Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 9 Issue 3 March 2025

Research Article

Lipid profile and other Biochemical changes in streptozotocon -induced diabetic rats fed on high concentrations of Bambara groundnut-flour (*Vigna subterranean*)

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Received: January 21, 2025

Published: February 07, 2025

Abstract

Background: The global prevalence of Diabetes mellitus and its estimated percentage growth make it a chronic disease of Public Health importance, with a focus on how best to reduce the alarming high care cost and seek cheaper alternative and acceptable management options, among which is the utilization of readily accessible and culturally acceptable food-based nutritional therapy. Plant foods such as Bambara groundnut (BGN) is available, cheap and acceptable across regions with previous studies suggestive of the presence of bioactive compounds that can be useful in non-communicable disease therapy, hence the aim of this work to establish the effects of administration of high concentrations of BGN extract on the biochemical parameters in streptozotocin-induced diabetic rats.

Methodology: BGN were sourced from an open market and processed into pellets that were oven-dried at 600C and stored for later use. Forty-two male albino rats with weights that ranged between 134 and 247 grams were procured and acclimatized before inducing Insulin resistance using 10% fructose diet. In order to induce type-2 diabetes mellitus in these rats, Streptozotocin (STZ) was used intra-peritoneally and those with T2DM, administered with prepared BGN formulations for twenty-eight days, at the end of which blood samples were collected from the killed animals for biochemical assessment.

Results and Discussion: When compared with the values obtained from the standard control group, the BGN-administered group exhibited lower random blood glucose values, significantly lower MDA value (12.77 mg/g versus 10.98 mg/g), lower total bilirubin value, significantly lower total cholesterol value (113.34 mg/dl versus 110.25 mg/dl) and lower mean urea level (14.20 mg/dl versus 12.98 mg/dl).

Conclusion: BGN formulation at higher concentration had better hypocholesterolaemic effects, better hypoglycemic effect, showed higher potency in free radical mop up, exhibited milder anti-inflammatory activities, and had higher potency in the renal tissue protection, when compared to a standard anti-diabetic drug. It is therefore possible that the incorporation of BGN in the nutritional management of T2DM can be of immense health benefit

Keywords: Biochemical; Bambara Groundnut; Diabetic Rats

Introduction

Diabetes mellitus is a disease of global concern due to the high prevalence and the projected population that can be affected in future if drastic prophylactic actions are not [1]. The effect of the disease on the pancreas results in the organ damage and various other systemic effects that manifest as multi-organ failures if unchecked [2]. The problem is worse in various resource poor countries even though same magnitude of damage has been recorded in developed countries with loss of body functions and productivity on the increase as a result [3].

Diabetes mellitus diagnosis, treatment, follow-up and prevention consumes significant resources [4]., and this is coupled with the associated loss in productivity, compromised quality of post-care life [5]. This humongous cost is more noticeable in low income setting whose resources cannot sustain a resilient health system [6]. This makes it imperative for less expensive care alternatives to be sort for in the management of diabetes and its multi-organ damage in such poor income countries.

Cheaper management alternatives cannot be achieved if complementary strategies that targets adherence to acceptable dietary intake, regular and graded exercises and other lifestyle modifications that target amelioration of the risk factors that can reduce diabetic mellitus incidence, prevalence and complications are in place [7]. One of the trending dietary management protocols is the use of Diabetic Medical Nutrition Therapy, which involves nutrition personalization and insulin intake that relates to the individual's lifestyle [8], and this therapy have been found efficacious [9].

In furtherance to this, natural sources of nutrients from plants and plant foods, documented to be of medicinal values have been recommended by the WHO for use as a cheaper therapeutic and preventive approach to diabetes mellitus management [10]. These medicinal plants include Bambara groundnut (*vigna subterranean*), [11] and which contains macronutrients [12-14], and micronutrients and macronutrients in good amounts [15]. The phytochemicals and bioactive compounds present in this legume include phenolics [16,17]; dietary fibres [18]; fatty acids [19] and peptides and amino acids [18,20]. The biological actions of these phytochemicals include anti-inflammatory, antioxidant, antimicrobial, activities, stimulation of immune system, reduction of platelet aggregation, modulation of hormone metabolism and detoxification of enzymes [21,22]. The dietary fibres can reduce obesity, lower blood lipids, and boost immunity [23]. When the phenolic compounds are expose to the biological system *invivo,* they have been reported to inhibit the activities of α-amylase and α-glucosidae which enhances glycogenolysis [24]. Other anti-hyperglycemic action of Bambara groundnut is the acceleration of glucose uptake in the peripheral tissues in diabetic rats [25,26]. The thrust of this work was to evaluate the lipid profile changes that occurs when diabetic rats consume high concentration of Bambara groundnut flour.

Collection and preparation of the plant food material

Vigna subterranean seeds were located in a regular market, and with the aid of a plant taxonomist, identified. The purchased seed samples were washing, and soaking in water for 10 min, then rinsed, boiled and oven dried at a temperature of 60^0C. When the weight remained constant, the dry weight was then processed into fine flour before being pelletized and oven dried at 60^0C until a constant weight was obtained. The pelletized feed, was then stored for future use in an airtight container.

Experimental animals

Male albino rats whose weights ranged from 134-249 grams were procured from a reference breeder. Fourty-Two (42) of these rats whose weights were recorded weekly, were group in sets of eight recognized by indelible marker on each tail marked 1 – 8 and placed per cage for four sets (A, B, C and D). The rats were kept in the same house where the day-light cycle of 12 hours each was maintained and the room temperature kept at 27^0C-30^0C. All the rats were given rats' feeds and water *ad libitum* for one week, until the experiment was commenced. Ethical protocols guiding laboratory animals according to the NRC guideline [27] was adopted in this work.

Induction of Insulin Resistance using low Fructose Diet

Low dose fructose diet constituted by the dissolution of 30 g of fructose in 300 mL of water was administered to the albino rats *ad libitum* for fourteen days, to induce Insulin resistance.

