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ORIGINAL ARTICLE

Effect of Livingstone Potato (*Plectranthus esculentus* N.E.Br) on Hepatic Glucose-6-Phosphophate Dehydrogenase Activity of Streptozotocin Induced Diabetic Rats

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ABSTRACT

Context To determine the effect of livingstone potato (*Plectranthus esculentus* N.E.Br) on hepatic Glucose-6-phosphate dehydrogenase (G6PD) activity in streptozotocin induced diabetic rats. **Methods** The G6PD activities in the liver homogenates of the rats and chemical analysis of the test feeds were determined using standard techniques. **Results** The diabetic control rats had significant alteration (P<0.05) of their hepatic G6PD activities compared with the non-diabetic rats. Intake of the test feed by the diabetic rats of group 3, resulted in significant (P<0.05) amelioration of their hepatic G6PD activities in comparison with the diabetic control rats. Chemical analysis of the test feed revealed that it contained considerable amounts of tannins, saponins, alkaloids, antioxidants and their cyanide contents were below the toxic level for humans. **Conclusion** The study shows ameliorative potentials of livingstone potato on the hepatic G6PD activity of diabetic rats which is attributed to its antioxidant/polyphenolic constituents.

INTRODUCTION

Glucose 6 phosphate dehydrogenase (G6PD) catalyzes the first step in the pentose phosphate pathway (conversion of glucose 6-phosphate to 6-phosphogluconate) with the generation of NADPH required for the maintenance of reduced glutathione and for bile acid synthesis [1].

The NADPH that is provided by this enzyme is the main source of reducing equivalents for the lipogenic pathway thereby making this enzyme a crucial lipogenic enzyme [2]. G6PD also plays an important role in preventing oxidative damage to human cells by participating in the generation of antioxidants. The NADPH generated by this pathway is used as an electron donor for multiple intracellular reactions such as the regeneration of reduced glutathione (GSH), an important intracellular antioxidant, thereby offering protection to cells against oxidative stress [3].

Umesh *et al*. [4] reported a decrease in the hepatic glucose-6-phosphate dehydrogenase activity of streptozotocin diabetic rats. Similarly, Debidas *et al*. [5] reported that a reduction in the G6PD activity in diabetic rats contributed to a reduction in metabolism via the gluconate oxidative pathway.

In our previous study [6], we described for the first time, the anti-diabetic potentials of Livingstone potato in streptozotocin induced diabetic rat models. However, there is no experimental evidence presently available in literature with regard to its effect on the G6PD activity of diabetic rats.

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Since supplementation of therapeutics with plant based antioxidants/polyphenols could have chemoprotective role in the management of diabetes mellitus [7] and in a bid to further understand the mechanism of anti-diabetic action of Livingstone potato, this study was set out to investigate the phytochemical composition, antioxidant capacity and effect of Livingstone potato on the hepatic G6PD activity of streptozotocin diabetic rat models.

MATERIALS AND METHODS

Plant Materials

The *Plectranthus esculenta* varieties were obtained at harvest from National Root Crops Research Institute, Umudike, Nigeria. They were identified by NRCRI, Umudike that has Livingstone potato as a National Mandate as well as by a Taxonomist in Michael Okpara University of Agriculture, Umudike, Nigeria and deposited in their herbarium for authentication.

Chemicals

Streptozotocin, β-Nicotinamide Adenine Dinucleotide Phosphate-Sodium Salt, Triethanolamine Hydrochloride, Glucose-6-phosphate monosodium salt, magnesium chloride salt, 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical and Quercetin used were products of Sigma and Aldrich Chemical Company, United Kingdom. Other chemicals used were purchased from Hoslab, Umuahia, Abia State, Nigeria and were of analytical grade.

Processing of the Plant Materials

The samples were properly washed, chopped and oven dried at 60°C for 48 hours to constant weight. The dry samples were then processed to flour and incorporated into the standard rat feeds at 19.55% incorporation.

