Hematological and biochemical changes in diabetic rats fed with fiber-enriched cake

Ochuko L. Erukainure\textsuperscript{a,b,c,*}, Osaretin A.T. Ebuehi\textsuperscript{b}, Folasade O. Adeboyejo\textsuperscript{a}, Muhammad Aliyu\textsuperscript{c}, Gloria N. Elemo\textsuperscript{a}

\textsuperscript{a} Food Technology Division, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria
\textsuperscript{b} Department of Biochemistry, University of Lagos, Idi Araba, Lagos, Nigeria
\textsuperscript{c} Hussain Ebrahim Jamal (H.E.J.) Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

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Abstract

\textbf{Background:} There have been emerging interests in functional foods, which fall in the gray area between conventional foods and medicine, over the years owing to their health benefits. In this study, the effect of fiber-enriched cake, produced from selected fruits, on the hematology and serum biochemistry of diabetic rats was investigated.

\textbf{Materials and methods:} Diabetes was induced by an intraperitoneal injection of alloxan. Treatment lasted for 14 days, after which the rats were sacrificed humanely. Blood was collected by cardiac puncture and some of it was centrifuged to obtain serum. The serum was analyzed to evaluate alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatinine, urea, albumin, and lipid profiles. The uncentrifuged blood was analyzed for hematological profile.

\textbf{Results:} The rats fed on the cake had increased levels of red blood cells, hemoglobin, packed cell volume, red blood cells, and platelets compared to the untreated diabetic rats. Elevated levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatinine, urea, and albumin were observed in the diabetic rats; this was significantly reduced in the cake-fed group. Induction of diabetes led to increased levels of cholesterol, low-density lipoprotein, and triglyceride, and a low level of high-density lipoprotein. These results were significantly reversed, except for the triglyceride level, in the cake-fed group.

\textbf{Conclusion:} These results indicate the potentials of the fiber-enriched cake as a functional food with a therapeutic effect against hematological and biochemical changes associated with diabetes mellitus. Consequently, such a cake can serve as an adjunct to dietary therapy for diabetes.

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\textbf{Keywords:} Dietary fibers; Function enzymes; Functional food; Hypcholesteremia

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by abnormalities in carbohydrate, protein, and lipid metabolism. This often occurs as a result of inadequate insulin release resulting in type 1 diabetes or insulin insensitivity as seen in type 2 diabetes.\textsuperscript{1} If left uncontrolled, DM can lead to hyperglycemia and increase the risks of atherosclerosis, thereby leading to cardiovascular complications.\textsuperscript{2} Other complications include retinopathy, nephropathy, and neuropathy.\textsuperscript{3} Most pharmacological treatments of DM are based on either oral hypoglycemics and/or insulin therapy, which has many side effects such as weight gain, hypoglycemia, gastrointestinal disturbances, and hypersensitivity reactions.\textsuperscript{4} These have been of tremendous interest to health practitioners. Alternative therapies, especially those involving medicinal plants, for management of DM has long been in practice.\textsuperscript{5} These plants are rich sources of phytochemicals and dietary fibers, which serve as nutraceuticals and ingredients for functional foods.\textsuperscript{5}

There have been emerging interests in functional foods, which fall in the gray area between conventional foods and medicine, over the years owing to their health benefits. Functional foods are foods that are enriched with nutraceuticals derived from natural foods and can be added to other foods to impart specific health benefits. Of particular interest are the dietary fibers. The health benefits of dietary fibers have already been reported. Slavin reported that consistent consumption of 30–50 g/d of dietary fibers from whole food sources assists in lowering blood glucose concentrations. Galisteo et al showed that dietary fiber improves insulin sensitivity in individuals with type II diabetes. This paper reports the effects of a fiber-enriched cake on the hematology and serum biochemistry of alloxan-induced diabetic rats that fed on such a cake.

