Full Length Research Paper

Effect of traditional diets on oxidative stress and lipid profile of alloxan induced diabetic rats

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The objective of this study was to compare the effect of recommended diet namely, plantain porridge and beans with other traditional Nigerian diets on oxidative stress and lipid profile in alloxan diabetic rats. Therefore, in the design, six groups (1 non diabetic and 5 diabetic) of 7 rats each, were accordingly treated: the normal and diabetic controls were fed normal rat pellets; garri with afang soup, pounded yam with edikang ikong soup, ekpang nkukwo, and the control diet (plantain porridge with beans) respectively, were fed to the other diabetic groups. The diets and water were given *ad libitium* and this lasted for 15 days after which biochemical indices of oxidative stress and lipid profile were respectively determined in liver homogenates and serum of the experimental animals. Superoxide dismutase (SOD) activity significantly increased (p<0.05) in all diet fed rats relative to the diabetic control. Although the increase was highest in the control diet, it was not significant (p>0.05) with respect to the traditional diets. The reverse was the case with Glutathione peroxidase (GPx) activity, which was significantly decreased in the control diet compared to the traditional diets and diabetic control. Yet the extent of lipid peroxidation (malondialdehyde, MDA) was seen to be highest in the control diet. The effects of the traditional diets and the control diet on lipid profile were similar. These traditional diets could be more effective in amelioration of oxidative stress in dietary management of diabetes mellitus.

Key words: Traditional diets, oxidative stress, lipid profile, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a complex disorder characterized by chronic hyperglycemia which results from malfunction in insulin secretion and/or insulin action, both causing impaired metabolism of glucose, lipids and proteins (Mayfield, 1998; Kim et al., 2006). It is prevalent worldwide and known to be one of the major causes of death. The prevalence of diabetes in urban African communities is increasing with ageing of the population and changes in lifestyle associated with urbanization and westernization. However the prevalence is still low in traditional rural communities, about 1-2%, except in some specific high risk groups, where about 1-3% or more adults have diabetes (Sobngwi et al., 2001). The combination of rising prevalence of diabetes and the high rate of long-term complications in Africans is believed to lead to a drastic increase of the burden of diabetes on health systems of African countries in the very near future.

The chronic hyperglycemia of diabetes is associated with long-term complications viz- dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels. These complications are being initiated and propagated by oxidative stress processes. Several workers have reviewed the role of oxidative stress (Table 1) in diabetes mellitus (Zozulinskaet al., 1998). Oxidative stress is characterised by an increased concentration of O2- derived products that provoke critical and most times irreversible, cell injury. Oxygen reduction leads to the synthesis of reactive intermediate com-pounds collectively known as reactive oxygen species (ROS) and peroxidative derivatives of polyun-saturated fatty acids (PUFA) such as conjugated dienes, lipid hydroperoxides and malonyl dialdehyde (MDA). In diabetes, an altered oxidative metabolism is a consequence either of the chronic exposure to

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Table 1. Effect of diets on oxidative stress indices of alloxan diabetic rats.

Group	Liver SOD activity (U/g tissue)	Serum GPx activity (unit/ml)	Liver MDA Conc. (U/g tissue)
Normal control (rat pellets)	1457.67+95.56	910.90+39.78	89.28 + 5.08
Diabetic control (rat pellets)	848.51+50.66 a	1240.22+63.89 a	165.28+ 9.03 a
Diet 1 (garri with afang soup)	1124.43 + 40.00 b	1170.14+ 99.44	309.33+10.74 b
Diet 2 (pounded yam with edikang ikong)	1110.19+ 26.98 b	1133.12+ 98.81	320.86+11.48 b
Diet 3 (ekpang nkukwo)	1037.50 + 53.12 b	1124.08+61.00	345.55+20.58 b
Diet 4 (reference - plantain with beans)	1147.76+33.06 b	735.48 + 64.15	364.34+18.15 b

Values are expressed as Mean ± SD, n = 7, a = $p<0.05$ vs Normal control, b = $p<0.05$ vs Diabetic control and values with identical superscripts within a column are not significantly different ($p>0.05$).

hyperglycaemia or of the absolute or relative insulin deficit. Insulin regulates several reactions involved in oxido-reductive metabolism (Baynes, 1991; Sinclair, 1993).

