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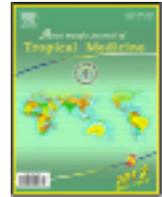
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Attenuation of oxidative stress and hepatic damage by some fermented tropical legume condiment diets in streptozotocin−induced diabetes in rats

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ABSTRACT

Objective: To investigate the modulatory effect of fermented legume condiments diet on oxidative stress in streptozotocin (STZ) induced diabetic rats. **Methods:** Adult male Wistar rats were randomly divided into six groups with six animals in each group. Diabetes was induced by intraperitoneal injection of STZ (35 mg/kg b.w.). After being confirmed diabetic, the rats were fed with fermented Bambara groundnut, Locust bean and Soybean diets for 14 days. The plasma was obtained after 14−day treatment and analyzed for hepatic damage marker enzymes (AST, ALT and ALP) and *in vivo* antioxidant indices. **Results:** The diabetic untreated rats showed elevated ($P<0.05$) levels of AST, ALT, ALP and malondialdehyde with reduced activities of glutathione−S−transferase, catalase as well as plasma reduced glutathione, vitamin C and total protein content. However, treatment of diabetic rats with fermented legume condiments diets for 14 days significantly ($P<0.05$) reversed the above parameters towards normalcy, suggesting their modulation of oxidative stress, which may be due to their high phenolic content and antioxidant capacity. **Conclusions:** The attenuation of oxidative stress and protection of hepatic tissue damage by the legume condiment diets in STZ induced diabetic rats compare favourably with that of metformin, a well known oral hypoglycemic drug.

1. Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia, resulting from insufficient or inefficient insulin secretion, with alterations in carbohydrate, protein and lipid metabolism[1]. Diabetic complications are linked to hyperglycemia induced oxidative stress which eventually overcomes the endogenous antioxidant defense system through glucose autoxidation, induction of non−enzymatic glycosylation of various macromolecules and generation of reactive oxygen species. Increasing evidence in both experimental and clinical studies suggests oxidative stress as being involved in the pathogenesis and progression of diabetic tissue damage[2]. However, strategies to reduce hyperglycemia and its attendant free radical−induced oxidative stress have been employed in diabetes management with recent

report suggesting antioxidant treatment to be beneficial[3]. Renewed attention in recent decades to alternative natural therapies has stimulated a new wave of research interest in traditional practices. Plant foods and products; which are used in folklore, are now being investigated and employed as alternatives in diabetes management. The therapeutic properties of these plant based food/products have been attributed to their phytochemical constituents such as polyphenols, which possess strong antioxidant properties and thus could help to reduce/protect against hyperglycemia induced oxidative stress in diabetes.

The liver being rich in mitochondria to perform metabolic functions is a crucially important organ and in a chronic hyperglycemic state, liver oxidative stress is considered as a relevant process[4]. There is possibility of liver damage in diabetes due to increased gluconeogenesis and ketogenesis. This disease is also grossly reflected by profound changes in protein metabolism, a negative nitrogen balance and loss of nitrogen from most organs[5], which might be accounted for by enhanced catabolism of both liver and plasma proteins.

Tropical legumes with high phenolic content and strong antioxidant properties abound in Africa south of the Sahara

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where they are largely consumed and are traditionally major component of many food preparations[6]. In African folklore, legumes are component of many preparations used for the treatment/management of diabetes and cardiovascular conditions. These legumes seeds are also fermented to produce several food products which constitute significant proportion of the diets of many people south of the Sahara. Previous research has shown that fermentation significantly increases the polyphenol content and antioxidant properties of the legumes through the liberation of polyphenol aglycones by the catalytic action of microbial β –glucosidase during production of the fermented food products[7].

Hence, this study sought to investigate the effect of consumption of polyphenol–rich fermented tropical legume condiment supplemented diets on the antioxidant status and hepatic tissue damage in streptozotocin (STZ)–induced diabetic rats.

2. Materials and methods

2.1. Materials

Bambara groundnut (*Vigna subterranea* L. Verdc), African locust bean (*Parkia biglobosa*) and Soybeans (*Glycine max* L. Merrill) TGx 1903–3F were sourced locally from the Erekesan main market at Akure, Ondo–State, Nigeria. The identification and authentication was done at the Department of Biology, Federal University of Technology, Akure, Nigeria. Unless stated otherwise, all the chemicals and reagents used were purchased from Sigma Chemical Co. (St. Louis, MO), while the water was glass distilled.

