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Comparative Analysis of the Biochemical Changes in Streptozotocin (STZ) Induced Diabetic Rats Fed Differently on Cocoyam, Soya Bean and Bambara Groundnut Flour

Uro-Chukwu, H.C [a,b,c*], Ezekwe, A.S [d] and Uro-Chukwu, F.C.U [c]

[a] *Department of Biochemistry, Coal City University, Enugu, Nigeria.*
[b] *Department of Community Medicine, Ebonyi State University, Abakaliki, Nigeria.*
[c] *Institute of Nutrition, Nutraceuticals and Public Health Research and Development, Nigeria.*
[d] *Department of Medical Biochemistry, Rivers State University, Nkpolu, Port Harcourt, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author UCHC did topic design, Proposal writing, literature search, draft manuscript. Author EAS did topic design, statistical analysis, draft manuscript. Author UCFCU did data entering and literature search. All authors read and approved the final manuscript.

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**Corresponding author: Email: hurochu@gmail.com;*

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ABSTRACT

Background: Diabetes mellitus is a predominant non-communicable disease in both developing and high income countries causing multiple organ damage and disabilities worldwide. The orthodox approach to managing the disease is confronted with myriads of challenges making alternative cheaper and culturally acceptable methods unavoidable. Global experts have suggested the use of plants with medicinal values, resulting in researches into plants foods with health benefits. Cocoyam (CYN), soya bean (SB), and Bambara groundnut (BGN) are documented plant foods whose bioactive constituents have series of biochemical effects in diabetic animals. The essence of this study was to compare the biochemical changes occurring in different sets of streptozotocin-induced diabetic rats fed with CYN, SB and BGN.

Methodology: CYN, SB and BGN were packaged in airtight containers after undergoing processing, pelletization and grinding into fine flours. Fourty two healthy male albino rats, were acclimatized for one week before the induction of Insulin resistance and Type 2 Diabetes using 10% fructose diet and intraperitoneal injection of streptozotocin respectively. The recorded weights of the rats were between 134 and 247 g. The intervention formulations were administered for 28 days, following which blood samples were collected from the killed animals for biochemical analysis.

Results and Discussion: The study results showed a more potent lipid peroxidation amelioration with the intervention formulations when compared to the standard control, and that among the formulations, BGN and CYN-fed groups outperformed the SB-fed group. The intervention formulations also showed stronger anti-inflammatory properties than the standard control, with the SB-fed group exhibiting the best. CYN and SB equally exerted hypolipidemic effects unlike in the BGN-fed rats. All the formulations had similar urea and uric acid concentration levels which were lower than observed with the antidiabetic drug, implying a better renal protective capacity in the flour-fed rat groups.

Conclusion: The hepato-renal protection and hypolipidemic effects arising from the administration of BGN, SB and CYN were better than in the standard control, with SB and CYN being generally more efficacious. Hence soybean and cocoyam flour can be useful adjuncts in the nutritional and clinical management of patients with Type 2 diabetes mellitus.

Keywords: Diabetic rats; biochemical activities; cocoyam; bambara groundnut; soya bean.

1. INTRODUCTION

Diabetes mellitus affects pancreatic and extra pancreatic organs resulting in increased concentration of blood glucose that causes damage to tissues and hence presenting as a disease in vast number of people in the world [1]. Due to multiple organ involvement, complications that increases care cost and disability arise [2]. In both low and high income countries, the prevalence of this disease and the complications are high though more cases now occur in developing countries [3].

The obvious challenge is the coinciding high burden of preventable diseases and other extraneous factors such as insufficient infrastructure, lack of medical products, inefficient health insurance system and inadequate number of health care professionals in low/medium income countries [4]. The sudden emergence of pandemics and other health system resilience possess more danger to fighting diabetes mellitus with orthodox medicine [5], hence the dire need for affordable and culturally acceptable effective management strategies in developing countries. Among the alternative management recommendations are change in lifestyles including dietary patterns, regular exercise and guided adolescent nutrition, all of which have been found to be effective in addressing diabetes mellitus and its complications [6-8].

