



Effects of Consuming Different Varieties of Bambara Nut (*Vigna subterranea*) Seeds on Liver and Kidney of Diabetic and Non-diabetic Subject

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Authors' contributions

This work was carried out in collaboration among between authors. Authors AM and DHM designed and wrote the protocol of the study, and performed the study experiments. Author AM managed the analyses of the study and the literature searches. Author DHM performed the statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Bambara nut seeds have been reported as a good source of food with high fiber and nutritional contents, and is consumed by persons with diabetes as one of the dietary therapy. However, the plant seeds are of different varieties and whether they might have vary biological effects on renal and liver functions is not fully known. This has prompted the study to evaluate effects of consuming different varieties of Bambara Nut seeds on liver and kidney of both diabetic and non-diabetic rats.

Methodology: Four Bambara nut seeds varieties were dehulled and each heated at 60°C, cooled then grounded into flour using a mechanical grinder. The flours were used to formulate feeds which were given to different diabetic and non-diabetic rats' groups as follows; group A (ALK01 feed), group B (ALK02 feed), group C (ALK03 feed), group D (ALK04 feed), group E (Basal feed), and group F (Normal animal's feed). The animals were fed for 28 days then sacrifice, blood collected and serum separated and used for hepatic and renal parameters analysis.

Results: The results of the study showed decrease levels of serum total protein and albumin, and

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elevation of AST and ALT activities as well as urea and creatinine concentrations in diabetic rats fed basal and normal diets, whereas, reverse in these parameters were recorded from diabetic rats fed varieties of Bambara nut seeds' formulated feeds.

Conclusion: Findings from the study suggest consumption of Bambara nut seeds of different varieties is safe and has ability to ameliorate diabetic hepatic and renal failure in a variety dependent manner where; ALK 04 is more likely the most effective.

Keywords: Bambara nut; seeds; varieties; hepatoprotective; nephroprotective; diabetic rats.

1. INTRODUCTION

Diabetes mellitus, a metabolic disorder which is resulted by inability of the pancreas to produce insulin or respond of cells to insulin, it is characterized by an elevation of glucose in the blood. The incidence of the disease in African nations have been reported to be on the increase where changes in the type of diets consumed has been reported as one of the major contributor [1]. The earlier low prevalence of diabetes mellitus in Africa has been attributed to Africa's rich biodiversity and high consumption of natural foods [2].

Uncontrolled diabetes mellitus have led to an increase in the number of diabetes related complications leading to kidney and liver failure, hence compromises their functions. Diabetic nephropathy is defined as diabetes with impaired glomerular filtration rate and has been a strong predictor of mortality in patients with diabetes [3], it also increases the demand for renal replacement therapies, like dialysis and kidney transplants [4]. Patients with diabetes mellitus developed renal failure, especially after many years of disease progression [5,6]. This was due to the fact that, over time, the high levels of sugar in the blood of diabetic patients damages millions of tiny filtering units (nephrons) within each kidney, which eventually leads to kidney failure [7].

Diabetes mellitus has been found to be strongly associated with the development and progression of liver injury with worse clinical outcomes, including; reduced survival, more severe liver failure-related complications etc [8]. Liver functions as a "glucostat". Its plays a vital role in the maintenance of blood glucose level but under prolong diabetes, this function is compromised. In diabetes state, hyperglycemia affects liver function by altering the metabolism of carbohydrates, lipids and proteins leading to abnormalities like non-alcoholic fatty liver disease which progress to cirrhosis and finally hepatocellular carcinomas [9]. This has been

found to be due to increase oxidative stress and aberrant inflammatory response in diabetic subject which activates transcription of pro-apoptotic genes and damages hepatocytes [9].

