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# Modulatory effect of fibre-enriched cake on alloxan-induced diabetic toxicity in rat brain tissues

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ABSTRACT

Diabetes is a metabolic disorder characterized by hyperglycaemia and it is fast becoming a scourge in sub-Saharan Africa. The nutritional properties of developed fibre-enriched cake and its protective potential against diabetic induced neurotoxicity in rats were investigated. Fibre-enriched cake was developed from selected fruits and analysed for its nutritional and sensory attributes. Rats were induced with diabetes by a single intraperitoneal injection of alloxan and treated with the formulated cake. After 14 days treatment, the rats were sacrificed by cervical dislocation. Their brain tissues were accessed for reduced glutathione (GSH), catalase, superoxide dismutase (SOD) activities, protein content and lipid peroxidation as well as lipid profiles which cover for total cholesterol, triglycerides, HDL and LDL. Induction of diabetes led to significant reduction ($p < 0.05$) of GSH, catalase, SOD activities and protein content. Feeding on the formulated cake led to their significant increase. Decreased lipid peroxidation, total cholesterol, LDL and triglycerides, and increased concentration of HDL were also observed on feeding with the cake. These results indicate an antioxidant protective potential of the fibre-enriched cake against diabetic-induced brain toxicity. Thus, it can serve as an adjunct to dietary therapy for diabetes.


## 1. Introduction

Diabetes have been described as a growing scourge which affects more than 12 million people in sub-Saharan Africa (SSA), causing a major drain on her health resources already overburdened by other infectious diseases [19]. It is a metabolic disorder characterized by hyperglycaemia and is associated with long-term vascular complications such as retinopathy, nephropathy, cardiopathy, and neuropathy [20]. Oxidative stress has been reported to play a major role in these complications. A number of studies have implicated hyperglycaemia-induced oxidative stress in the aetiology of a variety functional and structural disorder in the central and peripheral nervous system [13,20,10].

Over the years, it has been documented that medicinal plants are very effective in the treatment and management of diabetes [22]. The combination of two basic central factors, food and medication has been attributed to their utilization [14]. These plants are major source of fibre with tremendous health benefits [9]. The health benefits of dietary fibre have been reported in several studies. Diabetes prevalence has been shown to correlate with fibre intake among various populations [32,31]. Its consistent consumption has been reported to cause a reduction in blood glucose concentrations [31]. In previous study we

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reported the hypoglyceamic and antidiabetic effect of fibre-enriched snacks in diabetic rats [8].

This paper is a continuation of our study on the effect fibre-enriched snacks on diabetes. It aims at reporting the nutritional properties of the cake and its protective potential against diabetic induced neurotoxicity in rats.

## 2. Materials and methods

### 2.1. Plant materials

Banana (*Musa species*), oranges (*Citrus sinensis*), watermelon (*Citrullus lanatus*), pineapple (*Ananas cosmosus*) and pawpaw (*Carica papaya*) were purchased from Ketu fruit market, Ketu, Lagos, Nigeria. They were processed into fibre paste as described by Erukainure et al. [10,9].

### 2.2. Production of high fibre cake

High fibre cake was produced as described by Erukainure et al. [9].

### 2.3. Proximate analysis

The proximate nutritional qualities of the cake sample were carried out according to the method of AOAC [3], which covers for total protein, ash, fat, crude fibre, carbohydrates and reducing sugar, respectively.

### 2.4. Sensory attributes

Sensory evaluation was conducted on the developed cake according to the method described by Ihekoronye and Ngoddy [17]. It was compared to readily available commercial cake. They were given the reference codes YLQ and PGE for the developed and commercial cake respectively. The coded samples were presented to a 10-men panellist to evaluate for the attributes: colour, texture, taste, crumb grains, mouth feel, aroma, and overall acceptability. Scores were given to the scales: (9) extremely acceptable, (8) very acceptable, (7) moderately acceptable, (6) slightly acceptable, (5) neither acceptable nor unacceptable, (4) slightly unacceptable, (3) moderately unacceptable, (2) very unacceptable and (1) extremely unacceptable.