2.2. Animals

The handling and use of the animals were in accordance with NIH Guide for the care and use of laboratory animals. Adult male Wistar rats weighing 180–200 g used for this experiment were purchased from the breeding colony of the Department of Biochemistry, University of Ilorin, Nigeria. The rats were maintained at 25 ℃ on a 12 h light/dark cycle with free access to food and water. They were acclimatized under these conditions for two weeks prior to the commencement of the experiment. The experimental study was approved by the Institutional Animal Ethical Committee of the Federal University of Technology, Akure, Nigeria.

2.3. Induction of diabetes

The animals were subjected to overnight fast prior to the induction of diabetes. STZ freshly prepared in citrate buffer (0.1 M, pH 4.5) was administered intraperitoneally (*i.p.*) at a single dose of 35 mg/kg body weight. Diabetic state was checked 72 h after induction with STZ. Blood samples were taken by tail vein puncture and glucose levels was monitored using an auto–analyzer (Fine test Auto–coding TM). Animals with blood glucose ≥ 200 mg/dL after 72 h were considered diabetic and were used in the study.

2.4. Experimental design

The rats were randomly divided into six groups comprising six animals per group as given below:

Group Ⅰ: normal control rats, receive citrate buffer (pH 4.5) (1 mL/kg, *i.p.*) fed with basal diet (44% skimmed milk, 42% corn starch, 4% mineral & vitamin premix and 10% oil); Group Ⅱ: diabetic control rats, diabetic rats fed with basal diet; Group Ⅲ: diabetic rats treated with metformin (25 mg/kg body weight) fed with basal diet; Group Ⅳ: diabetic rats fed formulated diet containing fermented Bamabara groundnut condiment (17% skimmed milk, 19% corn starch, 4% mineral & vitamin premix, 10% oil and 50% fermented condiment); Group Ⅴ: diabetic rats fed formulated diet containing fermented African locust bean condiment (17% skimmed milk, 28% corn starch, 4% mineral & vitamin premix, 10% oil and 42% fermented condiment); Group VI: diabetic rats fed formulated diet containing fermented soybean condiment (17% skimmed milk, 31% corn starch, 4% mineral & vitamin premix, 10% oil and 38% fermented condiment).

The experiments lasted for fourteen days after which the animals were sacrificed by cervical dislocation and the blood samples were collected by cardiac puncture.

2.5. Analytical procedures

The plasma was assayed for liver damage marker enzymes; Alanine aminotransferase (AST), Aspartate aminotransferase (ALT) and Alkaline phosphatase (ALP) using commercially available kits (Randox Laboratories UK). Plasma lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids[8]. Protein was estimated by the method of Lowry *et al*[9] using bovine serum albumin as standard. Glutathione (GSH) content was determined according to the method of Ellman[10]. Glutathione–S–transferase (GST) activity was estimated according to the method of Mannervik and Guthenberg[11]. Catalase (CAT) activity was assayed following the method of Beers and Sizer[12]. Plasma ascorbic acid content was estimated according to Benderitter *et al*.[13].

2.6. Preparation of extracts

The extract was prepared according to a modified method of Chu *et al*[14]. 10 g of the ground sample was extracted with 80% acetone (1:5, w/v) and filtered (filter paper Whatman No. 2) under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 45 ℃ until about 90% of the filtrate had been evaporated and then lyophilized to obtain a dry extract. The extract was kept at - 4 ℃ prior to analysis.

2.7. Total phenol determination

The total phenol content was determined according to the method of Singleton *et al*[15]. Briefly, appropriate dilutions of the extracts were oxidized with 2.5 mL 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 ℃ and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

2.8. ABTS radical scavenging ability

The total antioxidant capacity was determined based on 2,2–azinobis(3–ethylbenzothiazoline–6–sulfonate radical (ABTS$^{\bullet+}$) scavenging ability of the extracts according to the method described by Re et al[16]. ABTS$^{\bullet+}$ was generated by reacting ABTS aqueous solution (7 mM) with $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 16 hr and adjusting the Abs 734 nm to 0.700 with ethanol. 0.2 mL of appropriate dilution of the extracts was added to 2.0 mL ABTS$^{\bullet}$ solution and the absorbance were measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated using TROLOX as the standard.

