RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2013.601.605

Fibre-enriched Cake Attenuates Lipid Peroxidation, Hyperlipidemic and Modulates Redox Activities in Renal Tissues of Diabetic Rats

[1,2]Ochuko L. Erukainure, [2]Osaretin A.T. Ebuehi, [1]Folasade O. Adeboyejo, [1]Viola A. Nwachukwu, [3]Muhammad Aliyu and [1]Gloria N. Elemo

[1]Food Technology Division, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria

[2]Department of Biochemistry, University of Lagos, Idi Araba, Lagos, Nigeria

[3]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

Background: Oxidative stress has been demonstrated in several studies to play a major role in diabetic complications and pathophysiology including nephropathy. The aim of this study is to investigate the protective effect of feeding fibre-enriched cake on diabetes-induced oxidative stress and hypercholesterolemia in renal tissues of rats. **Materials and Methods:** Diabetes was induced by a single intraperitoneal injection of alloxan. Treatment lasted for 14 days, after which the rats were sacrificed by cervical dislocation. Renal tissues were used for the assessment of glutathione (GSH), catalase, superoxide dismutase (SOD) activities, protein content and lipid peroxidation as well as lipid profiles. **Results:** 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. Feeding on the cake led to decrease in lipid peroxidation, total cholesterol, LDL and triglycerides and increased concentration of HDL. **Conclusion:** These current findings suggest that the increased oxidative stress and hyperlipidemia in the renal tissues of alloxan-induced diabetic rats was effectively reduced and controlled by feeding on fibre-enriched cake corroborating and enhancing the established role of dietary fibre.

Key words: Fibre, oxidative stress, diabetes, hypolipidemia and antioxidant

Pharmacologia 4 (11): 601-605, 2013

INTRODUCTION

Diabetes mellitus has been described as a metabolic disorder which affects carbohydrate, lipid and protein metabolism. This is due to inadequate insulin release resulting to type 1 diabetes or insulin insensitivity as seen in type 2 diabetes (Barar, 2000; Auslander *et al*., 2002). It is characterized by hyperglycemia, which has been found to increase the generation of Reactive Oxygen Species (ROS). Increased ROS production combined with insufficient up regulation of antioxidant defense mechanisms leads to oxidative stress (Wrighten *et al*., 2009), which has been demonstrated in several studies to play a major role in diabetic complications and pathophysiology including nephropathy (Ugochukwu and Cobourne, 2003).

According to reports by Forbes *et al*. (2008), possible enzymatic and non-enzymatic sources of ROS in the diabetic renal pathology are autoxidation of glucose, transition metal-catalyzed Fenton reactions, Advanced Glycation End products (AGEs), polyol pathway flux, mitochondrial respiratory chain deficiencies, xanthine oxidase activity, peroxidases, Nitric Oxide Synthase (NOS) and NAD(P)H oxidase. Management of diabetic-induced oxidative stress poised by the increased production of the ROS in renal tissues might be protective and/or therapeutic against diabetic kidney damage (Ugochukwu and Cobourne, 2003).

Various plants have been reported to exhibit antioxidant activity, which protect against the damage caused by Reactive Oxygen Species (ROS). These plants are major source of fibre which has been shown to have tremendous benefits in the management of coronary heart disease, diabetes, obesity and some forms of cancer (Slavin, 2005; Nomura *et al*., 2007). The antioxidant properties of plant fibre have also been reported (Sakac *et al*., 2009).

This study aims at investigating the protective effect of feeding fibre-enriched cake on diabetes-induced oxidative stress and hypercholesterolemia in renal tissues of rats.

MATERIALS AND METHODS

Plant materials: Fruits with high fibre were identified as banana (*Musa species*), oranges (*Citrus sinensis*),

Corresponding Author: Ochuko L. Erukainure, Food Technology Division, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria

© 2013 Science Reuters, UK

watermelon (*Citrullus lanatus*), pineapple (*Ananas cosmosus*) and pawpaw (*Carica papaya*). The fruits were purchased from Ketu fruit market, Ketu, Lagos, Nigeria.