Induction of Type 2 Diabetes Mellitus using STZ

The rats were fasted overnight, after administering the 10% fructose diet for six days, and the blood glucose tested. All but nine normoglycemic rats were injected by intraperitoneal route with streptozotocin (STZ), which was prepared by the dissolution of 1 g of the STZ in 50 mL of freshly prepared buffer, where the buffer consisted of sodium citrate buffer, 0.1M, pH 4.5. The volume of the extract used was based on standard methods [28,29]. Prior to the commencement of the feeding with the intervention formulations, determination of the blood glucose levels at 72 hours and day 12 post STZ administration, was conducted in all the STZ-treated rats, using blood glucose meter (Acu-check active^R). Each of the control groups were assigned eight of the STZ-induced diabetic rats by random selection, except the nine normoglycemic rats which formed the normal control group. All the groups were exposed to the various interventions for 28 days, as follows: Groups A and B received commercial rat feed at 4.48 kg per group, for the 28 days, Group

C was given metformin at 200 mg/kg, given as 0.002 ml per Kg body weight by oral route per day with the aid of an oral dispenser and Group D administered with Bambara groundnut flour (50%) blend with commercial rat feed (50%) based on the method used by Nnadi and colleagues [30]. The groups were as follows

- **Group A:** Normal Control on commercial rat feed
- **Group B:** STZ-induced Diabetic rats administered with commercial rat feeds (Negative Control)
- **Group C:** STZ-induced Diabetic rats administered with Metformin (Standard Control)
- **Group D:** STZ-induced diabetic rats administered with *Vigna subterranean* intervention feed

Biochemical analysis

Estimation of blood glucose, lipid profile, liver function, kidney function and myocardial function tests

After administering the intervention formulations for 28 days, the rats were fasted overnight and the blood collected for the determination of the blood glucose levels with the aid of glucometer (Acu-check active[R]). The lipid profile was equally estimated with the aid of Randox assay diagnostic kits for the total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) according to the method by Tietz [31], while the low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol values were obtained from the formula of Friedewald and colleagues [32]. The protocol of Jin and colleagues [33], was adopted for the liver function tests which included assessment of the serum bilirubin, total protein, serum glutamate oxaloacetate transaminase, ALT, and serum glutamate pyruvate transaminase, AST. The kidney (urea, and creatinine) and myocardial (lactate dehydrogenase, and creatin) function tests were also conducted.

Estimation of antioxidant enzyme system [Superoxide dismutase (SOD), Catalase, (CAT), Glutathione Peroxidase (GPx), Reduced and Oxidized Glutathione (GSH) and Glutathione-S-Transferase (GST)] Activities.

The antioxidant enzyme system which included the superoxide scavenging activity was determined by the modified NBT reduction method[34], while the catalase activity was evaluated using the spectrophotometric method [35]. In the case of the glutathione peroxidase activity the method by Paglia and Valentine [36] was adopted by using the erythrocyte hemolysate. The oxidized and reduced glutathione were estimated using the Beutler, and colleagues [37] procedure, with the Erythrocyte GSH values expressed as mg/g GSH/Hb and the result expressed in mg/g.

Estimation of Lipid peroxidation

To determine the lipid peroxidation, the Malonyldialdehyde (MDA) free radical scavenging activity was measured according to the method of Ohkawa and colleagues [38].

Statistical analysis

The Statistical package for social sciences (SPSS Inc., Chicago, IL) version 20.0, was used to analyze the data after expressing the results obtained in triplicate as standard error of mean. One-way analysis of variance (ANOVA) was used for the mean comparison while the Tukey`s post-hoc test, was applied in the determination of the mean difference, with P<0.05 used to denote statistical significant.

Results

Figure 1 Graph showing the mean values of the Random Blood sugar and body weight of the rats in the various intervention groups over week 1 to week 4 of the interventions.

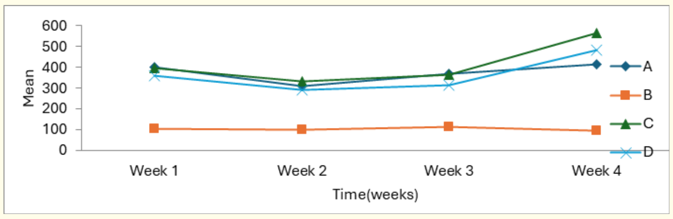

Figure 1: Random blood Glucose.

Figure 2 showed that the SOD in the normal control and Bambara groundnut-fed group had the least mean values. Similarly, the mean insulin values followed similar trend.

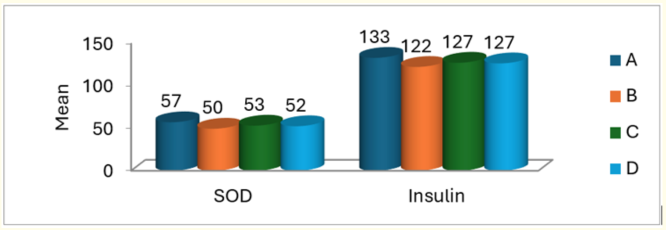

Figure 2: Bar chart of the SOD and Insulin Concentrations in the various rat groups.

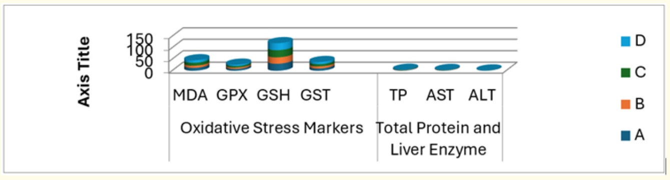

Figure 3: The chart of the mean values of Oxidative stress markers and total protein and liver enzymes among the various treatment groups.

Figure 3 showed that the mean MDA, GPX, GSH and GST values were least in the normal control, followed by that in the Bambara ground nut-fed group. The Total protein and AST and ALT means values showed similar pattern.

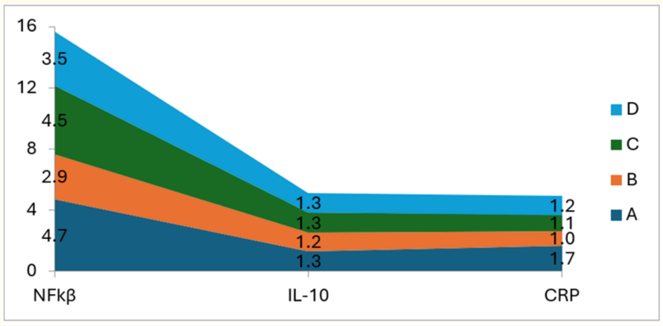

Figure 4: Graph of the mean values of the Inflammatory Markers in the various treatment groups.