Both diabetic kidney and liver failure are among the most common long-term diabetes-related complications leading to mortality of patients with diabetes. Improving both kidney and liver failure in diabetes mellitus is very important, based on the fact that the function of this organs are critical to overall health of an individual. Healthy feeding (diet) by a diabetic patients is recommended as one of the legs of the tripod of diabetes management [10,11,12]. Where consumption of diet with low glycemic index (GI) and rich fiber was recommended as one of the ways of achieving low plasma glucose excursion [13], as well as improving renal and hepatic functions [14,15]. Low GI foods, such as legumes was found to provide slower, more consistent source of glucose to the bloodstream, thereby stimulating less insulin release and promote metabolisms of lipids, carbohydrate and proteins [15,16]. Legumes like beans and their products have been reported to have low GI [17,18], and was recommended as one of the major component of daily dietary therapy for persons with diabetes [19].

Bambara nut (*Vigna subterranea* L. Verdc or *Voandzeia subterranea*) is one of the legumes referred to as beans. It is an indigenous leguminous African crop that is grown across the continent [20]. Is commonly found in Nigeria and known locally as; "Okpa" (Igbo), "Epa-roro" (Yoruba) and "Kwaruru" or "Gurjiya" (Hausa). There are about seven varieties of Bambara nut which is mainly recognized by their seed-color or design. These include: black seed, red seed, cream/black eye seed, cream/brown eye seed, cream/no eye seed, speckled/flecked/spotted purple seed and brown (light or dark) seed [21]. The plant's seeds have been reported as a good source of food with high fiber and nutritional contents [22,23] and is being consumed locally by persons with diabetes as one of the dietary

therapy [24]. However, the plant seeds are of different varieties and whether they might have vary biological effects on renal and liver functions is not fully known. This has prompted the study to evaluate effects of consuming different varieties of Bambara Nut seeds on liver and kidney of both diabetic and non-diabetic rats.

2. MATERIALS AND METHODS

2.1 Materials

In this study, the following materials were used; plastic cages consisting of feeding container and water bottle for rats, On-call plus glucometer, weighing scale, spectrophotometry, centrifuge and oven.

2.1.1 Experimental plant sample

The different varieties of Bambara nut (*Vigna subterranea L Verdc*) seeds were purchased directly from farmers in Alkaleri LGA of Bauchi State, North-East Nigeria. They were identified by a taxonomist at the Institute of Forestry, Jos, Plateau State. The four varieties were identified as ALK01 (cream/black eye seed), ALK02 (light brown seed), ALK03 (cream/no eye seed), and ALK04 (black seed). The seeds were dehulled then heated at 60°C, cooled and grounded into flour using a mechanical grinder. The flour were kept at 25°C in an air tight bags until needed.

2.1.2 Feed formulation

The formulated feed containing flour of Bambara nuts seeds' varieties composed of the following: Bambara nut seed flour (56%), Cray-fish (20%), vegetable oil (5%), rice bran (4%), sucrose (10%), Vitamin/mineral mixture (5%), respectively. The control feed used was animal's feed (Grower's mash) which represented a well-balanced diet consisting of significant percentage of carbohydrate, protein, fats (lipid), vitamins and minerals while the basal feed composed of same ingredients as the experimental feeds except that Bambara nut seed flour was replaced with corn-flour (56%) as prepared by Olunmi et al [25]. The Bambara nut seeds' varieties formulated feeds were tag as; ALK01 feed, ALK02 feed, ALK03 feed and ALK04 feed.

2.1.3 Experimental animal

A total of sixty (60) male albino rats were used in the study. They were grouped into 12 groups of 5 rats each promptly labelled A, B, C, D, E and F.

Group A-D are rats fed different varieties of Bambara nut seeds' formulated diet, group E; rats fed basal diet while group F; rats fed normal diet (Animal's feeds). The rats were acclimatized for two weeks before induction of diabetes and commencement of the study. During this period they were fed normal animal's feed and tap water before placing them on the varieties of Bambara nut seed formulated diets. Each rats' group was fed with a specific feed and tap water daily.

