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Extract of Fermented and Non-Fermented Seeds of *Parkia Biglobosa* Attenuates Hyperglycaemia and Related Nephropathy in Type 2 Diabetes Mellitus Animals

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ABSTRACT

Background: *Diabetes mellitus persists as a major global health challenge despite several interventions. Remedies from natural plant products such as Parkia biglobosa (PB) seeds promises to be an effective alternative with no or fewer side effects. This study sort to assess and compare the total protein, fatty acid profile, phytochemical, antioxidant and antihyperglycaemic effect of fermented (FSE) and non-fermented (NSE) seed extract of PB in streptozotocin-nicotinamide induced (STZ-N) diabetic rats.*

Methods: *Proximate composition (total proteins and lipids), fatty acid composition (by Soxhlet extraction with petroleum ether followed GC-MS) as well as phytochemical constituent and radical scavenging activity were performed on FSE and NSE. Type 2 diabetes mellitus was induced in rats by a single intraperitoneal injection of STZ (65 mg/kg b.w) followed by nicotinamide (110 mg/kg b.w) 15 minutes afterwards. Diabetic rats were orally treated with FSE and NSE (at 100 mg/kg b.w and 250 mg/kg b.w) or glibenclamide daily for 28 days. The antihyperglycemic effect was evaluated using biochemical parameters such as fasting blood glucose levels (FBG), lipid profile and kidney function parameters.*

Result*: Protein and crude fat levels in the fermented were higher than non-fermented (12.5% and 97.6%, respectively). Polyunsaturated fatty acids were present in both. NSE had higher phenolic content and antioxidant activity. The 250 mg/kg b.w of NSE and FSE reduced serum glucose levels by 53% and 42%, comparable to 39.5% of glibenclamide. total cholesterol, triglycerides, LDL and VLDL cholesterol levels were reduced, with increased HDL levels. Further improvement in kidney function parameters was observed compared to the diabetes control.*

Conclusion: *This study showed that both non-fermented and fermented seed extract of PB exerted antihyperglycemic effects and had potent therapy for type 2 diabetes and related nephropathy.*

Keywords
Diabetes mellitus, *Parkia biglobosa,* Nephropathy, streptozotocin-nicotinamide model.

Introduction
Diabetes mellitus (DM) is a disease of global concern affecting numerous people around the world. DM and its debilitating complications, including nephropathy, continue to increase persistently amidst several pharmacological and medical interventions [1,2]. It is estimated that 450 million people live with diabetes, with about 5.1 million related deaths annually worldwide [3]. The prevalence is expected to double by 2030 from the current global values of 8.3 to 17.6% [4,5], not counting the many undiagnosed cases globally. In sub-Saharan Africa, about 21.5 million people live with diabetes, with about half a million diabetes-related deaths in 2013 [6]. As DM continue to ravage the population, so does the related micro- and macro-vascular complications, including nephropathies.

Modern scientific techniques and potent drugs developed to combat the disease come at a high cost and several undesirable side effects [7,8]. The major classes of oral antidiabetic medications include biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, and α-glucosidase inhibitors [9]. These setbacks, coupled with a growing interest in natural products and alternative therapy, have increased the demand for medicinal plants [7].

Parkia biglobosa (Jacq.) G.Don (PB; family *Mimosoideae*), or African locust bean plant, is a multi-purpose legume of African origin [10,11]. Its name varies from country and local language; *dawadawa* (Ghana, Nigeria), *soumbala* (Burkina Faso, Mali), *afitin* (Benin), *iru* (Nigeria), *kinda* (Sierra Leone) and *netetou* (Gambia). *Dawadawa,* fermented seeds of PB, constitutes the primary economic value of the plant as it is widely eaten and hence commercialised throughout West Africa as a diet condiment with small quantities crumbled into traditional soups, stews and sauces during cooking to enhance taste and flavour [11,12].

PB is a perennial deciduous plant primarily found in savannah and woodland areas [11,13] and have found traditional use as food and medicine in many traditional communities in Africa [10]. The various parts of the plant are use in the ethnopharmacological management of many diseases: the bark (from trunk and roots), leaves, fruits and seeds, are processed into decoctions which are used in treating a range of diseases, including malaria; hypertension etc., and even wounds [11]. Builders et al. [14] and Ogunyinka et al. [15] have confirmed its hepatoprotective and antimalarial properties, respectively. Other studies [16,17] have also reported its ability to treat hypertension and some bacterial infections. Oyedemi et al. [18] also reported its antidiabetic and antihyperlipidemic property of the stem back of PB, which is of interest to the current focusing on the seeds.

Some studies by Odetola et al. [19] and Fred-Jaiyesimi & Abo [20] on the fermented seeds sold in open markets have revealed the presence of antidiabetic phytoconstituents (including tannins, sterols, saponins and alkaloids) within the most widely used product, *dawadawa* (fermented African locust beans). Fermentation with fungal species (e.g. *Saccharomyces* spp.) degrades antinutritional factors, improved digestibility and makes available essential secondary metabolites [21] further improving pharmacological activity. The current study assessed and compared the antidiabetic effect of phytoconstituents in locally fermented and non-fermented seed extracts on hyperglycaemia in streptozotocin (STZ)-nicotinamide-induced type 2 diabetes in rats. It also assessed the effect of fermentation on the phytochemical, proximate nutrient composition, and fatty acid content on PB seeds. The rational for the comparative study was to popularize the use of the unfermented seeds which is underutilized currently and offer an alternative to many people who despise the use of fermented seeds for its pungency.

Materials and Methods
Chemicals
Streptozotocin, citrate buffer and nicotinamide were obtained from AK Scientific (CA, USA), and normal saline (0.9% w/v) was obtained from Intravenous Infusions (Accra, Ghana). Glibenclamide (Daonil, New Zealand) was used as standard drug. All reagents for phytochemical, free radical scavenging activity and proximate analyses were obtained from Sigma-Aldrich (USA). Reagents for biochemical analyses were obtained from ELITECH (France). All chemicals and reagents were of analytical grade.