Figure 4 showed that NFkB was least in the normal control and the Bambara groundnut-fed group. The standard control had the highest mean NFkB, IL-10 and CRP values.

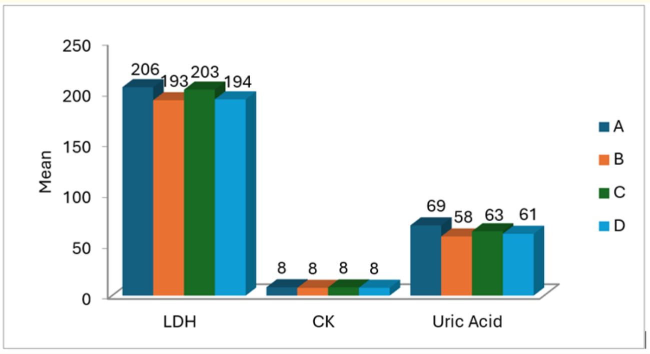

Figure 5: Serum LDH, CK and Uric Acid.

In the LDH, Group B and D had the least values with Groups A and C having the highest values. For the CK, the values across the groups were same but for the uric acid, Group A had the highest value followed by Group C, then D and B the least.

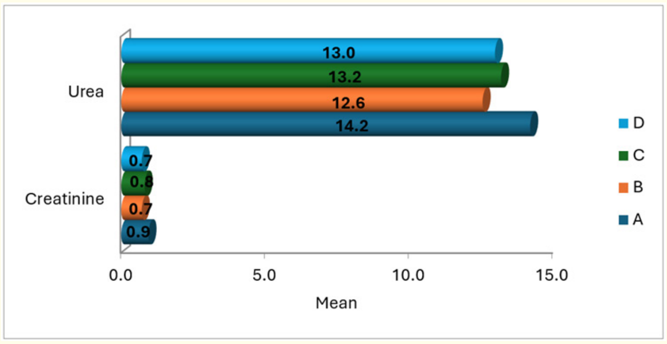

Figure 6: Creatinine and Urea.

The mean urea value for Group A was the highest, with Groups B, C and D having approximately similar values. The creatinine values in Group A were higher than values obtained for Groups B, C and D.

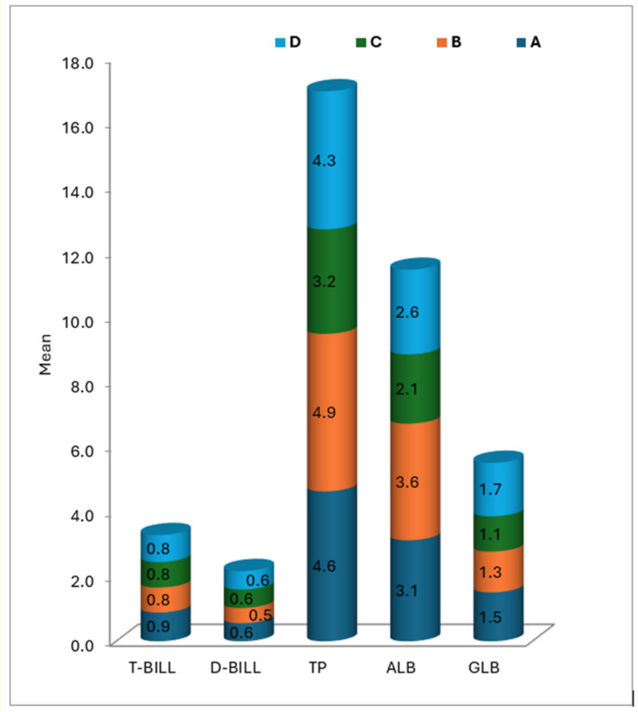

Figure 7: Serum bilirubin and Protein.

There was no significant difference in the total bilirubin and direct bilirubin mean values across groups but the total protein showed that Group C had the least value and B highest with A and D values being in the approximate range.

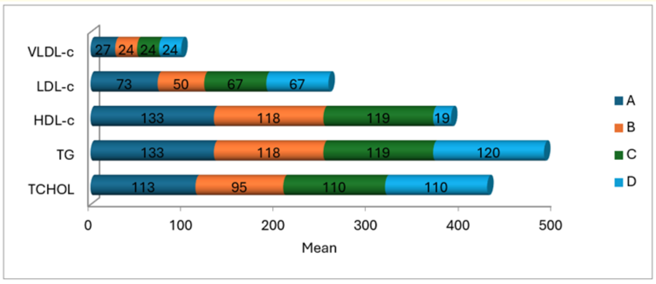

Figure 8: Serum Lipid Profile.

For the total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol Group A had the highest mean values, while the values for Group D were among the least.

Discussion

Effect on blood glucose

The result from this study revealed that mean random blood glucose values were observed to be lower in group on Bambara groundnut formulation with same trend over time even though the mean value difference was only statistically significant in week 2 when compared with the negative control (Figure 1). When the intervention group was compared against the standard and diabetic control groups, the former exhibited lower random blood glucose values throughout the duration of intervention than the two control groups (Figure 1), signifying that the intervention flour formulations had better blood glucose than the anti-diabetic drug, metformin. The non-linearity of the findings might be due to blood glucose fluctuations which also occur during the management of diabetic patients on anti-diabetic medications, resulting in multiple anti-diabetic drug applications [39]. The possible explanations for the hypoglycemic effects of the formulations might be as a result of the presence of the phenolic compounds in bambara groundnut formulations responsible for the activation of the 5' adenosine monophosphate-activated protein kinase (AMPK) pathway, and the inhibition of gluconeogenesis and improvement of glycogenesis in tumor necrosis factor-α (TNFα) - treated insulin-resistant mouse hepatocytes [40]. It also causes the elevation of the α-amylase and α-glucosidase inhibitory activity in streptozotocin-induced diabetic rats [41]. These actions are hypoglycemic in nature [42,43].

