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Total Antioxidant Capacity, Nutritional Composition and Inhibitory Activity of Unripe Plantain (*Musa paradisiacae*) on Oxidative Stress in Alloxan Induced Diabetic Rabbits

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Abstract: The antioxidant composition of unripe plantain and its free radical scavenging activity on alloxan induced diabetic rabbits and on DPPH radical was investigated. 10 male rabbits weighing between 1.58 and 1.88 kg were used for this study. Diabetes was induced in the experimental rabbits with alloxan (35 mg/kg body weight. ip). Group 1 rabbits served as the control groups and they received normal rabbit feeds. Group 2 rabbits were the animals of group 1 which were made diabetic by the injection of alloxan and they also received normal rabbit feeds while group 3 rabbits (test groups) were the animals of group 2 which were later fed with unripe plantain at a dosage of 25 kg/kg body weight/day for 4 weeks. The duration of the experiment was 7 weeks and the weights of the animals in each group were recorded daily throughout the experiment while the blood glucose levels, malonaldehyde, catalase and glutathione were recorded on a 2 weeks interval. The results show that the diabetic rabbits placed on unripe plantain diet had an increase in their body weights, glutathione and catalase levels but a decrease in malonaldehyde and blood glucose levels after 4 weeks of unripe plantain intake when compared with the control ($p<0.05$). Correlation analysis carried out revealed that glutathione correlated negatively with malonaldehyde and glucose ($r = -0.77$ and -0.89), but positively with catalase and body weight ($r = 0.60$ and 0.70). Malonaldehyde correlated negatively with catalase and body weight ($r = -0.44$ and -0.72) but positively with glucose ($r = 0.86$). The antioxidant composition of the methanolic extracts of the unripe plantain flour as determined by the quantities of peroxidase and quercetin present was 52±0.00% peroxidase and 5.32 ug/ml quercetin while its free radical scavenging activity on DPPH radical was 78.57±0.00%. Analysis of the proximate and phytochemical composition of the unripe plantain flour showed that it contained 3.16±0.04% protein, 0.21±0.003% lipid, 52±2.82% moisture, 5.5±0.42% ash, 1.58±0.04% tannin, 1.82±0.05% saponin, 1.37±0.05% alkaloid and 0.98±0.00% flavonoid. These findings suggest that raised blood glucose level in diabetics could deplete cells of their antioxidant status by decreasing the glutathione and red cell catalase levels leading to an increased malonaldehyde level which is a marker of oxidative stress. In addition, unripe plantain intake by a diabetic could exert a free radical scavenging activity by restoring the altered antioxidant status since itself could serve as a natural source of antioxidants.

Key words: Diabetes, nutritional composition, rabbits, unripe plantain flour, free radical scavenger, oxidative stress, total antioxidant capacity

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases with hyperglycemia. World wide, an estimated 150 million people are affected by diabetes mellitus (Expert committee on the diagnosis and classification of diabetes mellitus, 1998) and this number is likely to reach 300 million by the year 2025 if successive strategies are not implemented for its prevention and control (King *et al*., 1999).

In recent studies, some evidence suggest that oxidative stress may play some role in the etiology of diabetes and its complications (Shin, 1998). Nourooz-zadeh *et al*. (1997) has reported an altered balance between Reactive Oxygen Species (ROS) production and antioxidants.

Though insulin therapy is used for the management of the disease, there are still draw backs like insulin resistance (Piedrola *et al*., 2001), as well as of its high cost which are not affordable in the poor economic community. Treatment with sulphonylureas and biguanides are also associated with side effects (Rang *et al*., 1991).

In India, use of herbal drugs based on *Ayurvada* has been commonly practiced for a long time and it is less

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expensive. The herbal drugs are considered to be less toxic with fewer side effects when compared with synthetic drugs (Geetha *et al*., 1996; Rao *et al*., 2003).
In addition, dietary management of diabetes has been helpful and such diets used in the management of diabetes include beans, breadfruit and in Nigeria, unripe plantain diet.
However the mechanism by which unripe plantain flour ameliorates diabetes mellitus has not been fully investigated. There's indication that this could be through antioxidant activity since some of the phytochemical constituents could serve as antioxidants. Also the unripe plantain could have a low glycaemic response when consumed. This present work is aimed at investigating the above.

