mineral mixture</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cooked Bambara groundnut</td>
<td>-</td>
<td>280</td>
<td>560</td>
</tr>
</tbody>
</table>

Group A - Standard formulated diet composition
Group B - 50:50 ratio of corn starch and cooked Bambara nut based diet
Group 3 - Whole cooked Bambara nut based diet
Biochemical Analysis

Lipid peroxidation was determined according to the method of [14]. Catalase (CAT) activity was determined according to the method of [15]. Superoxide dismutase (SOD) activity in microsomes was determined according to the method of [16]. The method of [17] was followed in estimating the level of reduced glutathione (GSH).

Lipid peroxidation determination

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) present in the test sample.

Procedure

The tissue homogenates were appropriately diluted using 5ml of distilled water. An aliquot of 0.4 ml of the sample was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. This was then cooled in ice and centrifuged at 3000 g. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5$ m$^{-1}$cm$^{-1}$.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance} \times \text{Volume of mixture}}{E_{532\text{nm}} \times \text{volume of sample} \times \text{mg protein}}$$

Superoxide dismutase determination

Procedure

Microsome (0.1ml) was diluted in 0.9ml of distilled water to make a 1 in 10 dilution of microsome. An aliquot of 0.2ml of the diluted microsome was added to 2.5ml of 0.05M carbonate buffer pH 10.2 to equilibrate in a cuvette and the reaction started by the addition of 0.3ml of 0.3M adrenaline. The reference cuvette contained 2.5ml of carbonate buffer, 0.3ml of substrate (adrenaline) and 0.2ml of distilled water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Where $A_0$ = absorbance at time, $t = 0$

$A_3$ = absorbance at time, $t = 150$ seconds

$$\% \text{ inhibition} = \frac{100 - \text{(increase in absorbance for substrate)}}{\text{(increase in absorbance of blank)}} \times 100$$
1 unit of SOD activity is defined as the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 minute.

**Catalase Determination**

**Procedure**
The supernatant (1ml) fraction of the homogenate was mixed with 19 ml distilled water to give a 1:20 dilution. The assay mixture contained 4ml of H$_2$O$_2$ solution (800μmoles) and 5 ml of phosphate buffer, pH 7.0. Thereafter 1 ml of properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide contents of the withdrawn sample were determined.

\[
\text{H}_2\text{O}_2 \text{ consumed} = 800\mu\text{moles} - \text{H}_2\text{O}_2 \text{ concentration remaining}
\]

\[
\text{Catalase activity} = \frac{\text{H}_2\text{O}_2 \text{ consumed}}{\text{mg protein}}
\]

**Reduced glutathione determination**

**Procedure**
An aliquot of the sample was deproteinated by the addition of an equal volume of 4% sulfosalicyclic acid. This was centrifuged at 4,000g for 5minutes. Thereafter, 0.5 ml of the supernatant was added to 4.5 ml of Ellman’s reagent. A blank was prepared with 0.5 ml of the diluted precipitating agent and 4.5 ml of Ellman’s reagent. Reduced glutathione, GSH, is proportional to the absorbance at 412 nm.

\[
\text{GSH Consumed} = 245.84 - \text{GSH remaining}
\]

**Statistical analysis**
The results are expressed as Mean ± standard deviation. Analysis of variance was used to test for differences in the groups. Differences were considered to be statistically significant at P<0.05.
RESULTS

Figure 1: Graph showing Concentration of Malondialdehyde (nmol/ml) in Plasma and Heart of Non-diabetic and Diabetic rats

Groups with different lettering (a, b, c, d) differ significantly (P ≤ 0.05) from each other.

A₁ – Non-diabetic group
A₂ – Diabetic group
B₁ – Non-diabetic group fed 50:50 ratio of corn starch and cooked Bambara groundnut based diet
B₂ – Diabetic group pretreated with 50:50 ratio of corn starch and Bambara groundnut based diet
C₁- Non-diabetic group fed whole cooked Bambara groundnut based diet
C₂- Diabetic group pretreated with whole cooked Bambara groundnut based diet

