Antioxidant Effect of Three Varieties of Premature Plantain (*Musa Paradisiaca*) On Oxidative Stress in Alloxan-Induced Diabetic Rats

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ABSTRACT

Effect of antioxidant activities of three different species of premature plantain on oxidative markers in alloxan-induced diabetic rats was studied. Adult albino rats were divided into 5 groups (n=5) and treated as follows: Group 1- Non- diabetic control; Group 2- diabetic control. Both Groups 1 and 2 received standard commercial animal feed only. Group 3 - diabetic rats fed with premature plantain diet (sample A), Group 4 - diabetic rats fed with premature plantain diet (sample B) and Group 5 - diabetic rats fed with premature plantain diet (sample C). Blood concentration of Malonylaldehyde, glutathione, catalase, sugar, and weight of rats were recorded from each group on the first and last week of the experiment. The proximate analysis and evaluation of in vitro antioxidant activity of the premature plantain samples were undertaken. The diabetic rats placed on premature plantain diet had a decrease in their blood glucose and malonylaldehyde levels with corresponding increase in their weight, glutathione and catalase levels compared with diabetic control rats. The premature plantain extracts also had a high scavenging activity of DPPH radical and high total antioxidant activity. The finding from this study suggested that premature plantain intake may improve the antioxidant status of rats. The three different species of premature plantain used in the study had no significant difference in their nutrients.

KEY WORDS: Antioxidant, oxidative stress, premature plantain, diabetic rats, *Musa paradisiaca*.

INTRODUCTION

Diabetes is the third ranking cause of death and remains one of the most crippling of all diseases. This is because of its chronicity and complication (WHO, 2003). In addition to the degenerative complications, diabetes causes blindness and is associated with acute infections. Until the roles of the multiple factors implicated in the pathogenesis are clearly defined, the treatment will probably remain palliative. The importance of research directly at etiology of diabetes cannot be overemphasized since it may ultimately lead to the prevention and / or cure of the disease.

In recent studies, some evidence suggests that oxidative stress may play some role in etiology of diabetes and its complications.

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Nouroz et al (1997) has reported an altered balance between Reactive Oxygen Species (ROS) production and antioxidants.

Though insulin therapy is used for management of the disease, there are still draw backs like insulin resistance as well as high cost. In addition, most drugs that are being used in the management of the disease have been associated with serious side effects. This has led to an increase in research into medicinal plants with anti-diabetic potential because the herbal drugs are considered to be less toxic with fewer side effects compared with synthetic drugs (Omoregie and Osagie, 2006).

In addition, dietary management of diabetes has been helpful. Unripe plantain is usually consumed by Nigerian diabetics to reduce post-prandial glucose level (Ayodele and Godwin, 2010). Plantain (*Musa paradiaca*) belongs to the natural order of plantaginacea which contains more than 200 species, 30 of which have been reported to be of domestic use (FAO, 1990). Plantain is one the highly nutritious food that is consumed in the world. However, the mechanism by which the unripe plantain ameliorates diabetes mellitus has not been fully investigated.

An indirect way of assessing the level of oxidation stress is to measure the level of antioxidant compounds present in body fluids. Oxidative stress is related to the levels of antioxidant compounds and enzymes. The concentration of these compounds correlates with the balance between their intake and production and their uses during the inhibition of free radical compounds (Blanck, 2003). Free radicals are simply molecules with impaired electrons, and thusly with these free electrons can easily catalyze reactions that may be harmful to the body. Antioxidants help neutralize highly reactive molecules before they can do damage. This work was aimed at investigating the antioxidant activity and effect of three different varieties of *Musa paradisiaca* on oxidative stress in diabetic rats.

