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Modulation of lipid peroxidation, hypolipidemic and antioxidant activities in brain tissues of diabetic rats by fibre - Enriched biscuits

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ABSTRACT

Objective: To investigate the effect of feeding fibre - enriched biscuit on the antioxidant and hypolipidemic activities in brain tissues of diabetic rats. **Method:** Diabetes was induced by a single intraperitoneal injection of alloxan. Treatment lasted for 14 d, after which the rats were sacrificed by cervical dislocation. Brain tissues were used for the assessment of GSH, catalase, SOD and lipid peroxidation as well as lipid profiles. **Result:** Induction of diabetes led to a significant decrease in GSH level, elevated SOD and catalase activities. These were significantly modified by the biscuits. There was an elevated level of malondialdehyde in the brain tissues of the untreated diabetic rats; this was significantly reduced by the biscuits. There was a significant decrease in HDL and a significant increase in LDL levels, total cholesterol and triglycerides in the untreated (diabetic) rats. Feeding with fibre - enriched biscuits led to decrease in the levels of total cholesterol, triglyceride, LDL - cholesterol and caused a significant increase in the levels of HDL. **Conclusions:** These results suggest a therapeutic and protective effect of the fibre - enriched biscuits against diabetic - induced brain toxicity in rats.

1. Introduction

Diabetes mellitus has been described a chronic disorder of carbohydrate metabolism resulting from inadequate insulin release (type 1 diabetes; T1D) or insulin insensitivity (type 2 diabetes; T2D). If left uncontrolled, diabetes could result in hyperglycemia[1] and several complications including retinopathy, nephropathy, neuropathy, and microvascular damage to the cerebral artery[2]. Experimental evidence shows that increased production of reactive oxygen species (ROS) play a major role in these complications[3]. In normal physiological conditions there is a balance between the synthesis of free radicals and the activities of anti-oxidant pathways to protect the organism, of which imbalances favoring the former results to oxidative stress[3]. Hyperglycemia-induced oxidative stress has been identified as potential contributors to diabetes-induced brain aging, leading to cellular and molecular damage[4,5].

Plants are recognized as a wonderful source for medicines. It is estimated that there are about 1 200 species of plants are used as folk medicines for the treatment and management of diabetes[6]. These plants are rich sources of dietary fibre.

Dietary fibre has been reported to have tremendous health benefits. Epidemiological studies reveals that its increased intake is associated in the management and treatment of several diseases, including reduced risk of coronary heart disease, diabetes, obesity, and some forms of cancer[7,8]. Consumption of 30 to 50 g/d of fibre from whole food sources consistently have been reported to assist in lowering blood glucose concentrations[9]. Dietary fibre has also been shown to improve insulin sensitivity in individuals with type II diabetes[10]. The vast majority of epidemiological and intervention studies show that high fiber diets help improve glycaemic control and reduce the need for insulin in diabetic subjects[11].

This chapter aims at investigating the antioxidant and hypolipidemic effect of feeding fibre - enriched biscuit on

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brain tissues of diabetic rats.

2. Materials and methods

2.1 Plant materials

Fruits with high fibre were identified as banana (*Musa species*), oranges, watermelon (*Citrullus lanatus*), pineapple (*Ananas cosmosus*) and pawpaw (*Carica papaya*). The fruits were purchased from Ketu fruit market, Ketu, Lagos, Nigeria. They were rinsed with tap water and peeled. Juice extractor was used to extract the juice from the oranges, pineapple and watermelon, leaving behind the fibres. 400 g of each fibre were weighed and blended together with 400 g of pawpaw and banana respectively in a warring blender for 10 min to produce fibre paste.

2.2 Production of high fibre biscuits

500 g of wheat flour was weighed into a plastic bowl, to which 225 g of fibre paste was added. 50 g of margarine and 10 g of baking powder were also added. They were mixed with a mixer to form dough. The dough were spread on a clean flat surface and cut into fine circles of biscuits. The biscuits were transferred into metal trays greased with margarine and allowed to bake in an oven for 30 min at a temperature of 150 ℃. After preparation the biscuits were allowed to cool, wrapped with foil paper and stored under refrigerated condition.