Plant Preparation and Extraction
Dried seeds of PB were obtained locally from Ejura Market, Ashanti Region, Ghana. They were identified and authenticated at the Department of Herbal Medicine, KNUST, Kumasi, Ghana (KNUST/HM1/2018/S001). The traditional method of fermentation, that involved use of wild strains of microbial fermenters was adopted. This involved the moistening of the dried seeds, followed by rapping in polyethene sheet for 3 days. Non-fermented and fermented seeds of *Parka biglobosa* were air-dried and pulverized into powder using an electronic mill (Christy Lab Mill, England). Both plant materials were stored in air-tight plastic bags and stored at 4 ^{o}C.

One hundred grams sample was extracted in 500 mL distilled water by maceration. The mixture was left to stand for 48 hours at room temperature with regular shaking by hand. The supernatant was filtered, and residue resuspended in 500 mL distilled water and re-extracted. The supernatant was pulled and lyophilized by a vacuum freeze dryer (Labconco, UK). The extracts were designated as fermented (FSE) and non-fermented (NSE) seed extracts of PB.

Phytochemical Analysis
FSE and NSE were subjected to qualitative phytochemical evaluation [22,23], and quantitative contents of phenols and tannins (by the Folin–Ciocalteu method) [24,25]; were performed using standard procedures previously described (Table 1). The screening included the following phytochemicals; alkaloids, flavonoids, glycosides, saponins, tannins, sterol and triterpenoids.

Table 1: Test for the Presence of Phytochemicals.

Phytoconstituent	Test	Observation
Tannins (Braymers' Test)	2 ml extract + 2 ml H_2O + 2-3 drops of $FeCl_3$ (5%)	Green precipitate
Flavonoids	1 ml extract + 1 ml $Pb(Ac)_4$ (10)	Yellow colouration
Saponins	5 ml of extract + olive oil (few drops)	Emulsion forms
Sterols (Salkowski Test)	2 ml extract + 2ml $CHCl_3$ + 2 ml conc H_2SO_4	A reddish-brown ring at the junction
Triterpenoids	2 ml extract + 1 ml chloroform + 3 ml conc H_2SO_4	Grey colour
Glycosides (Salkowski's Test)	2 ml extract + 2 ml conc H_2SO_4	Reddish brown colouration
Alkaloids (Dragendorff's Test)	2 mL of the extract + 1% H_2SO_4 + Dragendorff's reagent (drops)	Orange or orange-red precipitate

Antioxidant Activity (radical scavenging activity)

2,2-Diphenyl-1-picrylhyrazyl (DPPH) is reduced from purple to yellow when there is a reaction with an antioxidant compound. This colour change is measured at the absorbance 517 nm using a UV/VIS spectrophotometer [26]. About 0.5 g of extracts were dissolved volume of 10 mL distilled water followed by centrifugation at 10,000 rpm for 15 minutes. 0.2 mL of the supernatant was added to 0.2 mL distilled water and 6 mL of 0.004% DPPH (2,2-diphenyl-1-picrylhyrazyl). The resulting mixture was vortexed and incubated at room temperature in the dark for 30 minutes. The absorbance of the blank solution and then the reaction mixture was measured at 517 nm. Distilled water was used as blank This process was carried out in duplicates for each sample.

DPPH radical scavenging activity (% inhibition) was calculated as:

$$\% \, Inhibition = \left[1 - \left(\frac{As}{Ao}\right) \times 100\right]$$

A_s = Absorbance of sample
Ao = Absorbance of DPPH solution diluted to some volume of distilled water

Proximate Nutritional Analyses

Protein Determination

The standard Kjedahl method of nitrogen analysis was used with slight modification to determine the percentage total protein present in the samples. Total protein was determined by determining the amount of nitrogen in the sample. In this procedure, the organic material is oxidized using strong sulphuric acid with the nitrogen converted to ammonium ions [27]. Briefly, 2 g of digested at 300 °C followed by distillation and titration. The percentage concentration of protein in the samples was calculated using this equation

$$\% \, Total \, Protein = \frac{100 \times (Va - Vb) \times N \times 0.01401 \times 6.25 \times 100}{W \times 10}$$

Va = titre value of sample N = molecular weight of nitrogen
Vb = titre value of blank W = weight of sample

Fat Content: Soxhlet Extraction

The Soxhlet system was used to achieve total fat extraction of samples based on the methods previously described [28]. About 5 g of powdered sample was extracted using petroleum spirit (B.P 40-60°C). The flask with the fats was heated in the oven at 105 °C to evaporate the solvent, cooled to room temperature in a desiccator and weighed. This process was carried out in duplicates for each sample. The weight of fat/oil collected was calculated using this equation:

$$\% \, Fat \, (dry \, basis) = \frac{fat(oil) \, collected \times 100}{Weight \, of \, sample}$$

$$\therefore \% \, Fat \, (dry \, basis) = \frac{[(weight \, of \, flask + oil) - (weight \, of \, flask)] \times 100}{Weight \, of \, sample}$$

Fatty Acid

Fatty acid analysis was conducted using the method adopted by Parry et al. [29]. 1 mg of sample was added to 1 mL of 0.1 M sodium hydroxide methanol (NaOH-MeOH). The mixture was vortexed for 1 min and allowed to stand for 5 min. 1 mL of 4% hydrochloric acid methanol (HCl-MeOH) was added and vortexed again for 1 min and allowed to stand for 5 min. 1 mL of distilled water was added and afterwards 1 mL of hexane. The samples (1 µL) were subjected to both GC-FID and GC-MS analysis for fatty acid compositions using the PerkinElmer GC Clarus 580 Gas Chromatograph interfaced to a Mass Spectrometer PerkinElmer (Clarus SQ 8 S) equipped with ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane) fused capillary column (30 × 0.25 µm ID × 0.25 µm DF) and conditions previously described by Parry et al. [29].