Production of fibre paste: The fruits 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. Four hundred gram of each fibre were weighed and blended together with 400g of pawpaw and banana respectively in a warring blender for 10 minutes to produce fibre paste.

Production of high fibre cake: Wheat flour (500 g) was weighed into a plastic bowl, to which 400 g of fibre paste was added. 100 g of margarine, 12 g of baking powder and 2 eggs were also added. They were mixed with a mixer to form dough. The dough was transferred into 2 baking pans greased with margarine and allowed to bake in an oven for 30 min at a temperature of 150°C. After baking, the cakes were allowed to cool and removed from the pans. They were wrapped with foil paper and thereafter preserved at 4°C for further use.

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 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.

Induction of diabetes: Diabetes was induced by a single intraperitoneal injection of 180 mg kg^{-1} 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^{-1}) were separated and used for the study.

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

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

Preparation of tissue homogenates: The organs (kidney) 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°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 (Lowry *et al*., 1951).

Determination of oxidative stress parameters: Lipid peroxidation was determined by measuring malondialdehyde (MDA) formed by thiobarbituric acid reaction (TBAR) (Chowdhury and Soulsby, 2002). Catalase (CAT) activity was estimated by measuring the rate of decomposition of H_2O_2 (Aebi, 1983). The level of Superoxide Dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972). While the method of Ellman (1959) was adopted in estimating the activity of reduced glutathione (GSH).

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 Friedewald *et al*. (1972).

Statistical analysis: Statistical significance was established using one-way analysis of variance (ANOVA) and data were reported as Mean+SD. Significant difference was established at $p<0.05$. Statistical analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL).

RESULT

There was no significant difference in the amount of food consumed by the experimental groups as shown in Fig. 1.

Table 1 depicts the antioxidant activities of the experimental groups. Induction of diabetes caused a significant reduction ($p<0.05$) of the GSH level, feeding on the formulated cake led to 60.18% restoration which was significant ($p<0.05$). Reduced levels of catalase and SOD activities were also observed in the untreated diabetic group. These were significantly ($p<0.05$) increased in the cake-fed group.

© 2013 Science Reuters, UK

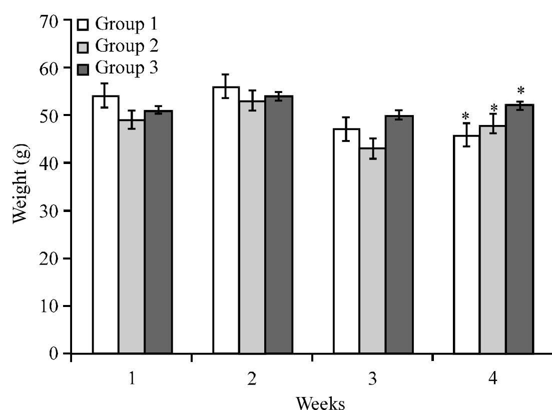

Fig. 1: Weight of feed consumed by experimental groups, values = Mean±SD, n = 6

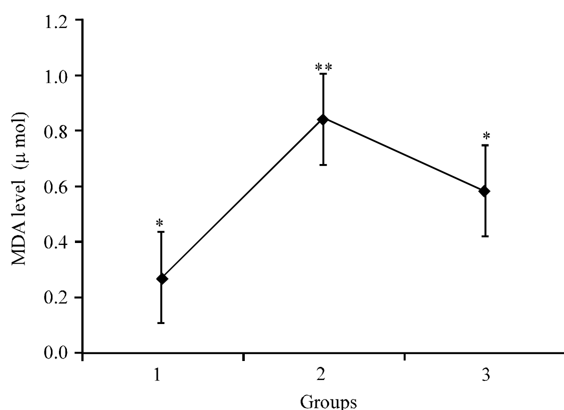

Fig. 2: MDA levels of renal tissues of experimental groups, values = Mean±SD, n = 6,*Significantly different ($p<0.05$) as compared to group 2. MDA: Malondialdehyde, **Significantly different ($p<0.05$) as compared to group 1 and 3

