International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:9, No:3, 2015

Antioxidant Activity of Germinated African Yam Bean (*Sphenostylis stenocarpa*) in Alloxan Diabetic Rats

Nneka N. Uchegbu

***Abstract*—**This study was conducted to investigate the effect of the antioxidant activity of germinated African Yam Bean (AYB) on oxidative stress markers in alloxan induced diabetic rat. Rats were randomized into three groups; control, diabetic and germinated AYB – treated diabetic rats. The Total phenol and flavonoid content and DPPH radical scavenging activity before and after germination were investigated. The glucose level, lipid peroxidation and reduced glutathione of the animals were also determined using standard technique for four weeks. Germination increased the total phenol, flavonoid and antioxidant activity of AYB extract by 19.14%, 32.28% and 57.25% respectively. The diabetic rats placed on germinated AYB diet had a significant decrease in the blood glucose and lipid peroxidation with a corresponding increase in glutathione ($p<0.05$). These results demonstrate that consumption of germinated AYB can be a good dietary supplement in inhibiting hyperglycemia/ hyperlipidemia and the prevention of diabetic complication associated with oxidative stress.

***Keywords*—**African Yam Bean, Antioxidant, Diabetes, Total phenol.

I. Introduction

DIABETES is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to the development of neuropathy. Diabetes mellitus is a metabolic disorder characterised by hyperglycemia, abnormal insulin secretion, altered metabolism of lipids and carbohydrates [1]. It is becoming the third "killer" of the health of mankind along with cancer, cardiovascular diseases [2]. The prevalence of diabetes mellitus is expected to reach up to 4.4% in 2030, and the occurrence was found to be high in India, China and USA [3]. Among all the cases of diabetes, type 2 diabetes was found to be more prevalent [4]. Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defences. Reference [5] reported that oxidative stress plays a major role in the pathogenesis of diabetes. This increased oxidative stress is accompanied by a decreased antioxidant capacity. Hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood. According to the latest studies hyperlipidemia leads to oxidative stress [6]. Usage of antioxidants helps in reducing risks of oxidative damages in diabetic and hyperlipidemic patients [7]. There is growing interest towards natural antioxidant from herbal sources [8]. Legume seeds are a rich source of many substances with antioxidant properties, including plant phenolics. Phenolic compounds do not only effectively prevent oxidation in foods; they also act as protective factors against oxidative damage in the human body. African yam bean (AYB) is an herbaceous leguminous plant occurring throughout tropical African [9]. It is one of the lesser-known and under-utilized legumes. AYB is a potential source of bioactive compounds with antioxidant activities. With the aim of improving the nutritive value of legumes, preparation techniques including germination have been developed to significantly raise the bioavailability of their nutrients [10]. Extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell component take place during the germination. Vitamins and secondary compounds, many of which are considered beneficial as antioxidants, often change dramatically during the germination. Previous work carried out on AYB seed showed that there is scanty information on the antioxidant properties of AYB seed. In this present work, both antioxidant, free radical scavenging of germinated AYB and influence of antioxidant activity by measuring the level of blood glucose concentration, lipid peroxidation and non protein sulphydryl group in the liver tissues of diabetes induced high cholesterol fed rats were investigated.

N. N. Uchegbu is with the Institute of Management and Technology, Enugu, Nigeria (Phone: +2348034080388; e-mail: uchegbu.nneka@yahoo.com).

II. Materials and Methods

Dried African yam bean seed (*Sphenostylis stenocarpa*) were purchased from Ogbete Main Market in Enugu State – Nigeria. The samples were contained in plastic sealed and stored in refrigerator at 4^{o}C before germination.

A. Germination Process

The African yam bean was soaked in 250 ml of water containing 0.7% sodium hypochlorite solution for 30 minutes at room temperature. The seeds were drained off, watered to neutral pH, and soaked in distilled water for 5 hours and the hydrated seeds were placed under wet muslin cloth and left to germinate for 3 days at room temperature (28^{o}C) without direct contact with sunlight [11]. The sprouted seeds were oven dried (Gallenkemp 1H – 100 model, UK) at 60^{o}C for 4 hours and ground to pass 0.18 mm sieve to obtain the flour which was packaged. The non sprouted seed was ground, sieved and packaged. This served as control.