### 2.5. 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 (Ladoke feeds) and allowed to adapt for one week. They were provided water *ad libitum* and maintained under standard laboratory conditions of natural photoperiod 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. The approval number from the Animal Institutional Ethical Committee is UL/CMUL/IEC 2011/1003.

### 2.6. 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.

### 2.7. Experimental design

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

Group 1 – normal rats + pelletized mouse chows.
Group 2 – diabetic (untreated).
Group 3 – diabetic + high fibre cake.

Treatment lasted for two weeks. At the end of the feeding trials, the rats were fasted overnight and sacrificed by cervical dislocation.

### 2.8. Preparation of tissue homogenates

The brain tissues were removed, rinsed in ice-cold 1.15% KCl solution to wash off excess blood, blotted dry with filter paper. 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 °C to get the mitochondrial fraction. The supernatant (post-mitochondrial fraction) was decanted and stored at −4 °C 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 [24].

### 2.9. Determination of oxidative stress parameters

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formed by thiobarbituric acid reaction (TBAR) [6]. Catalase (CAT) activity was estimated by measuring the rate of decomposition of $H_2O_2$ [2]. The level of superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich [26]. While the method of Ellman [7] was adopted in estimating the activity of reduced glutathione (GSH).

### 2.10. Determination of hypolipidemic activities

Tissue total cholesterol, triglyceride and high density lipoprotein (HDL) were measured by enzymatic colorimetric method using Randox kits according to manufacturer's protocol. The concentration of low-density lipoprotein (LDL) cholesterol was calculated by the formula of Friedwald et al. [12].

### 2.11. Statistical analysis

To address the biological variability, each set of experiments was repeated at least three times ($n = 3$) for proximate analysis and six times for experimental rats

**Table 1**
Proximate composition of fibre-enriched cake.

| Parameter | Fibre-enriched cake (%) |
|---|---|
| Moisture | 10.49 ± 0.18 |
| Ash | 2.97 ± 0.11 |
| Fat | 14.09 ± 0.15 |
| Protein | 12.48 ± 0.02 |
| Crude fibre | 10.43 ± 0.10 |
| Dietary fibre | 1.69 ± 0.02 |
| Nitrogen free extract | 49.54 ± 0.23 |
| Reducing sugar | 20.41 ± 0.07 |

*Note*: Values = mean ± SD; $n = 3$.

**Table 2**
Sensory attributes of developed fibre-enriched.

| Cake attributes | YLQ | PGE |
|---|---|---|
| Colour | 6.50 ± 0.67 | 8.3 ± 0.67 |
| Texture | 6.10 ± 0.70 | 7.70 ±0.82 |
| Taste | 5.00 ± 1.00 | 7.90 ± 0.56 |
| Crumb grains | 5.20 ± 0.87 | 7.60 ± 0.84 |
| Mouth feel | 5.80 ± 1.46 | 7.80 ± 0.63 |
| Aroma | 5.60 ± 0.66 | 7.90 ± 0.56 |
| Overall acceptability | 6.2 ± 0.92 | 8.8 ± 0.63u |
| Ranking sum | 20 | 10 |

*Note*: Values = mean ± SD; $n = 10$.

($n = 6$). Differences between the groups were analysed by one-way analysis of variance (ANOVA) with the aid of SPSS software (SPSS Inc., Chicago, IL, USA) standard version 17. The $p$ values of <0.05 were considered statistically significant for differences in mean using the least of significant difference. Data were reported as mean ± standard deviation.

## 3. Results

Results of the proximate analysis as depicted in Table 1 showed higher concentrations of nitrogen free extracts (NFE), reducing sugar, fat, crude protein and crude fibre with NFE being the highest. The moisture content was observed to be moderate, while lower values where observed for ash and dietary fibre.

Table 2 shows the sensory attributes of the fibre-enriched cake. Except for colour and taste which were slightly acceptable by the panellists, other attributes of the fibre–enriched cake (YLQ) were rated neither acceptable nor unacceptable compared to commercially available cake (PGE) which served as the control.