2. Materials and methods

2.1. Plant materials

Fruits such as banana (Musa species), oranges (Citrus Sinensis L.), watermelon (Citrullus lanatus), pineapple (Ananas comosus), and pawpaw (Carica papaya) were identified to have high fiber contents. The fruits used for the study were purchased from the Ketu Fruit Market, Ketu, Lagos, Nigeria. They were rinsed with tap water and peeled. Juice was extracted from oranges, pineapples, and watermelons using a juice extractor, leaving behind the fibers. The amount of each fiber weighed was 400 g, which were blended together with pawpaw and banana (400 g each) in a waring blender for 10 minutes to produce a fiber paste.

2.2. Preparation of a high-fiber cake

A fiber-enriched cake was produced according to the method described by Erukainure et al. Wheat flour (500 g) was taken into a plastic bowl, to which 400 g of the fiber paste was added. In addition, 100 g of margarine, 12 g of baking powder, and two eggs were added to this mixture. They were mixed with a mixer to form a dough. The dough was transferred into two baking pans greased with margarine and allowed to bake in an oven for 30 minutes at a temperature of 150°C. After baking, the cakes were allowed to cool and removed from the pans. They were wrapped in a foil paper and stored at 4°C.

2.3. Mineral analysis

Part of the cake was blended, from which 2 g was digested with concentrated nitric acid. The resulting solution was evaporated to dryness and dissolved in 100 mL deionized water. The solution was analyzed for mineral elements (calcium, potassium, iron, and zinc) using an atomic absorption spectrophotometer (AAnalyst 200, PerkinElmer) and a flame photometer (Jenway PFP7).

2.4. Phytochemical analysis

To have an idea of the active constituents of the formulated cake, its phytochemical properties were determined using standard methods.

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 and allowed to adapt for 1 week. They were provided with water ad libitum and maintained under standard laboratory conditions of natural photo period of a 12-hour light–dark cycle. Care of all animals was in accordance with the National Law on Animal Care and Use. 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 body weight of alloxan monohydrate in normal saline water in a volume of about 3 mL. After 72 hours 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 the following three groups, each consisting of six animals: Group 1—normal rats; Group 2—diabetic (untreated) rats; and Group 3—diabetic + high-fiber cake-fed rats.

The rats were monitored daily for food and water intake, and body weight. Their blood glucose levels were monitored on a weekly basis with a glucometer. Treatment lasted for 2 weeks. At the end of the feeding trials, the rats were fasted overnight and sacrificed humanely by cervical dislocation.

2.8. Collection of blood and preparation of serum

Blood was collected from each rat with a 5 mL syringe and needle by cardiac puncture, and transferred into clean EDTA and plain centrifuge tube bottles as soon as it was collected, to prevent lysing.

Part of the blood sample was centrifuged at 3000 rpm for 10 minutes, and the serum (supernatant) was transferred into labeled sample bottles. They were stored at 4°C to maintain enzyme activity.

2.9. Determination of hematological parameters

Hematology profile, which covers hemoglobin level (HGB), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC), platelets (PLT), lymphocytes (LYMP), and neutrophils (NEU), was determined using a Synchron CX5 autoanalyzer according to the manufacturer’s protocol.
2.10. Determination of serum biochemistry (liver and renal function enzymes, lipid profile)

Blood serum was used for the evaluation of biochemical parameters, including urea, creatinine, total bilirubin, conjugated bilirubin, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, using commercial kits from Randox Laboratories, UK, according to the manufacturer’s protocol.

Serum’s total cholesterol, triglyceride, and high-density lipoprotein (HDL) were measured by the enzymatic colorimetric method using Randox kits. The concentration of low-density lipoprotein (LDL) cholesterol was calculated using the formula of Friedwald et al.\textsuperscript{20}

Atherogenic Index (AI) and Atherogenic Index of Plasma (AIP) were calculated as described by Takasaki\textsuperscript{21} and Onat et al,\textsuperscript{22} respectively.

2.11. Statistical analysis

To address the biological variability, each set of experiments was repeated at least three times for $n = 3$ (phytochemical/mineral analysis) and $n = 5$ (experimental rats). Differences between the groups were analyzed 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 significance difference, and data were reported as mean ± standard deviation.