2.1.4 Induction of diabetes

Type I diabetes was induced in rats by intraperitoneal injection of Streptozotocin (STZ) at a dose of 60 mg/kg body wt in 0.1 M citrate buffer (pH 4.5). After 72 hours, blood glucose levels were checked to identify the onset of diabetic hyperglycemia; rats with fasting blood glucose levels ≥ 200 mg/dl were considered diabetic and selected for the study which were grouped as follows:

Group A₁: non-diabetic rats fed with ALK01 feed
Group A₂: diabetic rats fed with ALK01 feed

Group B₁: non-diabetic rats fed with ALK02 feed
Group B₂: diabetic rats fed with ALK02 feed

Group C₁: non-diabetic rats fed with ALK03 feed
Group C₂: diabetic rats fed with ALK03 feed

Group D₁: non-diabetic rats fed with ALK04 feed
Group D₂: diabetic rats fed with ALK04 feed

Group E₁: non-diabetic rats fed with basal feed
Group E₂: diabetic rats fed with basal feed

Group F₁: non-diabetic rats fed with normal feed
Group F₂: diabetic rats fed with normal feed

2.2 Hepatic Parameters Determination

Total Protein was determined by Bradford [26] method. Exact 25 μ l of serum, standard solution, and distilled water was each pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 1000 μ l of assay reagent was added to the content in all the tubes, mixed by inversion and incubated at 37°C for 5 minutes. Then, absorbance of the test and standard were read against the blank at 540 nm. Total Protein (mg/dL) = Abs. of sample/standard x 750 mg/dL

Albumin was determined by Doumas *et al* [27] method. Exact 5 μ l of serum, standard solution,

and distilled water was each pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 1000 µl of assay reagent was added to the content in all the test tubes, mixed by inversion and incubated at 37 °C for 5 minutes. Then, absorbance of the test and standard were read against the blank at 630 nm. Albumin (µmol/L) = Abs. of sample/standard x 5 g/dl x 144.9 µmol/L

Aspartate and alanine aminotransferases activity was assayed by Reitman and Frankel [28] method. A 1000 µl of test reagent was pipetted into a clean separate test tubes labelled as sample test and blank test. Then, 100 µl of serum was added to the content in the test tube whereas 100 µl of distilled water was added to the blank test, mixed by inversion and then incubated at 37°C for 10 minutes. Absorbance of test was read against the blank at 340 nm. AST activity (U/L) = Absorbance/min x 1750, and ALT activity (U/L) = absorbance/min x 1745.

2.3 Renal Parameters Determination

Urea was determined by Jendrassik and Grof [29] method. Exact 5 µl of serum, standard solution, and distilled water was each pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 1000 µl of assay reagent 1 was added to the content in all the test tubes, mixed by inversion and incubated at 37°C for 5 minutes. Then, 1000 µl of assay reagent 2 was added to the content in all the test tubes, mixed by inversion and incubated at 37°C for 5 minutes. Absorbance of the test and standard were read against the

blank at 580 nm. Urea (mmol/L) = Abs. of sample/standard x 50 mg/dl x 0.1663 mmol/L..

Creatinine was determined by Weatherburn [30] method. Exact 100 µl of serum, standard solution, and distilled water was each pipetted into a clean separate test tubes labelled as sample test, standard test and blank test. Then, 1000 µl of assay reagent was added to the content in all the test tubes, mixed by inversion and then absorbance of the test and standard were read against the blank at 492 nm. Creatinine (µmol/L) = Abs. of sample/standard x 2 mg/dl x 88.4 µmol/L.

2.4 Statistical Analysis

Data from the experiments were expressed as mean ± standard deviation (SD). Means were analyzed by one way analysis of variance (ANOVA) and then compared by Duncan's multiple range test (DMRT) [31]. Significant difference was accepted at $P < .05$. The statistical analysis was conducted using the computer software, statistical package for the social sciences (IBM SPSS version 21).

3. RESULTS

3.1 Different Varieties of Bambara Nut Seeds Used

Fig. 1. shows the different varieties of Bambara nut seeds used in the study. About four varieties were used and identified as; ALK01 (cream/black seed), ALK02 (light brown seed), ALK03 (cream/no eye seed), and ALK04 (black seed) respectively.