MATERIALS AND METHODS

Chemicals: Quercetin and DPPH (2,2-diphenyl-1-picrylhydrazyl) used were products of Sigma Chemical Company (UK). Peroxidase used was purchased from Horseradish. All other chemicals used were purchased from Associated Laboratories, Aba, Abia State, Nigeria.

Plant materials: Unripe plantain used was bought locally from the market in Umuahia, Abia State, Nigeria. It was thoroughly washed, peeled and freeze dried in a freeze drier for 48 h.

Preparation of plant materials for analysis: The peeled portion of the unripe plantain was ground into flour using a food processor and the flour was then used for analysis.

Proximate composition of unripe plantain flours: Moisture, crude protein, crude fat and total carbohydrates were analyzed according to the AOAC methods (1990). The values reported are means of triplicate samples with their standard deviations.

Phytochemical composition of unripe plantain flour: The gravimetric method of Harbone (1973) was used in the determination of the total alkaloid content while the AOAC method (1984) was used in the determination of other phytochemical constituents of the sample.

Assay of DPPH radical scavenging activity: The free radical scavenging activity of the plantain extract was determined using the modified method of Blois (1985). 1 ml of different concentrations (500, 250, 125, 62.5, 31.25 ug/ml) of extracts and standard quercetin were added to 1 ml of 0.3 mm DPPH in methanol to bring the final concentration of 250, 125, 31.25 and 15.62 ug/ml. The mixture was vortexed and incubated in a dark chamber for 30 min and the absorbance read at 517 nm against a DPPH control which contained 1 ml of methanol.

The Percentage Inhibition was calculated as:

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

Assay of total antioxidant activity: The total antioxidant activity was measured according to the method described by Hsu *et al*. (2003). 0.2 ml of peroxidase + 0.2 ml of H_2O_2 (50 um) + 0.2 ml ABTS (100 um) + 1 ml distilled water were mixed together and left in the dark to form a bluish green complex.
After adding 1 ml of methanolic plantain flour extract, the absorbance was measured at 734 nm to represent the total antioxidant activity.

Animal experiments

Selection of animals and their care: 10 matured rabbits weighing between 1.58 and 1.88 kg were used for this experiment. Animals were acclimatized for a period of 7 days to the laboratory conditions prior to the experiment. Rabbits were housed in colony cages with 2 rabbits per cage at room temperature with 12 h light and dark cycle and they had free access to drinking water and their diets.

Chemicals: Alloxan used was obtained from Sigma and Aldrich. Malonaldehyde derivative (1,1,3,3 tetraethoxypropane) and Stock Glutathione used were also obtained from Sigma and Aldrich Chemical Company, UK. All other chemicals used for the animal experiments were bought from Associated Laboratories, Aba, Nigeria and were of analytical grade.

Induction of diabetes: Rabbits were fasted for 24 h before injection of a freshly prepared solution of alloxan intra-peritoneally at a dosage of 35 mg/kg body weight. This single dose of alloxan produced type 1 diabetes having fasting blood sugar level of 155±10.71 mg/dl after 10 days of injection of alloxan and this diabetic state was maintained throughout the duration of the experiment.

Experimental procedure

The rabbits were divided into 3 groups as follows:
(Group 1) Control group: The animals of this group received normal rabbit feeds. After feeding them for about 1 week, their body weights and fasting blood sugar levels were taken. Other parameters which included glutathione, malonaldehyde and whole blood catalase levels were also taken and recorded.

(Group 2) Diabetic rabbits without unripe plantain feed: At the expiration of 1 week, alloxan was injected intraperitoneally into the control group and they formed group 2 animals. The animals were confirmed diabetic after estimation of their fasting blood sugar level, 2 weeks after injection of alloxan. An animal was

considered to be diabetic if it had a fasting blood sugar level > 115 mg/dl. Other parameters which included body weight, catalase, plasma glutathione and malonaldehyde were also taken and recorded.

