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Full Length Research Paper

The effects of copper supplement on zinc status, enzymes of zinc activities and antioxidant status in alloxan-induced diabetic rats fed on zinc over-dose diet

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The aim of this study was to investigate the effect of copper supplementation on over-dose zinc in experimental diabetes. Male alloxan-induced diabetic Wistar albino rats of 12 weeks of age were divided into three groups. The first group received a diet containing 54 mg zinc/kg (control group), the second group received a diet containing 231 mg zinc/kg (Zn group), and the third group received a diet containing 231 mg zinc/kg supplemented with copper (30 mg/kg diet) (Zn+Cu group). Body weight gain of all rats was recorded regularly over a period of three weeks. On day 21, after overnight fasting, animals were sacrificed and blood glucose, zinc concentration, and amylase, aldolase, lactate dehydrogenase and alkaline phosphatase activities and parameter antioxidants were determined on tissues and serum samples. Body weight gain of copper supplementation diabetic animals at the end of three weeks of dietary manipulation was significantly lower with a percentage of 41.09% than that of zinc over dose diabetic animals. The administration of copper significantly altered blood glucose with a percentage of 22.98%, serum and tissues zinc concentration ($P < 0.01$) and all enzymes zinc dependants in animals. Copper added significantly increased glutathione (GSH) concentration ($P < 0.05$) and glutathione peroxydase (GPx) activity ($P < 0.01$) in rats. In contrast, liver malondialdehyde (MDA) and testis glutathione S transferase (GST) activity levels were lower with a percentage of 13.52 and 29.78%, respectively. There is no statistically change in liver GST activity in (Zn+Cu) group. It was concluded that supplementation of copper diet have significantly reduced the zinc status, disrupt the activity of zinc-dependent enzymes and altered in the chemical and oxidative parameters in diabetes.

Key words: Diabetic rats, alloxan, zinc status, copper, antioxydants.

INTRODUCTION

Life would not be possible without a large number of 'trace' elements, each serving critical roles in metabolism and function. Trace elements are necessary for normal function and are therefore associated with morbid

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deficiency states. They are also commonly toxic when present in excess (Huffman and O'Halloran, 2001). Zinc and copper are two of the most metabolically active and intensively investigated trace metal nutrients. The established biochemical role of zinc is as a component of more than 300 metalloenzyme (Atukorala and Waidyanatha, 1987) by participating in their structure or in their catalytic and regulatory actions. It is a structural ion of biological membranes and closely related to protein synthesis. The concept of zinc fingers explains the role of zinc in gene expression and endocrine function, and mechanisms of action of zinc involve the effects of the metal on DNA synthesis, RNA synthesis, and cell division. Zinc also interacts with important hormones involved in bone growth such as somatomedin-c, osteocalcin, testosterone, thyroid hormones, and insulin (Maria et al., 2002).

Oxidative stress is caused by a relative overload of oxidants, reactive oxygen species zinc-containing enzymes participate in many components of macronutrient metabolism, particularly in cell replication. In addition, zinc-containing enzymes such as carbonic anhydrase and lactate dehydrogenase are involved in exercise metabolism while superoxide dismutase protects against free radical damage (Parvaneh et al., 2009). There are characteristic and specialized systems of zinc absorption, transport and excretion in human organism. The synergic and antagonist effects of minerals with zinc influence the quantum of zinc in a certain organ (Richard et al., 2001). Cu is also an essential micronutrient required by all life forms. Cu is a transition metal and hence involved in a variety of biological processes, embryonic development, mitochondrial respiration, regulation of hemoglobin levels as well as hepatocyte, neuronal functions and free radical detoxification.

Cu is vital for normal healthy functioning of organisms (Krupanidhi et al., 2008). Dietary copper, absorbed in the stomach and upper intestinal tract, reaches liver as a complex with serum proteins, albumin or transcuperin or the amino acid histidine. Liver is the major store house for intracellular copper need to be maintained in a complex state so as to prevent the oxidative damage caused by free copper to DNA, proteins and membrane components (Krupanidhi et al., 2008). Because copper is a redox metal, unfettered copper is a potential oxidant (or reductant) of cellular proteins, lipids and nucleic acids. This property mandates copper to be in a bound form and not a free ion in blood, extracellular fluids or cytosol. Of its two major valencies, (Cu (I) and Cu (II)), Cu (I) behaves as a progenitor of free radicals. Metal ion, Cu (I) also has the potential to antagonize Zn (II) (Huffman and O'Halloran, 2001).

Oxidative damage due to free radicals is associated with vascular disease in people with type 1 and those with type 2 diabetes mellitus (DM). There are several potential sources of increased free radical production in diabetes including auto-oxidation of plasma glucose, activation of leucocytes and increased transition metal bioavailability. The radical-scavenging antioxidant activity of the serum of people with either type 1 or 2 DM is lower than that of age-matched controls. This may be attributed to lower blood orate, vitamin C or vitamin E or other factors including the trace elements. Several reports underlie the key role of micronutrient status in patients with type 1 or 2 DM. Correction of Zn deficiency in patients with type 1 DM by zinc over dose also leads to decreased lipid peroxidation and improvements in glucose homeostasis. Therefore, Zn act in normalizing glycemia and is postulated to function as antioxidants, a restored Zn status in people with type 2 DM may counteract the deleterious effects of oxidative stress and help to prevent complications associated with diabetes (Richard et al., 2001). Therefore, the aim of this study was to examine the effects of dietary copper supplementation and its effect on diabetic pathology observed in zinc over dose rats by evaluating body weight gain, zinc status, some zinc dependant enzyme activities and antioxidants status in alloxan induced diabetic rats.

MATERIALS AND METHODS

Animals and diet

Male albino (Wistar) rats of two to three months of age, weighing 220 to 300 gm were housed in standards cages. Humidity and temperature were controlled with a 12 h light/dark cycle. Animals were left to feed and were given water *ad libitum*. After one week, rats were injected intraperitoneally with a freshly prepared alloxan monohydrate solution (Alloxan; Sigma, UK) at a dose of 150 mg/kg of body weight (Awadallah and Dessoukey, 1977) to induce diabetes. Seven days after alloxan administration serum glucose concentration was measured. All alloxan-treated animals in which glucose concentration in the serum was lower than 11 mmol/L (1.98 g/L) were excluded from the experiment. The animals were divided into three groups (10 rats per group) after the stabilization of diabetes. The first group received a diet containing a 54 mg zinc/kg diet (adequate zinc, group control), the second group received a diet containing a 231 mg $ZnSO_4.7H_2O$/kg diet (Dorota et al., 2011) (zinc over-dose, Zn group) and the third group received a diet containing a 231 mg $ZnSO_4.7H_2O$/kg diet supplemented with copper (30 mg $CuSO_4.5H_2O$/kg diet) (Bremner et al., 1976) (Zn+Cu group). The composition of the diet was similar to that described previously by Southon et al., (1984). Rats were maintained on the appropriate experimental diet *ad libitum* for 20 days. Body weight gain was monitored weekly and at the time of sacrifice (after 1, 2 and 3 weeks of disease). Animals were fasted overnight on day 20, and on day 21 given access to food for two periods of 1 h between 11.00 to 12.00 h and 17.00 to 18.00 h so that time of feeding on day before they were sacrificed was similar for all groups.

Rats were then sacrificed between 11.00 and 12.30 h on day 22. One animal from each group was sacrificed approximately at the same time by exsanguinations from the heart whilst under diethyl-ether anaesthesia. Blood was transferred into ice cold centrifuge tubes and a portion was taken for whole-blood glucose analysis which was performed immediately after exsanguinations. The

Table 1. Body weight gain, serum zinc and tissues zinc concentrations of diabetic rats fed adequate zinc diet (control), zinc over dose diet (Zn) and zinc over dose diet supplemented with copper (Zn+Cu) at the end of experimental period.