B. Extraction

A 200 g of both the sprouted and non sprouted flour samples were defatted separated by stirring with 100 ml of 70 % acetone at 25^{o}C for 24 hours and filtering through Whatman No. 4 filter paper, following the method of [12]. The residues were further defatted with an additional 50 ml of 70% acetone,

as described above, for 3 hr. The solvent of the combined extract was evaporated under reduced pressure, using a rotary vacuum evaporator (RE 300, Yamato, Tokyo, Japan) at 40°C and the remaining water was removed by Lyophilization (4KBTxL-75; Virtis Benchtop K, New York, USA). The obtained dry powder was stored in an air tight polythene bag at 0°C until it was used.

C. Determination of Total Phenol

Total phenol content of the sample was determined using the method of [13]. Sample (50 µL) was put in test tubes and the volume was made up to 500 µL using distilled water. Then, 250 µL of folin-ciocalteu reagent was added into test tube followed by 1.25 ml of 20 % sodium carbonate solution. The tube was vortexed before incubated in the dark for 40 minutes. Absorbance was read at 725 nm using spectrophotometer.

D. Determination of Total Flavonoids

Aluminium chloride colorimetric method was used for flavonoids determination [14]. Each plant extracts (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 g ml^{-1} in methanol.

E. Determination of DPPH Free Radical Scavenging Activity

DPPH scavenging activity was carried out by the method of [15]. 250 µg/ml of African Yam bean seed extract with methanol was dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1 mM ethanol solution of DPPH (1,1, Diphenyl-2-picrylhydrazyl) was added to each of the test tubes and were shaken vigorously. It was allowed to stand at 35°C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample and measured at 517 nm. A radical scavenging activity was expressed as 1 % scavenging activity and was calculated by the following formula.

$$\text{Radical scavenging activity \%} = \frac{\text{OD control} - \text{OD sample}}{\text{OD Control}} \times 100$$

F. Animal and Diet

Adult wistar –albino rats weighting between 150 – 200g purchased from the Department of Animal Science, University of Nigeria Nsukka, Enugu State, Nigeria were used in the experiments. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions, in their individually and partly restricted metabolic cages (16 hours before the experiments, they were fasted overnight, but allowed free access to water). Six rats were used for each group of study.

G. Induction of Diabetes

Diabetes mellitus was induced by a single interperitonial injection of ice cold alloxan monohydrate freshly dissolved in normal saline (2%) at a dose of 180mg/kg body weight [16]. Single intraperitonial injection of normal saline was given to animal in the control group. After 7 days, the fasting blood glucose (FBG) level of test animal was measured and only rat with FBG level more than 220mg/dl were used for the study.

III. Experimental Design

Eighteen rats were divided into three groups, each consisting of six rats.

Group 1: Normal control, rats were non-diabetes induced and were given normal diet.

Group 2: Rats were diabetes induced fed with high cholesterol diet.

Group 3: Rats were diabetes induced fed with high cholesterol diet plus germinated AYB meal.

After four weeks of treatment, the blood was collected and the animal decapitated. The study was performed in accordance with the International Guidelines regarding animal experiment.

A. Determination of Blood Glucose Levels

Blood glucose concentration (mg/100ml) were determined using a glucometer – elite commercial test (Bayer), based on the glucose oxidase method. Blood samples were collected from the tip of tail after 16 hours fasting with free access to water. The blood sugar level was determined on the 1st, 2nd, 3rd and 4th week of the experiment.

B. Determination of Lipid Peroxidation (LPO) on Liver

The method of [17] with slight modification was used to determine lipid peroxidation in tissue samples. The liver of decapitated rat was exercised and chilled in ice – cold 0.9% NaCl Solution. A 1.0 g of wet tissue was weighted and homogenized in 9 ml of 0.25 M sucrose using a Teflon homogenizer to obtain a 10% suspension. The cytosolic fraction was obtained by a two – step centrifugation first at 1000x g for 10 minutes and then at 2000x g for 30 minutes at 4°C. A volume of the homogenate (0.20ml) was transferred to a vial and was mixed with 0.2ml of an 8.1% (w/v) sodium dodecyl sulphate solution. A 1.50ml of a 0.8% (w/v) solution of TBA and the final volume was adjusted to 4.0ml with distilled water. Each vial was tightly capped and heated in boiling water bath for 60 minutes. The vials were then cooled under running water.

Equal volumes of test samples and 10% TCA were transferred into a centrifuge tube and centrifuged at 1000x for 10 minutes. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental protocol except the TBA solution was replaced with distilled water due to the peroxidative effect of alloxan monohydrate on tissue: (liver of alloxan monohydrate – diabetic rats were used as positive control). The lipid peroxidation was calculated on the basis of the molar extinction coefficient of

MDA (malondialdehyde) and expressed as nm MDA/s protein [18].