Fig. 1. Different varieties of Bambara nut seeds used. Legend: (a) ALK01 (cream/black seed), (b) ALK02 (light brown seed), (c) ALK03 (cream/no eye seed), and (d) ALK04 (black seed)

3.2 Effect of Consuming Different Varieties of Bambara Nut Seed on Rats Liver

The result of feeding non-diabetic and diabetic rats with different varieties of Bambara nut seed formulated feeds on liver function is presented in Figs. 2 & 3. There was no significant difference in total protein concentration of non-diabetic rats fed different varieties of Bambara nut seeds formulated feeds with those fed the basal and normal diets except rats group fed ALK01 of the plant seed varieties. However, when the protein values of diabetic rats fed different varieties of the plant's seed formulated feeds are compared with those fed basal and normal diets, a significant different ($P < 0.05$) was observed.

Albumin levels seem to be elevated in non-diabetic rats group fed ALK04 formulated seed variety compare to those fed other seeds varieties as well as normal and basal diets. However, a fall in albumin levels was recorded by the study particularly in diabetic rats fed basal and normal diets as well as some of those rats that were fed plant's seed formulated feeds particularly ALK02 variety. In another dimension, the albumin levels for non-diabetic and diabetic rats fed ALK04 formulated feed were not significantly different whereas, diabetic rats fed ALK01 formulated feed had their albumin level elevated compared to the non-diabetic rats fed same diet (Fig. 2b).

The study also recorded an increased in the activities of hepatic serum enzymes; ALT and AST in diabetic rats' groups fed basal and normal

diets in comparison to the non-diabetic rats fed basal and normal diets as well as those fed different formulated feeds of the Bambara nut seeds' varieties. However, it was observed that, both non-diabetic and diabetic rats that fed ALK03 and ALK04 seeds varieties, had no significant difference in their enzymes activities. The result of the activities of the hepatic enzymes; ALT and AST for non-diabetic and diabetic rats is presented in Fig. 3 (a & b).

3.3 Effect of Consuming Bambara Nut Seed Formulated Feed on Renal Indices of Rat

Fig. 4 (a & b) present serum concentrations of both urea and creatinine of non-diabetic and diabetic rats fed different varieties of Bambara nut seeds' formulated feeds. The study observed a fluctuation in the decrease levels of urea for rats fed different varieties of the plant seed formulated feeds as those fed normal feed whereas, those fed basal diet had their urea levels elevated.

The result of the study for feeding non-diabetic and diabetic rats with different varieties of Bambara nut seed formulated feeds on serum creatinine is shown in fig. 4b. There was no significant difference in creatinine levels between non-diabetic rats fed different varieties of Bambara nut seed formulated feeds and those fed normal diets. However, a significant different ($P < 0.05$) was observed for creatinine values between non-diabetic and diabetic rats fed same diets as well as among rats fed different varieties of the plant seed formulated feeds.