Table 1: Antioxidant activities of renal tissues of experimental groups

	Groups (µmol)		
Parameters	1	2	3
GSH	15.73±1.76*	4.42±1.00**	11.10±2.65*
SOD	237.60±8.81*	89.14±3.61**	144.28±12.26*
Catalase	1589.32±58.98*	596.31±24.20*	965.09±82.04

Mean±SD<n: 6, *Significantly different ($p<0.05$) as compared to group 1 and 3, **Significantly different ($p<0.05$) as compared to group 2. GSH: Reduced glutathione, SOD: Superoxide dismutase

Increased lipid peroxidation was observed in the untreated diabetic rats as presented in Fig. 2. This was significantly ($p<0.05$) reduced by feeding on the formulated cake.

The total protein content of the untreated diabetic rats was observed to be significantly reduced ($p<0.05$) as shown in Fig. 3. Feeding on the formulated cake led to a 15.02% increase of the content.

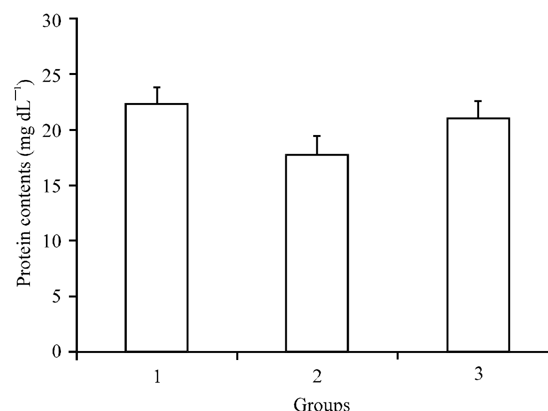

Fig. 3: Protein contents of renal tissues of experimental groups, values = Mean±SD, n = 6, *Significantly different ($p<0.05$) as compared to group 1 and 3

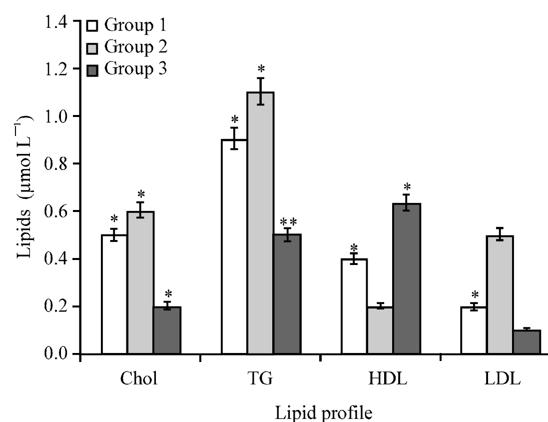

Fig. 4: Lipid profile of renal tissues of experimental groups, values = Mean±SD, n = 6, *Significantly different ($p<0.05$) as compared to group 2, **Significantly different ($p<0.05$) as compared to group 1 and 3. Chol: Cholesterol, TG: Triglyceride, HDL: High density lipoprotein cholesterol, LDL: Low density lipoprotein cholesterol

Induction of diabetes led to significant increases ($p<0.05$) in the total cholesterol (chol), triglyceride (TG) and low density lipoprotein cholesterol (LDL) levels as shown in Fig. 4. These were significantly reduced on feeding on the formulated cake. Reduced level of High Density Lipoprotein cholesterol (HDL) was also observed in the untreated diabetic group, this was significantly increased in the cake-fed group.

© 2013 Science Reuters, UK

DISCUSSION

Oxidative stress has been suggested in both experimental and clinical studies to play a major role in the pathogenesis of diabetes mellitus (Ugochukwu and Cobourne, 2003). Glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins leads to the production free radicals which causes a simultaneous decline of antioxidant defense mechanisms (Maritim *et al*., 2003). This study reports the antioxidant and antilipemic effect of fibre-enriched cake produced from selected fruits on renal tissues of diabetic rats.