Table 2: Activities of Superoxide dismutase (%) in Plasma and Heart of Non-diabetic and Diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>3.08±0.32ᵇ</td>
<td>3.03±0.31ᵇ</td>
</tr>
<tr>
<td>A₂</td>
<td>1.89±0.12ᵃ</td>
<td>1.92±0.23ᵃ</td>
</tr>
<tr>
<td>B₁</td>
<td>4.07±0.31ᵇ</td>
<td>5.01±1.20ᵇ</td>
</tr>
<tr>
<td>B₂</td>
<td>4.90±0.31ᵇ</td>
<td>5.98±1.24ᵇ</td>
</tr>
<tr>
<td>C₁</td>
<td>5.01±1.31ᵇ</td>
<td>6.44±1.13ᵇ</td>
</tr>
<tr>
<td>C₂</td>
<td>7.62±1.12ᶜ</td>
<td>9.81±1.61ᶜ</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD; values in the same column with different superscript are significantly different at P ≤ 0.05.
A₁ – Non-diabetic group
A₂ – Diabetic group
B₁ – Non-diabetic group fed 50:50 ratio of corn starch and cooked Bambara groundnut based diet
B₂ – Diabetic group pretreated with 50:50 ratio of corn starch and Bambara groundnut based diet
C₁ - Non-diabetic group fed whole cooked Bambara groundnut based diet
C₂ - Diabetic group pretreated with whole cooked Bambara groundnut based diet

Table 3: Activities of Catalase (µmol/mg protein) in Plasma and Heart of Non-diabetic and Diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>7.14±1.12(^{bc})</td>
<td>7.05±1.15(^{b})</td>
</tr>
<tr>
<td>A₂</td>
<td>5.31±1.33(^{a})</td>
<td>5.30±1.31(^{a})</td>
</tr>
<tr>
<td>B₁</td>
<td>7.33±1.44(^{bc})</td>
<td>7.31±0.73(^{b})</td>
</tr>
<tr>
<td>B₂</td>
<td>7.55±1.12(^{bc})</td>
<td>7.82±0.82(^{b})</td>
</tr>
<tr>
<td>C₁</td>
<td>8.13±1.05(^{bc})</td>
<td>7.97±1.78(^{b})</td>
</tr>
<tr>
<td>C₂</td>
<td>10.47±1.34(^{d})</td>
<td>9.65±0.69(^{c})</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD; values in the same column with different superscript are significantly different at P ≤ 0.05.

A₁ – Non-diabetic group
A₂ – Diabetic group
B₁ – Non-diabetic group fed 50:50 ratio of corn starch and cooked Bambara groundnut based diet
B₂ – Diabetic group pretreated with 50:50 ratio of corn starch and Bambara groundnut based diet
C₁ - Non-diabetic group fed whole cooked Bambara groundnut based diet
C₂ - Diabetic group pretreated with whole cooked Bambara groundnut based diet

Table 4: Concentration of Reduced glutathione (mg/g) in Plasma and Heart of Non-diabetic and Diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>2.07±1.12(^{b})</td>
<td>2.91±0.67(^{b})</td>
</tr>
<tr>
<td>A₂</td>
<td>1.03±1.33(^{a})</td>
<td>1.08±0.32(^{a})</td>
</tr>
<tr>
<td>B₁</td>
<td>3.09±0.33(^{b})</td>
<td>3.05±0.93(^{b})</td>
</tr>
<tr>
<td>B₂</td>
<td>3.17±1.12(^{b})</td>
<td>4.95±0.56(^{bc})</td>
</tr>
<tr>
<td>C₁</td>
<td>3.43±0.33(^{b})</td>
<td>6.22±1.78(^{c})</td>
</tr>
<tr>
<td>C₂</td>
<td>5.01±1.32(^{c})</td>
<td>7.99±0.22(^{d})</td>
</tr>
</tbody>
</table>
Results are expressed as mean±SD; values in the same column with different superscript are significantly different at P ≤ 0.05.

A₁ – Non-diabetic group.
A₂ – Diabetic group.
B₁ – Non-diabetic group fed 50:50 ratio of corn starch and cooked Bambara groundnut based diet.
B₂ – Diabetic group pretreated with 50:50 ratio of corn starch and Bambara groundnut based diet.
C₁ – Non-diabetic group fed whole cooked Bambara groundnut based diet.
C₂ – Diabetic group pretreated with whole cooked Bambara groundnut based diet.

A significant increase (P ≤ 0.05) in lipid peroxidation was observed in the Plasma and heart of groupA₂ compared with groupA₁. Non significant decrease in lipid peroxidation was observed in the plasma and heart of groupB₂ compared with groupB₁. Significant decrease (P ≤ 0.05) in lipid peroxidation was observed in the heart and plasma of groupC₂ compared with groupC₁.

A significant decrease (P ≤ 0.05) in CAT, SOD activities and GSH concentration was observed in the heart and plasma of groupA₂ when compared with groupA₁. In the plasma and heart of groupB₂ compared with B₁ a non significant increase in CAT, SOD activities and GSH concentration was observed, a significant increase (P ≤ 0.05) in CAT, SOD activities and GSH concentration was observed when groupC₂ was compared with groupC₁.