Anti-hyperglycaemic study

Animals

Forty-four adult albino rats, weighing between 120 and 250 g, purchased from University of Ghana Medical School, Korle-Bu and housed in the animal holding facility of the Department of Biochemistry and Biotechnology, Kumasi. The animals were kept in stainless steel cages suitably lined with wood shavings and maintained at room temperature with a 12 h light-dark cycle. They were fed on laboratory chow (Agricare, Kumasi) and normal tap water *ad libitum*. They were acclimatized to the laboratory conditions for two weeks before the commencement of the study. All protocols were accessed and approved by a veterinarian at the Faculty of Veterinary Medicine, KNUST, Kumasi. All animal experiments were performed per the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (Washington, USA) and the guide for the care and use of laboratory animals [30].

Induction of Diabetes in Rats

All experimental rats were subjected to an overnight fast prior to induction of diabetes. Twenty-four animals were given a single intraperitoneal (i.p.) injection of freshly prepared STZ solution using citrate buffer (0.1 M, pH 4.5) at dose of 65 mg/kg b.wt.,

Table 2: Experimental grouping in Diabetic study.

Groups	Group Name	Treatment
I	Normal control	Normal animals; no drug/extract treatment, 1 mL/kg b.wt of distilled water daily
II	100 mg/kg b.wt NSE	Normal rats treated with 100 mg/kg b.wt NSE once daily
III	250 mg/kg b.wt NSE	Normal rats treated with 250 mg/kg b.wt NSE once daily
IV	100 mg/kg b.wt FSE	Normal rats treated with 100 mg/kg b.wt FSE once daily
V	250 mg/kg b.wt FSE	Normal rats treated with 250 mg/kg b.wt FSE once daily
VI	Diabetic control	N-STZ induced diabetic rats
VII	Diabetes + Glib	N-STZ induced diabetic rats, treated with standard drug, glibenclamide (10 mg/kg b.wt once daily)
VIII	Diabetes + 100 mg/kg b.wt NSE	N-STZ induced diabetic rats, treated with 100 mg/kg b.wt NSE once daily
IX	Diabetes + 250 mg/kg b.wt NSE	N-STZ induced diabetic rats, treated with 250 mg/kg b.wt NSE once daily
X	Diabetes + 100 mg/kg b.wt FSE	N-STZ induced diabetic rats, treated with 100 mg/kg b.wt FSE once daily
XI	Diabetes + 250 mg/kg b.wt FSE	N-STZ induced diabetic rats, treated with 250 mg/kg b.wt FSE once daily

All treatment for 28 days; each group consisted of four rats (n=4); NSE – non-fermented seed extract; FSE – fermented seed extract; N-STZ – nicotinamide-streptozotocin

followed by a single i.p. injection of freshly prepared nicotinamide solution in normal saline at dose of 110 mg/kg b wt., 15 minutes after STZ injection (31,32). After 72 hours, the fasting blood glucose (FBG) levels were measured using OneTouch Ultra2 metre and test strips. Animals with FBG ≥ 11.00 mmol/L were considered diabetic.

Experimental Grouping

Table 2 shows the experimental grouping for the anti-diabetic study. Eleven [11] groups of 4 animals per group were used for the study. Each group was marked exclusively on their tails using permanent markers for easy identification in the experimental grouping. Their body weight was taken into consideration to ensure uniformity in the groups. Extract and glibenclamide treatment lasted 28 days.

Effect of Treatment of Body Weight and Glucose Levels

Animals were weighed at the commencement of the study (D0) and at the end of every 4 days till day 28 (D4, D8, …., D28). The treatment effect on percent change in body weight was accessed using the formula below. The effect of treatment of fasting blood glucose was assessed at commencement (D0) and every 7th day (D7, 14, 21, 27 and 28 before sacrifice) using a OneTouch Ultra2 with test strips. Tails of animals were punctured with a sterile needle, and blood was used f

$$\% \text{ Change in Body Weight} = \frac{(Weight)n-(Weight)0}{(Weight)0} \times 100$$

Haematological, Serum Preparation and Biochemical Analyses

All animals were sacrificed on the 29th day following an overnight fast by light ether anaesthesia. The animals were quickly slit at the neck, and blood samples were collected into gel activated tubes for biochemical analyses and EDTA tubes for haematological analyses. Blood samples in gel activated tubes were allowed to clot and centrifuged at 5000 rpm for 15 min. Serum obtained were subjected to biochemical analyses using Flexor E chemistry analyzer (Vital Scientific, Japan) using ELITECH (France) reagents. Tests performed included total proteins (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, potassium, triglycerides, total cholesterol (TC), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high density lipoproteins (HDL) and glucose (FBG).

Haematological assessments were performed using the Sysmex Haematology System (USA). The parameters including Haemoglobin (HGB), Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Platelet count, Lymphocytes, Neutrophils, Haematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Plateletcrit, Platelet Distribution Width (PDW) and Platelet Larger Cell Ratio (P-LCR) were determined.

Statistical Analysis

GraphPad Prism 8 for Windows was used to analyse the data. Results were expressed as the mean ± standard error mean (SEM). Data were assessed by two-way analysis of variance followed by Tukey post hoc test. Values for which $p \leq 0.05$ were considered as statistically significant.

Results

Nutritional and Phytochemical Composition of Parkia biglobosa

Table 3 shows the protein and fat content of fermented and non-fermented seeds measured in the study. Raw and defatted protein in the fermented seed exceeded the non-fermented by 12.5% % and 38.9%, respectively. The amount of fat in fermented seed (25.46%) was nearly twice that of the non-fermented (12.91%).