ANIMAL EXPERIMENTS

Selection of Animals

Thirty male albino rats of the wistar strain (140-208g) obtained from the animal house of the Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria were used for the study. The animals were kept in metabolic cages in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria. They were acclimatized for two weeks to their diets prior to the commencement of the experiment and were maintained under a constant 12-h light and dark cycle and a room temperature of 27-30°C. The National Institutes of Health Principles of Laboratory Animal Care [8] were observed.

Induction of Diabetes

Freshly prepared solution of streptozotocin (0.1g dissolved in 5 ml of freshly prepared sodium citrate buffer 0.1 M, pH 4.5) was injected intraperitoneally to the rats at a dosage of 65mg/kg body weight at fasting state. Blood was collected from the tail vein and the blood glucose concentration was analyzed prior to the commencement of the dietary feeding using a blood glucose meter (Double G glucometer, USA). The STZ-treated rats with fasting blood glucose levels > 200 mg/dl after seven days of induction of STZ and with evidence of glycosuria were considered to be diabetic and used for the study.

Experimental Procedure

The experimental rats with stable diabetic condition were then divided into 2 subgroups (groups 2 and 3) comprising of six animals per group while the non-diabetic group formed the first group as follows:

Group 1. Normal rats fed standard rat pellets (Non-diabetic control)

Group 2. Diabetic control rats fed standard pellets

Group 3. Diabetic rats fed Livingstone potato incorporated feeds.

BIOCHEMICAL ANALYSIS

Their diets and water were both administered *ad libitum* for 28days after which the rats were stunned by blow, sacrificed and their liver was removed, washed with ice-cold physiological saline immediately and stored at -20°C until analyzed. Ten percent homogenate (w/v) of the liver was prepared in 150 mM KCl using a homogenizer at 4°C and centrifuged for 15 min at 4°C (9) and the supernatant was analyzed for glucose-6-phosphate dehydrogenase activity. The specific activity of NADP linked glucose-6-phosphate dehydrogenase activity (EC 1.1.1.49) was determined using the method of Noltmann *et al.* [9, 10].

DPPH Radical Scavenging Assay

The method of Blois [11] was used with modifications. A measured amount (5 g) of each sample was dissolved in 100ml of methanol to give a stock concentration of 50mg/ml and left for 3 to 4 hrs. The mixture was filtered with Whatmann No 1 filter paper through vacuum pump. The resulting solution was diluted with methanol to final concentrations of 50, 100, 150, 200 and 250μg/ml respectively. Finally, 0.1ml of 0.3mM DPPH (in methanol) was added to each of the reaction mixtures and the whole setup was well shaken and left in the dark for 30mins before the absorbance was read spectrophotometrically at 517nm against the DPPH control that contained 1 ml of methanol only in place of the extract. The percentage scavenging activity was calculated as:

[(Absorbance of control - Absorbance of sample)/Absorbance of control]*100.

Quercetin was used as the standard for this assay.

Reducing Power Assay

The method of Hsu *et al.* [12] was used with modification. A measured amount (2 g) of each sample was dissolved in 40 ml of methanol to give a stock concentration of 50mg/ml and left for 3 to 4 hrs. It was filtered with Whatmann No 1 filter paper through vacuum pump, made up to 50 ml with methanol to give a concentration of 40 mg/ml and diluted to suitable concentrations: 5, 10, 15, 20 and 25 mg/ml for reducing power assay. One ml of each extract + 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium hexacyanoferrate solution (1% in water) were placed in a test tube and reacted for 20 min at 50°C. The tubes were cooled with crushed ice and an aliquot of 0.5 ml trichloroacetic acid (10% in water) was added. After centrifugation at 3000 g for 10 min, an aliquot (1 ml) of the supernatant was mixed with an equal volume of distilled water + 0.1 ml of ferric chloride (0.1% in water) and the set up was allowed to react for 10 min before the absorbance was measured at 700nm using a UV spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power. Quercetin was used as the standard.