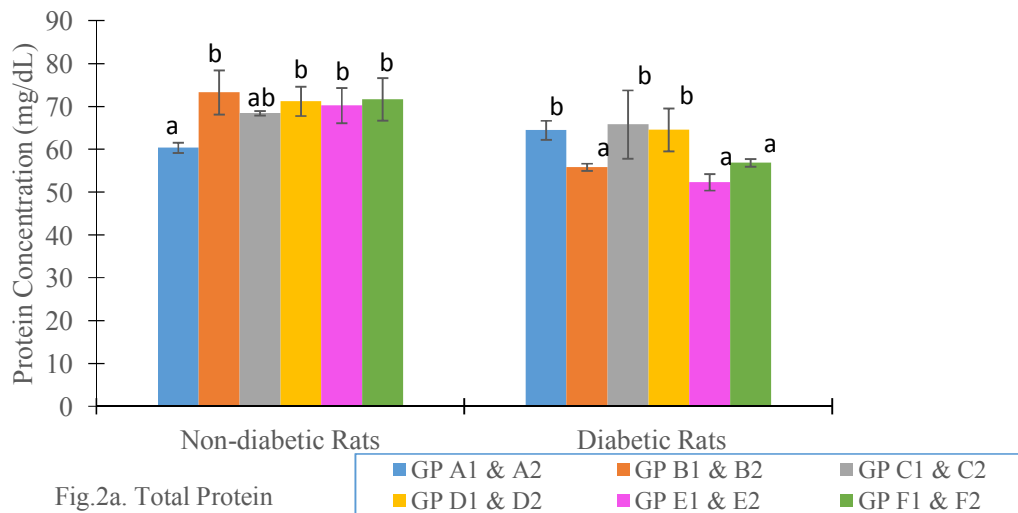


Fig.2a. Total Protein

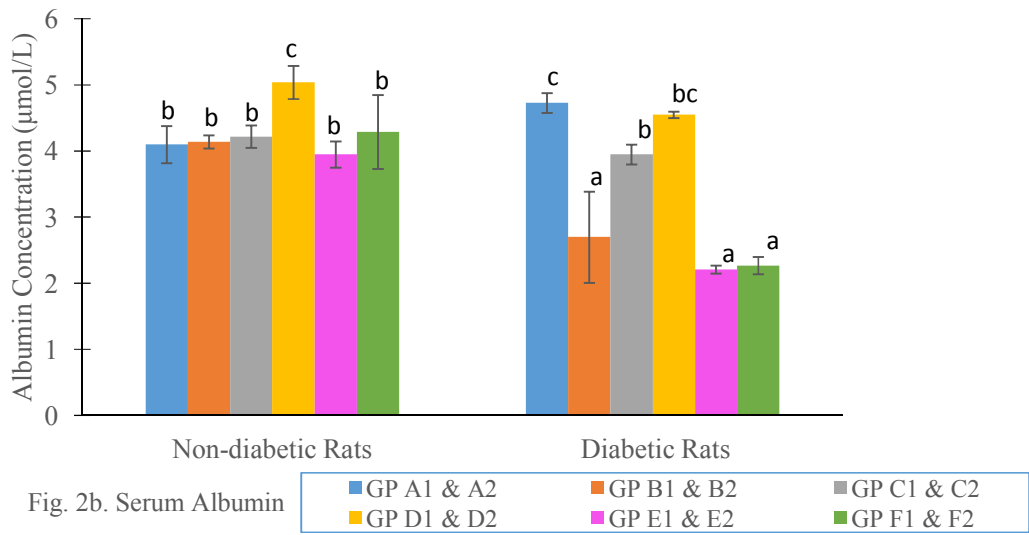


Fig. 2b. Serum Albumin

Fig. 2. Impact of feeding different varieties of Bambara nut seed formulated feeds on hepatic indices of non-diabetic and diabetic rats

Bars with different superscript are significantly different at P < 0.05

- Group A₁: non-diabetic rats fed with ALK01 feed Group A₂: diabetic rats fed with ALK01 feed
- Group B₁: non-diabetic rats fed with ALK02 feed Group B₂: diabetic rats fed with ALK02 feed
- Group C₁: non-diabetic rats fed with ALK03 feed Group C₂: diabetic rats fed with ALK03 feed
- Group D₁: non-diabetic rats fed with ALK04 feed Group D₂: diabetic rats fed with ALK04 feed
- Group E₁: non-diabetic rats fed with basal feed Group E₂: diabetic rats fed with basal feed
- Group F₁: non-diabetic rats fed with normal feed Group F₂: diabetic rats fed with normal feed