Other strategies equally canvassed by global experts are consumptions of food plants with health benefits [9]. Food plants such as Bambara groundnut (*Vigna subterranean*), credited with quality nutrients and phytochemicals [10-12], which have proven bioactivity including anti-oxidative actions [13-15]. These bioactivities in Bambara groundnut include hypoglycemic control in diabetic rats [16]. Similarly cocoyam also possesses hypoglycemic control *In vivo*

[17,18] and this action has been linked to the presence of some bioactive compounds [19- 21]. It has also been reported that Soya Bean (*Glycine max. (L) Merrill*) with its rich quantity of macro and micro-nutrients [22], and bioactive compounds also exerts antioxidant activity [23]. This study aims to evaluate the comparative biochemical effects of the consumption of these plants foods in streptozotocin-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Bambara groundnut and soya bean purchased from the local market were processed according to established protocols before grinding them into fine flours that were made into pellets and stored in airtight containers for use later in the experiment. Cocoyam was also locally purchased and peeled after cleaning, before soaking them in water to reduce the starch content and oxalates. It was further boiled and dried inn an oven. When the weight was constant, it was ground and made into pellets that were equally stored for use later in the work.

2.2 Experimental Animals

Albino male rats numbering Fourty-two and weighing between 134 and 249 grams, were placed in sets of eight per cage, with each rat marked 1 – 8 on the rat tail. To ensure a natural and conducive experimental condition the room temperature was maintained together with a 12 hours of light and 12 hours of darkness. All ethical guidelines in animal experiment were observed [24].

2.3 Induction of Insulin Resistance Using Low Fructose Diet

After acclimatization for one week, the rats were induced with 10% fructose diet, to develop insulin resistance by giving the fructose diet *ad libitum* for two weeks. To establish the glycemic state of the rats, the blood glucose level was checked using *Acu Chek*^{R} glucometer at the end of two weeks. The blood glucose level in all the rats were normal.

2.4 Induction of Type 2 Diabetes Mellitus using STZ

To induce Type 2 Diabetes mellitus in the insulin resistant rats, 1 gram of the Streptozotocin, was dissolved in 50 mL of freshly made buffer (sodium citrate buffer, 0.1M, pH 4.5) and administered intraperitoneally to thirty-three of the fourty-two rats [25, 26]. Blood glucose concentrations were determined at 72 hours and on the 12th day post-STZ with the aid of glucometer. The remaining normoglycemic nine rats were designated as the normal control group (Group B). The diabetic rats were designated as the standard control (Group A), negative control (Group B), and intervention groups (Group D) by random selection. The different groups were subjected to the various interventions for a total of 28 days, namely: Group C received oral metformin at a dosage of 200 mg/kg administered daily using an oral dispenser at 0.002 ml per kg body weight; Group D received a blend of 50% commercial rat feed and 50% intervention flour, as per the methodology outlined by Nnadi and colleagues [27]. Groups A and B were fed a commercial rat diet.

The groups were as follows:

Group A: STZ-induced Diabetic rats administered with Metformin (Standard Control)

Group B: Non-diabetic control fed on commercial rat feed (Normal Control)

Group C: STZ-induced Diabetic rats administered with commercial rat feeds (Negative Control)

Group D: STZ-induced diabetic rats administered with *Vigna subterranean* intervention feed, Cocoyam or Soya bean intervention feeds.

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

The rats were fasted all the night at the end of the 28 days, at which time the weights were recorded while blood was collected total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) in compliance with the guidelines [28], using Randox assay diagnostic kits. Subsequently the amounts of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol were derived from standard formula [29]. The liver, kidney and cardiac biochemical function tests were all determined following standard procedures.

2.4.2 Estimation of lipid peroxidation

The protocol described by Ohkawa and colleagues [30] was used to determine the level of Malonylaldehyde (MDA) and the results recorded.

2.5 Statistical Analysis

All the generated data were analysed using the statistical package for social sciences (SPSS Inc., Chicago, IL) version 20.0 with Tukey's post-hoc test was used to determine whether the difference between means was statistically significant at P<0.05. The means were compared using a one-way analysis of variance (ANOVA).

3. RESULTS

3.1 Biochemical Activities

3.1.1 Effect on oxidative stress markers

The result in Fig 1 showed that the MDA values were higher in the Standard and Negative controls compared to the groups fed with the intervention formulations. The MDA values in Bambara groundnut and Cocoyam-fed groups were lower than in the soya bean-fed group. Similarly the SOD values in the Standard and Negative controls were equally higher than in the groups on intervention formulations.