Biochemical activities

Effect on oxidative stress markers

Oxidative stress reduces the capacity of the antioxidant system to cope with the neutralization of the damaging cellular radicals and with higher free radicals in the system, an imbalance between this antioxidants and free radicals occur [44]. Chronic diseases such as diabetes mellitus reduces the antioxidant potentials of individuals [45], resulting in changes in the biochemical parameters such as decreased levels of reduced glutathione (GSH) and increased levels of oxidized glutathione (GSSG), to an extent that a GSH/GSSG ratio, a redox ratio, that is indicative of oxidative stress occurs [46,47]. Other antioxidant system parameters such as levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities scavenging are lowered in both the plasma and erythrocytes of diabetes patients [48]. Indicators of overwhelming free radical effects are presence of malondialdehyde (MDA) which is indicative of lipid peroxidation [49], presence of carbonyls and advanced glycation end products (AGEs) which are indicative of protein glycation in diabetic patients [50] have been reported [51]. When antidiabetic drugs such as metformin and plant extracts containing antioxidants are administered the oxidative stress indicators are reduced while the antioxidant body systems are increased [52].

In this research work, a comparative study of the MDA levels in the normal and negative controls was 9.29 mg/g and 12.65 mg/g, respectively (Figure 3), showing an increase of 36.06% in the level of MDA in the non-treated diabetic group, which arose due to unchallenged stress induced by hyperglycemia [49,52,53]. When the group administered with Bambara groundnut flour was assessed, the MDA value of 10.98 mg/g, which was significantly lower than the standard control value of 12.77 mg/g, (Figure 3), showing that the potency of the intervention formulation in free radical mop up was higher than when antidiabetic drug was used. However it was equally seen that the MDA value for the standard control, when compared to that of the negative control, was not significantly different, unlike previous reports which documented a significant reduction in lipid peroxidation products in diabetic patients receiving antidiabetic medications [52,54,55]. One possible explanation for this is the occurrence of metformin toxicity at certain dose margin in STZ-induced diabetic rats [56]. The GPx had same pattern as the MDA but the values obtained for the GSH, GST and SOD had no significant difference among the groups (Figures 2 and 3) as earlier documented in some other studies [52].

Effect on inflammatory biomarkers

Hyperglycemic states result in the activation of Nuclear Factor kappa B (NF-kB) and the enhancement of pro-inflammatory cytokines [57]. In this work, the mean values of NFkB and c-reactive proteins, showed significant difference among the groups (Figure 4). The 1.24 ng/ml value of the c-reactive proteins in the group fed on Bambara groundnut flour intervention, was higher than the 0.97 ng/ml reported in the normal control group. When compared with the 1.67 ng/ml gotten in the standard control group, there was a differences which were statistically significant (Figure 4). This finding implied that the inflammatory processes were milder in Bambara groundnut-treated group than when antidiabetic drug was used.

For the NFkB values, the normal control had a value of 2.91 x 10^{-6} mg/g, which was lower than the 4.51 x 10^{-6} mg/g, seen in the negative control and the 4.73 x 10^{-6} mg/g seen in the standard control group. The value in the Bambara ground nut-fed diabetic rats was 3.54 x 10^{-6} mg/g, which when compare with the standard and negative control groups, was significantly lower (Figure 4). The values obtained for the interleukin (IL)-10 for the groups, were equally statistically significant, with the Bambara groundnut-fed group having lower value (1.27 x 10^{-7} mg/g) than the negative (1.31 x 10^{-7}mg/g) and standard controls (1.33 x 10^{-7}mg/g). Levels of c-reactive are correlated with features like hyper-insulinaemia and Insulin resistance [58]. The high level of C- reactive protein reported in this work is in agreement with previous works that documented increased c-reative protein in diabetic patients [59,60].

Effect on liver enzymes and liver proteins

Enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) are high in hepatic diseases and other cases of liver injury [61], with ALT being involved primarily in liver diseases, [62] while AST though sensitive in liver injury, is seen in other tissues in like on the other hand is seen in the hepatocyte cytoplasm and mitochondria and some other tissues besides the liver [63]. Diseases of the pancreas and the kidney in addition to that of the liver shows raised GGT [64], which equally rises in some other disease conditions [65]. Raised GGT, reactive oxygen species and antioxidant like glutathione are interrelated [66] such that it can be used as an indicator of chronic diseases such as diabetes mellitus [67].

This work showed an AST values of 0.788 mg/g; 0.86 mg/g, 0.83 mg/g and 0.81 mg/g for normal, standard, negative and intervention groups respectively with differences that were statistically significant from the normal but not with one another for the last three groups. The ALT values showed no significant difference in the values of all the groups. It is possible that the deposition of fatty tissues in the liver could be the reason for raised liver enzymes since there is an association between fatty liver and metabolic syndrome [68].

This work recorded higher levels of AST which corroborates with previous findings in diabetic patients who showed raised liver enzymes especially in uncontrolled fasting blood sugar [69] and in metabolic syndrome [70,71]. The raised liver enzymes in this work was associated with raised random blood glucose. The group on Bambara groundnut intervention had slightly lower AST value signifying some degree of protective effect on the hepatocytes in diabetic rats.

There was no significant changes in the total liver protein (TP) among the groups in this work, possibly because the oxidative stress markers (GST, GSH and SOD) and the liver enzyme, ALT were not significantly affected thereby making TP values not to change significantly. However the total serum protein showed significant difference between the diabetic rat groups and rats in normal control.

Effect on bilirubin

Bilirubin, has free radical scavenging activity when present at certain concentrations [72,73], and this function is utilized as the benefit in non-communicable diseases [74]. The concentration at which bilirubin performs such physiological role is at a moderately high level [75,76]. since both the antioxidant and anti-inflammation effects are manifest at such points [77].

In this work, the various groups` mean bilirubin values had differences but such difference in mean were not significant. The total and direct bilirubin mean values were the lowest in the normal control, while that of the total bilirubin in the standard control group was higher than that in the group on Bambara groundnut. The opposite was the case, for direct bilirubin. The mean value in the negative control was the highest, which was in consonance with previous study that reported increased level of direct bilirubin in diabetic patients [78] and initial high levels of all subtypes in impaired fasting glucose and new-onset T2D, with a subsequent lowering of such levels in situations of chronic hyperglycaemia [78]. It has been revealed that hyperglycaemia results in oxidative stress that up-regulates heme oxygenase-1, with a resultant increased production of bilirubin which incidentally is mopped up by the increasing high reactive oxygen species in chronic hyperglycemia, thereby causing the decreased bilirubin [78,79]. Hence serum bilirubin is low in diabetes mellitus of long standing [77].