**DISCUSSION**

A significant increase (P ≤ 0.05) in lipid peroxidation was observed in the plasma and heart of groupA₂ when compared with groupA₁. The increase observed in lipid peroxidation in the heart and plasma of Diabetic rats is consistent with the reports of\(^{18,19}\) Oxidative stress in diabetes coexists with a reduction in antioxidant capacity, which can increase the deleterious effect of free radicals. Alloxan and the product of its reduction, dialuric acid, establishes a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of Beta-cells.\(^{20}\) Lipid peroxidation, owing to free radical activity plays an important role in the development of complications of diabetes.
Increased lipid peroxidation as a consequence of free radical activity have been reported in diabetes. Elevations in blood and tissues levels of thiobarbituric acid reactive substances, mainly malondialdehyde, are very reliable indices of oxidative stress and lipid peroxidation.\cite{21} In the plasma and heart of groupB\textsubscript{2} compared with groupB\textsubscript{1}, a non significant decrease in lipid peroxidation was observed. Significant decrease (P ≤ 0.05) in lipid peroxidation was observed in groupC\textsubscript{2} compared with groupC\textsubscript{1} in the heart and Plasma. The observation in this work however agrees with earlier works carried out by\cite{22} who reported decrease in lipid peroxidation level with *Gymnema montanum* pretreatment in alloxan induced diabetic rats. This suggest that 50:50 ratio of corn starch and cooked Bambara nut based diet and whole cooked Bambara groundnut based diet may be effective in preventing experimentally induced diabetes mellitus as well as reducing the free radical induced complications. The profiles of malondialdehyde (Figure 1) observed in the rats clearly indicate that cooked Bambara groundnut may prevent against oxidative stress.

Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O\textsubscript{2} and to the less reactive species H\textsubscript{2}O\textsubscript{2}.\cite{23} In the plasma and heart of groupA\textsubscript{2} compared with groupA\textsubscript{1}, a significant decrease (P ≤ 0.05) in superoxide dismutase activity was observed. This study is in agreement with earlier reports by\cite{24} who reported a decrease in SOD activity in the heart of diabetic rats compared with non diabetic rats. This observation is further substantiated by the elevated malondialdehyde levels (Figure 1). The reduced activity of superoxide dismutase in the plasma and heart could be as a result of increased demand for this enzyme in deactivating the high influx of reactive oxygen species generated due to the induction of diabetes. In the plasma and heart of groupB\textsubscript{2} compared with groupB\textsubscript{1}, SOD activity was non significantly increased. Significant increase (P ≤ 0.05) in SOD activity was observed in groupC\textsubscript{2} compared with groupC\textsubscript{1} in the heart and Plasma. This is similar to the findings of\cite{24} which showed that pretreatment with *Terminalia arjuna* improves antioxidant status by increasing SOD activity in the heart of alloxan induced diabetic rats. Bambara groundnut contain flavonoids e.g anthocyanins and kaempferol.\cite{25} investigated the anthocyanins present in Bambara groundnut which are delphinidin-3-O-β-glucoside, petundin-3-O-β-glucoside and malvidin-3-O-β-glucoside). The experimental evidence demonstrating anthocyanin benefits for diabetes and pancreatic disorders has also accumulated in recent years and again the efficacy is attributed to the multiple, simultaneous, biological effects those pigments cause in the body, including prevention of generation of free radicals, decreased lipid peroxidation, reduced pancreatic
swelling and decreased blood sugar concentrations in urine and plasma.\textsuperscript{[26]} In all \textit{Vigna} species the prevalent flavonoid appears to be keampferol.\textsuperscript{[27]} Kaempferol has been shown to have an array of antioxidant effects in vitro and in vivo. At low concentrations, it acts as a superoxide scavenger, specifically against the highly reactive hydroxyl radical and peroxynitrite species. At high concentrations, it increases the activity or expression of antioxidant enzymes such as superoxide dismutase, catalase and heme oxygenase-1.\textsuperscript{[28]} The development of diabetes has been indicated to be in part due to the tissue damage by increased oxidative stress.\textsuperscript{[29,30]} Since flavonoids has an antioxidative effect, those contained in Bambara groundnut might have exerted a protective role in the development of diabetes by reducing oxidative stress. Polyphenolic compounds are known to possess antioxidant activity and thus able to boost biological resistance against the deleterious effect of reactive oxygen species.