3.2 Effect on Inflammatory Biomarkers

The comparative values of the standard control, and other groups on intervention formulations showed lower values of the inflammatory biomarkers in the latter groups. Among the groups on the intervention formulations, soya bean flour triggered the least inflammatory reaction with the least mean value of CRP, followed by Bambara groundnut and then cocoyam. The mean value of IL-10, an anti-inflammatory biomarker, did not differ.

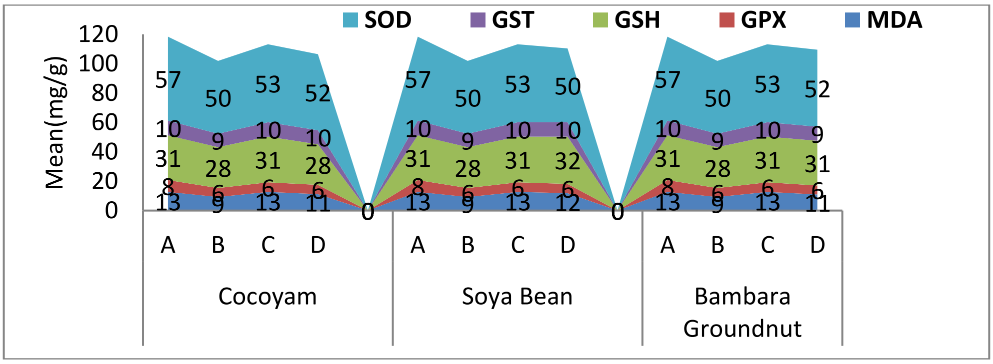

Fig. 1. Graph showing the values of oxidative stress markers in various groups of diabetic rats fed with cocoyam, soybean, and bambara groundnut

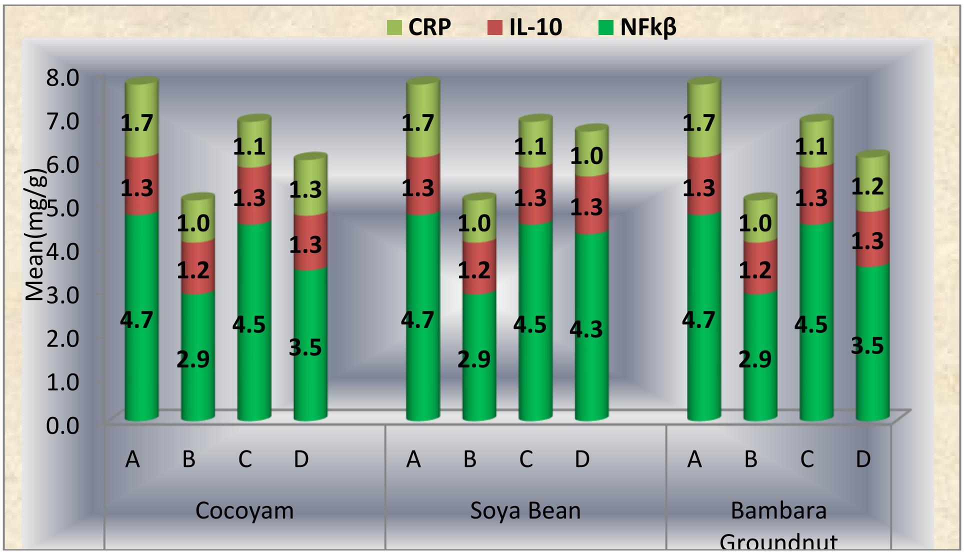

Fig. 2. Graphs showing the mean values of inflammatory biomarkers found in various groups

3.3 Effect on Bilirubin and Serum Proteins

Fig 3 revealed that although the total and direct bilirubin levels were lowest in the normal control group, there were no significant differences in the bilirubin mean values between the groups.

3.4 Effect on Serum Lipid Profile

Fig 4 revealed that among the intervention flour, rats fed with bambara groundnut flour had higher levels of total cholesterol and triglycerides than did the cocoyam and soya bean groups, however, these groups' values were still lower than those of the Standard and negative control groups. In comparison to the normal control, the VLDL-c and LDL-c values were lower than in the intervention groups, with cocoyam having the lowest.