Parameter	Experimental groups		
	Control (n=10)	Zn (n=10)	Zn+Cu (n=10)
	Mean SEM	Mean SEM	Mean SEM
Body weight gain (g/day)	3.99±0.51	4.38±0.44	2.58±0.88**
Zinc serum(µg/100ml)	90.30±3.40	147.12±9.21	93.20±12.0**
Zinc liver (µg/g dry weight)	28.59±1.18	35.48±2.40	29.50±3.43*
Zinc kidney(µg/g dry weight)	35.19±3.14	45.98±7.72	40.09±6.35*
Zinc pancreatic(µg/g dry weight)	59.82±5.21	76.32±8.52	61.30±3.82**
Zinc testis(µg/g dry weight)	81.70±4.05	170.50±7.08	89.92±5.83***

*(P < 0.05) ; **(P < 0.01) ; ***(P < 0.001).

remaining blood was centrifuged for 10 min at 3,000 revolutions/min and the serum was utilized for serum zinc, amylase, aldolase, lactate dehydrogenase and alkaline phosphatase assays. The liver, kidney, pancreas and testis of each animal was removed, washed with ice-cold physiological saline solution, dried and processed for biochemical measurements. Homogenates were prepared on ice in the ratio of 4 g tissue in 16 ml of phosphate buffer, pH 7.5, containing 1 mmol/l Na_2EDTA. For each sample, 10 µl of 500 mmol/l BHT in acetonitrile was added to prevent formation of new peroxides during the assay. The homogenates were centrifuged at 20,000 × g for 15 min at 4°C and frozen at -70°C until analysis.

Analytical methods

Blood glucose was estimated by the glucose oxidase method using commercial test kit for glucose. Serum amylase, aldolase, lactate dehydrogenase and alkaline phosphatase activity were determined using commercial test kits for amylase (Ying Foo 1998), aldolase (Feissli 1966), lactate dehydrogenase (Pesce and Kaplan, 1984) and alkaline phosphatase (Bowers and Macomb, 1966). Dried liver, pancreas, testis and kidney were heated in silica crucibles at 480°C for 48 h and the ash taken up in hot Ultrex nitric acid (11.7 M) for Zn analysis by atomic absorption spectrophotometer (SHIMADZU AA-6200) China (Kechrid and Bouzerna, 2001). The accuracy of zinc recovery was checked using standard reference materials; bovine liver and wheat flour. These standards were prepared and analyzed in similar conditions to the test items to assess recovery. The recovery of zinc in the standard reference material exceeded 96%. Zinc in serum was analysed after a twenty-fold dilution of the serum by flame atomic absorption spectrophotometer (SHIMADZU AA-6200). Zinc standards were prepared from a 1 mg/ml zinc nitrate standard solution (BDH) using 5% glycerol to approximate the viscosity characteristics, and to avoid zinc contamination from exogenous sources. All tubes were soaked in HNO_3 (10% v/v) for 16 h and rinsed with double distilled water. Glutathione (GSH) concentration was measured utilizing the method described by Weckbercker and Cory (1988). Malondialdehyde (MDA) concentration was determined by spectrophotometry method of Okhawa et al. (1979). GSH-Px (EC 1.11.1.6) activity was measured spectrophotometrically at 340 nm by the Pinto and Brtley method (1969), as regards the activity glutathione S transferase, it was determined by the Habig et al. (1974) method. The reported data are the means of measurements and their standard error of mean (SEM) values. For statistical evaluation, the Student's t-test and P < 0.05 was considered the limit for the statistical significance.

RESULTS

Table 1 summarizes the body weight gain, serum zinc, liver zinc, kidney zinc, pancreatic zinc and testis zinc concentrations of the animal groups studied. Body weight gain of diabetic zinc over dose animals (Zn) at the end of three weeks of dietary manipulation was significantly higher than those of adequate zinc diabetic rats (AZ) by 4.38 ± 0.44 to 3.99 ± 0.51. Compared with adequate zinc diabetic group, zinc over dose diabetic group had high serum zinc, liver zinc, kidney zinc, and pancreatic zinc and testis zinc levels. Copper significantly decreased the body weight gain (P < 0.01), serum zinc (P < 0.01), liver zinc and kidney zinc (P < 0.05), pancreatic zinc (P < 0.01) and testis zinc (P < 0.001) levels of zinc over dose diabetic rats (Table 2). Blood glucose was lower of zinc over dose diabetic rats than those of zinc adequate diabetic animals by 1.61 ± 0.01 to 2.08 ± 1.15. However, serum amylase, aldolase, lactate dehydrogenase and alkaline phosphatase activity of diabetic zinc over dose diabetic group (Zn) were higher than those of zinc adequate diabetic group (control). Supplementation of the diet of zinc over dose alloxan diabetic rats with copper significantly reduced amylase (P < 0.001), aldolase (P < 0.05), lactate dehydrogenase (P < 0.01) and alkaline phosphatase (P < 0.001) activities (Table 2).

The liver malondialdehyde (MDA), liver glutathione (GSH) and testis GSH levels in zinc over dose diabetic group (Zn) were increased compared to the adequate zinc diabetic group. Supplemented with copper showed a significantly reduction of liver MDA level (P < 0.001) and elevated liver GSH (P < 0.05) and testis GSH levels (P < 0.05) (Table 3). The liver glutathione S transferase (GST) and testis GST activities in zinc over dose diabetic group

Table 2. Mean blood glucose and enzymes of zinc activities of diabetic rats fed adequate zinc diet (control), zinc over dose diet (Zn) and zinc over dose diet supplemented with copper (Zn+Cu) at the end of experimental period Dry

Parameter	Experimental groups		
	Control (n=10) Mean SEM	Zn (n=10) Mean SEM	Zn+Cu (n=10) Mean SEM
Blood glucose (g/l)	2.08±1.15	1.61±0.01	1.98±0.01**
Serum amylase (U/l)	623.21±71.22	765±14.2	541.8±24.7***
Serum alkaline phosphatase (U/l)	212.31±12.90	259.8±31.9	231.8±167***
Serum lactate dehydrogenase (U/l)	1132.60±47.16	1262.3±63.4	1134±365**
Serum aldolase (U/l)	6.05±0.29	7.8±0.1	6.8±0.15*

*(P < 0.05) ; **(P < 0.01) ; ***(P < 0.001)

Table 3. Antioxidant enzyme activity levels and concentrations of GSH and MDA in liver and testis in the diabetic rats fed adequate zinc diet (control), zinc over dose diet (Zn) and zinc over dose diet supplemented with copper (Zn+Cu) at the end of experimental period.

Parameter	Experimental groups		
	Control (n=10) Mean SEM	Zn (n=10) Mean SEM	Zn+Cu (n=10) Mean SEM
Liver GSH (nM/mg protein)	32.92±1.57	45.92±2.74	50.53±5.26*
Testis GSH (nM/mg protein)	24.58±1.30	34.01±0.90	35.6±1.70*
Liver MDA (nM/ mg protein)	182.41±34.2	212.9±69.5	184.1±54.6***
Liver GST (µM/g protein)	0.38±0.03	0.44±0.08	0.42±0.16
Testis GST (µM/g protein)	0.41±0.07	0.47±0.03	0.33±0.08***
Liver GPx (mU/g protein)	51.4±3.60	38.18±2.93	41.5 6±2.60**
Testis GPx (mU/g protein)	33.4±2.60	24.7±1.12	37.15±0.05***

*(P < 0.05); **(P < 0.01); ***(P < 0.001).