MATERIALS AND METHODS

Collection of Samples

Three bundles of three varieties (samples) of premature plantain were collected from different locations in Akwa Ibom State of Nigeria as follows: sample A (from Effiat Offot, Uyo LGA); sample B (from Attai Obio Offot, Itu LGA) and Sample C (from Nwut Usung Itam, Itu LGA). They were identified and confirmed to belong to be varieties of *Musa paradisiaca* by a botanist from the department of botany and ecological studies, University of Uyo, Nigeria. The local names (in Ibibio) of the samples are as follows: sample A (Okoyo), sample B (Odurosuk) and Sample C (Eba-Oboikpa).

Preparation of Plantain Flour

The premature plantain from each of the samples (A, B and C) was peeled, sliced, sun dried to a constant weight and ground into flour. The flour was sieved, stored in an air tight container and kept at room temperature for further use.

Preparation of plantain extract for antioxidant assays

The preparation was done according to the modified method of Bios (1985). Each of the processed premature plantain flour (1 g) and methanol (20 ml) were thoroughly mixed and left over night. Each of the mixture was filtered and made up to 25 ml with methanol. Each of the methanolic premature plantain extract (40 mg/ml) was serially diluted to suitable concentrations of 250, 125, 62.5, 31.25 and 15.52 (µg/ml) which were used for the DPPH (2,2, diphenyl-1-picnyl hydrazine) radical scavenging test while the concentrations of 10, 20, 30, and 40 mg/ml were used for total antioxidant activity assays.

Proximate Analysis of Premature Plantain Flour

The percentage moisture, crude protein, crude fibre, crude fat and total carbohydrate of each species were analyzed according to AOAC (1990) methods.

Assay of DPPH radical scavenging activity

The free radical scavenging activity of each species of premature plantain extract was evaluated using the Biois' (1985) method with some modifications. One ml of different concentrations (500, 250, 125, 62.5 and 31.25 µg/ml) of each sample extracts and standard vitamin C were added to 1 ml of 0.3 mM DPPH in methanol to bring the final concentrations to 250, 125, 62.5 and 31.25 µg/ml. Each mixture was vortexed and incubated in a dark chamber for 30 mins and the absorbance read at 517 nm against DPPH control that contained 1 ml of methanol. The percentage scavenging activity of each species of premature plantain was calculated as

% scavenging activity =

$$\frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Assay of total antioxidant activity

The total antioxidant activity was evaluated according to the method of Kirkoynan (2003) by determining the scavenging activity of the extract on ABTS (2.2, azinobis 3- ethylbenzlthiazoline-6- sulfonic acid) radical. A measured quantity (0.2 ml) of peroxidase (4.4 units /ml) + 0.2 ml of H_2O_2 (50 µM) + 0.2 (ABTS, di-ammoniun salt, 100 µM) + 1 ml distilled water were mixed together and left in the dark to from a bluish green complex. After adding 1 ml of plantain extract, the absorbance was measured at 734 nm to represent the total antioxidant activity. The total antioxidant activity was calculated as follows:

Total oxidant activity =

$$\frac{1 - (\text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Experimental Animals

Fifteen adult albino rats weighing 110-120 g were used for the study. The animals were acclimatized for a period of 7 days to the laboratory conditions prior to the experiment in line with University's animal ethics guidelines. They were housed in well ventilated cages (3 rats per cage) with 12 hr light- dark cycle. They had free access to drinking water and were fed (*ad libitum*) with standard pellet feed.

Induction of Diabetes

Rats were fasted for 24 hrs before intraperitoneal injection of freshly prepared alloxan tetrahydrate (sigma, USA) solution at a dose of 153 mg/kg body weight. Blood glucose from tail vein was tested three days after using one-touch glucometer. Rats with blood glucose level greater than 15 mmol were considered to be diabetic and were used in the diabetic groups of rats.

Administration of Extracts

The rats were divided into 5 groups and treated as follows: Group 1- Non- diabetic control, Group 2- diabetic control. Both Group 1 and 2 received standard commercial animal feed only. Group 3 - diabetic rats fed with premature plantain diet (sample A), Group 4 - diabetic rats fed with premature plantain diet (sample B) and Group 5 - diabetic rats fed with premature plantain diet (sample C). Blood concentration of Malonylaldehyde, glutathione, catalase, sugar, and weight of rats were

recorded from each group on the first and last week of the experiment.