Catalase catalyzes H$_2$O$_2$ to form water and molecular oxygen.\textsuperscript{[31]} Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. This enzyme is located in the peroxisomes. A significant decrease (P ≤ 0.05) in catalase was observed in the plasma and heart of groupA$_2$ rats when compared with groupA$_1$. This is similar with the findings of\textsuperscript{[18]} in which plasma catalase activity significantly decreased in diabetic rats compared with non diabetic rats. Reduced activities of the antioxidant enzyme systems, often results from the progressive glycation of the enzymatic proteins in diabetes.\textsuperscript{[32]} Numerous reports indicate variations in antioxidants levels in diabetic patients.\textsuperscript{[33]} In the plasma and heart of groupB$_1$, a non significant increase in catalase activity was observed. Significant increase (P ≤ 0.05) in catalase activity was observed in groupC$_2$ compared with groupC$_1$ in the heart and Plasma. Similar results was observed by\textsuperscript{[24]} in the heart of pretreated diabetic rats with \textit{Terminalia arjuna}. In this study, pretreatment with cooked Bambara groundnut based diet improved the activities of catalase in diabetic rats. Suggesting that 50:50 ratio of corn starch and cooked Bambara nut based diet, and whole cooked Bambara groundnut based diet appears to alter oxidative stress which may be owing to the compensatory mechanisms of the antioxidant potential of the plant.

Reduced glutathione functions as a direct free radical scavenger, a co-substrate for glutathione peroxidase and a co-factor for many enzymes.\textsuperscript{[34, 35]} GSH is known to protect the
cellular system against the toxic effect of lipid peroxidation.\textsuperscript{[34]} GSH is a ubiquitous intracellular peptide with diverse functions that include detoxification, antioxidant defence, maintenance of thiol status and modulation of cell proliferation. GSH is synthesized in the cytosol of all mammalian cells in a tightly regulated manner.\textsuperscript{[35]} Significant decrease (P ≤ 0.05) in reduced glutathione concentration was observed in the plasma and heart of groupA\textsubscript{2} when compared with groupA\textsubscript{1}. Similar findings was reported by [36] in which significant decrease in GSH was observed in diabetic rats compared with non diabetic rats. Depletion in reduced glutathione concentration could be due to the alloxan injected in the rats, which acts as a xenobiotic and an inducer of diabetes. Xenobiotics are known to deplete antioxidants as they are consumed in the course of scavenging reactive species generated. The reduction in glutathione concentration could lead to a devastating decrease in total antioxidants status of the animals because glutathione helps in recycling cellular antioxidants, inhibits free radical damage and plays a key role in the detoxification of harmful compounds.\textsuperscript{[37]} The observation in this work is in line with earlier works carried out by.\textsuperscript{[36]} In the plasma and heart of groupB\textsubscript{2} compared with groupB\textsubscript{1}, a non significant increase in GSH concentration was observed. Significant increase (P ≤ 0.05) in GSH concentration was observed in groupC\textsubscript{2} compared with groupC\textsubscript{1} in the plasma and heart. These findings is in agreement with that observed by\textsuperscript{[22]} where Gymnema montanum pretreatment resulted in significant increase (P ≤ 0.05) in GSH concentration in the plasma of alloxan induced diabetic rats. Other researchers also reported that Bambara groundnut contains cysteine, glutamic acid and glycine which are needed for the synthesis of GSH.\textsuperscript{[38]} Dietary supplementation with GSH precursor amino acids can restore GSH synthesis and lower oxidative stress and oxidative damage in the face of persistent hyperglycemia. The amino acids present in Bambara groundnut may be responsible for synthesis of GSH would be the possible explanation for the non significant increase and significant increase observed as a result of pretreatment with 50:50 ratio of corn starch and cooked Bambara nut based diet, and whole cooked Bambara groundnut based diet. Also the fact that B\textsubscript{1} and C\textsubscript{1} non diabetic rats fed 50:50 ratio of corn starch and cooked Bambara nut based diet and whole cooked Bambara groundnut based diet has significantly higher GSH content compared with A\textsubscript{1} fed control diet further supports the presence of precursors required for GSH synthesis in the sample.

CONCLUSION
In conclusion, results indicate that pretreatment with Cooked Bambara groundnut based diet can reduce free radical mediated oxidative stress in experimental diabetic rats. However,
pretreatment with whole cooked Bambara groundnut based diet is more effective in reducing oxidative stress and diabetes when compared with 50:50 ratio of corn starch and cooked Bambara nut based diet as reflected in the increased activities of antioxidant indicating the effectiveness of the nut is concentration dependent. Hence, Cooked Bambara groundnut could be a good nutraceutical for the prevention of early onset of diabetes in Man.

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REFERENCES