2.3 Animals

Eighteen male albino rats of wister strain weighing about 150 – 200 g were used for the study. They were fed on standard rat pellet diet and allowed to adapt for one week. They were provided water *ad libitum* and maintained under standard laboratory conditions of natural photo period of 12 h light – dark cycle. The animals used in the present study were maintained in accordance with the approval of the Animal Ethical Committee, University of Lagos, Lagos, Nigeria

2.4 Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 180 mg/kg of alloxan monohydrate in normal saline water in a volume of about 3 mL. After 72 h of alloxan injection, the diabetic rats (glucose level > 250 mg/dL) were separated and used for the study.

2.5 Experimental design

The rats were divided into three groups, each consisting of six animals.

Group 1 – Pelletized mouse chows

Group 2 – Diabetic (Untreated)

Group 3 – Diabetic ± fibre biscuits

The rats were monitored daily for food and water intake, and body weight. Treatment lasted for two weeks after induction. At the end of the feeding trials, the rats were fasted overnight and sacrificed by cervical dislocation.

2.6 Preparation of tissue homogenates

The organs (brain) were removed, rinsed in ice-cold 1.15% KCl solution to wash off excess blood, blotted dry with filter paper and weighed. They were homogenized in four parts of homogenizing buffer and centrifuged at 10 000 rpm for 15 min in an ultracentrifuge at a temperature of −2 ℃ to get the mitochondrial fraction. The supernatant (post-mitochondrial fraction) was decanted and stored at −4 ℃ for subsequent analysis. Each time the supernatant was outside the freezer, it was kept in ice bags.

The protein content of the tissue fractions of the organs were determined by Lowry's method using bovine serum albumin (BSA) as standard[12].

2.7 Determination of oxidative stress parameters

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formed by thiobarbituric acid reaction (TBAR)[13]. Catalase (CAT) activity was estimated by measuring the rate of decomposition of H_2O_2[14]. The level of superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich[15]. While the method of Ellman[16] was adopted in estimating the activity of reduced glutathione (GSH).

2.8 Determination of hypolipidemic activities

Tissue total cholesterol, triglyceride and high density lipoprotein (HDL) were measured by enzymatic colorimetric method using Randox kits. The concentration of low-density lipoprotein (LDL) cholesterol was calculated by the formula of Friedwald *et al*[17].

2.9 Statistical analysis

Statistical significance was established using one-way analysis of variance (ANOVA), and data were reported as mean ± standard deviation. Significant difference was

established at $P < 0.05$. Statistical analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL).

3. Results

3.1 Weight of animals and food consumed

The consumption pattern varied among the groups as depicted in Figure 1. No significant difference was observed in the weight of feed consumed in all treatment groups during the first and second weeks. In the third week however, there was significant difference ($P<0.05$) among the groups.

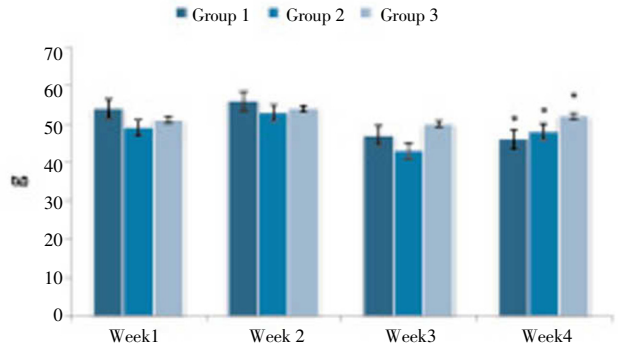

Figure 1. Weight of food consumed. Values = mean ±SD; n = 5. *values are significantly different.

No significant difference was observed in the weight of the animals during the course of the experiment. However, there were weight losses in all groups except group 3 (Figure 2).

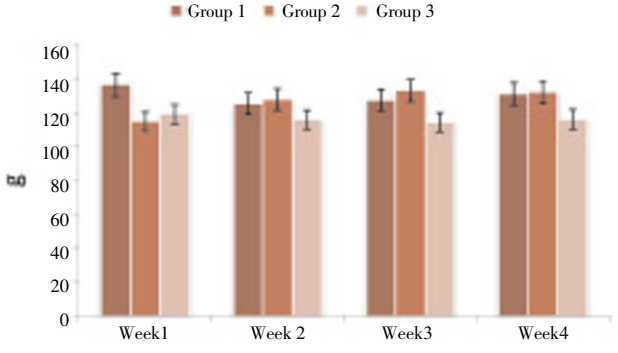

Figure 2. Weight of rats. Values = mean ±SD; n = 5.