2.9. Data analysis

Results were expressed as mean ± standard deviation ($n = 6$) and the mean was compared using one–way analysis of variance (ANOVA) followed by Duncan's multiple range test, using statistical package for Social Science (SPSS) 10.0 for Windows. The significance level was taken at $P<0.05$.

3. Results

Table 1 summarizes the effect of fermented tropical legume condiment supplemented diets on plasma AST, ALT, ALP and plasma total protein levels in normal and diabetic animals. After the 14 days of experiment, the activities of plasma enzymes such as AST, ALT, ALP and total protein were significantly ($P<0.05$) elevated in diabetic control group but were found to return to normal levels upon treatment with metformin and fermented legume condiment diets. However, diets containing fermented locust bean (Ⅴ) and soybean (Ⅵ) caused significant ($P<0.05$) reduction in AST activities when compared with the normal control group and their reduction in the ALT activities compares favourably with that of the metformin treated group (Ⅲ).

As shown in Figure 1, plasma GST activity reduced significantly ($P<0.05$) in the diabetic control group (Ⅱ) compared to the normal control group (I). However, there is a significant ($P<0.05$) increase in the activity of this enzyme in the metformin treated (Ⅲ) and fermented legume condiment diets fed groups (Ⅳ–Ⅵ). With fermented legume treated groups exhibiting significantly higher GST activity when compared to the normal control group.
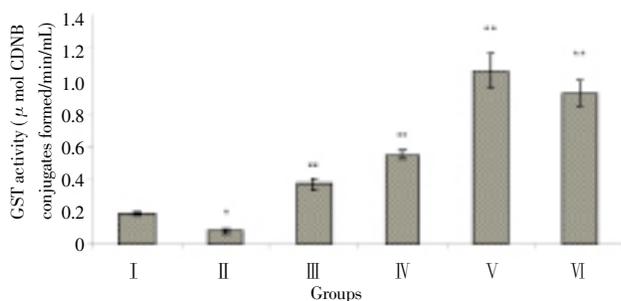
Figure 1. Effect of some fermented legume condiment diet on plasma GST activity in diabetic rats.
*$P < 0.05$ compared with the corresponding value for normal control animals. **$P < 0.05$ compared with the corresponding value for diabetic control animals.

Furthermore, as shown in Figure 2, the plasma CAT activity reduced significantly ($P<0.05$) in the diabetic control group (Ⅱ) as compared to the normal control group (Ⅰ). The Figure also revealed a significant increase in the activity of this enzyme in the metformin treated group (Ⅲ) as well as both fermented locust bean (Ⅴ) and soybean treated (Ⅵ) groups (except fermented bambara treated group). And these compare favourably with that of the normal control group.
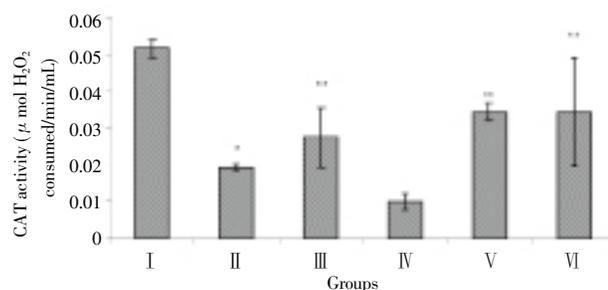
Figure 2. Effect of some fermented legume condiment diet on plasma CAT activity in diabetic rats.
*$P < 0.05$ compared with the corresponding value for normal control animals. **$P < 0.05$ compared with the corresponding value for diabetic control animals.