Percentage change in weight was calculated as;

Final weight – Initial weight x 100

Initial weight

The percentage growth rate was calculated as;

Final weight – Initial weigh x 100

Experimental Duration

The percentage change in fasting blood glucose was calculated as;

Final fasting blood glucose level – Initial fasting blood glucose level x 100

Initial fasting blood glucose

Determination of Malonylaldehyde MDA

The method of Health and Parker (1968) with slight modification was employed. A measured amount (0.2 ml) of blood plasma was added to 3 ml of glacial acetic acid followed by addition of 3 ml of thiobarbituric acid (TBA) solution. The mixture was placed in boiling water for 15 mins and allowed to cool before being read spectrophometrically at 520 nm. One percent TBA was prepared by dissolving 1 g of TBA in 100 ml of the 2% sodium hydroxide. The standard curve was plotted using the MDA derivatives (1, 1, 3, 3 tetraethoxypropane).

Determination of Whole Blood Glutathione

This was done using the method of Fleming (1997). The principle is based on the determination of reduced glutathione in each dilution by the measurement of the absorbance of colored solution developed within 5 mins of generation of Elman's reagent at 430 nm wavelength.

Determination of Plasma Glucose

The principle of oxidation of β- D glucono 1,5 Lactone with the release of hydrogen peroxide by glucose oxidase which later hydrolyze gradually β -D gluconic acid was employed using a Randox kit for the assay. The absorbance of the mixture was measured at 625 nm using ortholidine as the color reagent.

Determination of Red Cell Catalase Activity

The method of Cohen et.al (1970) was employed. The activity of the enzyme that catalyzed the decomposition of hydrogen peroxide H_2O_2 was monitored using potassium tetraoxomaganate VII ($KMnO_4$). Fifty (50µl) of sample was added to to a test tube. H_2O_2 was then added to the tube and incubated on ice for 3 mins. Then H_2SO_4 was added to stop the reaction. Finally $KMnO_4$ was added and absorbance was recorded at 480 nm.

$2MnO_4^- + 5\ H_2O_2 + 6H^+ = 5O_2 + 2Mn^{+2} + 8\ H_2O$

In this assay, 1 unit of enzyme activity k = 0.00693 Where $k = \log \frac{So}{S_2} \times \frac{23}{t}$

So = Absorbance of standard – absorbance of blank

S_2 = Absorbance of standard – absorbance of sample

T = time interval

STATISTIC ANALYSIS

Data was subjected to analysis of variance using SPSS software. Results are presented as means ± SEM. Differences between means were considered to be significant at $p < 0.05$ using the Duncan Multiple Range.

RESULTS

Results of the proximate analysis of premature plantain flour are shown on Table 1

Table 1: Proximate composition of premature plantain flour of the three varieties

	Percentage composition		
	SAMPLE A	SAMPLE B	SAMPLE C
Ash	4.00±0.00	3.4± 0.01	3.51± 0.04
Carbohydrate	36.50 ± 0.49	35.04 ± 0.08	36.32 ±0.05
Protein	3.12 ± 0.02	3.25 ±0.02	3.16 ± 0.01
Lipid	0.50 ± 0.10	0.40 ± 0.00	0.46 ± 0.02
Crude fibre	5.91 ± 0.91	5.84 ±0.92	5.85 ± 1.09
Moisture	49.96 ±0.01	51.41±0.03	50.44 ±0.04

The three species premature plantain analyzed had almost the same proximate composition. The percentage of carbohydrate contents ranged from 35.04% to 36.50%. They also had low lipid and protein values.