Table 3: Protein and Fat Content of seeds of *P. biglobosa*

	Fermented	Non-fermented	p-value
Proteins Raw (% conc)	28.32 ± 3.72	25.17 ± 2.85	0.6102
Proteins Defatted (% conc)	42.42 ± 4.37	30.54 ± 3.76	0.0028
Fat (% conc)	25.46 ± 2.91	12.91 ± 1.72	0.0018

Table 4 shows conjugated linolenic acid was the most abundant fatty acid in the non-fermented seed, while 9-Methyl 5, 12-octadecadienoate fatty acid was highest in the fermented seed. The fatty acid composition shows that non-fermented is a source of more diverse types of fatty acid than fermented seeds. Moreover, the percentage concentration of each type of fatty acid discovered was higher in the non-fermented than its fermented seeds.

Table 4: Fatty acids composition of Fermented and Non-fermented seeds of *P. biglobosa.*

Fatty Acids (% Conc)	Non-fermented	Fermented
Palmitic acid (C16:0)	17.39	17.05
Oleic acid (C18:1)	28.01	16.74
Stearic acid (C18:0)	23.57	10.44
Arachidonic acid (C20:4)	4.39	2.8
Arachidic acid (C20:0)	4.91	1.89
Methyl-6-cis-9-cis-11-trans-octadecatrienoate (C18:3)	4.02	2.37
γ- linolenic acid (C18:3)	1.88	0
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)	4.6	0
Heneicosanoic acid, methyl ester (C21:0)	11.23	0
Conjugated linolenic acid (C18:2)	51.23	0
6,9,12,15-Docosatetraenoic acid, methyl ester (C12:4)	2.27	0
Vaccenic acid (C18)	0	6.51
9-Methyl 5,12-octadecadienoate (C18:2)	0	37.59
Nonadecanoic acid, methyl ester (C19:0)	0	4.61

Table 5 shows the radical scavenging activity and phytochemical composition of seeds and extracts observed in the study. Antioxidant percentage inhibition of the NSE was significantly greater than that of the FSE: 17.09 % and 74.33%, respectively. The phytochemicals included phenolics and tannins. Total phenolics were 2.41 mg/g in FSE and 6.44 mg/g in NSE. A very low number of tannins was observed in both samples.

Table 5: Antioxidant and Phytochemical composition of *P. biglobosa* seeds.

Test	Fermented		Non-fermented		p-value
	Raw	Extract	Raw	Extract	
Saponins	-	-	-	-	
Glycosides	-	-	+	+	
Tannins	+	+	+	+	
Flavonoids	-	-	-	-	
Alkaloids	+	+	+	+	
Sterols	-	-	-	-	
Triterpenoids	+	-	+	-	
Antioxidants (% inhibition)		17.09 ± 2.01		74.33 ± 5.31	<0.0001
Total Phenolics (GAEmg/g)		2.41 ± 0.18		6.44 ± 1.017	0.1643
Tannins (GAEmg/g)		0.078 ± 0.00		0.10 ± 0.001	>0.9999

Effect of Aqueous Extract of Non-fermented (NSE) And Fermented (FSE) on Diabetic Rats

Effect of Treatment on Glucose Levels

The FBG levels of rats were assessed every 7 days and on the 28th day of the experiment before termination. There was no significant change in FBG levels among the normal rats (Group I) and those treated with NSE and FSE (Group II-V) only. However, there were significant changes in glucose levels of diabetic rats from day zero (0) to day twenty-eight [28] after treatment ($p<0.05 – 0.001$). Non-fermented seed extract (NSE) had the highest effect on glucose levels than the fermented seed extract (FSE) and glibenclamide. Diabetic animals treated with 100 mg/kg FSE expired within the experimental period, perhaps due to excessive hyperglycaemic condition (Figure 1 and Table 6).

Table 6: Glucose levels at termination.

Days	FBG (mmol/dL)	
	0	28
Normal	5.50 ± 0.46	6.00 ± 1.00
100 mg NSE only	5.93 ± 0.39	6.00 ± 0.40
250 mg NSE only	6.23 ± 0.24	5.33 ± 0.35
100 mg FSE only	6.08 ± 0.23	5.30 ± 0.21
250 mg FSE only	5.97 ± 0.41	5.47 ± 0.37
DM only	24.17 ± 3.40[a]	23.67 ± 0.99[a]
DM + Glib	23.77 ± 1.28[a]	14.37 ± 2.05[ab]
DM + 100 mg NSE	18.98 ± 2.18[a]	12.33 ± 1.02[b]
DM + 250 mg NSE	23.23 ± 2.05[a]	11.15 ± 0.84[b]
DM + 250 mg FSE	26.27 ± 3.75[a]	15.00 ± 2.34[ab]

Statistical significance: a - $p<0.05 – 0.001$ from Normal; b - $p<0.05 – 0.001$ from DM only. NSE – non-fermented seed extract; FSE – fermented seed extract; DM – Diabetes mellitus; Glib – Glibenclamide

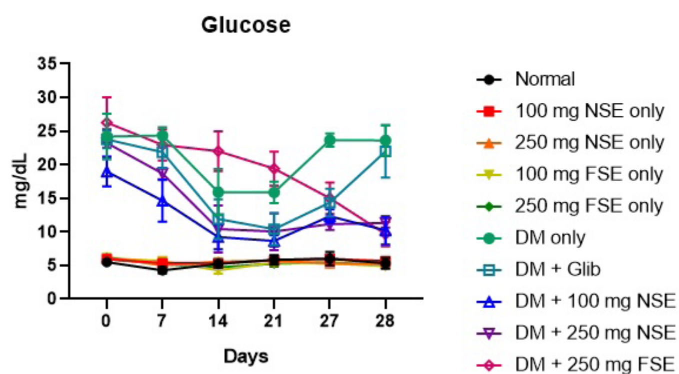

Figure 1: Effect of Treatment on Glucose Levels of diabetic rats

Effect of Treatment on Body Weight

The effect of treatment on the rats' body weight was measure and data presented in Figure 2 and Table 7. The normal and extract only treated groups recorded significant increases in body weight throughout the experiment. DM only and DM + glibenclamide groups recorded significant decreases. DM plus extracts showed significant body weight recoveries compared with DM only ($p<0.05 – 0.001$). On termination, animals with DM only and DM+ glibenclamide had a percentage reduction in the body by -10.18% and -11.92 %, respectively.