C. Estimation of Non-Protein Sulphydryl Group (Cellular GSH) in Liver

The method of [19] was used to estimate the GSH. 200 mg of liver was homogenized in 8.0ml of 0.02m EDTA in an ice bath. 0.5ml of tissue homogenate was precipitated with 2ml of 5% TCA and centrifuged at 3200xg for 20 minutes. After centrifugation 1ml of the supernatant was taken and added to 0.5ml of Ellmen's reagent (2,2-dinitro-5,5-dithiobenzoic acid) and 3ml of phosphate buffer (pH 8.0). Then the absorbance was measured and 412nm. The values were expressed at mg/100g tissue.

D. Statistical Analysis

Data was subjected to analysis of variance using the statistical package for social science (SPSS), version 15.0. Results were presented as mean ± standard deviations. 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 values are average of triplicate experiments ± standard deviation.

IV. Results and Discussion

TABLE I
TOTAL PHENOL AND FLAVONOID CONTENT AND DPPH IN GERMINATED AND NON GERMINATED AFRICAN YAM BEAN

Assay	Sample	Mean ± SD
Total phenol (mg/100g dry weight)	Germinated African yam bean	117.08±0.03
	Non Germinated African yam bean	98.27±0.11
Total flavonoid (mg/100g dry weight)	Germinated African yam bean	68.31±1.12
	Non Germinated African yam bean	51.64±0.01
DPPH (µg/ml)	Germinated African yam bean	48.92±1.22
	Non Germinated African yam bean	31.11±1.14

Value are means ± SD; n = 3. Mean values followed by different letters in a column are significantly different ($p<0.05$).

A. Total Phenol Content

Phenolic compound are a class of antioxidant agents which act as free radical terminators and scavenger [20] and many play a role in the prevention of certain diseases [21]. Table I shows that Total Phenol Content in germinated AYB is higher ($p<0.05$) than that of non-germinated AYB. This increase in the amount of phenolic compound after germination agrees with the work done by [22] which indicated that germination modifies the quantity and quality of phenolic compounds in legumes. Also [23] reported an increase of 46% in total phenols after germinating lupin seeds.

B. Total Flavonoid Content

Researchers have shown that flavonoids show antioxidant activity and their effect on human nutrition and health is considerable. The mechanism of action of flavonoids is through free-radical scavenging and protection against oxidative stress [20]. The flavonoid contents of the extracts of germinated AYB (68.31 mg/100g of dry weight) were higher ($p<0.05$) than that of non-germinated AYB (51.64mg/100g of dry weight). This findings might be due to the biochemical metabolism of seeds during germination which result to the production of secondary plant metabolites such as anthocynins and flavonoids [24]

C. DPPH Free Radical Scavenging Activity

DPPH is a stable nitrogen centred free radical which can be use to evaluate the antioxidant activity of natural products by measuring the radical quenching capacity in a relatively short period of time.

Reference [25] reported that antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as inflammation, neurodegeneration, ageing process, cancer and diabetes.

The free radical scavenging activity of germinated and non germinated AYB was tested by measuring their ability to quench the DPPH radical (Table I). The results showed that germinated AYB had the higher DPPH free radical scavenging ability (48.92 µg/ml) than non germinated AYB (31.11 µg/ml).

The high scavenging property of germinated AYB may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. DPPH absorbance is measured at 517nm. It does this adsorption when accepting an electron [26]. Therefore, germinated AYB could contain some substances which are electron donors that reacted with free radicals to convert them to more stable products and blocked the radical chain reaction.

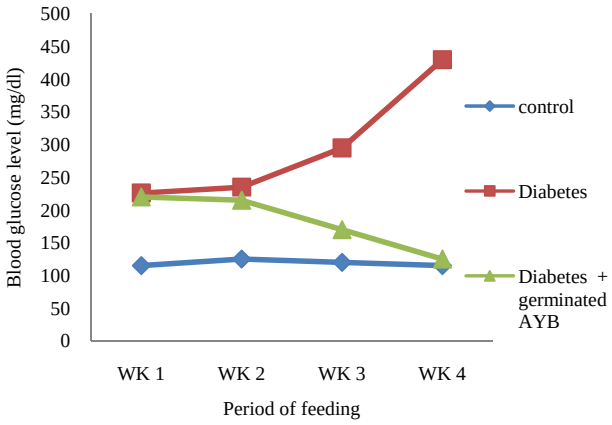

Fig. 1 Effect of germinated AYB diet on blood glucose levels in normal and alloxan – induced diabetic wistar rats for 4 weeks