Effect on serum lipid profile

The lipid profile assessment parameters using the triglycerides (TG), low density (LDL-c) and Very low density lipoproteins (VLDL-c) values in this report, revealed a non-significant difference among the various groups. However, the mean values of the total cholesterol were significantly different among the groups with the lowest values obtained in the normal control group (94.85 mg/dl). The mean value for total cholesterol, in the group administered with Bambara groundnut flour was significantly lower (110.25 mg/dl) when compared with the value obtained from the standard control (113.34 mg/dl) but the mean values of the high density lipoproteins (HDL-c) in the latter (133.25 mg/dl) was higher than the one obtained in the intervention group (19.26 mg/dl). The interpretation of these results implied that the hypolipidemic effect of the antidiabetic drug, metformin was comparatively better than the Bambara groundnut formulation. Dyslipidemia is a common presentation in patients with T2DM since both the triglycerides and the LDL-c are both elevated with reduced levels of high-density lipoproteins (HDL) [80]. As the biodegradation of the plasma triglycerides occurs, HDL levels are lowered, while the LDL concentrations are raised [81]. There is a positive association between hypertriglyceridemia and the development of insulin resistance and cause β-cell dysfunction [82]. There is also a significant correlation between serum triglyceride and blood sugar levels [83] and the subsequent occurrence of diabetic heart diseases[84.] Administration of potent antioxidant compounds such as taurine, have been associated with improved lipid profile, even in diabetic rats [85].

Effect on renal function

Diabetic patients with nephropathy presents with raised serum urea and creatinine levels, due to the poorly controlled diabetes, with a resultant renal pathology, which if not ameliorated can lead to renal failure [86]. Reports have identified renal pathology as one of the diabetic complications [87], with manifestations such as elevated plasma urea and creatinine [88]. Even though the mean values of the creatinine were not significantly different among the groups, there were notable changes in the urea levels among the groups in this work. The normal control mean urea value (12.55 mg/dl) was lower than that found in the group on Bambara groundnut formulation (12.98 mg/dl), which again was lower than the value in the standard and negative controls (14.20 mg/dl and 13.19 mg/dl respectively). The value differences between the intervention group and the standard and negative controls were statistically significant, implying that Bambara groundnut formulation had higher potency in the renal tissue protection than the anti-diabetic drug in diabetic rats. Previous reports have equally documented higher values in urea and creatinine levels in diabetic control compared to normal control, as seen in this study [89]. The mean value of the uric acid was comparatively significantly lower in bambara groundnut-fed diabetic rats than in the standard control, whose value was lower than in the negative controls. High values in uncontrolled diabetic rats have been reported in previous studies [90].

The possible explanation for the protection of renal tissue damage as demonstrated by the administration of Bambara groundnut formulation in diabetic nephropathy is the presence of polyphenols [91,92], whose *invivo* bioactivities such as reduction of transforming growth factor (TGF)-β expression, matrix metalloprotinease (MMP)-2/-9, and matrix-degrading enzymes [89] in hyperglycemia-induced renal damage. When the TGF-β is stimulated, there is excessive extracellular matrix, which leads to glomerular fibrosis and renal dysfunction [93]. Hence in inflammatory conditions, both the TGF-β and metalloprotinease are associated factors [94] that results in fibroblasts production and formation of fibrosis [95]. Therefore, once these deleterious actions of TGF-β and metalloprotinease are contained, diabetic renal failure can be arrested. Yassine and colleagues established that STZ-induced diabetic rats administered with polyphenol mix, maintained a normal renal architecture, showed less necrotic cells and less leukocytes infiltration [89].

Effect on cardiac function

Contraction of muscles is influenced by the presence of creatine kinase (CK) which is necessary for the breakdown of adenosine triphosphate to generate the needed energy [96], such that during some muscular activities and in cases of metabolic syndrome, the level of this enzyme appreciates [97]. In diabetic patients, raised levels of creatine kinase and lactate dehydrogenase (LDH) are used as biological markers of cardiac muscle damage and hence signifies impending diabetic cardiac complications [98]. When uncontrolled diabetes becomes chronic, cardiotoxic compounds such as advanced glycated end products (AGEs) are formed to initiate myocardial fibrosis, in a process mediated by the transforming growth factor (TGF)-β1 -dependent or independent pathway [99]. Findings from this work revealed that both the mean values of LDH and CK, was not significantly different among the groups, meaning that irrespective of the rats` diabetic status, and the various forms of interventions, these measured cardiac enzymes were not affected. This finding agreed with similar documented reports of no significant difference in the levels of CK and LDH values in metformin-treated T2DM when compared with normal control [100]. However some other studies reported slight increases in these enzymes [97], even in subclinical cases [98]. The raised CK and LDH were seen to be more pronounced in diabetic cases with metabolic syndrome [97]. It is likely that the short duration of this study might have accounted for the insignificant difference in the LDH and CK mean values among the groups, since studies have reported that glucose cardiotoxic effects in diabetic cases occur in chronic hyperglycemia [101]. Both this study and that of Kotb lasted for less than two months [100] and therefore couldn't have initiated cardiac cell damage.

Conclusion and Recommendation

Bambara groundnut flour when given to male streptozotocin-induced diabetic albino rats in significant amounts was as effective as standard antidiabetic drug in the regulation of blood cholesterol levels. Its other biochemical actions include significant mop up of free radicals, reduced hyperbilirubinaemia, hypoglycemic effects, and renal tissue protection.

These positive impact on the various biochemical parameters, is suggestive that addition of Bambara groundnut as a nutritional adjunct in the clinical management of type 2 diabetes mellitus.

Acknowledgements

We wish to acknowledge the technical support from Prof Ezekwe, Afamefula Sunday of the Department of Medical Biochemistry, Rivers State University, Nigeria, as well as Dr Chinedu Eleazu and Dr Uchechukwu Okorie both of Alex Ekwueme Federal University, Ndufu-Alike, Ikwo. The authors declare no conflicts of interest whatsoever in the course of this research work and manuscript production.

Authors` Contributions

- HCU: Conceptualized and conducted the work, and drafted the manuscript
- FNU: Data Entry and literature search
- FUU: Date Entry, Statistical Analysis and Proof reading.
- KO: Literature Search, Manuscript writing

Authors` Contributions

- HU: Topic design, Proposal writing, Literature search, draft manuscript
- AE: Topic design, Statistical analysis, draft manuscript
- UO: Study Analysis, Literature search
- CE: Manuscript writing
- FU: Data Entering and literature search

All authors read and approved the final manuscript.

Competing Interest

There is no competing interest.