Table 7: Percent Weight Changes in Body Weight at Termination.

	% Change in Body Weight
Normal	9.88 ± 3.17
100 mg NSE only	17.32 ± 6.25
250 mg NSE only	-0.80 ± 5.20[a]
100 mg FSE only	4.56 ± 4.54
250 mg FSE only	1.61 ± 3.49[a]
DM only	-10.18 ± 7.05[a]
DM + Glib	-11.92 ± 4.21[a]
DM + 100 mg NSE	6.27 ± 6.61
DM + 250 mg NSE	-3.00 ± 7.97[a]
DM + 250 mg FSE	11.413.14

Statistical significance: a $p<0.05 – 0.001$ from Normal; b $p<0.05 – 0.001$ from DM only. NSE – Extract of non-fermented dawadawa; FSE – Extract of fermented dawadawa; DM – Diabetes mellitus; Glib – Glibenclamide.

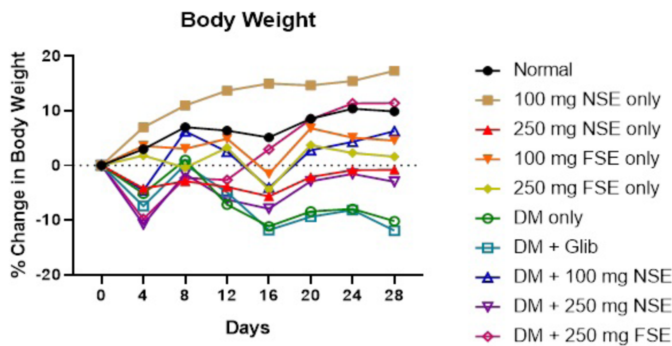

Figure 2: Effect of Treatment on Percent Change in Body Weight of rats.

Effect of treatment on Haematological parameters

The effect of treatment on the haematology of the rats were determined, and data presented on Table 8. Haematological parameters were unaffected significantly by treatment except for some variations in platelet levels.

Effect of Treatment on Biochemical Parameters

The effect of treatment on biochemical parameters of animals (Liver Function, Renal Function and Lipid Profile) were ascertained and data is shown in Table 9. Extract only treatments resulted in significantly decreased ALT and AST levels compared with normal ($p<0.05$-0.01). Further, hyperglycaemia caused by STZ-nicotinamide treatment resulted in increased levels of AST, ALT, creatinine and urea, indicating possible liver and kidney dysfunction. Co-treatment with NSE and FSE reversed the increases to near-normal. Treatment had no significant effect on the lipid profile of animals. However, increased glucose levels following STZ-nicotinamide treatment was reversed to near-normal by NSE and FSE extracts, better than standard glibenclamide.

Discussions

Fermentation involves extracellular reactions by microorganisms to transform food, usually legumes (and cereals), as a means of preservation or for developing desirable, sensual characteristics such as flavours and texture [33]. Fermentation of PB seeds to dawadawa has been reported to enrich the fermented product with proteins, amino acids, vitamins, fatty acids, increased antioxidant activity and eliminate or reduce antinutrients [34,35].

In the present study, protein content was observed to be higher in fermented seeds than in non-fermented. The protein content of both products was 28.3% in fermented and 25.2% in non-fermented (a percentage difference of 12.5% for defatted samples). This result is similar to that of Agblemanyo & Abrokwah [36], who observed a slightly higher mean percentage increase of 14.18% in protein content after fermentation of PB seeds, and Esenwah & Marcel [37], who also observed a mean percentage increase of 15.9% in the proteins of PB seeds after fermentation in 48 hours. This increase in the protein content could be due microbial mass accumulation during fermentation. It could also be attributed to the secretion of extracellular enzymes by fermentative microbes and the enzymatic hydrolysis of some protein inhibitors during the fermentation process [36]. The percentage increase in protein observed in the present study and the others mentioned could result from experimental differences. These results further support that fermented PB seeds are a good source of dietary protein and a cheap replacement of high-cost diary and meat proteins for low-income families [11].

The fat content of the fermented seed (25.5%) was observed to be almost two times higher than that of the non-fermented (12.9%). These results concur with the findings of Oboh et al. [34], who reported the fat content of fermented seed (26.8%) to be more than twice that of the non-fermented (13.2%). According to reports by Igbabul et al. [38] large fat globules are broken down extensively into several simpler fatty acid molecules by lipases during fermentation. This is reported to increase the fat content in the fermented products, coupled with the fact that fermenting microbes do not use the crude fat as energy substrate. Further, fat from dead microflora during fermentation could also contribute to this increase. In addition to this, Oboh et al. [34] reported that

Table 8: Effect of treatment on Haematological.