3.5 Effect on Creatinine and Urea

Fig 5 did not show any discernible differences in the creatinine values among the groups, but the intervention flour groups' urea concentration values were lower than those of the controls, suggesting that the formulations provided superior renal protection than the typical antidiabetic medication.

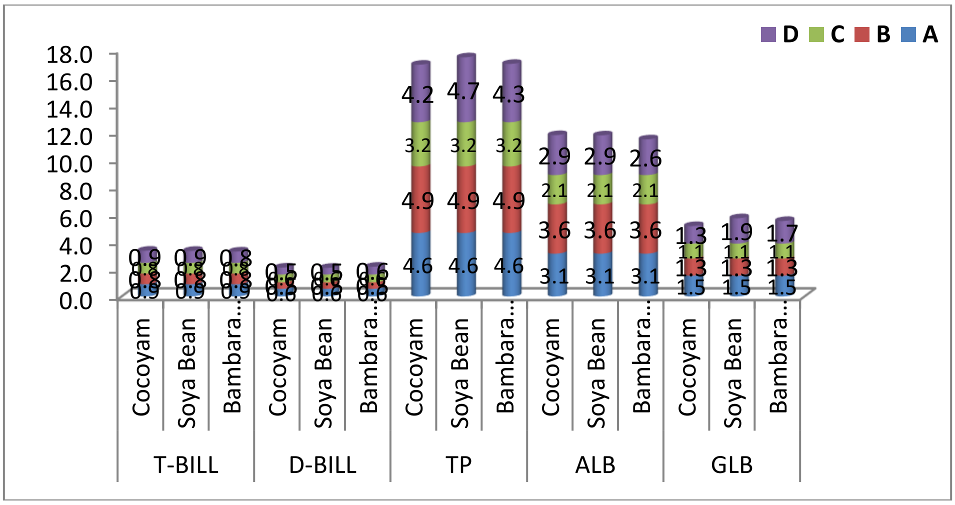

Fig. 3. Graphs showing the mean values of the serum proteins and bilirubin in the various groups