PHYTOCHEMICAL ANALYSIS

Determination of Alkaloids

The gravimetric method of Harborne [13] was used in the determination of total alkaloid content of the test feed. Five gram of each sample was dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4hours before filtering. It was evaporated to one quarter of its original volume and drop wise concentration of ammonium hydroxide was added to precipitate the alkaloids. The precipitate was filtered off with a pre-weighed filter paper and washed with 1% ammonium hydroxide solution. The precipitate was oven dried for 30 minutes at 60°C and re-weighed. The alkaloid content of the samples was determined by difference using the equation:

Percentage alkaloid = $(W_2 - W_1)/W*100$

Where W = Weight of sample; W_1 = Weight of empty filter paper; W_2 = Weight of paper + precipitate.

Determination of Flavonoid Content

Ten grams of each sample was extracted with 100 ml of 80% aqueous methanol at room temperature for 2 to 4

hours. The mixture was filtered through Whatmann No 1 filter paper. The filtrate was later transferred into a Petri dish, evaporated to dryness over a water bath (70°C) and weighed to constant weight [14].

Determination of Cyanide Content

The alkaline picrate method [15] was employed for the determination of cyanide contents of the test feed. A portion (5 g) of each sample was grinded into paste and dissolved in 50 ml distilled water in a corked conical flask. The extraction was allowed to stay for 12 hrs. The sample was filtered and the filtrate was used for cyanide determination. To 1 ml of the sample filtrate in a corked test tube, 4 ml of alkaline picrate (prepared by dissolving 1g of picric acid in 5g of Na_2CO_3 and made up to 100ml with H_2O) was added and incubated in a water bath for 5 minutes for colour development (reddish brown) and the absorbance was read at 490 nm after stabilizing the spectrophotometer with the absorbance of the blank (which contained 1 ml of distilled water and 4 ml of alkaline pirate solution). The cyanide content was extrapolated from a cyanide standard curve and the cyanide content was calculated using the formula:

Cyanide (mg/kg) = Absorbance* Gradient Factor* Dilution Factor / Weight of the sample

Determination of Tannins

The percentage composition of tannin in the test feed was determined using the method of AOAC [16]. The tannin content of the samples was calculated from a tannic acid standard curve and results were expressed as milligrams of tannic acid equivalence (TAE) per 100 g of dried sample.

Determination of Saponins

Saponin determination was done using the method of AOAC [16]. Saponin extraction was done using two different solvents. The first solvent, acetone, was used to extract crude lipid from the samples while the second solvent (methanol) was used for the extraction of the saponin proper. Two gram of the sample was folded into a thimble and put in a sox let extractor and a reflux condenser fitted on top. Extraction was done with acetone in a 250cm^3 capacity round bottomed flask for 3 hours, after which the apparatus was dismantled and another 150cm^3 capacity round bottomed flask containing 100cm3 of methanol was fitted to the extractor and extraction was carried on for another 3 hours. The weight of the flask was taken before and after the second extraction in order to make the change in weight. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove any remaining solvent in the flask. The flask was then allowed to cool and the weight of the flask taken. The saponin content of the sample was calculated as:

% Saponin = Weight of saponin / Weight of sample*100

STATISTICS

Data was subjected to analysis using the Statistical Package for Social Sciences (SPSS), version 15.0. Results were presented as the means ± standard deviations of duplicate or triplicate experiments. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P < 0.05$ using the Duncan Multiple Range Test.

RESULTS

The hepatic G6PD activities of the non-diabetic control, diabetic control and diabetic rats administered Livingstone potato incorporated feeds were 2.80±0.37, 1.69±0.69 and 2.21±0.42 respectively. There were significant increases ($P<0.05$) in the G6PD activities of the diabetic rats fed the test feeds compared with the diabetic control rats while the G6PD activities of the diabetic control rats were significantly lower ($P<0.05$) than that of the non-diabetic rats (Figure 1).