(Zn) were increased, compared to the adequate zinc diabetic group. However, the liver glutathione peroxydase (GPx) and testis GPx activities of zinc over dose animals was lower than those of adequate zinc animals. Addition of copper significantly reduced testis GST by 0.33 ± 0.08 to 0.47 ± 0.03 (P < 0.01) , elevated liver GPx, testis GPx activities by 41.5 6 ± 2.60 to 38.18 ± 2.93 and 37.15 ± 0.05 to 24.7 ± 1.12 (P < 0.001), respectively and non significantly variation liver GST activity (Table 3).

DISCUSSION

In our study, body weight gain was affected by copper by 2.58 ± 0.88 to 4.38 ± 0.44 (P < 0.01). It is demonstrated that high Cu in diet have damaged duodenal villi, therefore, have impact on nutrient absorption, depress food intake resulting in poor growth performance and decrease weight body (Rahman et al., 2001). Zinc concentrations in serum and tissues were assayed to confirm zinc over dose diet .The zinc absorption have strong connection with the copper absorption, because of their competition for the carrier (Dimitrova et al., 2010). It is suggested thus that copper was bounded to zinc and it resulted in depletion of zinc free level as confirmed by the result of low zinc concentration, which was found in serum. This growth retardation was due in part to a decrease in appetite and impaired protein synthesis (Kechrid et al., 2007a). Serum zinc, liver, pancreatic, kidney and testis zinc concentrations in rats fed zinc over dose diet were higher than that of adequate zinc group. Supplementation with copper (30 mg/kg) significantly reduced body zinc status. These findings indicated the effect of copper on decreased bioavailability of the zinc which is in agreement with investigation of Willis et al. (2005).

In the current study, when the time of feeding was strictly controlled and the amount of food eaten by each animal before an overnight fast was known to be similar, the mean fasting blood glucose concentration in rats fed

zinc over dose diet supplemented with copper were found to be higher than that of rats fed zinc over dose diet by 1.98 ± 0.01 to 1.61 ± 0.01 ($P < 0.01$). This suggests that copper caused reduction in level of free zinc (Dimitrova et al., 2010). Thus it exacerbated the ability of diabetic rats to utilize glucose. Results from previous studies showed an increased blood glucose concentration after intravenous injection with glucose in rats fed on zinc over dose diet (Kechrid et al., 2007b), suggesting a relation between carbohydrate utilization and the zinc status. A decrease in the plasma glucose concentration in Cu-supplemented may be due to decreased zinc intake. The decrease in Zn dependent enzymes activities of serum (amylase, aldolase, lactate dehydrogenase and alkaline phosphatase) in rats given over dose zinc diet supplemented with copper may be attributed to the decrease in serum zinc.

Zinc is essential for the activity of amylase (Savchenko et al., 2002), aldolase (Matthias and Georg, 1996), lactate dehydrogenase (Parvaneh et al., 2009), and alkaline phosphatase (Jamshid et al., 2011). It serves as one or more structural, regulatory or catalytic functions (Kechrid and Kenouz, 2003). In general, the present study indicated that some symptoms and signs associated with zinc deficiency and decrease metallo-enzyme in diabetic rats can be stimulated by supplementation with copper. The increase of GSH, MDA concentration and GST activity in animals fed Zn confirms an efficacious defense of the zinc against oxidative stress under diabetic conditions (Mariani et al., 2008). Zinc is also necessary to stimulate defense against reactive species oxygen and H_2O_2 that induce apoptosis (Ani et al., 2007). Supplementation with copper decrease MDA concentration and increase GSH, liver GPx activity by 184.1 ± 54.6 to 212.9 ± 69.5 ($P < 0.001$), 50.53 ± 5.26 to 45.92 ± 2.74 concentration ($P < 0.05$) and 41.5 6 ± 2.60 to 38.18 ± 2.93 ($P < 0.01$), respectively.

Liver GSH S-transferase activity in rats fed Zn+Cu was the same as in rats fed Zn, indicating this activity is unaffected by copper supplement. This result confirms the report of Stephen et al. (1982) who observed GSH S-transferase activity in liver unaffected by copper in rats. However, the decreased liver concentration of MDA in (Zn + Cu) fed ($P < 0.001$) is probably a result of decreased zinc by copper supplemented. Copper-supplemented cells accumulate glutathione through a decrease in glutathione efflux and not by an increase in GSH synthesis.

In contrast, elevated GSH levels tissues from rats exposed to zinc were caused by increases in GSH synthesis (Jonathan et al., 1989). Copper is essential for superoxide dismutase (SOD) activity. Different studies have confirmed that the production of hydrogen peroxide (H_2O_2) under the action of SOD (Joanny and Menvielle, 2005). Peroxide has been shown to react with Cu to form highly toxic hydroxyl radicals (Jonathan et al., 1989). An elevation in the activity of hydrogen peroxide metabolizing GSH peroxidase is believed to be an indication of increased cellular hydrogen peroxide flux. It has been suggested that increased GSH peroxidase activity may cause cells to develop higher levels of GSH (Jonathan et al., 1989).

REFERENCES

Ani M, Moshtaghie AA, Aghadavood M (2007). Protective effects of selenium and zinc on the brain acetyl cholinerterase activity in lead intoxified rat. Res. Pharm. Sci. 2:80-84.

Atukorala TMS, Waidyanatha US (1987). Zinc and copper content of some commen foods.J. Natn. Sci. Coun. Sri Lanka .1: 61-69.

Awadallah R, Dessoukey EA (1977). Serum enzymes changes in experimental diabetes before and after treatment with some hypoglycaemic drugs. Z Ernahringswiss. 16: 235.

Bowers GNJ, McComb RB (1966). A continuous spectrophotometric method for measurement the activity of serum alkaline phosphatase. Clin. Chem. 12:73.

Bremner I, Young BW, Mills CF (1976). Protective effect of zinc supplementation against copper toxicosis in sheep. Br. J. Nutr. 36:551.

Dimitrova A, Russeva A, Atanasova M, Strashimirov D (2010). Effect of zinc supplentation on some hematological parameters of spontaneously hypertensive rats. Trakia J. Sci. 8(2):61-65.

Dorota S, Barbara B, Andrzej T, Marzena K, Pawel R, Marek W (2011). The effect of zinc-and sulphate supplementation on tumor and hair concentrations of trace element (Zn, Cu, Fe, Ca, Mg, P) in Rats with DMBA-Induced Breast Cancer. Pol. J. Environ. Stud. 20(6):1585-1592.

Feissli S (1966). Quantitative determination of Aldolase .Klin. Wschr. 44: 390.

Habig H, Pabst MJ, Jakoby WB (1974). Glutathione S-transferase; the first step in mercapturic formation. J. Biol. Chem. 249:7130-7139.

Huffman DL, O'Halloran TV (2001). Function, structure, and mechanism of intracellular copper trafficking proteins. Ann. Rev. Biochem. 70: 677–701.

Jamshid GG, Karamouz H, Agdam H, Shahriar H, Zadeh AN, Rahim B, Nima K (2011). Effect of Inorganic Zinc Supplement on Activity of Alkaline Phosphatase Enzyme as an Index of Mucosal Functional in Small Intestine of Male Broilers. American-Eurasian J. Agric. Environ. Sci. 11(5):622-625.

Joanny F, Menvielle-Bourg (2005). Superoxide Dismutase (SOD), a Powerful Antioxidant, is now available Orally. Phytothérapie. 3:1-4

Jonathan H, Freedman, Maria RC, Jack P (1989). The Role of Glutathione in Copper Metabolism and Toxicity. J. Biol. Chem. 264(10):5598-5605

Kechrid Z, Amamra S, Dahdouh F, Bouzerna N (2007b). The Effect of CaNa2-EDTA on metabolism of Zinc and Carbohydrate as well as Some Biochemical Factors in Experimental Diabetes. Iranian J. Pub. Health. 36(1): 15-21.

Kechrid Z, Bouzerna N (2001). Effect of low zinc diet on Zn turnover in non-insulin dependent diabetic mice. Diabetes Metab. 27:580.