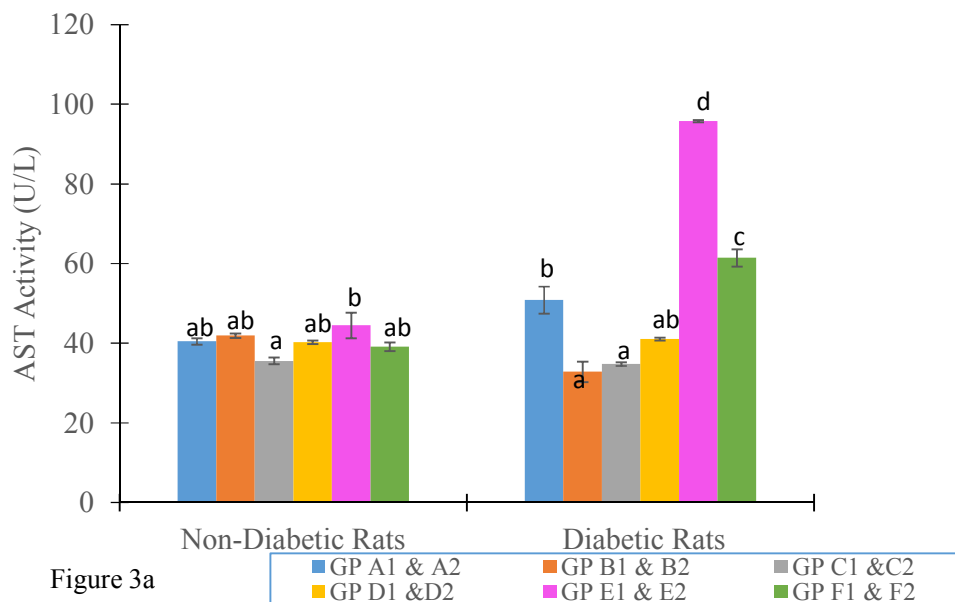


Figure 3a

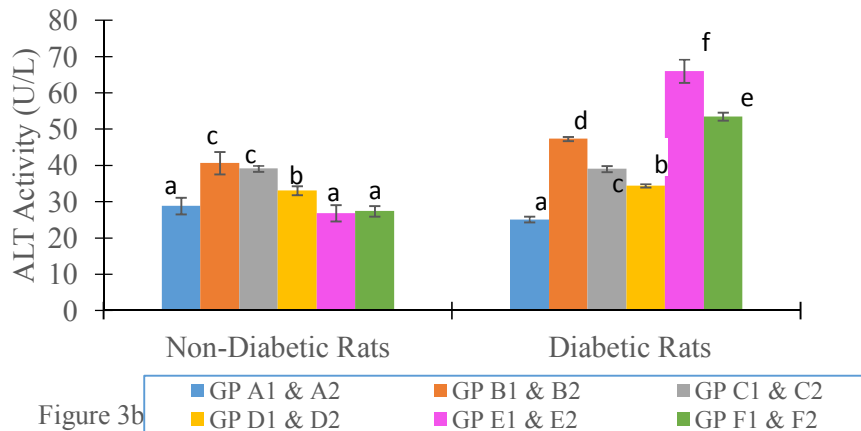
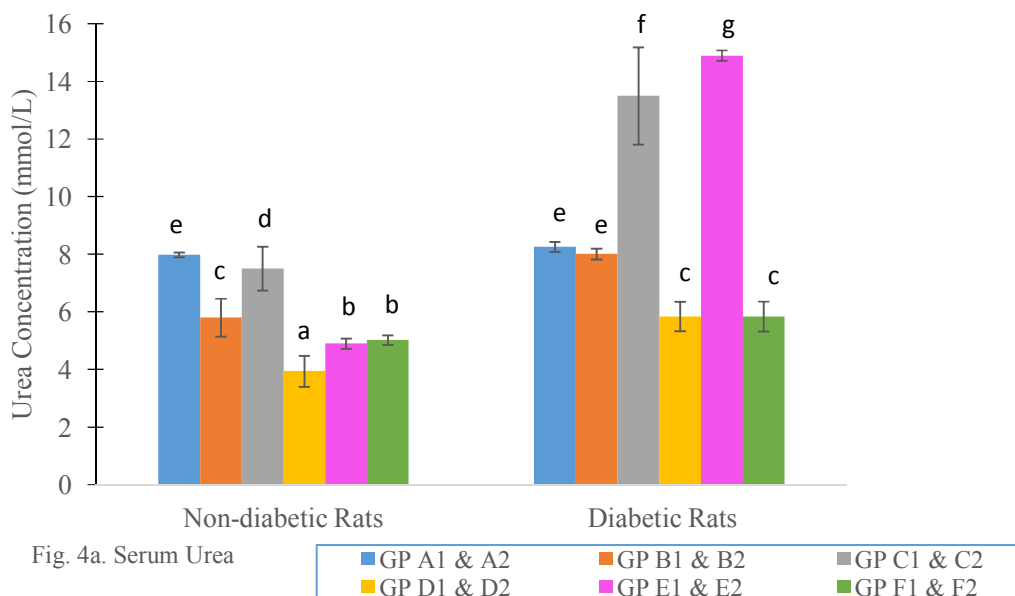