3. Results

3.1. Mineral composition

Table 1 depicts the composition of the studied minerals. A very high concentration of iron and calcium was observed, with concentration of iron being the highest. Zinc concentration was observed to be the lowest.

3.2. Phytochemical properties

Table 2 shows the composition of the studied phytochemicals. The saponin content of the cake was observed to be low compared to other phytochemicals. Phenol content was observed to be the highest, followed by flavonoid content.

3.3. Hematological indices

Results of the hematological indices are depicted in Table 3. Induction of diabetes led to a decrease in the WBC level; a significant ($p < 0.05$) reduction was also observed in the cake-fed rats. Altered levels of RBC, HGB, PCV, and PLT were observed in the untreated diabetic rats; feeding on the cake increased their levels significantly. A significant reduction was observed in the LYMP concentration of diabetic rats; feeding on the cake led to no significant increase. An increased level of NEU was observed in the untreated diabetic rats; a reduced level was observed in the cake-fed group, but it was not statistically significant.

3.4. Liver function enzymes

Induction of diabetes elevated the levels of the studied liver function enzymes, except for albumin (as depicted in Fig. 1). Feeding on the fiber-enriched cake resulted in significant ($p < 0.05$) reductions in the elevated levels.

3.5. Renal function enzymes

Elevated levels of the studied renal function enzymes were observed in the untreated diabetic rats, as shown in Table 4. Feeding on the fiber-enriched cake led to significant ($p < 0.05$) reductions in the elevated levels.
Fig. 2 shows an inverse correlation between serum creatinine level and HGB ($R^2 = 0.011$).

3.6. Lipid profile

Induction of diabetes led to increased levels of total cholesterol and LDL, as shown in Fig. 3. These were significantly ($p < 0.05$) reduced by feeding the rats with the fiber-enriched cake. Feeding on the cake was also observed to cause a significant ($p < 0.05$) elevation in the HDL level.

As depicted in Figs. 4 and 5, induction of diabetes led to increased AIP and AI, respectively, which were reduced significantly ($p < 0.05$) in the cake-fed group.

4. Discussion

Foods and nutrients play vital roles in the normal functioning of the body. Inclusion of dietary active compounds in human nutrition has been demonstrated to have tremendous health benefits and reduce the risk of chronic diseases such as colon cancer, obesity, diabetes, and diverticulosis.8,10 Their use has also been reported in the treatment and management of these chronic diseases.8,10 Dietary fiber is one such active compound.8–10 In this study, fibers from selected fruits were used to prepare a cake with the aim of studying its effect on the hematological parameters and serum biochemistry of diabetic rats.

The degree of anemia in diabetes patients can be associated with a number of factors, including glomerular filtration rate, urinary albumin excretion rate, and glycated h (HbA1c) levels.23 Anemia has been reported to be due to diminished erythropoietin production by failing kidneys and increased nonenzymatic glycosylation of RBC membrane proteins.24 In the present study, alterations in the RBC, HGB, and PCV levels of the diabetic rats suggest occurrence of anemia. The observed increase in these parameters on feeding the rats with the cake suggests its potency in the management of the ailment. This could be attributed to its phytochemical and mineral contents. The antioxidant properties of these phytochemicals, especially of phenols and flavonoids, have been reported in several studies.22 Their antisickling properties have also been reported.25 Thus, preventing oxidation of RBCs and HGB that often lead to hemolysis.26 It may also stimulate formation or secretion of erythropoietin in the stem cells of the animals, as evidenced by the increased level of RBC.27

![Liver function enzymes of the experimental groups](image1.png)

**Fig. 1.** Liver function enzymes of the experimental groups. ALB = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; T BIL = total bilirubin. Data are presented as mean ± SD; $n = 6$. *Statistically significant ($p < 0.05$) as compared with Group 1. **Statistically significant ($p < 0.05$) as compared with Group 2. ***Statistically significant ($p < 0.05$) as compared with Group 3.

![Correlation between creatinine level and hemoglobin level in the experimental groups](image2.png)

**Fig. 2.** Correlation between creatinine level and hemoglobin level in the experimental groups. HGB = hemoglobin level.