Kechrid Z, EL-Hadjla D, Naima L (2007a).The beneficial effect of vitamin E supplementation on zinc status, carbohydrate metabolism, transaminases and alkaline phosphatase activities in alloxan-diabetic rats fed on zinc deficiency diet . Int. J. Diabetes Metabolism. 15:46-50.

Kechrid Z, Kenouz R (2003). Determination of Alkaline Phosphatase Activity in Patients with Different Zinc Metabolic Disorders. Turk. J. Med. Sci. 33:387-391.

Krupanidhi S, Sreekumar A, Sanjeevi CB (2008). Copper & biological health. Indian J. Med. Res. 128: 448-461.

Maria J, Salgueiro BS, Marcela B, Zubillaga PhD, Alexis E, Lysionek

BS, Ricardo A, Caro PhD, Ricardo Weill, Eng and Jose´ R, Boccio PhD (2002). The Role of Zinc in the Growth and development of Children. Nutrition 18:510–519.
Mariani E, Mangialasche F, Feliziani FT, Cecchetti R, Malavolta M, Bastiani P, Baglioni M, Dedoussis G, Fulop T, Herbein G, Jajte J, Monti D, Rink L, Mocchegiani E, Mecocci P (2008). Effects of Zinc supplementation on antioxidant enzyme activities in healthy old Subjects. Exp. Gerontol. 43(5):445.
Matthias KD, Georg E (1996). Schulz Catalytic Mechanism of the Metal-dependent Fuculose Aldolase from *Escherichia coli* as Derived from the Structure. J. Mol. Biol. 259:458–466.
Okhawa H, Ohishi N, Yagi K (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351–358.
Parvaneh NA, Parichehr H, Shokofeh G (2009). Effect of Zinc Supplement on the Upper and Lower Trunk Strength on Athletics Women. Res. J. Int. Stud. 9:59-64
Pesce A , Kaplan A (1984). Lactate dehydrogenase. Clin Chem .The C.V. Mosby Co. St Louis. Toronto. Princeton. 438: 1124-1117.
Pinto RE, Bartley W (1969). The effect of age and sex on glutathione reductase and glutathione peroxydase on an aeortic glutathione oxidation in rat liver homogenates. Biochem J. 112 (11): 109-115.
Rahman Z, Faiza B, Ali MA, Essaddig AB, Mahjiub I, Zendah, Mehdi H, Masoud RM, Shoukat ASJ, Navid A (2001). Effects of Copper Supplement on Haematological Profiles and Broiler Meat Composition. Int. J. Agric. Biol. 03:203–205.
Richard A, Anderson PhD, Anne-Marie R, Nouri Z, Sylvia MD, Jean-Marc M, Abdelhamid Kerkeni PhD (2001). Potential Antioxidant Effects of Zinc and Chromium Supplementation in People with Type 2 Diabetes Mellitus. J. Am. Coll. Nutr. 20(3):212–218.
Savchenko A, Vielle C, Kang S, Zeikus Gg (2002). Pyrococcus furiosus α- amylase is stabilized by calcium and zinc. Biochemistry 41:6193-6201.
Southon S, Gee J, Johnson IT (1984). Hexose transport and mucosal morphology in the small intestine of the zinc-deficient rat. Br. J. Nutr. 52:371.
Stephen G, Jenkinson, Richard A, Lawrence, Raymond F, Burk, Darryl M, Williams (1982). Effects of Copper Deficiency on the Activity of the Selenoenzyme Glutathione Peroxidase and on Excretion and Tissue Retention of. 75 Se O32~12 .J. Nutr. 112:197-204.
Weckbercker G, Cory JG (1988). Ribonucleotide reductase activity and growth of glutathione- depended mouse leukaemia L 1210 cells in vitro. Cancer Lett. 40:257-264.
Willis MS, Monaghan SA, Miller ML, McKenna RW, Perkins WD, Levinson BS, Bhushan V, Kroft SH (2005). Zinc-induced copper deficiency: a report of three cases initially recognized on bone marrow examination. Am. J. Clin. Pathol. 123(1):125-131.
Ying Foo A (1998). Amylase measurement with 2-chloro-4nitrophenyl maltrotrioside as substrate. Clin. Chim. 272:137-147.

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Full Length Research Paper

Antioxidative properties and inhibition of some pro-oxidant induced lipid peroxidation by aqueous extract of two species of eggplants *Solanum macrocarpon* and *Solanum melongena*

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This study sought to determine the antioxidant activities and inhibitory effect of some egg plant species *Solanum macrocarpon* (yellow) and *Solanum melongena* (purple) on sodium nitroprusside and Fe^{2+} induced lipid peroxidation in rat's pancreas. The total phenolic, flavonoid and vitamin C content of the aqueous extracts were determined, as well as the antioxidant properties [ferric reducing antioxidant property (FRAP), Fe^{2+} chelating ability and 2,2-Azinobis(3-ethylbenzo-thiazoline-6-sulfonate) ($ABTS^{\cdot}$),1,1-diphenyl-2picrylhydrazyl (DPPH) and hydroxyl ($OH^{\cdot}$) radicals scavenging ability] inhibitory effect of the extracts on sodium nitroprusside (SNP) and Fe^{2+}-induced lipid peroxidation were also determined *in vitro.* The results revealed that there was no significant ($P > 0.05$) difference in the total phenol, total flavonoid, vitamin C and FRAP of both eggplant, while *S. melongena* had higher Fe^{2+} chelating ability and $ABTS^{\cdot}$ scavenging ability. The aqueous extracts from *S. macrocarpon* and *S. melongena* inhibited SNP and Fe^{2+}-induced lipid peroxidation in rat's pancreas in a dose dependent manner. The antioxidant properties of the aTherefore,aqueous extracts from *S. macrocarpon* and *S. melongena* eggplant species and their strong inhibition of SNP and Fe^{2+}-induced lipid peroxidation suggest that they could be a good antioxidants source in the management of degenerative diseases.

Key words: *Solanum* species, antioxidant properties, lipid peroxidation, degenerative diseases.

INTRODUCTION

Free radicals that cause cellular and metabolic injury have been associated with several health related problems including cancer, cirrhosis, ischemic reperfusion, diabetes, cardiovascular diseases and the process of aging (Fan et al., 2009; Sanaa et al., 2012). Recently, phenolics particularly flavonoids have gained much attention because of their high antioxidant activities and radical scavenging abilities with less toxicity than synthetic antioxidants (Degl'innocenti et al., 2008; Yuan and Liu, 2008). Although the phytochemicals have low potency as bioactive compounds as compared with pharmaceutical drugs, and their regular intake as a part of diet may have a noticeable long-term physiological effect (Prashant et al., 2011). Recent findings have revealed that antioxidants act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species (ROS), and therefore can enhance the immune defense and lower the risk of degenerative diseases (Pham-Huy and Pham-Huy, 2008).

Sodium nitroprusside (SNP) is an antihypertensive drug, which acts by relaxing smooth vascular muscle, consequently it dilates peripheral arteries and veins (Oboh et al., 2012). However, SNP has been implicated to cause cytotoxicity through the release of cyanide

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and/or nitric oxide (NO), and NO is involved in the pathophysiology of disorders like stroke, trauma, seizure disorders. NO could act independently or in cooperation with other ROS (Oboh et al., 2012). Moreover, iron has also been implicated as the most important pro-oxidant of lipids. Earlier report has revealed that Fe^{2+} accelerates lipid peroxidation by breaking down hydrogen and lipid peroxides formed by the fenton free radical reaction (Oboh et al., 2012).

In both developing and undeveloped countries, plants are been used to manage various degenerative diseases because phenolic compounds are an important group of secondary metabolites, which are synthesized by plants to adapt in biotic and abiotic stress conditions (infection, wounding, water stress, cold stress and high visible light).Fruits and vegetable are therefore being extensively explored to identify potential antioxidant-rich cultivar (Saniburu et al., 2010; Huang et al., 2012; Akanitapichat et al., 2010).