	Normal	100 mg NSE only	250 mg NSE only	100 mg FSE only	250 mg FSE only	DM only	DM + Glib	DM + 100 mg NSE	DM + 250 mg NSE	DM + 250 mg FSE
WBC	12.67 ± 1.59	14.83 ± 2.11	11.67 ± 1.82	10.80 ± 0.81	8.70 ± 0.32	8.80 ± 1.65	13.23 ± 3.00	15.63 ± 1.91	13.17 ± 0.47	12.87 ± 1.79
RBC	7.94 ± 0.60	7.51 ± 0.38	8.24 ± 0.45	6.74 ± 0.32	7.07 ± 0.08	5.53 ± 0.70	4.96 ± 1.22	7.42 ± 0.32	6.14 ± 0.71	6.61 ± 0.30
HGB	14.33 ± 1.12	12.40 ± 0.56	12.27 ± 0.41	12.75 ± 0.61	12.40 ± 0.29	10.97 ± 1.20	9.20 ± 1.89	12.43 ± 0.55	11.70 ± 1.22	13.07 ± 0.91
HCT	49.67 ± 3.58	42.90 ± 2.08	44.67 ± 1.44	41.55 ± 2.08	42.80 ± 0.55	40.83 ± 4.06	34.53 ± 5.96	43.93 ± 1.68	41.63 ± 3.37	45.53 ± 0.68
MCV	62.60 ± 0.35	57.20 ± 1.58	52.33 ± 0.56	61.63 ± 0.32	59.63 ± 0.97	74.30 ± 1.87	72.83 ± 6.50	59.28 ± 0.67	65.97 ± 2.72	62.70 ± 4.61
MCH	18.07 ± 0.22	16.50 ± 0.33	15.30 ± 0.61	18.93 ± 0.03	18.27 ± 0.24	19.90 ± 0.30	19.00 ± 1.01	16.75 ± 0.16	18.20 ± 0.78	18.30 ± 0.76
MCHC	28.83 ± 0.26	28.95 ± 0.25	27.40 ± 0.64	30.70 ± 0.18	29.57 ± 0.35	26.83 ± 0.33	26.27 ± 1.13	28.25 ± 0.21	28.70 ± 0.87	28.70 ± 1.14
PLT	702.33 ± 157.12	868.50 ± 77.91[a]	641.47 ± 282.42	893.25 ± 41.12[a]	991.33 ± 77.72[a]	849.33 ± 160.43	878.67 ± 86.98[a]	799.25 ± 77.37	740.00 ± 69.62	829.67 ± 27.94
RDW-SD	32.60 ± 1.68	35.13 ± 1.29	31.43 ± 1.02	31.30 ± 1.23	32.47 ± 0.75	61.57 ± 7.69	63.80 ± 15.92	37.15 ± 2.67	50.63 ± 13.81	44.43 ± 4.66
RDW-CV	12.77 ± 0.88	17.18 ± 0.84	15.63 ± 0.34	12.35 ± 0.70	13.63 ± 0.55	24.53 ± 4.08	25.90 ± 4.24	19.68 ± 2.93	23.67 ± 6.43	18.33 ± 2.45
PDW	9.13 ± 0.47	8.43 ± 0.30	8.07 ± 0.12	8.40 ± 0.18	8.93 ± 0.49	8.23 ± 0.52	8.07 ± 0.22	8.20 ± 0.18	8.17 ± 0.20	9.80 ± 0.91
MPV	7.70 ± 0.20	7.15 ± 0.21	7.17 ± 0.23	7.20 ± 0.09	7.53 ± 0.22	7.20 ± 0.31	7.00 ± 0.12	7.15 ± 0.10	7.10 ± 0.15	7.40 ± 0.42
P-LCR	9.20 ± 1.10	6.00 ± 1.16	5.20 ± 0.50	6.63 ± 0.60	8.20 ± 1.37	6.63 ± 1.38	5.50 ± 0.30	6.13 ± 0.48	7.00 ± 0.15	8.97 ± 1.69
PCT	0.54 ± 0.11	0.66 ± 0.08	0.68 ± 0.05	0.64 ± 0.03	0.64 ± 0.14	0.61 ± 0.11	0.62 ± 0.05	0.57 ± 0.05	0.50 ± 0.05	0.64 ± 0.05

Statistical significance: a - $p<0.05$ – 0.001 from Normal; b - $p<0.05$ – 0.001 from DM only. NSE – non-fermented seed extract; FSE – fermented seed extract; DM – Diabetes mellitus; Glib – Glibenclamide

Table 9: Effect of Treatment on some Biochemical Parameters.

	Normal	100 mg NSE only	250 mg NSE only	100 mg FSE only	250 mg DF only	DM only	DM + Glib	DM + 100 mg NSE	DM + 250 mg NSE	DM + 250 mg FSE
TP (g/L)	75.03 ± 4.31	70.53 ± 1.59	72.67 ± 5.86	71.40 ± 3.45	74.27 ± 4.08	66.63 ± 4.49	62.20 ± 1.06	72.28 ± 1.59	68.40 ± 4.76	66.60 ± 3.56
AST (U/L)	375.67 ± 51.27	279.53 ± 15.60[a]	348.07 ± 62.67	300.33 ± 13.60[a]	362.37 ± 54.19	322.77 ± 22.98	272.73 ± 65.16[a]	298.50 ± 18.17[a]	276.87 ± 22.52[a]	275.00 ± 39.15[a]
ALT (U/L)	107.27 ± 12.45	67.58 ± 2.41[a]	75.03 ± 4.08[a]	88.03 ± 6.37[a]	68.67 ± 3.38[a]	163.13 ± 8.40[a]	96.97 ± 10.92[b]	88.05 ± 6.86[b]	107.97 ± 1.67[b]	104.80 ± 7.23[b]
AST/ALT	3.63 ± 0.80	4.15 ± 0.33	4.47 ± 0.65	3.48 ± 0.30	3.87 ± 1.09	2.00 ± 0.26	2.90 ± 0.85	3.23 ± 0.45	2.60 ± 0.17	2.60 ± 0.21
Creatinine (µmol/L)	76.67 ± 4.71	59.93 ± 4.45	67.43 ± 14.52	84.20 ± 11.97	73.47 ± 7.46	101.17 ± 7.94[a]	87.53 ± 23.06	62.80 ± 9.37[b]	53.57 ± 4.80[b]	106.33 ± 9.26
UREA (mmol/L)	9.13 ± 1.25	7.90 ± 0.36	8.55 ± 0.31	8.06 ± 0.64	8.68 ± 1.09	15.68 ± 1.08[a]	13.54 ± 2.42	9.22 ± 1.15	11.82 ± 1.44	13.47 ± 2.19
Potassium (mmol/L)	3.47 ± 0.36	3.53 ± 0.26	2.89 ± 0.09	3.64 ± 0.11	3.86 ± 0.29	3.38 ± 0.10	3.57 ± 0.01	4.33 ± 0.49	3.69 ± 0.07	3.98 ± 0.25
TG (mmol/L)	1.30 ± 0.25	0.85 ± 0.12	0.82 ± 0.02	0.99 ± 0.03	1.02 ± 0.08	1.18 ± 0.30	0.68 ± 0.08[a]	0.82 ± 0.12	1.40 ± 0.28	0.71 ± 0.14
TC (mmol/L)	1.33 ± 0.06	1.18 ± 0.14	1.08 ± 0.09	0.91 ± 0.13	0.99 ± 0.14	1.13 ± 0.10	0.86 ± 0.12	0.88 ± 0.23	1.00 ± 0.06	1.04 ± 0.01
HDL-C (mmol/L)	0.11 ± 0.03	0.10 ± 0.01	0.08 ± 0.00	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.25 ± 0.18	0.60 ± 0.25
VLDL (mmol/L)	0.59 ± 0.11	0.39 ± 0.05	0.37 ± 0.01	0.45 ± 0.01	0.48 ± 0.03	0.54 ± 0.14	0.31 ± 0.04	0.37 ± 0.06	0.56 ± 0.14	0.43 ± 0.01
LDL (mmol/L)	0.63 ± 0.12	0.70 ± 0.14	0.60 ± 0.06	0.35 ± 0.13	0.40 ± 0.15	0.47 ± 0.15	0.45 ± 0.15	0.58 ± 0.05	0.37 ± 0.19	0.50 ± 0.06
GLU (mmol/L)	8.32 ± 1.50	5.85 ± 0.48	5.71 ± 0.41	5.77 ± 0.20	5.63 ± 0.50	31.00 ± 2.66[a]	18.13 ± 6.36[ab]	6.85 ± 0.52[b]	11.02 ± 6.11[b]	5.27 ± 0.51[b]