Figure 3 shows the effect of fermented tropical legume condiment supplemented diets on plasma reduced GSH level. The result revealed a significant ($P<0.05$) reduction in the plasma GSH level in the diabetic control group (Ⅱ) when compared with the normal control group (Ⅰ). However, treatment with metformin and feeding with the condiment supplemented diets caused a marked increase in the plasma GSH levels in the treatment groups (Ⅲ–Ⅴ) with the exception of group Ⅵ where no significant ($P>0.05$) difference with the diabetic control group is observed.
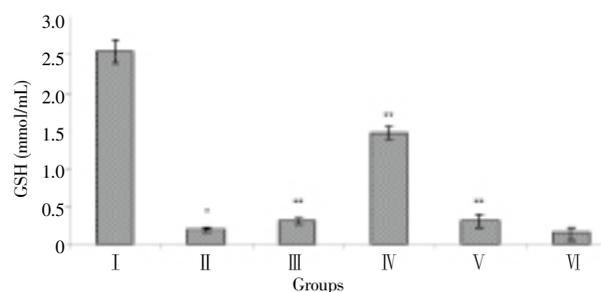
Figure 3. Effect of some fermented legume condiment diet on plasma GSH content in diabetic rats.
Data are expressed as mean ± S.D. ($n = 6$). *$P < 0.05$ compared with the corresponding value for normal control animals. **$P < 0.05$ compared with the corresponding value for diabetic control animals.

Likewise as revealed in Figure 4, significant ($P<0.05$) reduction in plasma ascorbic acid level was observed in the diabetic control group (Ⅱ) compared to the normal control group (Ⅰ). However, the groups treated with metformin (Ⅲ) and the fermented legume condiment fed groups (Ⅵ–Ⅵ) showed a significant ($P<0.05$) elevation in their plasma ascorbic acid level with the group fed fermented bambara groundnut (Ⅳ) having the highest ascorbic acid level.
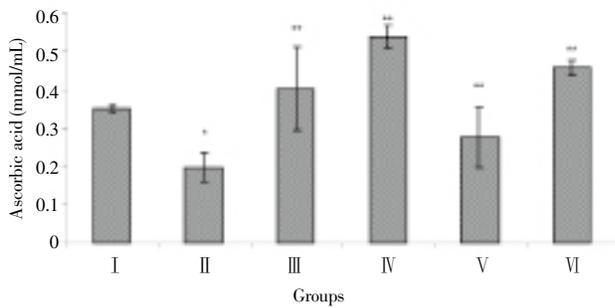
Figure 4. Effect of some fermented legume condiment diet on plasma ascorbic acid content in diabetic rats.
*$P < 0.05$ compared with the corresponding value for normal control animals. **$P < 0.05$ compared with the corresponding value for diabetic control animals.

Furthermore, there was a significant ($P<0.05$) elevation in plasma MDA in diabetic control group (Ⅱ) as compared with normal control group (Ⅰ). Treatment of diabetic rats with metformin (group Ⅲ) and feeding with fermented tropical legume condiments (Groups Ⅲ and Ⅳ) for 14 days resulted in a marked decreased ($P<0.05$) in plasma MDA (Figure 5).
The result of the total phenol content of the fermented legume

condiments is shown in Table 2. The results revealed that the phenolic content ranged from 120.21 mg/100g (bambara groundnut) to 169.46 mg/100g (soybean). Nonetheless, the total antioxidant capacity of the fermented legume condiments as typified by their ABTS radical scavenging ability (Table 2) ranged from 6.17 mmol. TEAC/100g (soybean) to 6.82 mmol TEAC/100g (locust bean).
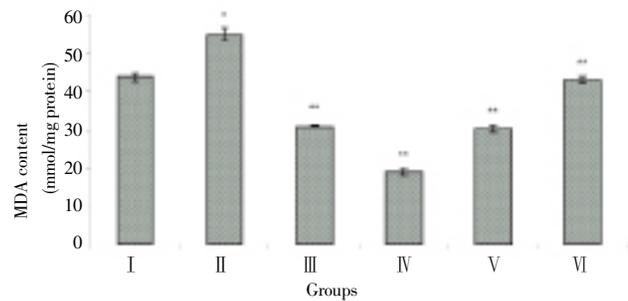
Figure 5. Effect of some fermented legume condiment diet on plasma MDA content in diabetic rats.
*$P < 0.05$ compared with the corresponding value for normal control animals. **$P < 0.05$ compared with the corresponding value for diabetic control animals.

Table 1
The effect of some fermented legume condiment diets on plasma ALT, AST, ALP and total protein content in STZ–induced diabetes in rats.