The toxic oxygen species produce serious derangement in cell metabolism, including DNA destruction, modification of proteins and also induced damage to membrane ion transporters and/or other specific proteins. Hydrogen peroxide produced in the course of oxidative stress participates in the development of diabetic vascular complications (Packer, 1993). As in similar conditions, oxidative stress in diabetes mellitus stems mainly from increased or excess production of free radicals and/or a sharp reduction of antioxidant defenses. It is well known that superoxide anion is the primary radical formed by the reduction of molecular oxygen that may lead to secondary radicals or reactive oxygen species (ROS), On the other hand, there is evidence that diabetes induces changes in the activities of antioxidant enzymes in various tissues (Ahmed, 2005). For instance experimental diabetic rats were shown to have decreased SOD activity in liver, kidney, heart, muscle and erythrocytes (Matkovics et al., 1982). Carmeli et al. (2004) also found that in healthy control subjects the levels of SOD activities in erythrocytes showed marked progressive increase with aging, especially after age 60, whereas in the diabetic individuals erythrocyte SOD activities were elevated to a much lesser degree with aging. Accordingly this enzyme has been used as a marker of oxidative stress.

One important effect of free radicals activity is lipid peroxidation (Morel et al., 1983) and the final product of this reaction is malonyldialdehyde (MDA), which is commonly accepted as the marker of the lipid peroxidative processes. Therefore measurement of serum MDA will assess the intensity of oxidative processes in diabetic patients as well as in other physiological and pathological conditions. Zozulinska et al. (1998), found the serum MDA in patients with type 2 diabetes significantly elevated. Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders including naturally occurring and chemically induced diabetes mellitus. Consequently, mechanisms in the formation of lipid hydroperoxides and biologically active metabolites, together with their effect on cellular structure and functioning are becoming of increasing importance to the study of diabetogenesis (Ahmed, 2005). Glutathione peroxidase (GPx) is an enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water. Carmeli et al. (2004) and Matkovics et al. (1982) have variously shown the enzyme's role in monitoring the severity of diabetes mellitus.

On the bases of the above, the present study used these indices of oxidative stress to monitor response to changes in diets of diabetic subjects. Dietary regimens in diabetes management are a major challenge in diabetic care both to the patient and the care giver firstly, due to the patients' inability to comply, given the monotony of the dietary prescriptions, and secondly that most recipes for the recommended diets are seasonal, relatively scarce and indeed expensive. It is imperative therefore to explore the option of a variety of food stuffs available in the traditional setting to whom the patients are culturally adapted to. We accordingly compared in this study, the effect of some traditional diets of the Efik and Ibibio in Cross River State of Nigeria with the regular recommended diets by health care givers.

MATERIALS AND METHODS

Animals and experimental design

Male Wistar rats (140-240 g) obtained from the animal house, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria, were used for the study.   The animals were kept in wooden cages with stainless wire mesh top in well ventilated animal house. Diabetes was induced by a single dose intra-peritoneal injection of 150 mg/kg body weight of alloxan after a twenty four hour fast (Esmerino et al., 1998). A week after injection of alloxan, diabetes was confirmed in alloxan-treated rats showing fasting blood glucose levels from 200 mg/dl (Kim et al., 2006). The experimental animals were then divided into six (6) subgroups as shown here and treated accordingly:

1. Normal rats fed normal rat pellets (normal control)

Table 2. Effect of diets on serum lipid profile of alloxan diabetic rats.

Group (mg/dl)	Normal control (rat pellets)	Diabetic control (rat pellets)	Diet 1 (garri with afang soup)	Diet 2 (pounded yam with edikang ikong)	Diet 3 (ekpang nkukwo)	Diet 4 (reference- Plantain with beans)
Triglyceride	73.66±11.37	114.50±70.47a	207.25±27.80b,c	111.88±19.03	160.76±9.03b	139.87±38.65
Total Cholesterol	63.35±5.31	70.01±8.71	81.71±20.64 b,c	66.42±10.24	66.48±13.80	63.21±4.89
HDL-Cholesterol	21.97±3.7	21.79±2.54	23.12± 4.97	26.36± 9.78	24.17±4.32	21.23±3.9
VLDL- Cholesterol	14.69±2.27	17.78±3.25	35.7±14.81 b,c	21.45± 4.81	26.52±10.15	23.67±7.12
LDL-Cholesterol	26.68±5.29	30.42±7.59	22.92±10.30 c	18.61±5.15 b	15.83±7.74 b	18.43±1.99 b

Values are expressed as Mean ± SD, n = 7, a = p<0.05 vs Normal control, b = p<0.05 vs Diabetic control and c = p<0.05 vs other diets.

2. Diabetic rats fed normal rat pellets (diabetic control)
3. Diabetic rats fed garri with afang soup (test diet 1).
4. Diabetic rats fed pounded yam with edikang ikong (test diet 2).
5. Diabetic rats fed ekpang nkukwo (test diet 3).
6. Diabetic rats fed plantain porridge with beans (diet 4 - reference diet).