Solanum species from the family of Solanaceae commonly known as eggplant, aubergine, guinea squash, brinjal, madapple, garden egg, pea, aubergine is an economically important vegetable crop of tropical and temperate parts of the world (Eun-Ju et al., 2012). It acts as a source of vitamins and minerals, making its total nutritional value comparable with tomato (Kwon et al., 2008). Apart from a source of food, eggplants are also used for medicinal purposes in both a traditional and modern sense for treating diseases like diabetes, bronchitis, asthma, dysuria, dysentery, ulcer (Kwon et al., 2008; Anosike et al., 2011).

Recently, there has been particular interest in the antioxidant ability and benefit of phytochemicals in food and vegetables (Kwon et al., 2008) which have been used for a large range of purposes, including nutrition, medicine, flavoring, beverages, fabric dying, smoking, and industrial uses. Although Solanaceae fruit sales in market are dominated by a single fruit species *Solanum melongena,* there are a considerable number of species and selections in the genus that have widely diverse shape, size, and colour. While information is available on phenolic contents, flavonoids, anthocyanins, antioxidants, antiglaucoma, and anti-diabetic activity of *S. melongena* eggplant fruit (Kwon et al., 2008; Scalzo et al., 2010), few information are reported for *Solanum macrocarpon* which is commonly found in Southwestern Nigeria known as “igbagba pupa”, or “Gboma” in Ghana which is used as sauce, stew and part of salad, but there are information on the leaf part which is been used as soup condiment in Nigeria (Oboh et al., 2005; Fasuyi, 2006; Ijarotimi et al., 2010), while the aqueous extract of the fruit had been shown to be nutritious, to lower high blood pressure, to treat constipation and lower hyperlipidaemia (Chinedu et al., 2011; Dougnon et al., 2012; Sodipo et al., 2013).

The present study sought to explore further and to compare the ability of these eggplant fruit species to prevent the various pro-oxidants: Fe(II) and sodium-nitroprusside induced lipid peroxidation in isolated rats’ pancreas *in-vitro* and its antioxidant properties.

MATERIALS AND METHODS

Sample collection

Fresh eggplants (*S. melongena* L.) and (*S. macrocarpon*) (200 g) each were purchased from Erekesan main market at Akure, Ondo State, Nigeria. The identification and authentication of the samples was carried out at the Crop, Soil, and Pest management (CSP) Department of the Federal University of Technology, Akure, Nigeria. The eggplant samples were sliced into pieces, sun-dried for 7 days and milled into powder using a Waring heavy duty blender (Waring Products Division, New Hartford, Connecticut, USA), and the powder was stored in an airtight plastic container. All the chemicals used were of analytical grade, and distilled water was used throughout the analysis.

Chemicals and equipment

Folin-Ciocalteu’s phenol reagent, gallic acid and anhydrous sodium carbonate used were products of Fluka (Buchs, Switzerland). Quercetin and DPPH, ascorbic acid and starch were products of Merck (Darmstadt, Germany), iron (II) sulphate, H_2O_2, Angiotensin-converting enzyme (ACE) and ABTS were products of Sigma (Aldrich, USA). Iron (III) chloride 6-hydrate and trichloroacetic acid are Fisher products. All other chemicals used were purchased from Rovet Scientific Limited, Benin City, Edo State, Nigeria. The distilled water used was obtained from the Chemistry Department at Federal University of Technology, Akure. Optical absorbance was measured with an ultraviolet (UV)-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom).

Aqueous extract preparation

1 g of each milled eggplant sample was soaked in 50 mls of distilled water for about 24 h. The mixture was filtered and the filtrate was centrifuged to obtain a clear supernatant liquid which was subsequently used for the various assays (0boh et al., 2010).

Total phenol determination

The total phenol content of the samples extract was determined by adding 0.5 ml of the extract to an equal volume of water, and 2.5 ml 10% Folin-Ciocalteau reagent (v/v) and 2.0 ml of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45°C for 40 min, and absorbance was measured at 726 nm (JENWAY 6305). Gallic acid was used as the standard phenol (Singleton et al., 1999).

Determination of total flavonoid content

The total flavonoid content of the extracts was determined using a slightly modified method reported by (Meda et al., 2005). Briefly, 0.5 ml of aqueous sample was mixed with 0.5 ml methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1 mol L^{-1} potassium acetate and 1.4 ml water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid content was calculated using quercetin as standard.

Determination of vitamin C content

Vitamin C content of the eggplant extracts was determined using the method of Benderitter et al. (1998). Briefly, 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4 \cdot 5H_2O$ in 100 ml of 5 M H_2SO_4) were added to 500 µl reaction mixture (300 µl of appropriate dilution of the extracts with 100 µl 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 h at 37°C, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm using a spectrophotometer. The vitamin C content of the extracts was subsequently calculated.

Absorbance of sample (Abs_{sam}) = (Abs_{sam} × $Conc._{std}$) / (Abs_{std} × $Conc._{sam}$)

Where Abs_{std} = Absorbance of standard (vitamin C); Abs_{sam} = Absorbance of sample; $Conc._{std}$ = Stock concentration of standard in mg/ml; $Conc._{sam}$ = Stock concentration of sample in g/ml.

Reducing power determination

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min, and 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against a reagent blank. A higher absorbance indicates a higher reducing power.

1,1-diphenyl–2 picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Gyamfi et al. (1999). Briefly, an appropriate dilution of the extracts was mixed with 1 ml of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm.

2,2-Azinobis(3-ethylbenzo-thiazoline-6-sulfonate) $ABTS^{\cdot}$ scavenging ability

The $ABTS^{\cdot}$ scavenging ability of the extracts was determined according to the method described by Re et al. (1999). $ABTS^{\cdot}$ was generated by reacting an ABTS aqueous solution (7 mmol L^{-1}) with $K_2S_2O_8$ (2.45 mmol L^{-1}, final concentration) in the dark for 16 h, and adjusting the absorbance at 734 nm to 0.700 with ethanol. 0.2 mL of appropriate dilution of the extracts was added to 2.0 ml $ABTS^{\cdot}$ solution, and absorbance was measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity was subsequently calculated.

Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the extracts was determined using a modified method by Puntel et al. (2005). Freshly prepared 500 µmol L^{-1} $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl of 0.1mol L^{-1} Tris-HCl (pH 7.4), 218 µl saline and the extracts (0 to 100 µl). The reaction mixture was incubated for 5 min before the addition of 13 µl of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer (JENWAY 6305).

$OH^{\cdot}$ radical-scavenging ability

The ability of eggplant extracts to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared aqueous extract (0 to 100 µl) was added to a reaction mixture containing 120 ml of 20 mM deoxyribose, 400 ml of 0.1 M phosphate buffer, 40 ml of 20 mM hydrogen peroxide and 40 ml of 500 mM $FeSO_4$, and the volume was made to 800 ml with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.5 ml of 2.8% TCA; this was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in spectrophotometer (JENWAY 6305):

%OH scavenging ability = ($Abs_{control}$ – Abs_{sam}) / $Abs_{control}$ × 100

Where $Abs_{control}$ = Absorbance of control, Abs_{sam} = Absorbance of sample.

Preparation of pancreas homogenates

The rats were decapitated under mild diethyl ether anesthesia, and the pancreas was rapidly dissected, placed on ice, and weighed. This tissue was subsequently homogenized in cold saline (1:10 wt/vol) with about 10 up-and-down strokes at approximately 1,200 rpm in a Teflon® (DuPont, Wilmington, DE)-glass homogenizer. The homogenate was centrifuged for 10 min at 3,000 g to yield a pellet that was discarded, and the low-speed supernatant (S1) was collected and kept for lipid peroxidation assay (Belle et al., 2004).