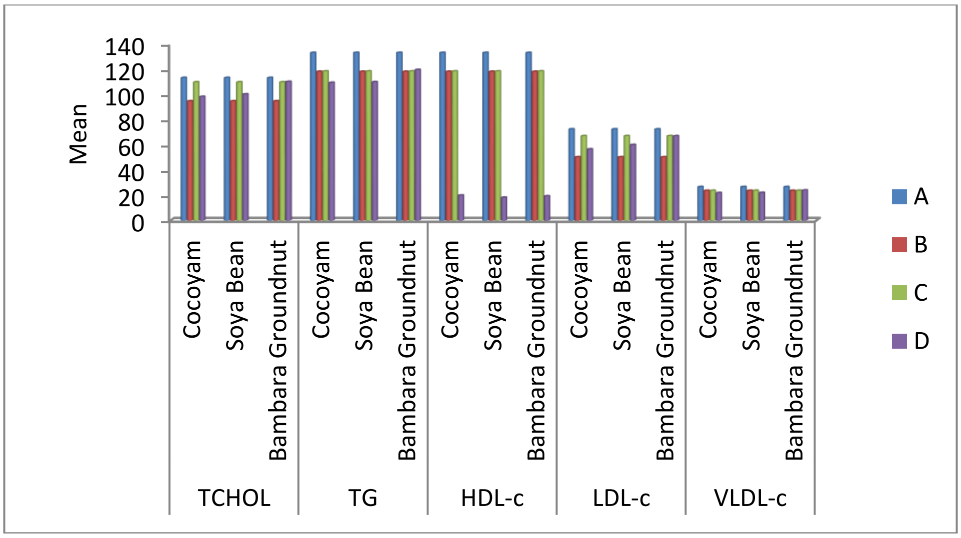

Fig. 4. Graphs showing the serum lipid profile of the various groups

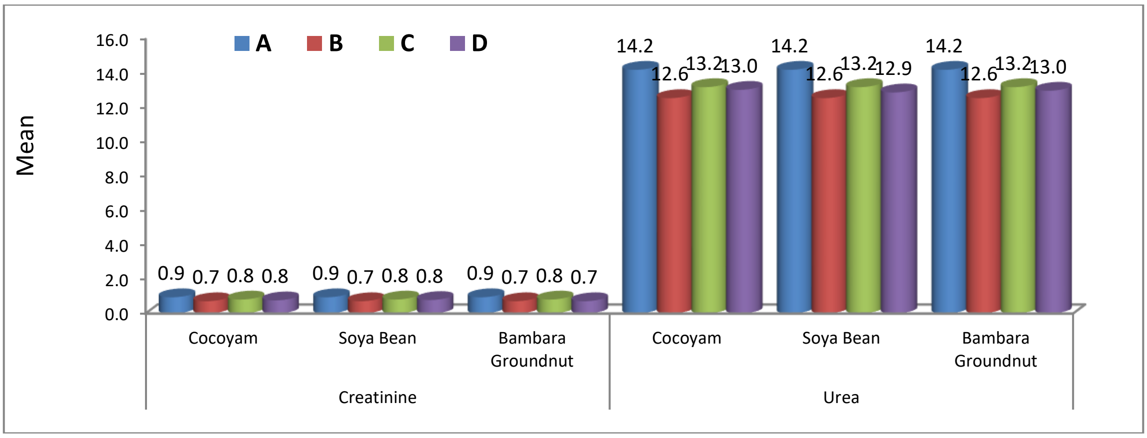

Fig. 5. Graph showing the mean serum urea and creatinine levels of the various diabetic rats and normal control

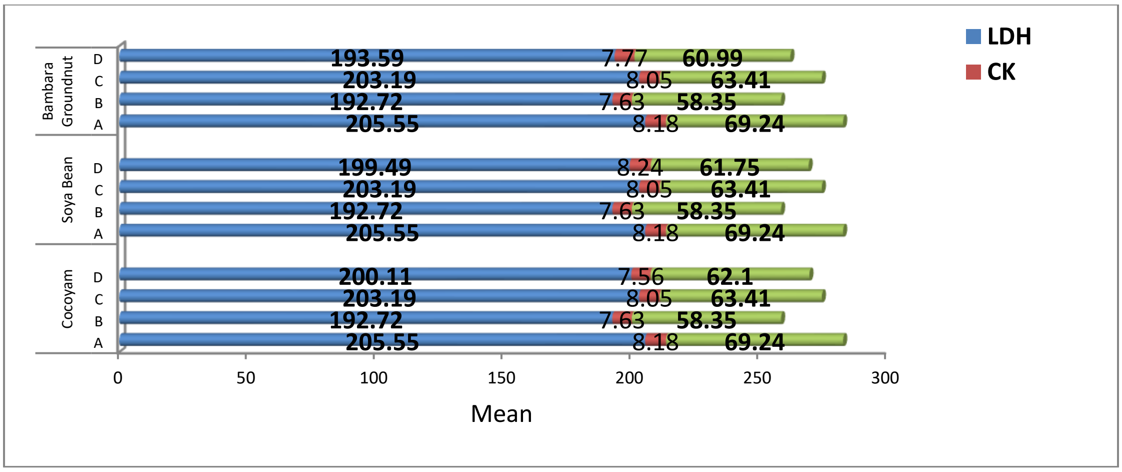

Fig. 6. Graph showing the mean serum levels of Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) in the various rat groups

3.6 Effect on Cardiac Functions

Fig 6 revealed absence of discernible variations in the average LDH and CK values among the different groups.

4. DISCUSSION

4.1 Biochemical Activities

4.1.1 Effect on oxidative stress markers

Anti-diabetic drugs and extracts with antioxidant activities can lower MDA and carbonylated protein levels while raising antioxidant levels, [31]. In this study, the standard and negative control groups had the highest levels of superoxide dismutase (SOD), while the experimental groups had lower levels. The mean MDA concentration for the soybean flour group was 9.29 mg/g, compared to 12.65 mg/g, in the negative control group, indicating an increase of lipid peroxidation by 36.06%. These findings align with previous studies which showed higher lipid peroxidation biomarkers in uncontrolled diabetes mellitus [31, 32]. The group treated with soybean, cocoyam and Bambara groundnut flour, had mean MDA values of 11.82 mg/g, 11.28 mg/g and 10.98 mg/g, respectively and these values in both the Bambara groundnut, soybean and cocoyam, were lower than that of the standard control (12.77 mg/g). These results imply that in comparison to metformin treatment, cocoyam, soya bean and Bambara groundnut interventions were more potent in lowering lipid peroxidation in diabetic rats (Fig 1). Comparatively, both Bambara groundnut and cocoyam flour outperformed soybean flour in lipid peroxidation amelioration efficacy (Fig 1). It appears from MDA values obtained from the