The diets and water were both given *ad libitum* for fifteen (15) days after which the animals were sacrificed and sera and liver tissues collected for biochemical assays.

Preparation and processing of the diet

The foodstuffs used for the preparation of the various traditional diets were bought at Ika Ika Oqua Market in Calabar, Cross River State of Nigeria. After initial washing and draining, these were used to prepare the experimental diets according to the indeginous methods including garri with "afang" soup, pounded yam with "edikan ikong" soup, "ekpang nkukwo", and plantain porridge with beans.

The proportions of the condiments used only, were modified from "A taste of Calabar" selected recipes by Ana (2000). Each of the diets was oven dried at 60°C to complete dryness then homogenized with a blender. The diets in the blended forms were wrapped in alluminium foils and kept in a microwave oven from where portions were withdrawn for daily feeding of the animals.

Sera and tissue preparation

At the end of the 15-day treatment the rats were after a 12 h fast euthanized under chloroform vapor and sacrificed. Whole blood was collected via cardiac puncture, emptied into plain tubes and allowed to clot for about two hours. The clotted blood was thereafter centrifuged at 3,000 rpm for 10 min and serum recovered from clotted cells and used for GPx activity and lipid profile analysis. The livers were also surgically removed and immediately perfused in heparinized saline (0.9% NaCl) to remove blood cells and thereafter blotted with blotting paper then stored frozen. Exactly 1 g of the tissue was weighed and thoroughly homogenized in 10ml of freshly prepared phosphate buffer (20 mM; pH 7.4). The homogenate was then centrifuged at 3,000 g for 10 min using table centrifuge (B. Bran Scientific and Instrument Company, England) and the supernatant decanted into clean tubes and used for MDA and SOD assays.

Biochemical assays

The assay kits for determination of these oxidative stress indices were obtained from Oxis Research TM 323 Vintage Park Drive, Suite B Foster City, CA 94404-1136 U.S.A. The assay methods for SOD activity and total (free and protein-bound) (MDA) concentration in the rat hepatocytes were based on the principles described by Nebot et al. (1983) and Gernard-Monnier (1998) respectively, whereas that for GPx activity was Colorimetric based on Paglia and Valentine (1967). Assay kits obtained from Randox Laboratories Ltd., Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom, Bt294QY were used to estimate serum lipids (Table 2) including total cholesterol, triacylglycerol and HDL- cholesterol based on the method of Tiezt (1990).

Statistical analysis

The results were analysed for statistical significance by one way ANOVA using the SPSS statistical program and Post Hoc Test (LSD) between groups using MS excel program. All data were expressed as Mean ± SEM. p values < 0.05 were considered significant.

RESULTS

Effect of diet on SOD activity

SOD activity of the diabetic control (848.51 ± 50.66) was significantly decreased at p<0.05 compared to the non-diabetic control rats (1457.67 ± 95.56). Upon a 15-day feeding both the traditional and reference diets caused significant increases (p<0.05) in SOD activity compared to the diabetic control. Although the increase in reference diet was higher than the traditional diets, this difference was however not significant (p>0.05).

Effect of diets on GPx activity

GPx activity of non diabetic rats (910.90 ± 39.78) was significantly lower at p<0.05 when compared with the diabetic control (1240.22 ± 63.89). Within the test groups, GPx activity was significantly increased (p<0.05) in the traditional diets: pounded yam with edikang ikong soup, (1133.12 ± 98.81), garri with afang soup (1170.14 ± 99.44), and ekpang nkukwo (1124.08 ± 61.00), relative to the reference diet (plantain porridge with beans) which had the lowest activity (735.43 ± 64.15).

Effect of diets on MDA concentration

There was observed significant increase in MDA level of diabetic control rats (165.28 ± 9.03) following alloxan treatment when compared to the non diabetic control (89.28±5.08). Within the diabetic test groups MDA concentration was highest in the reference diet (364.34±18.15) and significant ($p<0.05$) when compared to the levels in the traditional diets: garri with afang soup (309.33±10.74), pounded yam with edikang ikong soup (320.86±11.48) and ekpang nkukwo (345.55±20.58).

Effect on serum lipid profile

There was significant increase ($p<0.05$) in serum triglyceride (TG) of untreated diabetic rats relative to the non diabetic control. Within the diabetic test diets, the traditional diets, except garri with afang soup, had serum triglyceride levels within a close range with the reference diet, although these were significantly high ($p<0.05$) compared to the normal control value of (73.66 ± 11.37). There was non significant difference ($p>0.05$) in total cholesterol (TC) concentration between diabetic control and non diabetic control rats. The effect of the traditional diets (except garri with afang soup) on TC was similar to that of the reference diet and both compared well with the normal control. LDL-cholesterol concentration decreased significantly in all test groups and the extent of decrease (except garri with afang soup) compared well with the reference diet. No significant changes were observed in HDL and VLDL cholesterols following diet treatments. However VLDL cholesterol was significantly increased in rats fed diet 1 (garri with afang soup) compared to diabetic control and other diets.