D. Changes in Blood Glucose of Normal and Alloxan–Diabetic Rat

Fig. 1 illustrates the variation in blood glucose of normal control, diabetic control and germinated AYB extract – treated rat during 4 weeks period of study. The blood glucose levels of diabetic rats were significantly higher compared with those of the control group ($p<0.05$). After the administration of the germinated AYB meal, a significant decrease in blood glucose was observed compared with that of the diabetic group ($p<0.05$). It has been established that acute changes in blood

glucose level – both hyper- and hypo-glycemia have a marked, reversible, effect on gut mortality [27], that raised blood glucose in diabetics could deplete cells of their antioxidant status with concomitant increase in free radicals thereby precipitating oxidative stress. Thus germinated AYB with hypoglycemia potential may reduce oxidative stress indirectly by lowering blood glucose level and preventing hyperinsulinemia, and directly by acting as free radical scavengers.

TABLE II
EFFECT OF OXIDATIVE STRESS MARKERS IN CONTROL, HYPERLIPIDEMIC AND GERMINATED AYB EXTRACT TREATED DIABETIC RATS

Groups	Control	Alloxan + cholesterol	Alloxan + cholesterol + Germinated AYB extract 2.1g/kg bw
Lipid peroxidation n mol MDA release/mg protein	1.40 ± 1.03	2.01 ± 0.31[a]	0.99 ± 0.10[b]
GSH µg reduced GSH utilized /mm/mg protein	35.40 ± 2.00	21.51 ± 2.20[a]	32.30 ± 2.10[b]

Values are mean ± SD
MDA – Malondialdehyde, GSH – Reduced glutathione.

The results of antioxidant activity were shown in Table II. LPO and GSH serves as markers in studying the antioxidant activity. A significant elevation ($p<0.05$) in the concentration of MDA is observed in liver tissues of hyperglycemia/ hyperlipidemia rats. The germinated AYB extract given orally produced a significant reduction in LPO in liver (50.75%). An increase of (50.16%) in the level of GSH in liver tissue of hyperlipidemia diabetic rat was observed after administration of germinated AYB extracts.

The test animals which are both hyperglycemic and hyperlipidemic are prone to the development of oxidative stress. LPO and GSH is use to mark oxidative stress. Increased LPO, which causes oxidative stress, can lead to several pathogenesis like, inflammation, diabetes, aging, and renal failure [28]. The diabetic induced-high cholesterol fed animals showed increased LPO than in control animals. The animals treated with germinated AYB meal showed reduced LPO than diabetes induced animals fed only with high cholesterol food. GSH protect the cell constituents from oxidative damage [29]. Oxidative stress may lead to reduction in glutathione. Concentration of the marker was found to be increased in animals treated with germinated AYB extract.

V. CONCLUSION

The free radical scavenging activity of germinated AYB extract in diabetes – associated hyperlipidemia, its beneficial effect as an effective hypoglycaemic and antioxidant agent in reducing oxidative stress has been demonstrated in this study. The present study concluded that consumption of germinated AYB can be good dietary supplement in reducing the complications usually resulting from oxidative stress in diabetes and hyperlipidemia.