3.2 Lipid peroxidation

Figure 3 shows the MDA levels in the experimental rats. There was a significant increase ($P < 0.05$) in the MDA level of the untreated diabetic group signifying induction of lipid peroxidation. Feeding on fibre – enriched biscuits caused a significant reduction ($P<0.05$) of the MDA level.

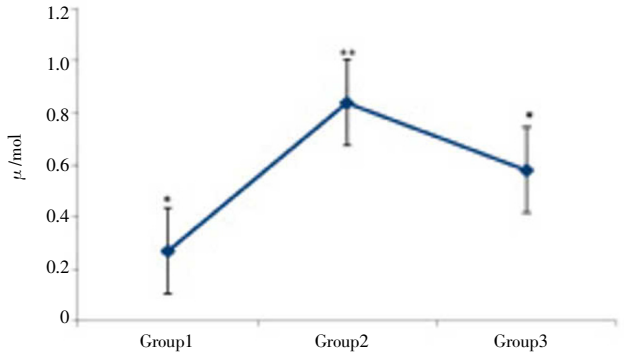

Figure 3. MDA levels of experimental groups. Values = mean ± SD; n = 5. *values are significantly different.

3.3 Antioxidant activities of brain tissues

The antioxidant activities in the brain tissues of the experimental groups are shown in Table 1.

Induction of diabetes led to a reduction of GSH level in the brain tissues of the untreated diabetic rats (group 2). Feeding with fibre – enriched biscuits increased the GSH level by 52.39% which was statically significant ($P<0.05$) (group 3).

No significant difference was observed in SOD activities in the brain tissues of the experimental groups. Induction of diabetes led to 32.46% increase of the SOD activity (group 2). However, feeding with fibre – enriched biscuits led to 48.41% reduction of the activity (group 3).

Elevated level of catalase was observed in the untreated diabetic rats (group 2). Feeding with fibre – enriched biscuits however, caused a reduction in the catalase level.

3.4 Tissue protein

Effect of feeding fibre – enriched biscuits on brain tissue protein concentration of the experimental groups is shown on Figure 4. There were significant difference ($P<0.05$) between the control and diabetic groups. An increased concentration was observed in the group fed fibre – enriched biscuits.

Table 1
Antioxidant activities of brain tissues of experimental groups.

Parameters	Group 1	Group 2	Group 3
GSH (μ /mol)	19.09±0.97*	5.38±0.53*	11.30±1.45*
SOD (μ /mol)	240.76±5.09	318.91±23.65	164.51±11.06
Catalase (μ /mol)	2 133.19±33.86	1 100.43±73.99	1 610.47±34.07

Values = mean ± SD; n = 5. * Significantly different ($P<0.05$).

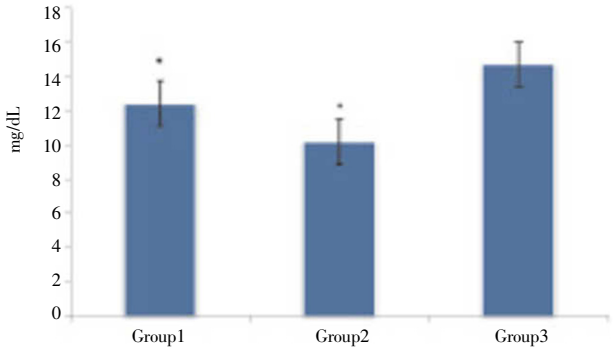

Figure 4. Protein contents of brain tissues tissues in experimental groups. Values = mean ± SD; n = 5. *Significantly different (P<0.05)

3.5 Hypolipidemic activities

Hypolipidemic activities of brain tissues of the experimental groups are shown in Figure 5. There was a significant decrease in HDL and a significant increase in the levels of LDL, total cholesterol and triglycerides in the untreated (diabetic) rats. Feeding with fibre – enriched biscuits produced a significant (P<0.05) decrease in the levels of total cholesterol, triglyceride, LDL-cholesterol and caused a significant (P<0.05) increase in the levels of HDL compared to the untreated group.