Chemical analysis of the test feed revealed that it contained 0.24±0.02% saponin, 0.28±0.02 mg/kg HCN, 0.44±0.01% alkaloid and 0.43±0.03% tannin (Figure 2).

The percentage scavenging activity of the Livingstone potato incorporated feed on DPPH free radical was 71.13±1.28 which was close to that of standard quercetin (73.46 ± 2.03) (Table 1).

The reducing power of the Livingstone potato incorporated feed on the average was 0.797±0.025 which was lower than that of standard quercetin (1.190 ± 0.328) (Figure 3).

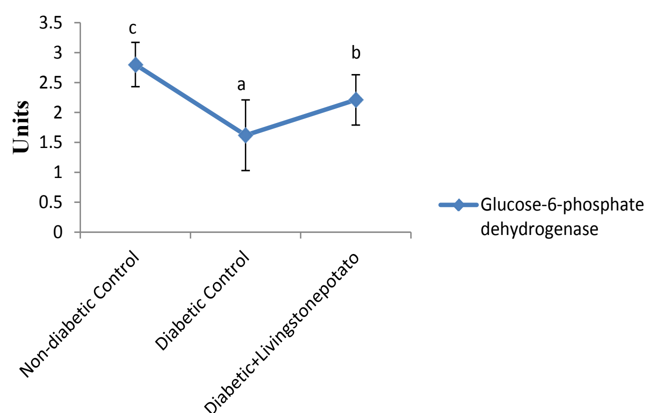

Figure 1. Glucose-6-phosphate dehydrogenase activity of rats. Values are the means ±SD of three determinations. [abc]Means with different superscripts for each parameter are significantly different ($P<0.05$): Unit definition: G6PD- µmol of $NADP^+$ reduced/min/g protein.

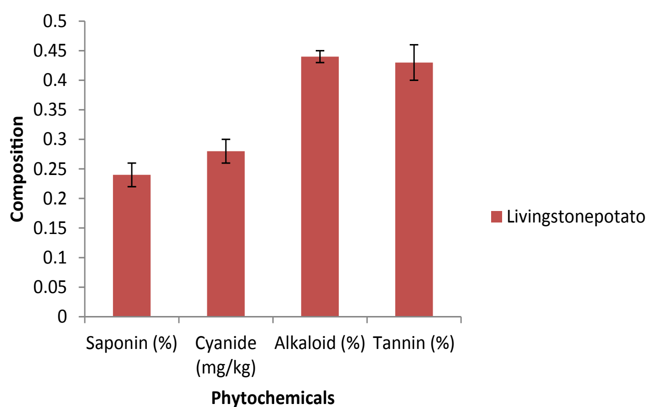

Figure 2. Phytochemical composition of livingstone potato incorporated feed. Values are the means ± SD of triplicate experiments.

Table 1. Inhibitory activity of Livingstone potato and quercetin on DPPH radical.

Samples	Percentage Inhibition
Livingstone potato	71.13 ± 1.28
Quercetin	73.46 ± 2.03

Values are the means ± SD of duplicate experiments

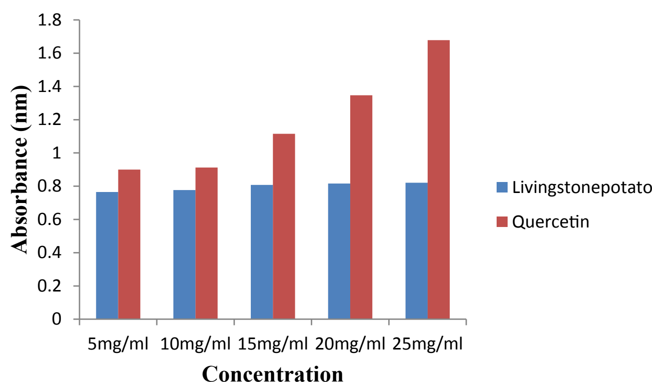

Figure 3. Reducing power of test feed and standard quercetin.