(Group 3) Diabetic rabbits after unripe plantain feed: At the expiration of 2 weeks of induction of diabetes into the animals of group 2, they were force fed with unripe plantain flour for a period of 4 weeks and they thus formed the animals of group 3. At the end of 4 weeks, their fasting blood glucose levels was estimated and recorded. Other parameters which included body weight, catalase, glutathione and malonaldehyde levels were also taken and recorded.

Determination of plasma malonaldehyde (MDA): The method of Health and Parker (1968) was used with slight modification. 0.2 ml of blood plasma was added to 3 ml of glacial acetic acid followed by 3 ml of thiobarbituric acid solution. The mixture was placed in boiling water for 15 min, allowed to cool before being read spectrophotometrically at 532 nm.

Preparation of thiobarbituric acid (TBA): 2% Sodium hydroxide was prepared by dissolving 2 g of sodium hydroxide in 100 ml of water. Then 1% TBA was prepared by dissolving 1 g of TBA in the 100 ml of the 2% sodium hydroxide. The standard curve was plotted using the MDA derivative (1,1, 3, 3 tetraethoxypropane).

Determination of whole blood glutathione: The principle was based on the determination of reduced glutathione in each dilution by the measurement of the absorbance of colored solution developed within 5 min of the generation of Elman's reagent at 430 nm wavelength.

Determination of plasma glucose: The principle of oxidation of β-D glucose to β-D glucono 1, 5 lactone with the release of hydrogen peroxide by glucose oxidase which later hydrolyses gradually to β-D gluconic acid was employed. The absorbance of the mixture was measured at 625 nm using ortholidine as the color reagent.

Determination of red cell catalase activity: The principle of Cohen *et al.* (1970) was made use of here by monitoring the rate of enzyme catalyzed decomposition of hydrogen peroxide (H_2O_2) using Potassium tetraoxomanganateVII ($KMnO_4$). 50 microlitre of sample was added to a test tube. H_2O_2 was then added to the tube and incubated on ice for 3 min. H_2SO_4 was used to stop the reaction. Finally, $KMnO_4$ was added and the absorbance recorded at 480 nm. In this assay,

$$1 \text{ unit of enzyme activity} = \frac{K}{0.00693}$$

where

$$K = \frac{So}{S_2} \times \frac{2.3}{t}$$

Where So = Absorbance of standard-absorbance, S_2 = Absorbance of standard-absorbance of sample. T = Time interval. The measured activities were normalized with the protein content of each sample.

Statistical analysis: Statistical analysis was conducted using the mean ± standard deviation of three experiments. The experimental design used was Completely Randomized Design while results were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition of unripe plantain flour: In the study carried out, the proximate composition of the locally consumed unripe plantain flour showed that it contained low quantities of ash which reflected the mineral contents of the plantain (Table 1). Plantains have been reported to contain low quantities of minerals (Ketiku, 1973).

Table 1: Proximate composition of unripe plantain flour

Proximate analyzed	Percentage composition
Ash	5.50±0.420
Carbohydrate	39.14±0.212
Protein	3.15±0.042
Lipid	0.21±0.028
Moisture	52.0±2.8

Each value in the table was obtained by calculating the mean ± std of 3 experiments carried out on the unripe plantain

The low fat contents obtained in the unripe plantain flours (Table 1) were in accordance with previous reports (Agunbiade *et al.*, 2006).

The low crude protein content obtained in the plantain flours (Table 1) were also in accordance with previous studies (Brakohiapa *et al.*, 2001). Since a healthy adult needs about 0.75 g of protein per kg per day, plantains alone cannot meet adult protein diet.

The low total carbohydrate obtained in the unripe plantain flour would be expected since unripe plantain contains large amount of starch and low sugar in its green stage (Table 1). Similar results have been reported by Ahenkora *et al.* (1998).

The moisture content was also found to be high and this is in agreement with earlier reports (Ketiku, 1973).