Standard control, that the toxicity of metformin at specific doses in streptozotocin-induced diabetic rats could be contributory to oxidative stress in our investigation. El-Nagger and colleagues [33] reported similar result by documenting an increase of MDA mean values in diabetic rats administered with metformin and linking such observation to metformin toxicity. For the glutathione (GSH), glutathione S-transferase (GST), and SOD, the mean values were enhanced in the intervention groups when compared to the normal control, but such changes were not significant. The glutathione peroxidase (GPx) demonstrated similar trend as the mean MDA values among the groups. Previous studies reported higher levels of antioxidants, catalase, and GSH in diabetic patients on anti-diabetic drugs compared to the untreated group [31].

4.1.2 Effect on inflammatory biomarkers

Cytokines are essential for the recruitment of leucocytes in both acute and chronic inflammation [34] and can be measured from different sources as proinflammatory and anti-inflammatory cytokines, with the former upregulated when hyperglycemia activates Nuclear Factor kappa B (NF-kB) [35]. This study discovered that soya bean flour elicited the least inflammatory response, with the lowest mean CRP value (1.03 ng/ml), followed by Bambara groundnut (1.24 ng/ml), and cocoyam (1.28 ng/ml) and these values were lower than found in the standard control (1.67 ng/ml) ((Fig 2). These changes were statistically significant, indicating that inflammatory processes were less severe in the intervention groups than when an antidiabetic medication was delivered. A similar pattern was observed for NFkB values when comparing the normal control (2.91 x10-6 mg/g), negative control (4.51 x 10-6 mg/g), standard control (4.73 x 10-6 mg/g), and groups fed with soya bean (4.29 x 10-6 mg/g), Bambara groundnut (3.54 x 10-6 mg/g) and cocoyam (4.46 x 10-6 mg/g). The detected variations were statistically significant (Fig 2). The mean value of IL-10, showed no significant difference among the groups (Fig 2). The comparative values of the standard control, and other groups on intervention formulations showed lower values of the inflammatory biomarkers in the latter groups and among the groups on the intervention formulations, soya bean flour triggered the least inflammatory reaction with the least mean value of CRP, followed by Bambara groundnut and then cocoyam. Numerous researchers, have found a strong association between CRP levels and other metabolic syndrome indicators such as hyperinsulinemia and insulin resistance [36, 37]. The current study found that diabetic rats had higher levels of CRP than non-diabetics, which have been corroborated by other studies [38]. Previous studies have associated anti-inflammatory properties with presence of bioactive compounds such as phenolic compounds [39], artemisinins [40], and stilbenes [41], which are present in soya bean, cocoyam and Bambara groundnut.

4.1.3 Effect on liver enzymes and liver proteins

Higher levels of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT) are seen in cases of liver cell injury and hepatic insulin resistance [42]. While ALT is a particular biomarker for liver injury, AST is found in hepatocyte cytoplasm, and some other tissues [43]. GGT is seen in several cell surfaces and hence connected with a variety of health disorders [44]. GGT, glutathione absorption, oxidative stress, and the risk of developing type 2 diabetes mellitus as a result of chronic inflammation are interlinked [45, 46].

In this study, there were variations in the mean values of AST when the control and intervention groups were compared and the difference were statistically significance. Different scholars posited a possible link between build-up of extra fats in the liver and metabolic syndrome [47]. The discovery of elevated AST levels in the negative control, in this study is consistent with previous findings indicating elevated liver enzymes in type 2 diabetes mellitus (T2DM) patients and that such elevation positively correlated with fasting blood glucose levels, body weight, and other metabolic syndrome factors [46, 48].