In this study, the observed reduced GSH and increased lipid peroxidation in the diabetic rats indicates oxidative stress. GSH is a major endogenous antioxidant, which counteracts free-radical-mediated damage and a marker of oxidative stress (Erukainure *et al*., 2011). It forms an important substrate for other enzymes which is involved in the free-radical scavenging. Enhanced lipid peroxidation (LPO) has been reported in diabetes (Davi *et al*., 2003). LPO is a marker of oxidative stress (Onyema *et al*., 2006). It also leads to oxidants production from many molecules and thus amplifying oxidative damage. Increased level of GSH and reduced level of LPO in the cake-fed groups indicates the antioxidant potentials of the snacks. The other studied biomarkers, SOD and catalase were observed to increase in the cake-fed groups also indicating their antioxidant potentials. These enzymes decrease with oxidative stress (Nandhini *et al*., 2005). They play important roles in the maintenance of physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals (Pari and Latha, 2004) and eliminating organic peroxides and hydroperoxides generated from glucose oxidation. Food fibres have been shown to reduce blood glucose level and increase insulin sensitivity (Moharib and El-Batran, 2008). Thus it could be deduced that the fibre enriched cake attenuated the oxidative stress by reducing the amount of blood glucose available for oxidation. Hence can be considered an antioxidant.

The observed reduction in protein concentration in the untreated diabetic group can be attributed to production of MDA-protein adducts due to lipid peroxidation (Jakus *et al*., 2000), which is also a biomarker of oxidative stress. The increased protein content on feeding on the formulated cake further indicates its antioxidant potentials against diabetes-induced oxidative stress. Thus, further buttressing the protective potential of the snack against oxidative stress.

The burden of dyslipidemia has been reported to be high in diabetic patients (Georg and Ludvik, 2003). Although, several factors may contribute to the development of diabetic nephropathy, hyperlipidemia is considered an independent and major determinant of its progression (Rosario and Prabhakar, 2006). It is characterized by higher concentrations of total cholesterol, LDL and triglycerides but lower levels of HDL (Bonnet and Cooper, 2000). This was also observed in the untreated diabetic rats thereby confirming the occurrence of hyperlipidemia. However, feeding on the formulated cake revealed a hypolipidemia effect as evidenced by the reduced levels of total cholesterol, LDL and triglyceride but a higher HDL level. This indicates the protective potential of the cake against diabetic dyslipidemia in renal tissues thus preventing and/or disrupting the development and progression of nephropathy. Food fibres have been reported to reduce hyperlipidemia by binding and reducing the production of bile acids (NAS, 2005). Another mechanism put forth by Spiller (1993) is the increased generation of propionate, which has been shown to reduce cholesterol levels and inhibit cholesterol synthesis. Thus, the observed hypolipidemic effect of the cake can be attributed to the fibre enrichment which shows useful potentials in the management of the ailment.

CONCLUSION

These current findings suggest that the increased oxidative stress and hyperlipidemia in the renal tissues of alloxan-induced diabetic rats was effectively reduced and controlled by feeding on fibre-enriched cake. Thus, indicating the protective potential of the cake against renal diseases in diabetes.

REFERENCES

Aebi, H.E., 1983. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Vol. 3, Academic Press, New York, USA., pp: 273-286.

Auslander, W., D. Haire-Joshu, C. Houston, C.W. Rhee and J.H. Williams, 2002. A controlled evaluation of staging dietary patterns to reduce the risk of diabetes in African-American women. Diabetes Care, 25: 809-814.

Barar, F.S.K., 2000. Essentials of Pharmacotherapeutics. 3rd Edn., S. Chand and Co. Ltd., New Delhi, India, ISBN-13: 9788121904445, Pages: 606.

Bonnet, F. and M.E. Cooper, 2000. Potential influence of lipids in diabetic nephropathy: Insights from experimental data and clinical studies. Diabetics Metab., 26: 254-264.