Phytochemical composition of unripe plantain flour: The phytochemical composition of the unripe plantain flour showed that it contained significant quantities of saponins, flavonoids, tannin and alkaloids (Table 2).

Saponins are known to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic

permeabilization of the intestine) properties (Price *et al*., 1987). However, the levels of saponin in the flour are quite too low to cause any deleterious effects.

Flavonoids, alkaloids and tannins are polyphenolic compounds with antioxidant properties. Phenolics have been associated with antioxidant properties of food (Robbins, 2003). It has been reported that phenolic compounds in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (Kirkosyan *et al*., 2003).

The present study shows that unripe plantain flour contains considerable amount of phenolics and this implies that it may be useful in relation to diseases involving free radical reactions.

Antioxidant activity of unripe plantain flour: The antioxidant activity of the methanolic extract of unripe plantain flour as determined in this study is presented in Table 3. The extract of the unripe plantain flour showed a remarkable antioxidant activity and this would be expected since analysis showed that it contained phenolics and phytochemicals which are high potency antioxidants with free radical scavenging activities. The results obtained show unripe plantain flour to be a potential natural source of antioxidants that could be of medicinal purposes in the treatment of ailments implicating free radicals and oxidative stress.

Table 2: Phytochemical composition of unripe plantain flour

Phytochemical	Percentage composition
Tannin	1.577±0.004
Alkaloid	1.37±0.048
Saponin	1.827±0.0042
Flavonoid	0.981±0.0014

Each value in the table is the average of 3 experiments ± standard deviation

Table 3: Total antioxidant activity of unripe plantain flour

Antioxidant	Activity
Peroxidase	52±0.00%
Quercetin	5.32ug/ml

The results are the means of triplicate experiments ± standard deviation

Table 4: Inhibitory activity of unripe plantain on DPPH radical

Free radical	Percentage inhibition
DPPH	78.57±0.06

The value in the table was derived by calculating the average of 3 experiments ± standard deviation

Inhibitory activity of unripe plantain flour: The high scavenging activity of the methanolic extract of the unripe plantain flour on DPPH radical is a major significant finding in this study (Table 4). This is attributable to the phenolic content and presence of other phytochemicals in the unripe plantain. However, we could not prove if the free radical scavenging activity came solely from the phenols present or other phytochemicals or a combination of both.

Animal experiments: There was a significant reduction in the reduced glutathione levels of the diabetics when compared with the control (Table 5). This depletion in blood glutathione is attributable primarily to the alloxan injected in the rabbits, a xenobiotic and an inducer of diabetes.

Both xenobiotics and normal metabolism are known to deplete antioxidants as they are consumed in the course of scavenging reactive species generated. The depletion in glutathione to the level that was observed in this work could lead to a devastating decrease in the total antioxidant status of the animals because glutathione helps in recycling cellular antioxidants, inhibits free radical damage and plays a key role in the detoxification of harmful compounds (Robert *et al*., 2000). This agrees with earlier works carried out by Dominquez *et al*. (1998) and Polidori *et al*. (2000) who reported reduced total plasma antioxidant capacity in uncontrolled diabetes. However, unripe plantain intake by the diabetic rabbits increased their glutathione status to near the control level and this is remarkable as this implies that unripe plantain diet could have an ameliorating effect on the altered antioxidant status of a diabetic.

The concentration of plasma Malonaldehyde (MDA) was shown to be significantly increased in diabetic rabbits without unripe plantain when compared with the control (Table 4). This was also attributed to the alloxan that was injected into the rabbits. Ceriello *et al*. (1998) have reported that diabetic patients show during the postprandial period, an increase in plasma malonaldehyde levels. However, the diabetic rabbits, when placed on unripe plantain diet also remarkably had a decrease in their plasma malonaldehyde levels (Table 5) when compared with the control, thus indicating the free radical scavenging activity of unripe plantain on oxidative stress in diabetics.