Groups	Treatments	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/dL)
Ⅰ	Control (Normal)	19.9±5.0	36.7±6.3	16.0±2.6	3.2±0.1
Ⅱ	Control (Diabetic)	32.9±1.7*	63.0±3.3*	60.2±9.6*	2.7±0.4*
Ⅲ	Diabetic + Metformin	30.8±9.5**	49.4±2.4**	23.2±5.6**	3.6±0.8**
Ⅳ	Diabetic + Bambara groundnut	30.2±4.4**	28.3±8.4**	56.6±2.7**	4.7±0.8**
Ⅴ	Diabetic + Locust bean	16.5±4.2**	15.1±4.6**	31.1±5.0**	3.3±0.1**
Ⅵ	Diabetic + Soybean	14.8±3.0**	13.5±3.6**	37.8±5.9**	2.7±0.9*

The data are expressed as mean±standard deviation ($n = 6$), * $P < 0.05$ compared with the corresponding value for normal control animals, ** $P < 0.05$ compared with the corresponding value for diabetic control animals.

Table 2
The total phenol content and ABTS radical scavenging ability of some fermented legume condiments.

Condiments	Total phenol content (mg/100g)	ABTS˙ scavenging ability (mmol TEAC/100g)
Bambara groundnut	120.21±5.00	6.78±1.30
Locust bean	130.47±4.20	6.82±2.00
Soybean	169.46±8.20	6.17±2.90

The data are expressed as mean±standard deviation ($n = 3$).

4. Discussion

Oxidative stress has been widely implicated in the etiology and progression of diabetes. And, STZ–exhibits it diabetological action through the production of free radicals causing damages to β –cells of the islets of Langerhans. STZ also causes damage to hepatocytes, nephrons and cardiomyocytes[17]. Hyperglycemia, a major characteristic of diabetes could also trigger oxidative stress through auto–oxidation of glucose as well as the production of advance glycation end products. Since numerous studies have shown that oxidative stress contributes to the development and progression of diabetes and related complications, therefore, it is clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications[18]. Recent reports have shown modulatory effect of plant and plant extracts with high phenolic contents on antioxidant status in diabetes[19].

Therefore, this study reports the effect of some fermented legume condiment diets on antioxidant status and hepatic damage in STZ–induced diabetic rats.
Aminotransferases (AST and ALT) mediate the catalysis of amino–transfer reactions and they are markers for clinical diagnosis of liver injury[3] while ALP is responsible for removing phosphate group from nucleotides and proteins. This enzyme is produced primarily in the liver and brain[20], and also used as marker of hepatic functions[21]. As revealed in this present study, the significant increase in the activities of plasma AST, ALT and ALP observed in diabetic control group as compared to normal control group was mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which is an indication of hepatotoxic effect of STZ[22] and consistent with previous studies which described increased liver oxidative stress in this model of experimental diabetes mellitus[2]. Furthermore, Khan *et al*[23] suggested that the elevation in serum AST and ALT activities

in diabetic rats could be related to excessive accumulation of glutamate and alanine in the serum of diabetic animals, as a result of amino acids mobilization from protein stores. However fermented condiment treated groups exhibited significant decrease in plasma AST, ALT and ALP activities which could be attributed to their ability to protect/ repair liver tissue damage. Fermented legume condiments are rich in antioxidant polyphenol phytochemicals with hepatoprotective properties[24].

Reduction in plasma protein level observed in diabetic control group may be due to microproteinuria which are important clinical markers of diabetic nephropathy and may be due to increased protein catabolism[25]. Also, formation of protein–MDA adducts may have contributed to the decrease in plasma protein level due to increased lipid peroxidation. However, the marked increase in the plasma total protein of the fermented legume diet treated group may be due to the hepatoprotective properties of their polyphenol constituents and also availability of their amino acids.

Hyperglycemia induced free radical production has been implicated in the disruption of endogenous antioxidant defense system in diabetes. In this present study, diabetes caused marked depletion of both enzymatic (GST and CAT) and non–enzymatic (GSH and ascorbic acid) antioxidants systems, which was restored after feeding with fermented legume condiment diet for 14 days. Reduced activities of plasma GST and CAT may be due to inactivation of these enzymes by ROS[26–31]. However, the increase in the plasma activities of these enzymes might be due to enhanced antioxidant status of the treated rats resulting from the condiments' high phenol content and free radical scavenging ability. GSH–metabolizing enzymes such as GST reduces lipid hydroperoxides through their Se–independent glutathione peroxidase activity and this enzymes can also detoxify lipid peroxidation end products such as 4–hydroxynonenal[32]. GST is also involved in the decomposition of hydrogen peroxide and other organic hydroperoxides to non–toxic products respectively, at the expense of reduced glutathione[33]. Catalase is chiefly involved in the detoxification of hydrogen peroxides produced from the action of superoxide dismutase and thus protects the tissue from highly reactive hydroxyl radicals.