Induction of diabetes led to significant reduction ($p < 0.05$) of GSH activities in brain tissues indicating oxidative stress as depicted in Table 3. This was significantly ($p < 0.05$) increased on feeding with fibre-enriched cake. SOD and catalase activities were also observed to

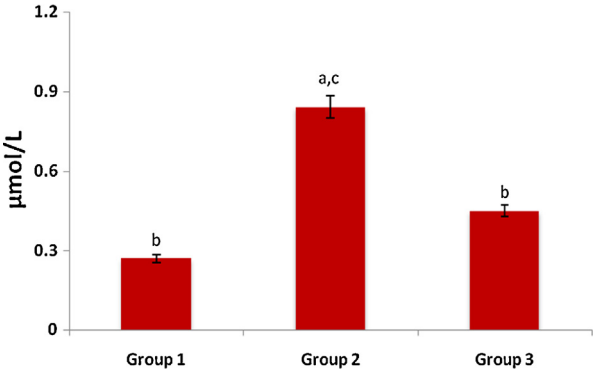


**Fig. 1.** MDA level of experimental groups. *Note*: values = mean ± SD; $n = 6$: (a) statistically significant ($p < 0.05$) as compared with group 1; (b) statistically significant ($p < 0.05$) as compared with group 2; and(c) statistically significant ($p < 0.05$) as compared with group 3.

significantly increase on induction of diabetes, these were significantly reversed on feeding with the developed cake.

Induction of diabetes significantly ($p < 0.05$) increased MDA level in the brain tissues as shown in Fig. 1. Feeding on fibre-enriched cake led to a significant ($p < 0.05$) reduction.

Increased cholesterol, triglycerides, LDL and reduced HDL were observed in the diabetic group as revealed in Fig. 2. A significant ($p < 0.05$) reverse was observed on feeding with fibre-enriched cake.

## 4. Discussion

DM is a metabolic disorder with tremendous effect on the peripheral and central nervous system [33,18]. Oxidative stress has been implicated in diabetic neuropathy and encephalopathy [29,15] as a result of hyperglycaemia. Owing to its cost of treatment and economic impact, there has been a shift to the development of novel and affordable therapies to combat the disease [9]. Of particular interests are the functional foods which fall in the grey area between conventional foods and medicine [21]. In this present study, fibre-enriched cake was developed from readily available selected fruits and its protective effect on diabetic neuropathy investigated.

The observed low moisture in the developed cake sample is of great advantage to most rural communities which lack access to basic storage facilities and electricity. This would reduce the cost of handling and storage [11]. The fat and protein contents were observed to be higher compared to our previous studies on fibre-enriched biscuits [9]. This may be attributed to the fortifying fruits and baking ingredients. This present study reports slightly higher crude fibre content compared to previous study on

**Table 3**
Antioxidant activities of brain tissues of experimental groups.

| Parameters | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| GSH (U/mg protein) | 19.09 ± 0.97$^{b,c}$ | 5.38 ± 0.53$^{a}$ | 7.54 ± 0.69$^{a}$ |
| SOD (U/mg protein) | 240.76 ± 5.09$^{b,c}$ | 318.91 ± 23.65$^{a,c}$ | 152.62 ± 3.52$^{a,b}$ |
| Catalase (U/mg protein) | 1610.47 ± 34.07$^{b,c}$ | 2133.19 ± 33.86$^{a,c}$ | 1020.92 ± 23.57$^{a,b}$ |

*Note*: Values = mean ± SD; $n = 6$: (a) statistically significant ($p < 0.05$) as compared with group 1; (b) statistically significant ($p < 0.05$) as compared with group 2; and (c) statistically significant ($p < 0.05$) as compared with group 3.