Lipid peroxidation and thiobarbituric acid reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa et al. (1979). 100 µl of S1 fraction was mixed with a reaction mixture containing 30 µl of 0.1 M (pH 7.4) Tris-HCl buffer, eggplant extracts (0 to 100 µl), and 30 µl of the pro-oxidant (70 µM SNP or 250 µM $FeSO_4$), and the volume was made up to 300 µl by water before incubation at 37°C for 1 h. The colour reaction was developed by adding 300 µl of 8.1% sodium dodecyl sulfate to the reaction mixture containing S1. This was subsequently followed by addition of 600 µl of acetic acid/HCl (pH 3.4) buffer and 600 µl of 0.8% thiobarbituric acid. This mixture was incubated at 100°C for 1 h. Thiobarbituric acid-reactive species (TBARS) produced were measured at 532 nm in the spectrophotometer (JENWAY 6305) and the absorbance was compared with that of a standard curve using malondialdehyde (MDA).

Statistical analysis

The results of the replicate readings were pooled and expressed as mean ± standard deviation. Student *t* test was performed and significance was accepted at $P \leq 0.05$ (Zar, 1984). EC_{50} was determined using linear and non-linear regression analysis.

RESULTS

In this study, the total phenol content (TPC) was determined in comparison with standard gallic acid, and the

Table 1. Total phenol, Total flavonoid, Vitamin C content and ABTS· of the aqueous extracts *Solanum macrocarpon* (Yellow eggplant) and *Solanum melongena* (Purple eggplant) species.

Samples	Total phenol (mg/GAEg)	Total flavonoid (mgQE/g)	Vitamin C (mg/ml)	ABTS (mmol/100 g TEA)	Reducing power (mg/g)
S. macro.	4.22±0.50[a]	2.55±0.20[a]	4.82±0.11[a]	1.87±0.10[a]	7.63±0.25[a]
S. melon.	4.05±0.41[a]	2.11±0.25[a]	4.50±0.17[a]	2.36±0.13[b]	7.40±0.26[a]

Data represent means of replicate determinations. Values with the same letter along the same column are not significantly different ($P < 0.05$).

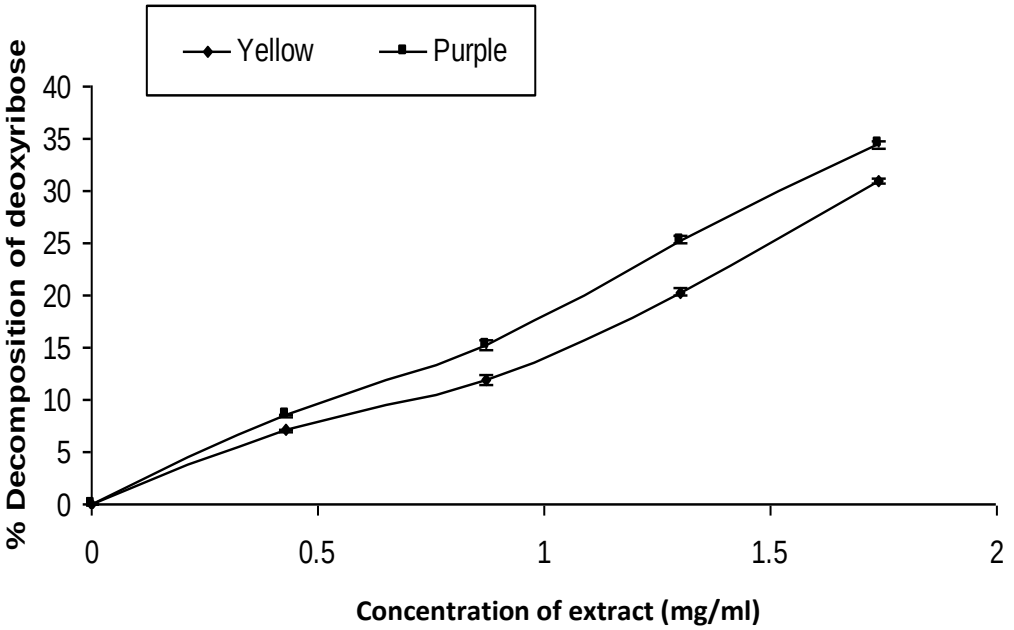

Figure 1. OH-radical scavenging ability of aqueous extracts of *S. macrocarpon* (yellow) and *S. melongena* (purple). Values represent mean ± standard deviation, $n = 3$ ($P < 0.05$).

results were expressed in terms of mg GAE/g of the extract. The TPC values obtained for *S. macrocarpon* and *S. melongena* eggplants were 4.22 and 4.05 mgGAE/g, respectively. The differences in the contents of the total phenolics (Table 1) in the extracts were not statistically significant ($P > 0.05$). Total flavonoids content (TFC) of the eggplants were determined in comparison with quercetin as a standard, and the results are shown as mgQE/g (Table 1). The TFC values obtained for *S. macrocarpon* and *S. melongena* eggplants were 2.55 and 2.11 mgGAE/g extract, respectively. The differences in the contents of the total flavonoids (Table 1) in the extracts were not statistically significant ($P > 0.05$). Furthermore, the vitamin C (in mg/g) content of both extracts was also assessed (Table 1). The results revealed that there was no significant change ($P > 0.05$) in the vitamin C content.

The reducing properties of the eggplants extracts determined at 700 nm are given in Table 1. The reducing power of *S. macrocarpon* was 7.63 and *S. melongena* 7.40. The ability of the aqueous extracts to reduce Fe^{3+} to Fe^{2+} were not statistically significant ($P > 0.05$).

The solanum species extracts were examined for their radical scavenging ability toward the stable free radical DPPH. The result is presented in Figure 1 and its EC_{50} (EC_{50} values represent eggplant concentrations required to scavenge 50% free radicals as presented in Table 2, a low EC_{50} translates to a higher antioxidant activity) Table 2. The results revealed that the radical-scavenging activities of both extracts increased with increasing concentration. However, as shown by the EC_{50} values, there was no significantly change ($P > 0.05$) in radical scavenging ability value of *S. melongena* eggplant extract (3.24 mg/ml) and *S. macrocarpon* eggplant extract (4.01 mg/ml).

The 2,2-Azinobis(3-ethylbenzo-thiazoline-6-sulfonate) $ABTS^{\cdot}$ scavenging ability of the aqueous extract was determined and reported as trolox equivalent antioxidant capacity. The result is presented in Table 1. The result revealed that *S. melongena* eggplant had a significantly

Table 2. EC_{50} of Fe^{2+} chelating ability,$OH^{\cdot}$ radical scavenging ability,DPPH radical scavenging ability of the aqueous extracts *Solanum macrocarpon* (yellow) and *Solanum melongena* (Purple eggplant) species.

Samples	Fe^{2+}-chelating ability (mg/ml)	OH-radical scavenging ability (mg/ml)	DPPH-radical scavenging ability (mg/ml)	Fe^{2+}-induced lipid peroxidation (mg/ml)	SNP-induced lipid peroxidation (mg/mL)
S. macro	2.87±0.33[a]	3.02±0.67[a]	4.01±0.59[a]	2.96±0.23[a]	1.31±0.73[a]
S. melon	3.40±0.20[b]	2.58±0.60[a]	3.24±0.21[a]	2.44±0.62[a]	1.37±0.59[a]

Data represent means of replicate determinations. Values with the same letter along the same column are not significantly different ($P < 0.05$).