Table 2: Inhibiting action on DPPH radical

Sample	**Percentage Inhibition**
Sample A	76.60 ± 0.0
Sample B	74.50 ± 0.0
Sample C	75.4 ± 0.0
Premature plantain flour and Vitamin C	
Sample A	45.10 ± 0.05
Sample B	46.02 ± 0.04
Sample C	45.02 ± 0.04

The premature plantain flour had 74.50 to 76.60 % range of inhibiting action on DPPH radical comparable to that Vitamin C (45.02 to 46.02%). They also possess a significant total antioxidant activity in addition (60.40 to 61.02) (Table 2 and 3).

Table 3: Total antioxidant activity of premature plantain flour

Sample	Activity
Sample A	61.02 ± 0.01
Sample B	60.40 ± 0.02
Sample C	61.00 ± 0.02

The results of animals studied indicate that the values of glutathione (GSH) in the non diabetic animals were higher than that of diabetic animals. The diabetic animals fed with premature plantain flour had higher glutathione, and catalase levels than control diabetic rats. The glucose and MDA levels were also lower, with increase in weight, than in the diabetic control rats as shown on Table 4.

Table 4: Indices of oxidative stress in diabetic rats

parameter	Non diabetic Rats	Diabetic Rats Control	Diabetic Rat + Premature Plantain Flour (PPF) ppf A	ppfB	ppfC
GSH (mg/dl)	60.03 ± 10.16[a]	26.04 ± 2.18[b]	46.05 ± 1.06[c]	45.08 ± 5.05[c]	46.04 ± 7.04[c]
MDA (mg/ml)	0.12 ± 0.02[a]	0.43 ± 0.14[b]	0.13 ± 0.02[c]	0.12 ± 0.04[c]	0.12 ± 0.03[c]
Catalase (umol/min/ml)	50.76 ± 10.11[a]	25.64 ± 10.32[b]	36.05 ± 10.71[c]	37.04 ± 11.81[c]	36.10 ± 10.52[c]
Glucose (mg/dl)	55.5 ± 12.65[a]	154. ± 10.71[b]	68.4 ± 12.22[c]	67.5 ± 11.41[c]	68.3 ± 12.32[c]
Weight (g)	160 ± 0.18[a]	86 ± 0.05[b]	132 ± 0.10[c]	131 ± 0.20[c]	132 ± 0.30[c]

Values with same superscript along each row are not significantly different (p< 0, 05). GSH = Glutathione, MDA = Malonyladehyde, ppf = premature plantain flour ppf A = premature plantain flour sample A, ' ppf B= premature plantain flour sample B, ppf C = premature plantain flour sample C

DISCUSSION

Out of thirty species of *Musa paradisiaca* (plantain) reported to be in domestic use (FAO, 1990), three species of the premature plantain were used in the study. The proximate composition of the three species showed no significant difference including other contents analyzed. The result showed each contained significant quantities of ash which reflects mineral contents of the plantain. Plantain is one of the highly nutritious foods that are consumed in the world. It is low in fat and crude protein when processed for consumption (Samuelsen, 2005). This is also confirmed in the present study. The moderate amount of carbohydrate analyzed from each species of premature plantain flour could also have a moderate glycemic index. The glucose levels in the diabetic rats fed with premature plantain diet were lower than that of diabetic control rats. Premature plantain diet had a beneficial effect on blood glucose level.

Dietary fibre is increasingly being recognized as a useful tool for the control of oxidative process in food products. And, consuming carbohydrate meal containing fat, protein and / or fibre will slow the rise in blood glucose by slowing down its entry into the small intestine where it is absorbed (Jenkin, 1981). Fibre also forms viscous solutions that slow glucose absorption from intestine. In addition, dietary fibre decreases the absorption of cholesterol from the gut in addition delaying the digestion conversion of starch to simple sugars, an important factor in the management of diabetes. Thus the high fibre content of the premature plantain flour, as observed in this study,

infer that premature plantain could be effectively utilized in the management of diabetes mellitus.