Table 5: Comparison of some parameters of oxidative stress in alloxan induced diabetic rabbits before and after unripe plantain intake

Parameter	Non-diabetic rabbits (Control)	Diabetic rabbits b/f unripe plantain intake	Diabetic rabbits after unripe plantain intake	p-value
GSH (mg/ml)	61.08±11.16	27.09±3.18	48.09±9.39	<0.05
MDA (mg/ml)	0.115±0.02	0.437±0.15	0.129±0.02	<0.05
Catalase (umol/min/ml)	51.78±11.15	155.8±10.71	68.4±12.58	<0.05
Glucose (mg/dl)	55.51±12.65	155.8±10.71	68.4±12.58	<0.05
Weight (kg)	1.73±0.15	0.86±0.05	1.317±0.098	<0.05

Reported values are the means ± standard deviations (n = 10). NS = Not Significant; S = Significant; GSH = Glutathione; MDA = Malonaldehyde; b/f = before

The depletion of whole blood catalase activity after injection of alloxan is another significant finding in this study (Table 5). The decreased concentration of red cell catalase is attributable in part to the reduced synthesis of this antioxidant enzyme (which functions in the detoxification of hydrogen peroxide) whose concentrations would have fallen with the alloxan that was injected into the animals. Some studies have reported no alterations in the activity of red cell catalase in diabetics (Dohi *et al*., 1998). However, this is in agreement with earlier reports by Udoh *et al*. (2007) and Tagami *et al*. (1992) who reported a decreased red cell catalase activity in diabetics. It is important to note at this point that the drastic decrease in the antioxidant status of the body could precipitate "oxidant stress" with a concomitant attack of reactive oxygen species or free radicals on cells of some target tissues or organs of the body. However, the diabetic rabbits placed on unripe plantain diet had an increase in their catalase concentrations and this again indicates the ability of unripe plantain diet to restore the altered antioxidant status of diabetics.

The concentration of fasting blood glucose was increased in the alloxan induced diabetic rabbits. Alloxan is known to destroy the β-cells of the islets of the langerhams of the pancreas that function in the regulation of insulin secretion and thus leads to an increase in the concentration of blood glucose. However, this parameter was decreased significantly in the diabetic rabbits placed on unripe plantain diet. This is in agreement with earlier works done by Gomathy *et al*. (1990) who reported a hypoglycemic action of the pectin present in the juice of plantain. Chhanda *et al*. (2006) have also reported that diminished serum insulin level in streptozotocin induced diabetic rats was recovered significantly after co-administration of methanolic extracts of *Euglena Jambolana* and *Musa Paradisiaca*. They noted that the fasting blood sugar level came towards the control level gradually after supplementation of the seed of *E. jambolana* and *M. paradisiaca* in separate ways.

The alloxan induced diabetic rabbits had a marked loss in body weight (Table 5). This would be expected as one of the effects of diabetes is body weight loss. With the destruction of the pancreatic cells by alloxan, there's deficiency of insulin leading to increased synthesis of ketone bodies which are excreted in urine. The increased synthesis of ketone bodies coupled with increased lipolysis leads to a severe body weight loss. However, the diabetic rabbits placed on unripe plantain diet had a remarkable gain in body weight (Table 5).

Correlation analysis carried out revealed that glutathione correlated negatively with malonaldehyde and glucose (r = -0.77 and -0.89), but positively with catalase and body weight (r = 0.60 and 0.70). Malonaldehyde correlated negatively with catalase and body weight (r = -0.44 and -0.72) but positively with glucose (r = 0.86). The free radical scavenging activity of unripe plantain in diabetics has been demonstrated in this study. There's indication that unripe plantain flour mimics insulin action by binding to specific receptors in the cell membrane of tissues (in a way similar to insulin) possibly at the α-subunits of receptors that are transduced to the β-subunits, promoting the rapid autophosphorylation of a specific tyrosine residue of each β-subunit and inducing a conformational change, one of which is decreased oxidative stress through the restoration of altered antioxidant status. This is approached from a biochemical point of view and is subject to further confirmation.

In addition, it has been found to be a good source of antioxidants and this property could be included to the purpose for which its been utilized in diabetics.

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