DISCUSSION

The decrease in the hepatic glucose-6-phosphate dehydrogenase activity of the diabetic untreated rats as observed in this study, could lead to decreased generation of NADPH needed for the regeneration of glutathione and other reducing agents needed for the maintenance of tissue integrity and oxidative balance, thereby leading to the induction of oxidative stress [17, 18].

One of the pathogenic mechanisms that explain the development of diabetic complications is oxidative stress resulting from increased generation of free radicals and impaired antioxidant defense system in diabetic conditions. Inhibition of these oxidative processes could prevent the onset and development of long-term diabetic complications [19, 20].

Thus, the increased activity of the hepatic G6PD activity of the diabetic rats given Livingstone potato incorporated feed is an indication of improvement in glucose utilization by the pentose phosphate pathway and also confirms our previous reports [6] that the major mechanism of anti-diabetic action of Livingstone potato is through its antioxidant scavenging mechanism.

Alkaloids and tannins are polyphenolic compounds with antioxidant properties. There are indications that apart from the scavenging of free radicals, polyphenols could also act by direct interactions with important cellular receptors or key signaling pathways leading to the modification of the redox status of cells which results in the triggering of a series of redox-dependent reactions [21]. In addition, tannins exert their action on carbohydrate metabolism through the inhibition of α-glucosidase and α-amylase, the key enzymes responsible for the digestion of dietary carbohydrates to glucose [19].Thus the considerable amount of these polyphenolic compounds in the Livingstone potato incorporated feed could be one explanation for the anti-diabetic potentials of Livingstone potato as previously reported [6].

Saponins are known to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic permeabilization of the intestine) properties [22]. The saponin content of the Livingstone potato incorporated feed as observed in this study could be one explanation for the anti-hypercholesterolemic and anti-hyperlipidaemic action of Livingstone potato as previously reported [6].

Cyanogenic glucosides are compounds that yield glucose, hydrogen cyanide and aldehyde or ketone upon hydrolysis with an acid or enzyme. The safe level of cyanide in foods as given by WHO/FAO is 10ppm (10mg/kg) [23]. According to Bolhius [24], 50-100mgHCN equivalent kg^{-1} cyanide was set as moderately toxic and above 100mg HCN equivalent kg^{-1} as dangerously toxic or lethal. Values obtained for cyanide in the Livingstone potato incorporated feed as observed in this study, were below toxic levels.

The DPPH assay is a widely accepted method for the determination of the antioxidant activities of various food systems. The method is widely used due to the relatively short time required for the analysis. As observed in this study, the methanolic extract of the Livingstone potato incorporated feed contained strong antioxidant activity which was close to standard quercetin.

The DPPH assay is limited to neutral and higher pH applications and also suffers from colour interference [25] and this informed the assay of the reducing power of the test feeds.

Reducing power assay is another widely accepted method that is employed in the assay of the antioxidant activities of various plants and it employs the reduction of Fe^{3+} to Fe^{2+} through electron transfer [26, 27]. The methanolic extract of the Livingstone potato incorporated feed was also observed to possess a strong reducing power, indicative of strong antioxidant activity though lower than the reducing power of standard quercetin and this strong antioxidant activity is attributed to the polyphenolic constituents of Livingstone potato.

Since several studies have implicated oxidative stress in the etiology of diabetes and its complications and inhibition of these oxidative processes by plant based antioxidants could prevent the onset and development of long-term diabetic complications [20], it is plausible to attribute the modulatory action of Livingstone potato on the glucose-6-phosphate dehydrogenase activity of diabetic rats to its antioxidant/polyphenolic constituents.

CONCLUSION

The study reveals the ameliorating action of Livingstone potato on hepatic G6PD activity of diabetic rat models which is attributed to its antioxidant/polyphenolic constituents.

Conflicts of Interest

The authors have no potential conflicts of interest.

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