![Lipid profile of the experimental groups](image3.png)

**Fig. 3.** Lipid profile of the experimental groups. Chol = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; and TG = triglyceride. Data are presented as mean ± SD; $n = 6$. *Statistically significant ($p < 0.05$) as compared with Group 1. **Statistically significant ($p < 0.05$) as compared with Group 2. ***Statistically significant ($p < 0.05$) as compared with Group 3.

![Renal function enzymes of the experimental groups](image4.png)

**Table 4** Renal function enzymes of the experimental groups.

<table>
<thead>
<tr>
<th>Parameters (mg/dL)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid</td>
<td>39.55 ± 14.95**</td>
<td>57.00 ± 12.40***</td>
<td>25.67 ± 1.24*</td>
</tr>
<tr>
<td>Urea</td>
<td>2.92 ± 0.43**</td>
<td>6.02 ± 3.78****</td>
<td>3.92 ± 1.68***</td>
</tr>
<tr>
<td>Creatinine</td>
<td>11.58 ± 0.97***</td>
<td>13.86 ± 5.12***</td>
<td>6.76 ± 3.25***</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD; $n = 6$. *Statistically significant ($p < 0.05$) as compared with Group 1. **Statistically significant ($p < 0.05$) as compared with Group 2. ***Statistically significant ($p < 0.05$) as compared with Group 3.
The reduced levels of WBC, PLT, and LYMP in diabetic rats indicate a suppression of the immune system. These cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by phagocytosis. They form part of the innate immune system, which is also an important mediator in the activation of the adaptive immune system. The reduced immunity can contribute to the various complications associated with DM. Except for WBC, the cake was able to raise the others to an appreciable level compared to the control, suggesting a boost in the immune system. This increase can be attributed to the observed micronutrient qualities of the cake. It has been reported that the micronutrient deficiencies may have direct effects on the functioning of immune cells. Zinc has been shown to enhance the phagocytic activity of macrophages and neutrophils. Its supplementation for 1–2 months has been reported to restore immune responses, reduce the incidence of infections, and prolong survival. Iron deficiency has been associated with reversible abnormalities of immune function. Reduced polymorph neutrophil function has been shown to be reversed by iron administration.

The reduced level of the activities of the liver and renal function enzymes observed in the cake-fed groups signifies a protective potential of the cake against hepatotoxicity and renal toxicity. Increased serum levels of these enzymes have been reported in diabetic rats. This elevation has been attributed to the release of these enzymes from the cytoplasm into blood circulation after rupture of the plasma membrane and cellular damage. An increased level of creatinine has been associated with a low level of HGB, often leading to anemia. The reduced creatinine level in the diabetic rats was observed to be inversely proportional to the HGB level, thus correlating with the anemic potential of the cake.

Diabetes has been associated with elevated levels of total cholesterol, triglycerides, and LDL, which are also risk factors of cardiovascular diseases. These elevated levels observed in diabetics have been linked to increased mobilization of free fatty acids from the peripheral fat depots. The observed reductions of these parameters in the cake-fed group indicate the antilipemic potentials of the cake, which could be attributed to the fiber fortification in it. In several studies, fiber intake has been reported to be associated with higher levels of HDL and LDL in the blood. A mechanism put forth by Spiller is the increased generation of propionate, which has been shown to reduce cholesterol levels and inhibit cholesterol synthesis. Anderson et al also stated that reduced insulin response and altered lipid absorption are the likely factors in the effects of dietary fibers on hyperlipidemia.

The low values of AI and AIP in the cake-fed rats are due to the observed high HDL level. High AIP is a sign of obesity and cardiovascular diseases. Thus, its low value further indicates the antiliglicemic potentials of the cake against diabetic dyslipidemia.

In conclusion, these results indicate the potentials of the fiber-enriched cake as a functional food with therapeutic protective effects against hematological and biochemical changes associated with DM. Thus, it can serve as an adjunct to dietary therapy for diabetes.

Conflicts of interest

All contributing authors declare no conflict of interest.

References