DISCUSSION

In the present study, it was observed that MDA level, a lipid peroxidation product and a marker of oxidative stress was elevated and SOD activity decreased both significantly in untreated diabetic rats compared to non diabetic control rats. These agree with previous reports of other researchers. For instance Mahdi et al. (2003) have demonstrated elevated plasma lipid peroxide levels in streptozotocin-induced diabetic rats along with a significant decrease in the anti-oxidant enzyme, superoxide dismutase activity. Whereas Oberley (1988) reported elevated serum lipid peroxide levels and diminished antioxidant status in diabetic subjects. Mahboob et al. (2005) observed a significant elevation in MDA level and decrease in glutathione and protein content in both male and female diabetic patients in comparison to non-diabetic controls. Oxidative stress occurs when there is an imbalance between production and scavenging of free radicals. Increase in lipid

peroxidation in diabetes mellitus is due to excess formation of free radicals. Reduced superoxide dismutase enzyme and lack of reduced glutathione are other causes for oxidative stress as is observed in this report.

Antioxidant enzyme-dependent defenses play important role in scavenging free radicals produced under oxidative stress, our data reveal that glutathione, an antioxidant was higher in the rats fed on the different traditional diets compared to those fed plantain porridge with beans (reference diet), this could probably be due to antioxidant nutrients present in the vegetables used in the traditional diets. Sailaja et al. (2003) reported that diabetic humans have shown increased lipid peroxidation and decreased levels of gluthathione peroxidase, but no significant changes in superoxide dismutase (SOD) activity was observed in type 2 diabetic patients (Kesavulu et al., 2000). Diabetic adult rats have also been reported to show an increase in MDA level, where as antioxidant enzymes such as reduced glutathione, superoxidee dismutase and glutathione peroxidase activities were markedly diminished in comparison to controls (Sailaja et al., 2003).

This study revealed combined hyperlipidaemia and hypertriglyceridemia in untreated diabetic condition similar to the reports by Idogun et al. (2007) among diabetic patients. Essien et al. (1992) had earlier reported mean total cholesterol level for diabetes mellitus to be slightly higher than those of controls. This wholly is in line with our observation in this study. The association of atherosclerosis and dyslipidaemia with diabetes was recognized as early as 1927 by Joslin. Premature and extensive atherosclerosis involving renal, peripheral and cardiovascular sites remain major complications of diabetes mellitus. In addition to hyperglycemia, systemic or local elevations in insulin may contribute to aberrant lipid metabolism and vascular wall function (Lyons, 1992). Alterations in serum lipid profiles are known in diabetics, which are likely to increase the risk of coronary heart disease (Laakso, 1996; Steiner 1999; Massing et al., 2001). Garg et al. (1988) suggested partial replacement of complex carbohydrates with monounsaturated fatty acids in the diet of patients with type 2 diabetes, which according to them, does not increase the level of LDL cholesterol, and may improve glycemic control and the levels of plasma triglycerides and HDL cholesterol. Also Chandalia et al. (2000) reported that high-fibre diet resulted in a lower fasting plasma total cholesterol concentration by 6.7%, a lower triglyceride concentration by 10.2% and a lower VLDL cholesterol concentration by 12.5%, the fasting plasma LDL cholesterol concentration was 6.3% lowered and no change in HDL cholesterol concentration. This probably may be the case with our diets in this study, where observably total and LDL cholesterols were significantly reduced upon feeding with the traditional diets, but without any change in HDL cholesterol. Research suggests that low fat diets are usually associated with modest weight loss, which can be

maintained as long as the diet is continued (Lichtenstein et al., 1994). Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease (Huang et al., 1988). Therefore lowering of serum lipid levels whether diet or drug induced would no doubt decrease the risk of vascular disease and related complications. This obviously would account for the observed hypolipidemic effects of these diets particularly due to the antioxidant action of the active agents in the soup vegetables. Many herbs and vegetables have variously been shown to have antihyperglycemic and antihyperlipidemic properties (Brown et al., 1993).

Conclusion

In conclusion traditional diets may be used alongside plantain porridge with beans by diabetic individuals given the semblance in their antioxidant and hypolipidemic properties reported in this study.

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