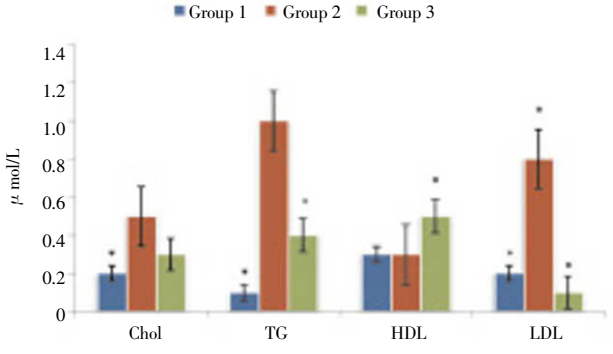

Figure 5. Hypolipidemic activities of brain tissues of experimental groups. Values = mean ± SD; n = 5. *Significantly different (P<0.05).

4. Discussion

Glucose is the principal metabolic fuel of the brain. The brain can neither synthesize nor store glucose for extended periods of time, it is therefore essential that proper glucose regulation be maintained to ensure its appropriate transport to the CNS[3]. Such processes are often disrupted in diabetics leading to hyperglycaemia which impairs the brain endogenous antioxidant defense system[18].

Induction of diabetes produced a marked oxidative impact, as confirmed by the significant increase of lipid peroxidation products (TBARs) and endogenous antioxidants *i.e.* SOD and CAT activities in the brain tissues. Lipid peroxidation (LPO) is a marker of oxidative stress[19]. It is initiated by free radicals attack to membrane lipids, generating large amounts of reactive products, which have been implicated in diabetes and its complications[20].

In this study, the significant increased level of MDA in the brain tissues of the untreated diabetic rats reflects an increase in lipid peroxidation. Thus, indicating a decrease in enzymatic and non-enzymatic antioxidant defense systems in diabetic rats. Previous studies by Arnal *et al*[21], and Cui *et al*[22] also reported an increased MDA level in brain tissues of diabetic rats. GSH is also a major endogenous antioxidant, which counteracts free-radical-mediated damage and a marker of oxidative stress[23]. It forms an important substrate for other enzymes which is involved in the free-radical scavenging. Its observed reduction in brain tissues of the untreated diabetic rats further reflects oxidative stress. Its increased level in the treated group indicates the antioxidant potentials of the biscuits.

The reduced SOD and CAT levels in the brain tissues of the treated diabetic rats could also be protective potentials of the biscuits to counteract the oxidative stress in the tissue. Their increased synthesis as observed in the untreated diabetic rats corresponds to previous studies by Onyema *et al*[19] and Erukainure *et al*[23] which suggests that these enzymes are synthesised in response to oxidative stress. These observed changes shows that the biscuits acted as an effective antioxidant, thus safeguarding the brain tissues against diabetes-induced oxidative damage. This could be attributed to the dietary fibre of the formulated biscuits. The antioxidant properties of dietary fibre have been reported and may be exploited as potential novel antioxidants[24].

Diabetes have been reported to be associated with dyslipidemia as evidenced by high cholesterol, particularly a high LDL and low HDL, and high triglycerides[24]. Chronic hyperglycemia promotes the glycation of LDL and increases the atherogenicity of LDL[25]. The observed hypolipidemic activity as indicated by reduced cholesterol, LDL, triglyceride, and increased HDL portrays a protective effect of the biscuits against diabetic – induced dylipidemia in brain tissues. This could be attributed to the dietary fibre present in the biscuits.

Dietary fibre may brin-g about the observed effects by the following: (1) slow uptake of sugars into the bloodstream by slowing gastric emptying and by coating the small intestine; (2) Decrease transit time by holding onto water and adding bulk to stool; (3) Adding bulk to food.

The present study suggests that the fibre – enriched

biscuits had a therapeutic protective effect against diabetic – induced toxicity in brain tissues of rats. The synergetic effect is revealed by reduced lipid peroxidation products (TBARs), decreased dyslipidemia, and increased antioxidant activities.

Conflict of interest statement

The authors report no conflict of interest.

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