The reduced AST values found in the intervention groups compared to the negative control was an indication that the intervention formulations had a hepato-protective impact on the diabetic rats' hepatocytes, even at high doses. When compared among the intervention groups, the soybean group had lower value than the Bambara groundnut and cocoyam groups. Eleazu and colleagues [49] reported that cocoyam flour pellets had a comparable protective effect to antidiabetic drugs in diabetic rats. The total liver protein (TP) levels in this study indicated no significant differences among the groups. The lack of substantial change in TP values could be due to the unaltered oxidative stress indicators (GST, GSH, and SOD) and the liver enzyme ALT, which kept TP values consistent throughout. However, there was a substantial difference in total serum protein levels between diabetic and control groups of the rats.

4.1.4 Effect on bilirubin

The mean values of total bilirubin did not differ significantly between the cohorts in this study; however, total bilirubin and direct bilirubin levels were significantly lower in the normal control group and higher in the negative control groups. The cohorts that consumed cocoyam flour and the standard control had similar total bilirubin levels (0.89 mg/dl), but a lower mean value of direct bilirubin (0.51 mg/dl), indicating that the antidiabetic medicines and cocoyam flour were equally effective. The total bilirubin mean value in the standard control category was higher than that of the Bambara groundnut (0.83 mg/dl) and soya bean-fed groups (0.88 mg/dl). In particular, the Bambara groundnut-fed group had lower mean total bilirubin levels than the soya bean-fed group, which in turn had lower mean values than the cocoyam-fed group. The presence of bioactive substances in these intervention diets may have increased the hepato-protective effects of such flours (Fig 3). Elevated bilirubin concentrations in the negative control group are consistent with Wang and team's findings, which indicated elevated levels of direct bilirubin in T2DM patients [50]. Serum bilirubin levels in all subtypes are increased in cases with impaired fasting glucose and newly diagnosed T2D but tend to drop with persistent hyperglycemia since in such disease states, an oxidative stress, which activates heme oxygenase-1, occurs resulting in the formation of bilirubin but as the hyperglycemia becomes chronic, the increased free radical production leads to higher bilirubin consumption, which leads to lower bilirubin levels [50, 51]. Studies indicating lower serum bilirubin levels in diabetic patients [52], could thus be attributable to extended hyperglycemia.

4.1.5 Effect on serum lipid profile

In this study, there were no significant differences in Triglyceride and Very low-density lipoprotein levels between the cohorts. The mean HDL-c levels were significantly lower in the groups fed soya bean (18.19 mg/dl), cocoyam (19.88 mg/dl), and Bambara groundnut (19.26 mg/dl) compared to the negative (118.53 mg/dl) and normal controls (133.25 mg/dl). Total cholesterol levels, on the other hand, varied significantly among the groups, with the normal control group having the lowest value (94.85 mg/dl), followed by the cocoyam flour (98.37 mg/dl) and soya bean flour (100.30 mg/dl) groups, both of which had lower values than the negative control group (109.94 mg/dL). These values were also lower than those in the Bambara groundnut flour group (110.25 mg/dl) and the normal control (113.34 mg/dl) (Fig 4). Although the hypolipidemic impact of the intervention feeds appears uncertain based on the results, the mean total cholesterol values in the cocoyam and soya bean-fed groups demonstrated a more favourable hypocholesterolemic effect than the standard antidiabetic drug and the Bambara groundnut flour-fed groups. T2DM is usually associated with dyslipidemia, which is defined by increasing triglyceride levels, decreased high-density lipoproteins (HDL), and increased low-density lipoproteins (LDL) [53]. Elevated plasma triglycerides result in HDL breakdown, resulting in lower HDL levels and a rise in LDL levels [54]. The HDL-C values were also lower than those of the Bambara groundnut flour group (110.25 mg/dl) and the normal control group (113.34 mg/dl) (Fig 4).

4.1.6 Effect on renal function

Several studies have identified renal impairment as a complication of diabetes [55], with increased plasma urea and creatinine levels in such cases [56]. In this study, the mean creatinine value did not demonstrate significant diversity among the groups, while the mean urea levels in the normal control group (12.55 mg/dl) did not differ statistically from the groups fed with the intervention formulations (Fig 5). Urea and creatinine levels rises in the serum of individuals with T2DM-induced nephropathy and once recognised and addressed by adequate interventions, can avert the progression to end-stage kidney failure [57]. With Bambara groundnut, soya bean, and cocoyam flour, the mean values of 12.98 mg/dl, 12.88 mg/dl, and 13.03 mg/dl, respectively, were lower than the standard values of 14.20 mg/dl and the negative control value of 13.19 mg/dl, and these values were statistically significantly different (Fig 5), indicating that these dietary interventions provided better renal tissue protection than antidiabetic medication in diabetic rats.