Fig. 3. Impact of feeding different varieties of Bambara nut seed formulated feeds on hepatic enzymes of non-diabetic and diabetic rats

Bars with different superscript are significantly different at P < 0.05

- | | |
|---|---|
| Group A ₁ : non-diabetic rats fed with ALK01 feed | Group A ₂ : diabetic rats fed with ALK01 feed |
| Group B ₁ : non-diabetic rats fed with ALK02 feed | Group B ₂ : diabetic rats fed with ALK02 feed |
| Group C ₁ : non-diabetic rats fed with ALK03 feed | Group C ₂ : diabetic rats fed with ALK03 feed |
| Group D ₁ : non-diabetic rats fed with ALK04 feed | Group D ₂ : diabetic rats fed with ALK04 feed |
| Group E ₁ : non-diabetic rats fed with basal feed | Group E ₂ : diabetic rats fed with basal feed |
| Group F ₁ : non-diabetic rats fed with normal feed | Group F ₂ : diabetic rats fed with normal feed |



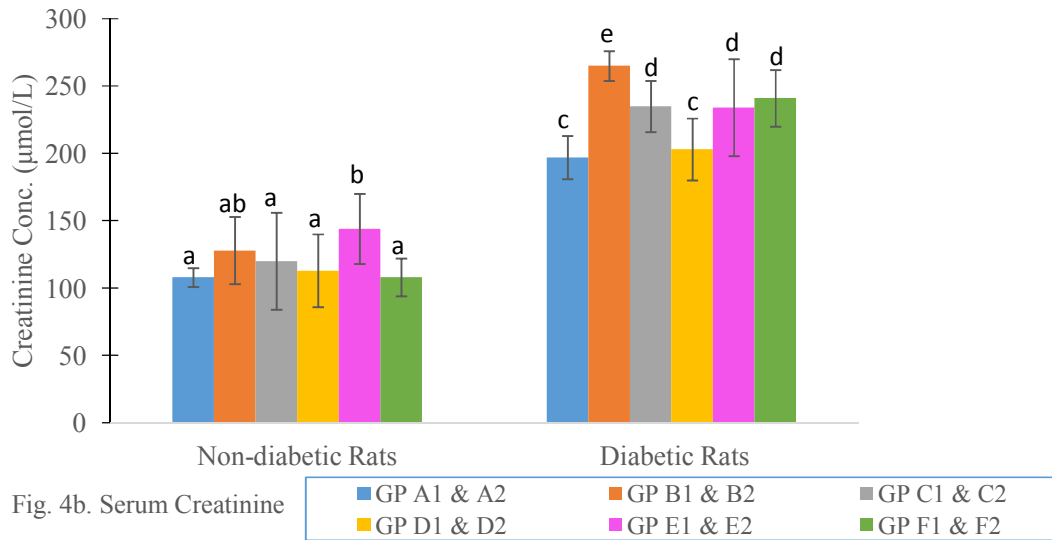


Fig. 4b. Serum Creatinine

Fig. 4. Impact of feeding different varieties of Bambara nut seed formulated feeds on renal indices of non-diabetic and diabetic rats

Bars with different superscript are significantly different at $P < 0.05$

- | | |
|---|---|
| Group A ₁ : non-diabetic rats fed with ALK01 feed | Group A ₂ : diabetic rats fed with ALK01 feed |
| Group B ₁ : non-diabetic rats fed with ALK02 feed | Group B ₂ : diabetic rats fed with ALK02 feed |
| Group C ₁ : non-diabetic rats fed with ALK03 feed | Group C ₂ : diabetic rats fed with ALK03 feed |
| Group D ₁ : non-diabetic rats fed with ALK04 feed | Group D ₂ : diabetic rats fed with ALK04 feed |
| Group E ₁ : non-diabetic rats fed with basal feed | Group E ₂ : diabetic rats fed with basal feed |
| Group F ₁ : non-diabetic rats fed with normal feed | Group F ₂ : diabetic rats fed with normal feed |