Epidemiological observations identified relationship between diets high in phytochemicals and a reduction in chronic disease. Further evaluations of these foods have led researchers to specific phytochemicals that are responsible for health benefits (Craigh, 1997; Thomas, 1999). Phytochemicals analyzed in this study include saponins, flavonoids, tannins and alkaloids. Saponins act as antioxidants that scavenge reactive oxygen and nitrogen. Saponins are known to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic permeabilization of intestine) properties (Price et al, 1987). However, the levels of saponins in the premature plantain are quite low to cause any deleterious effects.

Antioxidants are substances which when present in diet decreases the adverse effects of reactive species such as free radicals in the living human body.

The DPPH assay is a widely accepted method for the determination of antioxidant activities in various food substances according to Bios (1985). DPPH is a stable free radical in methanol or aqueous solution. The present study had high scavenging activity of the methanolic extract of premature plantain flour on DPPH. This may be attributable to the polyphenolic contents of the premature plantain flour.

The assay of the scavenging activities of extracts on ABTS/ H_2O_2/HRP discoloration has been reported to represent the total antioxidant activity of methanolic extracts of variety substances. ABTS assay is also stable and does not suffer from the color interference as DPPH assay. Oxidative stress is imposed on cells as a result of many factors including a decrease in antioxidant protection or failure to repair oxidative damage. Cell damage is caused by Reactive Oxygen Species (ROS) which are either free radicals, reactive anions containing oxygen atoms that can either produce free radicals or are chemically activated by them (Blanck, 2003). From the present study, result obtained showed that premature plantain flour could be used as a source of natural antioxidant in the treatment of ailment implicating free radicals and oxidative stress.

The depletion in the glutathione status of the diabetic rats is attributable primarily to the alloxan that was injected in the rats. The depletion in glutathione to the level observed in this work could lead to a devastating decrease in total antioxidant status of the animals because glutathione helps in recycling cellular antioxidants, inhibit free radical damage and also plays role in the detoxification of harmful compounds. Dominquez et al (1998) and Polidovi et al (2000) reported a reduced total plasma antioxidant capacity in uncontrolled diabetics. However, the administration of premature plantain to the diabetic rats resulted in significant amelioration of their glutathione status.

Malonyaldehyde (MDA) as biomarker for oxidative stress is one of the frequently used indicators for lipid peroxidation (Fleming et al, 1997). There was an elevation in the malonyladehyde levels of diabetic rats. Diabetic patients had an increase in plasma malonyladehyde levels during postprandial period (Cerello et al., 1998). However, the diabetic rats placed on premature plantain diet had a decrease in their plasma malonyladehyde levels. This is an indication of the free radical scavenging activity of

premature plantain on oxidative stress in diabetics. Catalase is in the blood and liver. Its function is assumed to be the destruction of oxygen peroxide.

Hydrogen peroxide is a product of a number of cellular oxidation reactions and catalase is believed to be present in cells in order to prevent H_2O_2 accumulation.

The depletion of whole blood catalase activity in the diabetic induced rats is another important finding in this study. Some studies have reported no alteration in the activity of red cell catalase in diabetes. However, the results obtained are in agreement with earlier reports of Udoh et al (2007) and Targami et al (1992) of a decrease in red cell catalase activity in diabetes. It is therefore important to note that drastic decrease in antioxidant status of the body could cause oxidant stress with a concomitant attack of reactive oxygen species or free radicals on some cells of some target tissues or organs of the body. However, the diabetic rats fed on premature plantain diet had an increase in their catalase concentrations. This shows that the premature plantain diet has ability to improve the altered antioxidant status of diabetics.

CONCLUSION

Raised blood glucose level in diabetes can deplete cells of their antioxidant status with concomitant increase in free radicals, thereby predicating oxidative stress. The guiding principles for the treatment of diabetes are early detection and prevention of complications. Healthy diet forms the foundation for good diabetes management. The free radical scavenging activity of premature plantain in diabetic has been demonstrated in this study. The premature plantain decreases the oxidative stress through the restoration of altered antioxidant status. Premature plantain flour has been found to be a good source of antioxidants. This property could be one of the justifications for its utilization in diabetes therapy.

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