REFERENCES

[1] D.K. Patel, R. Kumar, S.K. Rrasad, K. Sairam, and S. Hemalatha (2011). Anti-diabetic and in vitro antioxidant potential of hybanthus enneaspermus (linn). F. Muell in streptozotocin induced diabetic rats. Asian Pac. J. Trop Biomed. 1(4): 316 – 322.
[2] A. Chauhan, P.K. Sharma, P. Srivastava, N. Kumar and R. Dudhe (2010). Plants having potential anti-diabetic activity. A Review Der. Pharmacia Lettre. 2(3): 369 – 387.
[3] D.R. Patel, R. Kumar, D. Laloo and S. Hemalatha (2012). Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. Asian Pacific Journal of Tropical Disease. 239 – 250.
[4] A.K. Balaraman, J. Singh, S. Dash, T.K. Maity (2010). Antihypershycemic and hypolipidemic effect of melothria maderaspatana and coccinia indica in streptozotocin induced diabetes in rats. Saudi Pharm J. 18(3): 173 – 178.
[5] A.C. Maritime, R.A. Sandar and S.J.B. Watkin (2003). Diabetes, oxidative stress and antioxidants. A Review J. Biochem Mol. Toxicol. (17): 1216 – 1223.
[6] Y. Li, H.S. Yong, H. Gang, L. Wu and L. Guo-Wei (2008). Increasing oxidative stress with progressive hyperlipidemia in human: Relation between malondialdehyde and Atherogenic index. J. Clin. Biochem. Nutr. (43): 154 – 158.
[7] N. Yang (2006). Effect of antioxidant capacity on blood lipid metabolism and lipoproteins lipase activity of rats fed a high-fat diet. Nutrition (22): 1185 – 1191.
[8] R.A. Larson (1998). The antioxidants of higher plants. Phytochemistry. 27(4): 969 – 978.
[9] D. Porker (1992). Economic botany of sphenostylis (Leguminosae). Economic Botany. 46(3): 262 – 275.
[10] G. Urbano, P. Aranda, A. Vilchez, C. Aranda, L. Carbrera, T.M. Porres, and M. Lopez-Jurado (2005). Effect of germination on the composition and nutritive value of proteins in Pisum sativum L. Food Chem. (93): 671 – 679.
[11] A. Yasmin, A. Zeb, A.W. Khalil, G.M. Paracha and A.B. Khattak (2008). Effect of processing on antinutritional factors of red kidney bean (Phaseolus valgaris) grains. Food Bioprocess Technology 1(4):415 – 419
[12] K. Sowndheraraja, P. Shidduraju, and S. Manian (2011). Antioxidant and free radical scavenging capacity of the underutilized legumes (Vigna veillata L.) J. of Food Compt and Analy. (24):160 – 165.
[13] H.P.S. Makkar, M. Blummel, N.K. Borowy and K. Becker (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation method. Journal of Science and Food Agriculture. (61): 161 – 165
[14] C. Chang, M. Yang, H. Wen and J. Chem (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analy., (10): 178 – 182.
[15] M.S. Blois (1958). Antioxidant determination by the use of stable free radical. Nature. (81): 1199 – 2000
[16] Zheng Jianxian (1999). Functional food. Chemical Industry Press, Beijing. pp. 730-733.
[17] H. Ohkawa, N. Ohishi and K. Yagi (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry.; 95:351–358.
[18] J. Stocks, J.M. Gutteridge, R.J. Sharp and T.L. Dormandy (1974). Assay using brain homogenate for measuring the antioxidant activity of biological fluids. Clin. Sci. Mol. Med., 47:215 – 222.
[19] G.L. Ellmen (1959). Tissue sulfahydryl groups. Arch. Biochem. Biophys 82:70-77
[20] F. Shahidi and M. Naczk (1995). Phenolic compounds in grains. In: food phenolics; source, Chemistry Effect Applications, Technomic Publishing Company Inc., Lancaster P.A. pp. 3 – 39.
[21] A. Doss, M. Vijaya Santhi, V. Parivuguna and R Venkataswamy (2011). Antimicrobial effects of the Flavonoid fractions of Mimosa pudica L. Leaves. J. Pharm. Res. 4(5): 1438-1439.
[22] M.L. Lopez-Amoros, T. Hemandez and I. Estrella (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. J. Food Compos. Anal. (19): 277 – 283.
[23] M. Duenas, J. Hernandez, I. Estrella and D. Fernandez (2009). Germination as a process to increase the polyphenol content and antioxidant activity of Lupin seeds (Lupines angustifolius L.) Food Chem. (177):599 – 607.
[24] R. Randhr, Y.T. Lin and K. Shetty (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean

sprouts in response to peptide and phytochemical elicitors. Process Biochem. (39):637 – 647.
[25] J.M. Oke and M.O. Hamburger (2002). Screening of some Nigerian – medicinal plants for antioxidant activity using 2,2, Diphenyl picryl-Hydrazyl Radical. Afri J. Bio. Res (5): 77-79
[26] Y. Zou, Y. Lu and D. Wei (2004). Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. In vitro. Journal of Agricultural and Food Chemistry. (52):5032 – 5039.
[27] R.E. Schmidt (2002). Neuropathology and Pathogenesis of diabetic autonomic neuropathy. Inter. Neurobiol. (50): 257 – 292
[28] R. Karuna, S.S. Reddy, R. Baskar and D. Saralakumari (2009). Antioxidant potential of aqueous extract of phyllanthis amarus in rats. India J. Pharmacol (41): 64 – 67
[29] A. Rajasekaran and M. Kalaivani (2011). Antioxidant activity of aqueous extract of monascus fermented Indian variety of rice in high cholesterol diet fed – Streptozotocin diabetic rats, on in vivo study. Inter. J. of Current Sci. Res. 1(2): 35 – 38