Statistical significance: a - $p<0.05 – 0.001$ from Normal; b - $p<0.05 – 0.001$ from DM only. NSE – non-fermented seed extract; FSE – fermented seed extract; DM – Diabetes mellitus; Glib – Glibenclamide

increase in fat content in the PB seeds after fermentation could also be due to the metabolism of carbohydrates to fats during the fermentation process. Akindumila & Glatz [39] also suggested that some fungi are capable of anabolizing fats during fermentation.

After analyses, a number of fatty acids were identified to be present in both fermented and non-fermented PB seeds. The predominant fatty acids in both samples were polyunsaturated fatty acids (PUFA) followed by saturated fatty acids and monounsaturated fatty acids (MUFA). Some of the fatty acids observed in the present studies were also reported by Aremu et al. [40] and Ijarotimi and Keshinro [41] on PB seeds. However, in their research, they also reported other fatty acids such as behenic acid, myristic acid, lauric acid, linoleic and palmitoleic acids which were not observed in this study. The only monounsaturated fatty acid observed in both samples in comparatively high amounts was oleic acid (C18:1 *cis 9*). The saturated fatty acids observed in both samples were palmitic acid, stearic acid and arachidic acid. The fatty acid profile obtained for this study and their corresponding percentage compositions partially agree with reports from Aremu et al. [40] and Ijarotimi and Keshinro [41], as some fatty acids observed in the present study was not observed in theirs and vice versa. Further, the percentage compositions of fatty acids observed in all three studies differ slightly in some fatty acids and significantly in others. These differences could be due to seed origin and experimental methods employed. It has been shown that extraction methods influence the fatty acid profile obtained for a sample. This is exemplified in Wen et al. [42] study, who extracted oils from grape seeds of different origins (varieties) with supercritical carbon dioxide and petroleum ether. The same study reported differences in fatty acid profiles.

Several studies have reported that the phenolic content of plant foods is directly proportional to their antioxidant activity. Therefore, the higher the phenolic content, the more effective that plant food is against free radicals. According to Oboh et al. [43] and Nazarni et al. [44] phenolic content generally increases in legumes during fermentation due to the breakdown of bound phenolic compounds by hydrolytic enzymes, increasing the bioavailability of free phenolic compounds hence increasing the plant's potential antioxidant activity. These authors further reported an increase in total phenolics and antioxidant activities in their respective studies after fermentation. In contrast to these studies, a significantly higher phenolic content was observed in the NSE (6.44 mg/g) used in the present study than the fermented (2.41 mg/g). Consequently, the antioxidant activity of the non-fermented seed (74.33%) was observed to be much higher than that of the fermented (17.08%) inhibition. This observation in the present study, which disagrees with reports by studies mentioned above, could be attributed to the source and variety of the non-fermented and FSE used in the investigation, the differences in fermentation process and storage conditions.

Tannins, the water-soluble polyphenolic compounds responsible for the astringency of many plant foods was found to be present in very low amounts (less than 1%) in both samples. Tannin concentration generally decreases in the sample during the process of fermentation, as observed in the present study. This is usually a result of soaking, hydration and cooking of raw seeds, which precede fermentation. These processes reduce the level of antinutritional factors, which includes tannins, in fermented products [35]. Alkaloids and glycosides were also observed to be present in extracts of fermented and NSE of PB.

Several studies have provided numerous empirical evidence that supports the fact that STZ-nicotinamide-induced toxicity in animal models is characterized by a reduction in insulin production and hyperglycaemia [15]. STZ has a selective toxicity effect on β-cells by impairing glucose oxidation, decreasing insulin synthesis and secretion, and disrupting glucose transport and glucokinase activity [45]. In this study, STZ (65 mg/kg b.w) was injected intraperitoneally into experimental rats (Group VI to XI rats) followed by another intraperitoneal injection of nicotinamide (110

mg/kg b.w.) 15 minutes after. The nicotinamide exerts partial cytotoxic protection against STZ salvaging some β-cells of the pancreas through a complex mechanism [46,47]. This ensures that insulin production is eliminated, thereby inducing in experimental rats a diabetic condition similar to T2DM in humans [46]. This phenomenon, however, could be the basis for the observed steady drop in average blood glucose levels in the diabetic control group from 24.17 $mmol^{-1}$ to 23.67 $mmol^{-1}$, as shown in Table 6 because insulin is not completely deficient in STZ-nicotinamide induced diabetic rats. Comparing this observation to the report by Vessal et al. [48], who induced diabetes in experimental rats with STZ without nicotinamide (inducing T1DM), rather observed an increase in levels of FBG in diabetic control rats by the end of the experiment. This different experimental approach and its consequent result support the fact that pancreatic β-cell function is not completely eliminated when nicotinamide is administered.