4. DISCUSSION

Bambara nut seeds has been reported as a good source of food with high fiber and nutritional contents [22,23] and is being consumed locally by persons with diabetes as one of the dietary therapy [24]. However, the plant seeds are of different varieties and whether they might have varying biological effects on renal and liver functions is not fully known. This has prompted the study to evaluate effects of consuming different varieties of Bambara Nut seeds on liver and kidney functions of both diabetic and non-diabetic rats. Where it was found that, consumption of Bambara nut seeds of different varieties is safe and has ability to ameliorate diabetic hepatic and renal failure in a variety dependent manner. This was evidenced by the decreased levels of serum total protein and albumin, and elevated AST and ALT activities as

well as urea and creatinine in diabetic rats fed basal and normal diets, whereas, reverse in these parameters were recorded for the various diabetic rats' groups fed different varieties of Bambara nut seeds' formulated feeds.

Liver failure is a serious disease which is characterized by disturbances in its normal functions. It is clinically diagnosed by determining the serum activities of its enzymes particularly ALT and AST as well as some parameters like total protein etc. These enzymes are non-plasma specific enzymes and were reported to reach higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver, where ALT was reported of being the most specific liver injury marker and a more selective liver parenchymal enzyme [32].

In this study, increase in both liver enzymes' activities: ALT and AST as well as reduced total

proteins level of diabetic rats fed basal and normal diets support the notion that diabetes mellitus strongly promotes the development and progression of liver injury [8]. Suppressed activities of these enzymes and elevated level of total protein in diabetic rats fed various formulated seed varieties suggest their ability in protecting and enhancing liver function. On a general note, the difference observed in the activities of these enzymes between diabetic rats fed basal and normal diets against those fed plant's seed formulated feeds is an indication of the plant's seed ability in protecting and promoting the liver. This might be in line with a study where consumption of low glycemic index diet was reported to be able to reversed liver abnormality such as non-alcoholic liver fatty disease [17].

Kidneys are the major organs in metabolizing toxic compound besides liver where its failure can be ascertained by measuring the level of serum urea and creatinine. Uncontrolled diabetes mellitus was report to have caused kidney failure, hence compromises its functions. This was reported to be as a result of high levels of sugar in the blood of diabetic patients which damages millions of tiny filtering units (nephrons) within each kidney, eventually leading to kidney failure [7]. Decrease in albumin (which may be due to glycation, a biochemical process where glucose interacts with protein resulting to albuminuria) in diabetic rats fed basal and normal diets is an indication of diabetic nephropathy [33]. Diabetic nephropathy has been reported as a strong predictor of mortality in patients with diabetes [3]. A high blood level of urea is an indication of kidneys not working properly [34] which may agree with the high level recorded in this study for rats fed basal and normal diets.

In a study conducted by Juraschek et al [14], kidney failure was reported to have been reversed after feeding with diet containing low glycemic index. In a similar manner, one may suggest that elevated albumin and lower serum urea recorded in diabetic rats fed varieties of plant's seed formulated diets could have improved their kidney functions leading to the reduction of the serum urea and creatinine levels.

The findings of this study have to be seen in light of some limitations. The limitations of this study may include lack of exploring all the varieties in the current study which is as a result of poor access to the other varieties of the plant seeds. There are about seven varieties of the Bambara

nut seeds, however, four varieties were used in the study which are the ones growth and readily available in the study location. It is therefore recommended for further studying on the other varieties to help in bring out their medicinal potentials for pharmaceutical utilization.

5. CONCLUSION

In conclusion, findings from the study showed consumption of Bambara nut seeds of different varieties is safe and has the ability to protect both hepatic and renal abnormalities associated with diseases like diabetes in a manner that seem to be a variety dependent where; ALK 04 is more likely the most effective.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee."

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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