Bibliography

1. Sun H., *et al*. "IDF Diabetes Atlas: Global, Regional and Country-level diabetes prevalence estimates for 2021 and projections for 2045". *Diabetes Research and Clinical Practice* 183 (2022): 109119.

2. Van Belle TL., *et al*. "Type 1 diabetes: etiology, Immunology and therapeutic strategies". *Physiological Reviews* 91 (2011): 1.

3. Lin X., *et al*. "Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025". *Scientific Reports* 10 (2020): 14790.

4. Kirigia JM., *et al*. "Economic burden of diabetes mellitus in the WHO African region". *BMC International Health and Human Rights* 9 (2009): 6.

5. Ashcroft FM and SJH Ashcroft. "Insulin, Molecular Biology to Pathology". Oxford University Press (1992): 266-284.

6. Sobngwi E., *et al*. "Diabetes in Africans. Epidemiology and clinical specificities". *Diabetes Metabolism* 27.6 (2001): 628-634.

7. Piero MN. "Hypoglycemic effects of some Kenyan plants traditionally used in management of diabetes mellitus in eastern province". Msc thesis, Kenyatta University (2006).

8. Wylie-Rosett J and Delahanty LM. "Nutrition in the Prevention and Treatment of Disease (Fourth Edition)" (2017): 691-707.

9. Forouhi GN., *et al*. "Dietary and nutritional approaches for prevention and management of type 2 Diabetes, Science and Politics of Nutrition". *BMJ* (2018): 361.

10. Piero MN., *et al*. "The Role of Vitamins and Mineral Elements in Management of Type 2 Diabetes Mellitus: A Review South As". *Journal of Biological Sciences* 2.1 (2012): 107-115.

11. Oyeyinka SA and Oyeyinka AT. "A review on isolation, composition, physicochemical properties and modification of Bambara groundnut starch". *Food Hydrocolloids* 75 (2018): 62-71.

12. Arise AK., *et al*. "Influence of extraction methods on functional properties of protein concentrates prepared from South African Bambara groundnut landraces". *International. Journal of Food Science and. Technology* 50 (2015): 1095-1101.

13. Oyeyinka SA Tijani., *et al*. "Value added snacks produced from Bambara groundnut (*Vigna subterranea*) paste or flour". *LWT-Food Science and Technology* 88 (2018): 126-131.

14. Halimi RA., *et al*. "The potential of the underutilized pulse bambara groundnut (*Vigna subterranea* (L.) Verdc.) for nutritional food security". *The Journal of Food Composition and Analysis* 77.2 (2019): 47-59.

15. Olaleye AA., *et al*. "Chemical Composition of Bambara groundnut (*Vigna subterranea* L. Verdc)". *Bangladesh Journal of Scientific and Industrial Research* 48.3 (2013): 167-178.

16. Salawu. "Comparative study of the antioxidant activities of methanolic extract and simulated gastrointestinal enzyme digest of Bambara nut (*Vigna subterranean*) FUTA". *Journal of Research in Science* 1 (2016): 107-120.

17. Harris T., *et al*. "Flavonoids and tannin composition of Bambara groundnut (*Vigna subterranean*) of Mpumalanga, South Africa". *Heliyon* 4 (2018): e00833.

18. Yao DN., *et al*. "Nutritive Evaluation of the Bambara Groundnut Ci12 Landrace *Vigna subterranea* (L.) Verdc. (*Fabaceae*). Produced in Côte d'Ivoire". *International Journal of Molecular Sciences* 16.9 (2015): 21428-21441.

19. Baptista A., *et al*. "Characterization of protein and fat composition of seeds from common beans (*phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* L. Walp) and Bambara groundnuts (*Vigna subterranean* L. Verde) from Mozambique". *Journal of Food Measument and Characterization* 12 (2016): 1-9.

20. Carbonaro M. "Role of pulses in nutraceuticals, Eds., In Tiwari, B.K., Gowen, A., McKenna, B., Pulse Foods". *Academic Press* 11 (2014): 385-418.

21. Barbieri R., *et al*. “Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity”. *Microbiology Research* 196 (2017): 44-68.

22. Saxena M., *et al*. “Phytochemistry of medicinal plants”. *Journal of Pharmacognosy and Phytochemistry* 1.6 (2013): 168-182.

23. Montonen J., *et al*. “Whole-grain and fibre intaknd the incidence of type 2 diabetes”. *American Journal of Clinical Nutrition* 77.3 (2003): 622-629.

24. Iwai K., *et al*. “α-Glucosidase Inhibitory and Antihyperglycemic Effects of Polyphenols in the Fruit of *Viburnum dilatatum* Thunb”. *Journal of Agricultural and Food Chemistry* 54.13 (2006): 4588-4592.

25. Ruzaidi A., *et al*. “Hypoglycemic properties of Malaysia cocoa (Theobromacacao) polyphenols-rich extract”. *International Food Research Journal* 15 (2008): 305-312.

26. Chelangat M., *et al*. “Nutritional and Phytochemical Composition of Bambara Groundnut (*Vigna subterranean* L.. Verdc) Landraces in Kenya”. *International Journal of Agronomy* 2023.1 (2023): 9881028.

27. NRC (National Research Council) (1985) Guide for the care and use of laboratory Animals”. Bethesda (MD): *National Institute of Health* (1985): 8523.

28. Nair AB and Jacob S. “A simple practice guide for dose conversion between animals and human”. *J Basic Clinical Pharmacology* 7.2 (2016): 27-31.

29. Pneu-Dart. “Dosage Calculation” (2024).

30. Nnadi NN., *et al*. “Effects of Formulated Unripe Plantain and Millet Dietary Feeds in Alloxan-Induced Diabetic Albino Rats”. *International Journal of Innovative Research and Advanced Studies (IJIRAS)* 9.6 (2022).

31. Tietz NW. “Clinical Guide to Laboratory Tests. 3rd Edition. Philidelphia, PA: WB Saunders Company (1995).

32. Friedewald WT., *et al*. “Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge”. *Clinical Chemistry* 18 (1972): 499-505.

33. Jin XH., *et al*. “Effects of blue honeysuckle (Lonicera caerulea L.) extract on lipopolysaccharide-induced inflammation *in vitro* and *in vivo”*. *Experimental Eye Research* 82 (2006): 860-867.

34. Lalhminghlui K and Jagetia GC. “Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth *in vitro*”. *Future Science* 4.2 (2022): 283.