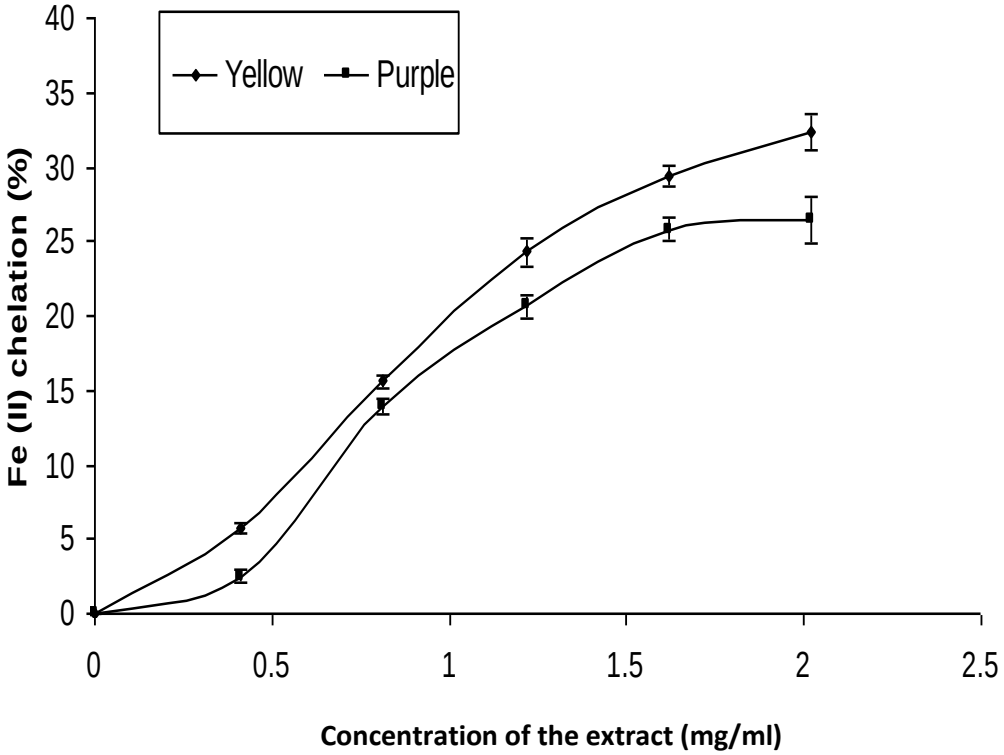

Figure 2. Fe^{2+}chelating ability of aqueous extracts of of *S. macrocarpon* (yellow) and *S. Melongena* (purple) eggplant. Values represent mean ± standard deviation, $n = 3$ ($P < 0.05$).

higher ($P < 0.05$) $ABTS^{\cdot}$ (2.36 mmol/100 g) radical scavenging ability. The plot of chelating% versus mg/ml was shown and its EC_{50} (Table 2). The result revealed that the extracts chelated iron in a dose dependent manner and judging by the EC_{50}, the *S. macrocarpon* eggplant extract at the highest concentration 2.02 mg/ml exhibited higher Fe^{2+} chelating 32.31% than the *S. melongena* eggplant extracts (26.43%) (Figure 2).

The hydroxyl radical ($OH^{\cdot}$) radical scavenging ability of the eggplant extracts are presented in (Figure 3), the extracts scavenged $OH^{\cdot}$ in a dose-dependent manner and judging by the EC_{50}. However *S. melongena* eggplant extract had the higher scavenging ability of 34.45% at the highest concentration (1.74 mg/ml) tested. The interaction of both extracts with Fe (II) induced lipid peroxidation in isolated rat's pancreas homogenates is presented in Figure 4. As shown, increased MDA (105.48%) produced due to incubation of the pancreas tissues in the presence of 250 µM Fe (II) was decreased by both eggplant extracts at the concentration range tested (1.25 to 6.25 mg/ml). However, there was no significant change ($P > 0.05$) in the ability of both extracts to inhibit Fe (II)-induced lipid peroxidation in the rat's pancreas. Incubation of rat pancreas homogenate in presence of SNP caused a significant increase ($P < 0.05$) in the MDA content 110.62% (Figure 5), however, the eggplant extracts inhibited MDA production in rats' pancreas homogenate in a dose dependent manner, with *S. macrocarpon* eggplant extract having higher inhibitory ability, inhibiting MDA production to 55.03% of control values at the highest

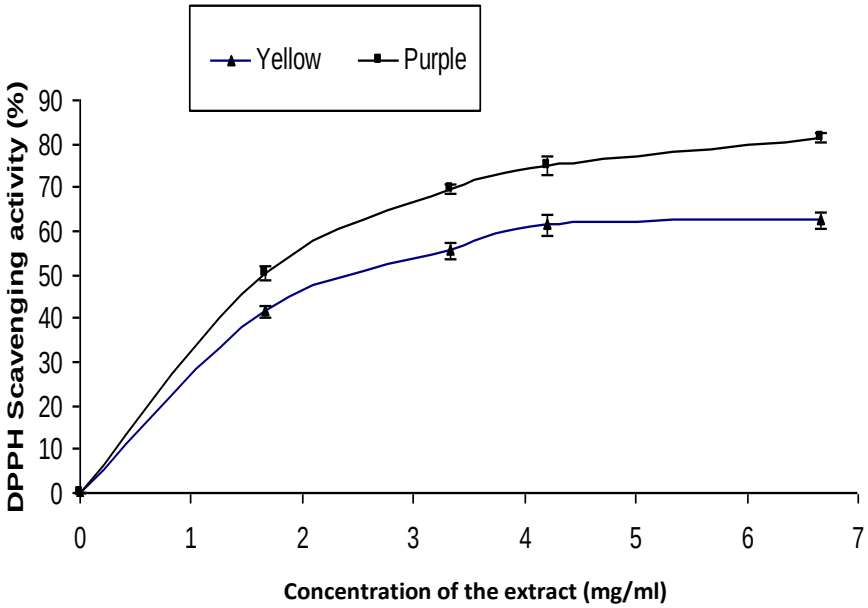

Figure 3. DPPH radical scavenging ability of aqueous extracts of *S.macrocarpon* (yellow) and *S.melongena* (purple) eggplant. Values represent mean ± standard deviation, $n = 3$ ($P < 0.05$).

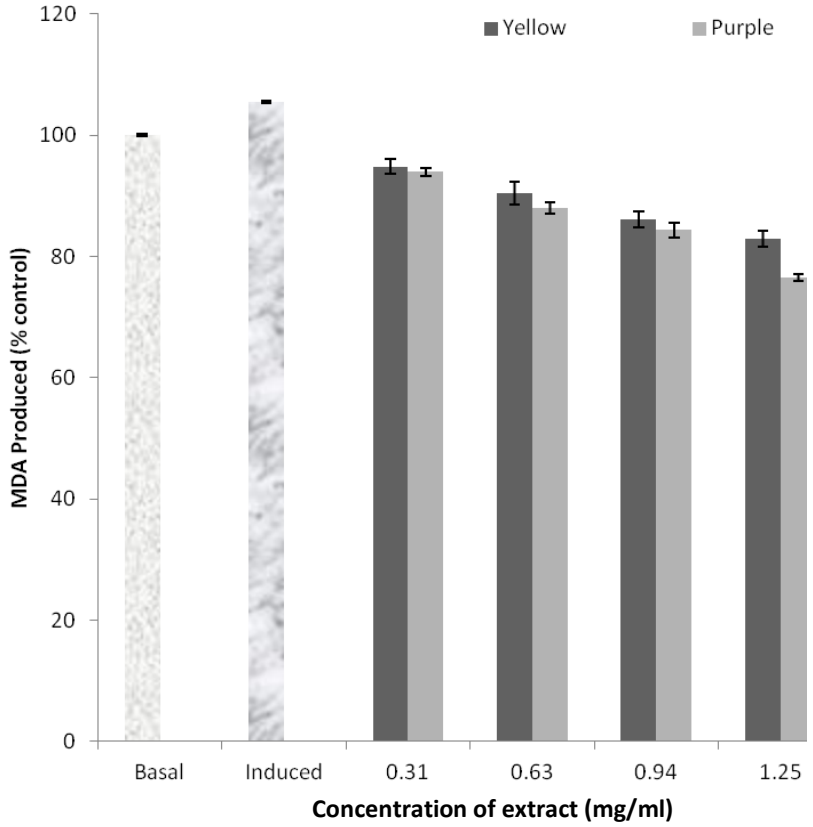

Figure 4. Fe^{2+}-induced lipid peroxidation in pancreas by aqueous extracts of *S.macrocarpon* (yellow) and *S.melongena*(purple) eggplant.Values represent mean ± standard deviation, $n = 3$ ($P<0.05$).