The antihyperglycaemic effect of PB seeds was compared with the standard glibenclamide, which has been used for many years to treat diabetes. The mechanism of action of glibenclamide as a sulfonylurea is to stimulate insulin secretion from pancreatic β-cells by inhibiting ATP-sensitive K^+ (K_{ATP}) channels in the plasma membrane. Sulfonylureas have a direct effect on β-cells exocytosis [49,50]. Therefore, glibenclamide oral administration to STZ-induced diabetic rats is known to decrease blood glucose levels by this mechanism. From the present study results, glibenclamide administration significantly ($p < 0.05$) reduced FBG levels by the end of the experiment. Similarly, the oral administration of both fermented and non-fermented seed extracts at a dosage of 250 mg/kg b.w once daily saw a significant decrease in FBG levels (~ 43% and 52% reduction at termination) in diabetic rats. Both PB seed extracts reduced the FBG to levels comparatively lower than with glibenclamide. This suggests that both fermented and non-fermented seed extracts have higher antihyperglycaemic and insulin-stimulatory effects. PB seeds extracts, similar to the glibenclamide, may have brought about its antihyperglycaemic effect through stimulation of surviving β-cells to increase insulin production. It is also suggested that the large decreases in FBG levels following extract treatment may be due to plant extracts having some extra-pancreatic antihyperglycaemic mechanism of action [19]. It can also be attributable to a possible antioxidant protective effect on the pancreas and alkaloids observed in the extracts [51].

Comparing the effect of different dosages of PB seed extracts on FBG levels in Group VIII diabetic rats, administered with 100 mg/kg b.w. NSE showed a significant ($p < 0.05$) reduction in FBG levels by day of termination (Table 5). Comparatively, this dosage produced the lowest FBG reductive effect by the day of termination. This lower percentage reduction could be attributed to the dosage (100 mg/kg b.w.) administered, leading to relatively lower β-cells stimulation and hence lower antihyperglycaemic effect compared to the higher dosages (250 mg/kg b.w.) and the glibenclamide. This suggests that the antihyperglycaemic effect of that plant extract is also dose dependent. Comparing the FBG reductive effect of the 250 mg/kg b.w. dosage, the non-fermented seed extracts showed a more significant antihyperglycaemic effect than the FSE. This correlates with the phytochemical content – phenolics and tannins – in the NSE than the fermented. These phytochemical compounds are confirmed to be potent bioactive compounds able to scavenge the free radicals induced by STZ induction. This activity against free radicals is reportedly antidiabetic, hence non-fermented PB seed extracts demonstrating a more potent antidiabetic activity [19,45]. Treatments of normoglycaemic rats (Groups II to V) with NSE and FSE at different dosages resulted in no significant change in FBG levels in rats after the 4-week study. This again correlates with the findings of Odetola et al. [19], who also reported a non-significant change in FBG in normoglycaemic rats.

According to the study by Odetola et al. [19], dehydration and body weight lose are associated with diabetes mellitus. Considerable reduction in body weight was, therefore, observed in diabetic groups. The diabetic rats treated with extracts of PB seeds over the experimental period rather appreciated in weight. The observation in the present study suggests that the PB seed extracts prevented weight loss because of high protein and fat content of the seed extracts. This deduction agrees with Krishnaveni et al. [45], who suggested that STZ-induced diabetes is characterized by severe weight loss due to the degradation of fats and proteins, which leads to muscle wasting. Therefore, high protein and fats found in PB seed extracts could have replaced those catabolized macromolecules. Further, the extracts could also stimulate appetite, hence improving food consumption and efficiency.

Administration of PB seed (fermented and non-fermented) extracts, glibenclamide saw a general reduction in triglyceride, and total cholesterol in plasma and tissues of diabetic rats was by the end of the experiment. This could be attributed to the fact that there is decreased activity of cholesterol biosynthesis enzymes or a low level of lipolysis in type II diabetic subjects when there is enough insulin stimulation [52]. A decrease in cholesterol is also associated with a decrease in LDL-C, VLDL-C and an increase in HDL-C, which was also observed in the present study, though not significant.

Serum transaminases (ALT and AST) are key pointers of hepatocyte injury [15,53]. These enzymes are usually abundant in the liver, where they play a central role in amino acid metabolism. STZ-toxicity has been characterized by changes in the permeability of the liver membrane and cellular leakage of ALT and AST from the hepatocytes into the blood stream [15]. In this study, the activities of these enzymes, as well as creatinine and urea of STZ-induced diabetic rats were significantly increased. This suggests a certain degree of damage to the liver and kidney after injection of STZ. However, extract treatment alone and in the diabetic group resulted in significant improvement in kidney and liver function, suggesting a possible protective effect attributable to the phytochemical constituents of the fermented and non-fermented seed extracts. The hepatoprotective effect of seed extract has been demonstrated in carbon tetrachloride model [54].

Conclusion

In summary, it is apparent from the result extrapolated that PB seeds – both non-fermented and fermented – possess antihyperglycaemic properties potent enough to protect against the advancement of T2DM and related complications and may have considerable potential for improving T2DM. The NSE however exhibited a higher antihyperglycemic action than the fermented in this study, suggesting that the therapeutic potentials of the non-fermented PB seeds may be higher. This study revealed phenolic compounds to be a major phytoconstituent of PB seeds. Antioxidant activity may represent mechanism that contributes to its antidiabetic activity.

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