35. Mukhtar A., *et al*. “Catalase Activity in Hot-Air Dried Mango as an indicator of Heat Exposure for Rapid detection of Heat Stress”. *Applied Sciences* 12.3 (2022): 1305.

36. Paglia DE and Valentine WN. “Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase”. *Journal of Laboratory and Clinical Medicine* 70 (1967): 158-169.

37. Beutler E., *et al*. “Improwed method for the determination of blood glutathione”. *Journal of Laboratory and Clinical Medicine* 61 (1963): 882-888.

38. Ohkawa H., *et al*. “Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction”. *Anal Biochemistry* 95.2 (1979): 351-358.

39. Azeez TA. “The pattern of antidiabetic drugs and glycemic control among type 2 diabetes patients in an endocrinology clinic in Lagos, Nigeria”.

40. Huang DW and Shen SC. “Caffeic acid and cinnamic acid ameliorate glucose metabolism via modulating glycogenesis and gluconeogenesis in insulin-resistant mouse hepatocytes”. *Journal of Functional Foods* 4.1 (2012): 358-366.

41. Huang DW., *et al*. “Gallic acid ameliorates hyperglycemia and improves hepatic carbohydrate metabolism in rats fed a high-fructose diet”. *Nutrition Research* 36.2 (2016): 150-160.

42. Oršolić N., *et al*. “Efficacy of caffeic acid on diabetes and its complications in the mouse”. *Molecules* 26.11 (2021): 3262.

43. Oboh G., *et al*. “Can gallic acid potentiate the antihyperglycemic effect of acarbose and metformin? Evidence from streptozto-cin-induced diabetic rat model”. *Archives of Physiology and Biochemistry* (2020).

44. Hussain T., *et al*. “The Role of Oxidative Stress and Antioxidant Balance in Pregnancy”. *Mediators of Inflammation* (2021): 9962860.

45. Maschirow L., *et al*. “Inflammation, coagulation, endothelial dysfunction and oxidative stress in prediabetes - Biomarkers as a possible tool for early disease detection for rural screening”. *Clinical Biochemistry* (2015).

46. Morel Y and Barouki. “Repression of gene expression by oxidative stress”. *Biochemical Journal* 342 (1999): 481-496.

47. Al-ansari RF., *et al*. “Serum levels of zinc, copper, selenium and glutathione peroxidase in the different groups of colorectal cancer patients”. *Caspian Journal of Internal Medicine* 11.4 (2020): 384-390.

48. He X., *et al*. "Metformin inhibits MAPK signaling and rescues pancreatic aquaporin 7 expression to induce insulin secretion in type 2 diabetes mellitus". *Journal of Biological Chemistry* (2021): 297.
49. Kattoor A., *et al*. "Oxidative Stress in Atherosclerosis". *Current Atherosclerosis* 19.11 (2017): 19-42.
50. Cakatay U. "Protein Oxidation Parameters in type 2 diabetic patients with good and poor glycaemic control". *Diabetes Metabolism* 31.6 (2005): 551-557.
51. Byrne NJ., *et al*. "Therapeutic potential of targeting oxidative stress in diabetic cardiomyopathy". *Free Radical Biology and Medicine* 169 (2021): 317-342.
52. Rabhi H., *et al*. "The Mediterranean diet biodiversity impact on metabolic and oxidative stress parameters in type 2 diabetes". *Genetics and Biodiversity* 6.2 (2022): 87-102.
53. Abdel-Raheem A., *et al*. "Oxidative Stress Markers as early predictors of diabetes complications in Type 2 diabetic patients". *Indian Journal of Physiology and Pharmacology* 66 (2022): 111-119.
54. Banik S., *et al*. "Attenuation of lipid peroxidation and atherogenic factors in diabetic patients treated with gliclazide and metformin". *Journal of Research in Medical Sciences* 23 (2018): 77.
55. Piccirillo F., *et al*. "Novel Antidiabetic Agents and Their Effects on Lipid Profiles: A Single Shot for Several Cardiovascular Targets". *International Journal of Molecular Sciences* 24.12 (2023): 10164.
56. El-Naggar SA., *et al*. "Metformin Causes Hepato-renal Dysfunctions in Obese Male Rats". *Brazilian Archives of Biology and Technology* 64 (2021): e21210188.
57. Shoelson SE., *et al*. "Inflammation and insulin resistance". *Journal of Clinical Investigation* 116.7 (2006): 1793-1801.
58. Nakano S., *et al*. "Small, dense LDL and high-sensitivity C-reactive protein (hs-CRP) in metabolic syndrome with type 2 diabetes mellitus". *Journal of Atherosclerosis and Thrombosis* 17.4 (2010): 410-415.
59. Pickup JC., *et al*. "Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes". *Life Sciences* 67.3 (2000): 291-300.
60. Mirza S., *et al*. "Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study". *Cytokine* 57.1 (2012): 136-142.
61. Hanley AJ., *et al*. "Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study". *Diabetes* 53.10 (2004): 2623-2632.
62. Giannini EG., *et al*. "Liver enzyme alteration: a guide for clinicians". *CMAJ* 172.3 (2005): 367-379.
63. Lee SH., *et al*. "Dietary phytic acid improves serum and hepatic lipid levels in aged ICR mice fed a high-cholesterol diet". *Nutrition Research* 27.8 (2007): 505-510.
64. Hanigan MH and Frierson HF. "Immunohistochemical detection of gamma-glutamyl transpeptidase in normal human tissue". *The Journal of Histochemistry and Cytochemistry* 44.10 (1996): 1101-1108.
65. Berk P and Korenblat K. "Approach to the Patient with Jaundice or abnormal liver tests. In Goldman`s Cecil Medicine: 24th Edition 1 (2011): 956-966.
66. Kim D-J., *et al*. "Serum gamma-glutamyltransferase within its normal concentration range is related to the presence of diabetes and cardiovascular risk factors". *Diabetic Medicine Journal Brain Diabetic Association* 22.9 (2005): 1134-1140.
67. Wang Y., *et al*. "Association between liver enzymes and incident type 2 diabetes in Singapore Chinese men and women". *BMJ Open Diabetes Res Care* 4.1 (2016): e000296.
68. Finelli C and Tarantino G. "What is the role of adiponectin in obesity related non-alcoholic fatty liver disease?" *World Journal of Gastroenterology* 19.6 (2013): 802.
69. Yazdi HB., *et al*. "Liver Dysfunction and Oxidative Stress in Streptozotocin-Induced Diabetic Rats: Protective Role of *Artemisia* Turanica". *Journal of Pharmacopuncture* 22.2 (2019): 109-114.
70. Islam S., *et al*. "Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults". *American Journal of Physiology-Endocrinology and Metabolism* 3.2 (2020).
71. Wang Y., *et al*. "Association between liver enzymes and incident type 2 diabetes in Singapore Chinese men and women". *BMJ Open Diabetes Res Care* 4.1 (2016): e000296.
72. Wu TW., *et al*. "Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins". *Biochemical Pharmacology* 51 (1996): 859-862.
73. Stocker L., *et al*. "Bilirubin is an antioxidant of possible physiological importance". *Science* 235.4792 (1987): 1043-1046.