GSH participates in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates. Therefore, the decrease in plasma GSH level in the diabetic control group might reflect a direct reaction between GSH and free radicals generated by hyperglycemia in diabetic state. This is consistent with GSH function to scavenge oxidants by binding them covalently[34]. However, the marked increase in the plasma GSH level of diabetic rats treated with diet containing tropical legume condiments might be due to their antioxidant phenolic content which exerts sparing effect on the plasma GSH in the diabetic rats. An antioxidant system based on thiols serves as a second line of cellular defense against oxidative damage mediated by reactive free radicals. The GSH and free thiol (SH) group present on albumin form a major contribution to the total thiol pool[35], and function by scavenging free radicals as well as by detoxifying various xenobiotics.

Furthermore, hyperglycemia–induced depletion in the endogenous antioxidant system in the diabetic rats may be responsible for the increased plasma MDA content which may cause loss of membrane fluidity, membrane integrity, and finally loss of hepatic cell functions[36]. Lipid peroxidation is an important parameter of oxidative stress and elevated plasma MDA levels in diabetic control group suggests enhanced lipid peroxidation, leading to hepatic tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. This peroxidative damage to membranes results in the leakage of enzymes, and metabolites into the blood circulation. However, the marked decrease in the plasma MDA content in the fermented legume condiment diet fed groups could still be attributed to their antioxidant phenolic content. Free radical scavenging is one of the major antioxidant mechanisms inhibiting chain reaction of lipid peroxidation.

Vitamin C is a potent endogenous antioxidant and acts as a strong reducing agent on exposure to free radicals. It could also prevent the binding of toxic free radicals to nucleic acid or proteins thus preventing free radical mediated damage to physiologically important biomolecules. The decreased level of vitamin C in diabetic rats may be due to the increased utilization of vitamin C as an antioxidant defense against increased ROS or a decrease in glutathione level; since glutathione is required for the recycling of ascorbic acid[37]. However, the significant increase in the plasma vitamin C content with treatment with fermented legume condiment diets could result from the sparing effect of vitamin C by the phenolic compounds in the fermented legume condiments diets.

The fermented legume condiments are rich in phenolic contents which compares favourably with other plant sources[14]. Our unpublished data revealed that Carvacrol, p–coumaric acid, vnillic acid, p–hydroxybenzoic acid, caffeic acid, ferulic acid, genistein, apigenin, shagaol, glycitein, kaempferol, luteolin, capsaicin, isorhamnetin, myricetin and rosmarinio acid were the predominant phenolic compounds in fermented bamabara groundnut. p–coumaric acid, vnillic acid, p–hydroxybenzoic acid, ferulic acid, genistein, apigenin, isorhamnetin and myricetin were the predominant phenolic compound in fermented locust bean while, p–hydroxybenzoic acid, daidzein, coumestrol, genistein and glycitein were predominant in fermented Soybean. Studies have shown that the majority of the antioxidant activities of plant foods are from phenolic compounds rather than from vitamins C, E and β–carotene[38–40]. The fermented legume condiment also demonstrated strong free radical scavenging activities by scavenging moderately stable ABTS radicals *in vitro*. Free radicals may play an important role in the causation and complications of diabetes mellitus. Alterations in the endogenous free radical scavenging defense mechanisms (associated with diabetes mellitus) could lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. Hence, steady supply of dietary antioxidants to augment or boost the endogenous antioxidant defense mechanisms could be one practical approach through which free radical mediated oxidative stress in diabetes mellitus may be curtailed. Phenolic compounds due to their structure are known to be involved in the healing process of free radical mediated diseases including diabetes[41]. Thus, the observed attenuation of oxidative stress in the diabetic rats treated with fermented legume condiment diets could be due to their phenolic constituents.

Consumption of phenolic–rich fermented tropical legume condiments could therefore be a practical dietary approach, through which oxidative stress arising from diabetes or its complications be manage.

Conflict of interest statement

The authors declare no conflict of interest.

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