Chowdhury, P. and M. Soulsby, 2002. Lipid peroxidation in rat brain is increased by simulated weightlessness and decreased by a soy-protein diet. Ann. Clin. Lab. Sci., 32: 188-192.

© 2013 Science Reuters, UK

Davi, G., F. Chiarelli, F. Santilli, M. Pomilio and S. Vigneri *et al*., 2003. Enhanced lipid peroxidation and platelet activation in the early phase of type 1 diabetes mellitus role of interleukin-6 and disease duration. Circulation, 107: 3199-3203.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.

Erukainure, O.L., J.A. Ajiboye, R.O. Adejobi, O.Y. Okafor and S.O. Adenekan, 2011. Protective effect of pineapple (*Ananas cosmosus*) peel extract on alcohol-induced oxidative stress in brain tissues of male albino rats. Asian Pac. J. Trop. Dis., 1: 5-9.

Forbes, J.M., M.T. Coughlan and M.E. Cooper, 2008. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes, 57: 1446-1454.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Georg, P. and B. Ludvik, 2003. Lipid and diabetes. J. Clin. Basic Cardiol., 3: 159-162.

Jakus, V., K. Bauerova, D. Michalkova and J. Carsky, 2000. Values of markers of early and advanced glycation and lipoxidation in serum proteins of children with diabetes mellitus. Bratisl Listy, 101: 484-489.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Maritim, A.C., R.A. Sanders and J.B. Watkins, 2003. Diabetes, oxidative stress and antioxidants: A review. J. Biochem. Mol. Toxicol., 17: 24-38.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Moharib, S.A. and S.A. El-Batran, 2008. Hypoglycemic effect of dietary fibre in diabetic rats. Res. J. Agric. Biol. Sci., 4: 455-461.

NAS, 2005. Dietary, Functional and Total Fiber. In: Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids, IOM (Ed.). Chapter 7, National Academies Press, Washington, DC., USA., pp: 339-421.

Nandhini, A.T.A., V. Thirunavukkarasu, M.K. Ravichandran and C.V. Anuradha, 2005. Effect of taurine on biomarkers of oxidative stress in tissues of fructose-fed insulin-resistant rats. Singapore Med. J., 46: 82-87.

Nomura, A.M., J.H. Hankin, B.E. Henderson, L.R. Wilkens and S.P. Murphy *et al*., 2007. Dietary fiber and colorectal cancer risk: The multiethnic cohort study. Cancer Causes Control, 18: 753-764.

Onyema, O.O., E.O. Farombi, G.O. Emerole, A.I. Ukoha and G.O. Onyeze, 2006. Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. Indian J. Biochem Biophys., 43: 20-24.

Pari, L. and M. Latha, 2004. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipidperoxidation in STZ diabetic male Wistar rats. BMC Complement. Altern. Med., Vol. 4, 10.1186/1472-6882-4-16

Rosario, R.F. and S. Prabhakar, 2006. Lipids and diabetic nephropathy. Curr. Diabetes Rep., 6: 455-462.

Sakac, M.B., J.F. Gyura, A.C. Misan and Z.I. Seres, 2009. Antioxidant properties of sugarbeet fibers. Sugar Ind., 134: 418-425.

Slavin, J.L., 2005. Dietary fiber and body weight. Nutrition, 21: 411-418.

Spiller, G.A., 1993. CRC Handbook of Dietary fiber in Human Nutrition. 2nd Edn., CRC Press, Boca Raton, Fl., USA.

Ugochukwu, N.H. and M.K. Cobourne, 2003. Modification of renal oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with extracts from *Gongronema latifolium* leaves. Clin. Chim. Acta, 336: 73-81.

Wrighten, S.A., G.G. Piroli, C.A. Grillo and L.P. Reagan, 2009. A look inside the diabetic brain: Contributors to diabetes-induced brain aging. Biochim. Biophys. Acta, 1792: 444-453.

© 2013 Science Reuters, UK