The elevated urea and creatinine levels reported in diabetic control rats when compared to normal controls, in this study, was consistent with previous research [58]. This study also showed that the lower mean uric acid level in diabetic rats fed with soya bean was not significantly different from the negative control group, whereas the higher uric acid level in the negative control, was consistent with previous findings in which plasma uric acid was elevated in chemically induced diabetic rats [59]. Diabetic rats fed with cocoyam and Bambara groundnut flour had significantly lower mean uric acid levels than the standard (69.24 mg/dl) and negative control groups (63.41 mg/dl), indicating that cocoyam and Bambara groundnut flour have superior renal tissue-protective effects over metformin in diabetic rats. Polyphenols have been linked to the positive effects of dietary treatments with soya bean, cocoyam, and Bambara groundnut flour in diabetic nephropathy rats [60]. Polyphenols have been experimentally shown to decrease the expression of TGF-β and matrix-degrading enzymes MMP-2/MMP-9 in renal damage induced by hyperglycemia, which arises from excessive extracellular matrix production stimulated by TGF-β, leading to glomerular fibrosis and eventual renal function deterioration [61, 62].

4.1.7 Effect on cardiac function

Creatine kinase (CK) is an important enzyme involved in the production of adenosine triphosphate during muscular contractions [63]. Its concentration is significantly higher during certain muscle exercises and in situations of metabolic syndrome [64]. Elevated CK and LDH values in T2DM patients indicate myocardial injury and are sensitive predictors of diabetic heart problems [65]. Prolonged hyperglycemia causes the formation of advanced glycated end products (AGEs) with cardiotoxic features, including cardiac fibrosis [66]. The fibrotic response can start from either the TGF-β1-dependent or independent pathway [67]. The results of this study show that there was no significant difference in the mean values of LDH and creatine kinase enzymes among the groups, implying that irrespective of the interventions, the cardiac enzymes concentrations, were not affected (Fig 6). This finding is congruent with the findings of Kotb and colleagues, who found no significant difference in CK and LDH levels in T2DM patients treated with metformin versus the control group [68]. In contrast, other studies have demonstrated a small increase in CK levels [64] or subclinically raised CK in T2DM people, particularly when accompanied by other components of metabolic syndrome [64]. The lack of significant difference in mean LDH and CK values across groups in this investigation could be ascribed to the duration of hyperglycemia, as prolonged exposure to high glucose levels predisposes cells, especially cardiac cells, to glucose-induced damage [68]. Both this trial and the one conducted by Kotb and colleagues [69] lasted 4 weeks and 8 weeks, respectively, which may not have been enough time for glucose toxicity to alter myocytes and circulating cardiac damage markers to become apparent.

5. CONCLUSION

Streptozotocin-induced diabetic rats exhibit changes in the biochemical parameters in response to soybean, Bambara groundnut, and cocoyam flour administration. These plant extracts not only had better lipid peroxidation amelioration properties than the conventional antidiabetic medication, but among the intervention formulations, Bambara groundnut and cocoyam had better amelioration than soybean. The lipid profile analysis reveals that soya beans and cocoyams had hypocholesterolemic effects, unlike the Bambara groundnut flour. Nevertheless, the reduction in question did not result in a favourable enhancement of very low-density lipoproteins (VLDL) or high-density lipoprotein cholesterol (HDL-c). In addition to the lower AST values in the intervention groups when compared to the standard controls, diabetic rats fed with cocoyam and Bambara groundnut flour had significantly lower mean uric acid and urea levels than the standard control. These findings showed that the intervention formulations had better hepato-renal protection potency than standard antidiabetic drug, with Bambara groundnut and cocoyam performing better than soybean. However, the hypercholesterolemic effects of Bambara groundnut flour on the diabetic rats pose a challenge which can be addressed by further research. Consumption of cocoyam and soya bean flour can be recommended as a possible adjunct in managing Type 2 Diabetes mellitus.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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