74. Troughton JA., *et al*. "Bilirubin and coronary heart disease risk in the Prospective Epidemiological Study of Myocardial Infarction (PRIME)". *European Journal of Cardiovascular Prevention and Rehabilitation* 14 (2007): 79-84.

75. Li X., *et al*. "Gut Microbiota Dysbiosis Drives and Implies Novel Therapeutic Strategies for Diabetes Mellitus and Related Metabolic Diseases". *Frontiers in Immunology* 8 (2017): 1882.

76. Gazzin S., *et al*. "A novel perspective on the biology of bilirubin in health and disease". *Trends in Molecular Medicine* 22.9 (2016): 758-768.

77. Dong H., *et al*. "Bilirubin increases insulin sensitivity in leptin-receptor deficient and diet-induced obese mice through suppression of ER stress and chronic inflammation". *Endocrinology* 155 (2014): 818-828.

78. Wang J., *et al*. "Serum bilirubin levels and risk of type 2 diabetes: results from two independent cohorts in middle-aged and elderly Chinese". *Scientific Reports* 7 (2017): 41338.

79. Rochette L., *et al*. "Redox functions of heme oxygenase-1 and biliverdin reductase in diabetes". *Trends in Endocrinology and Metabolism* 29.2 (2018): 74-85.

80. Wu L and Parhofer KG. "Diabetic dyslipidemia". *Metabolism* 63.12 (2014): 1469-1479.

81. Guérin M., *et al*. "Atherogenic role of elevated CE transfer from HDL to VLDL (1) and dense LDL in type 2 diabetes : impact of the degree of triglyceridemia". *Arteriosclerosis, Thrombosis, and Vascular Biology* 21.2 (2001): 282-288.

82. Briaud I., *et al*. "Lipotoxicity of the pancreatic betacell is associated with glucose-dependent esterification of fatty acids into neutral lipids". *Diabetes* 50.2 (2001): 315-321.

83. Hamzah SA. "Association between Lipid Profiles and Renal Functions among Adults with Type 2 Diabetes". *International Journal of Diabetes and Metabolic Disorders* 25 (2019): 234-138.

84. Rachek LI. "Free fatty acids and skeletal muscle insulin resistance". *Progress in Molecular Biology and Translational Science* 121 (2014): 267-292.

85. Wang G., *et al*. "Taurine attenuates oxidative stress and alleviates cardiac failure in type I diabetic rats". *Croatian Medical Journal* 54 (2013): 171-179.

86. Shlomo M., *et al*. "Diabetes Mellitus. "Willams textbook of endocrinology, 12th Edition". Philadelphia: Elsevier/Saunders (2011): 1371-1435.

87. Rosa MD., *et al*. "Autophagy in diabetic retinopathy". *Current Neuropharmacology* 14.8 (2016): 810-825.

88. Ali AA and Faris HA. "Prevalence and determinants of microalbuminuria among type 2 diabetes mellitus patients, Baghdad, Iraq, 2013". *SJKDT* 27.2 (2016): 348-355.

89. Yassine C., *et al*. "Investigation of the renal protective effect of combined dietary polyphenols in streptozotocin-induced diabetic aged rats". *Nutrients* 14.14 (2022): 2867.

90. Andonova M., *et al*. "Metabolic Markers Associated with Progression of Type 2 Diabetes Induced by High-Fat Diet and Single Low Dose Streptozotocin in Rats". Veterinary Sciences 10.7 (2023): 431.

91. Rana S., *et al*. "In Silico Target Identification and Validation for Antioxidant and Anti-inflammatory Activity of Selective Phytochemicals". *Brazilian Archives of Biology and Technology* 62.4 (2019).

92. Rudrapal M., *et al*. "Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism (s) of Action". *Frontiers in Pharmacology* 13 (2022): 806470.

93. Forbes JM., *et al*. "Diabetic nephropathy: where emodynamics meets metabolism". *Experimental and Clinical Endocrinology and Diabetes* 115.2 (2007): 69-84.

94. Cabral-Pacheco GA., *et al*. "The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases". *International Journal of Molecular Science* 21 (2020): 9739.

95. Catania JM., *et al*. "Role of matrix metalloproteinases in renal pathophysiologies". *American Journal of Physiology Renal Physiology* 292 (2007): F905-F911.

96. Field ML., *et al*. "Functional compartmentation of glycogen phosphorylase with creatine kinase and Ca2+ ATPase in skeletal muscle". *Journal of Theoretical Biology* 238.2 (2006): 257-268.

97. Al-Hail N., *et al*. "Creatine Kinase Is a Marker of Metabolic Syndrome in Qatari Women with and Without Polycystic Ovarian Syndrome". *Frontiers in Endocrinology* (*Lausanne)* 10 (2019): 659.

98. Frank M and Finsterer J. "Creatine kinase elevation, lactacidemia, and metabolic myopathy in adult patients with diabetes mellitus". *Endocrine Practice* 18.3 (2012): 387-393.

99. Leask A and Abraham DJ. "All in the CCN family: Essential matricellular signaling modulators emerge from the bunker". *Journal of Cell Science* 119 (2006): 4803-4810.

100. Kotb AS., *et al*. "Metformin ameliorates diabetic cardiomyopathy in adult male albino rats in type 2 diabetes". Minia Journal of Medical Research 33.4 (2022): 128-138.

101. Asmat U., *et al*. "Diabetes mellitus and oxidative stress-A concise review". *Saudi Pharmaceutical Journal* 24.5 (2016): 547-553.