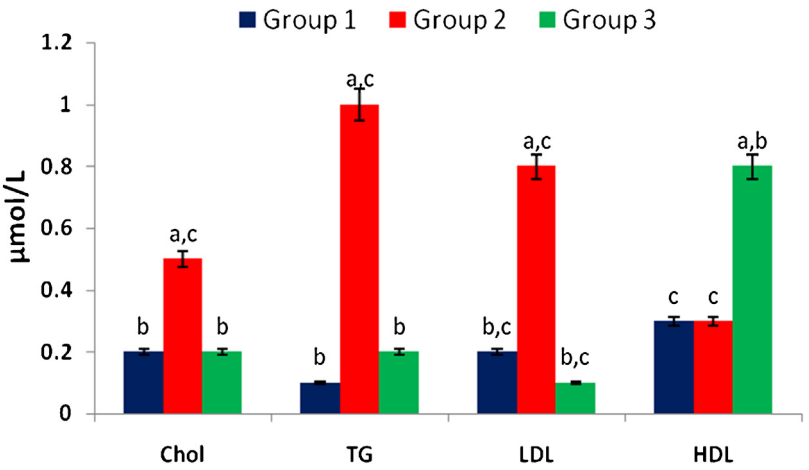


**Fig. 2.** Lipid profile of experimental groups. Note: values = mean ± SD; $n = 6$: (a) statistically significant ($p < 0.05$) as compared with group 1; (b) statistically significant ($p < 0.05$) as compared with group 2; and (c) statistically significant ($p < 0.05$) as compared with group 3.

fibre-enriched snacks [9]. However, the dietary fibre was much lower. Plants have been affirmed to be rich in fibre, particularly dietary fibre. Non-dietary fibre have been reported to have health benefits [27]. Its link between desired health effects have been directly correlated to its consumption in organized research studies [30].

Studies have shown that consumers' choice of food is greatly influenced by sensory characteristics of food particularly taste and flavour. In this present study, the formulated cake was presented to a 10-men panellist to evaluate for the selected attributes in comparison to a commercially available cake. The commercially available had a higher preference to the formulated. This can be attributed to familiarity with the product. This may also be coupled with the fact that they did not need so much training on sensory method of profiling [5]. Hughson and Boakes [16] and Labbe et al. [23] had concluded that training and experience increases the sensory ability.

GSH has been described as one of the most prominent nonenzymatic antioxidants which counteract free-radical mediated damage and a marker of oxidative stress [1,25]. It forms an important substrate for other enzymes which is involved in the free-radical scavenging. Its reduced activity in brain tissues of untreated diabetic rats portrays an incidence of oxidative stress. Its increased activity on feeding with the formulated cake indicates an antioxidant potential of the snack. The increased SOD and CAT activities in the brain tissues of untreated diabetic rats further suggests incidence of oxidative stress. These observed increments can be attributed to increased synthesis owing to induction of oxidative stress [28,1,10,8]. Their reduced activities on feeding with the formulated cake further reflect the antioxidant protective potential of the snack against diabetes-induced oxidative stress. Previous studies by Erukainure et al. [9] reported a reduction in blood glucose levels and increased serum level in diabetic rats fed with fibre-enriched snacks. Thus, the observed activities may be attributed to the ability of the formulated cake to reduce the amount of glucose level available for oxidation [9].

Lipid peroxidation has also been reported to be a major marker of oxidative stress. It is initiated by free radicals attack on membrane lipids, generating large amounts of reactive products that have been implicated in diabetes and its complications [4]. In this present study, induction of diabetes led to significant ($p < 0.05$) increase of lipid peroxidation products (TBARs) in brain tissues indicating oxidative stress. The reduced products on feeding with the formulated cake further portray the potential of the snack to attenuate and protect against diabetic induced brain toxicity.

Diabetes has been linked with hyperlipidemia as evidenced by high cholesterol, particularly high LDL and low HDL, and high triglycerides as seen in the untreated diabetic rats [15]. The observed hypolipidemic activity as indicated by reduced cholesterol, LDL, triglyceride, and increased HDL portrays a protective effect of the snack against diabetic-induced hyperlipidemia in brain tissues. This could be attributed to the fibre-enrichment.

## 5. Conclusion

Results from this study indicate an antioxidant protective potential of the fibre-enriched cake against diabetic-induced brain toxicity. As a functional food, the formulated snack can serve as an adjunct to dietary therapy for diabetes.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Transparency document

The Transparency document associated with this article can be found in the online version.

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