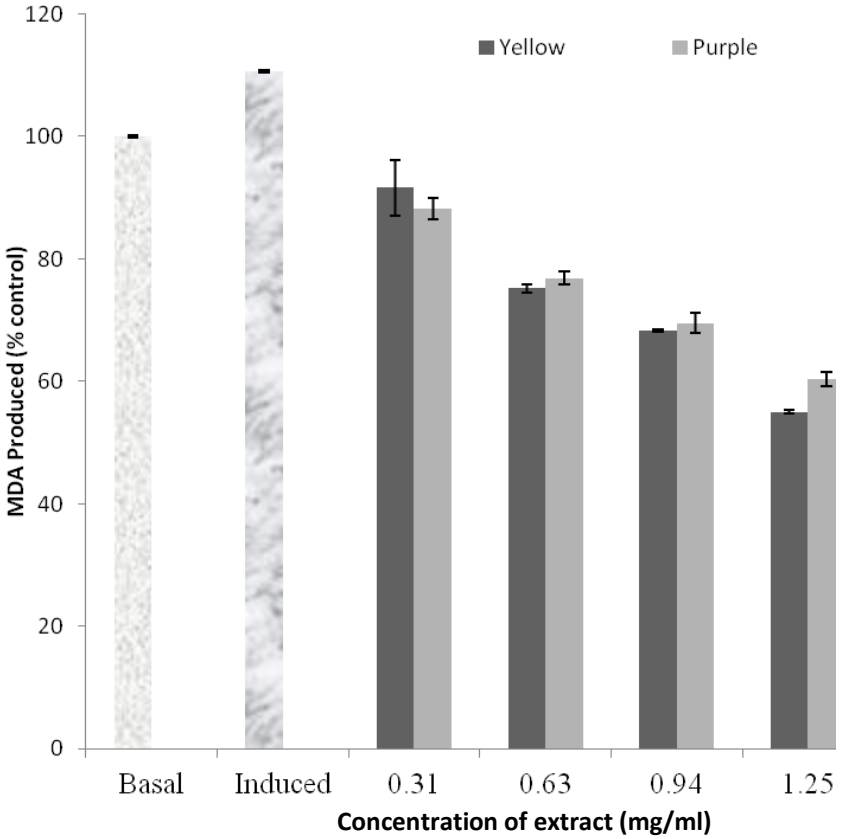

Figure 5. Inhibition of SNP-induced lipid peroxidation in pancreas by aqueous extracts of *S.macrocarpon* (yellow) and *S.melongena* (purple) eggplants.Values represent mean ± standard deviation, $n = 3$ ($P < 0.05$).

concentration (1.25 mg/ml) tested.

DISCUSSION

The antioxidant activities of phenolics are due to their redox properties. The phenol moiety (hydroxyl group on aromatic ring) helps them to work as reducing agents, hydrogen donors and singlet oxygen quenchers (Chua et al., 2008). Considering the heterogeneity of natural phenols and the possibility of interference from other readily oxidized substances, several methods including Folin–Ciocalteu, permanganate titration, colorimetry with iron salts and ultraviolet absorbance have been used for total phenol determination. But in most direct comparisons, the Folin–Ciocalteu method has been found preferable and is being used by many researchers. In this method, phenols form a blue colored phosphomolybdic–phosphotungstic–phenol complex in alkaline solution (Singleton et al., 1999). Polyphenols, particularly the flavonoids, are among the most potent plant antioxidants, the presence of high concentrations of polyphenolic phytochemicals in fruits and vegetables has been shown to increase antioxidant activities (Ghasemi et al., 2009).

There was no difference in the total phenolic and flavonoid contents of the eggplant species analysed. Total flavonoids of the solanum cultivars were determined in comparison with quercitin as standard. However, total phenols content reported from this studies is higher than that of fruits of *Myrica esculenta* (Rawat et al., 2011), but within the same range on aqueous extract of date plum persimmon (*Diospyros lotus* L.) fruits reported by (Hui et al., 2011). Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid is the main biologically active form of vitamin C. Ascorbic acid is widely distributed in plant cells where it plays many crucial roles in growth and metabolism. As a potent antioxidant, ascorbic acid has the capacity to protect renal injury (kensara, 2013), eliminate several different reactive oxygen species, keeps the membrane-bound antioxidant α-tocopherol in the reduced state, acts as a cofactor maintaining the activity of a number of enzymes (Arrigoni and De Tullio, 2002; Davey et al., 2000; Kleiln and Kurilich, 2000). Both species extract had high vitamin C compared to studies done on plantain pulp (Shodehinde and Oboh, 2012).

The reducing power of a compound may serve as an indicator of its antioxidant activity. The presence of

reductants such as antioxidant substances causes the reduction of Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrous form. Therefore, the reducing power of the sample could be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Manian et al., 2008). Aqueous extract with higher reducing power have better abilities to donate electrons. Free radicals form stable substances by accepting the donated electrons, resulting in the termination of radical chain reactions. It has been widely accepted that the higher the absorbance at 700 nm, the greater the reducing power. There was no difference statistically in the reducing power of the two species assessed. However, the species had a higher reducing power compared to *S. aethiopicum* species reported (Nwanna et al., 2013).

In this study, we investigated the 1,1-diphenyl–2 picrylhydrazyl free radical scavenging ability (DPPH) of eggplant extracts when compared with standard antioxidants ascorbic acid. It was observed that the scavenging activity was increased with the increasing concentration of the sample extract. The hydrogen donating ability of the bioactive compounds in the water extractable phytochemicals could be responsible for the DPPH scavenging ability of the eggplants. *S. melongena* exhibited higher scavenging ability than its counterpart, this could be as a result synergistic-like antioxidant effect of the total phenols, flavonoids constituents. However, the result from this studies is high compared to that of kinnow citrus cultivars (Khizar et al., 2011), ethanolic extract of some parts of *S. melongena* L. (Eun-ju et al., 2011) and also some of citrus juices (Ademosun and Oboh, 2012).

The radical scavenging ability of the aqueous extract of both species was further studied using 2,2-Azinobis(3-ethylbenzo-thiazoline-6-sulfonate)$ABTS^{\cdot}$ which is a moderately stable nitrogen-centred radical species. The ABTS radical-based model of radical scavenging ability has the advantage of being more versatile due to the minimal spectral interference as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Re et al., 1999) and reported as trolox equivalent antioxidant capacity (TEAC). The results revealed that *S. melongena* eggplant extract had higher $ABTS^{\cdot}$ scavenging ability than *S. macrocarpon*, which compliment DPPH ability reported earlier.

Incubation of the pancreas tissues in the presence of 25 µM Fe (II) increased malondialdehyde (MDA) produced in the isolated pancreas homogenates when compared with the basal tissues homogenate. These findings agree with the earlier reports of (Oboh et al., 2012) in that Fe (II) is a potent pro-oxidant. The increased lipid peroxidation, in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze an electron transfer reaction that generates reactive oxygen species which is formed from H_2O_2 through the Fenton reaction. Iron also decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, which favours the propagation of lipid oxidation (Oboh et al., 2012a). Nevertheless, both extracts at the concentration range tested caused a significant decrease in the MDA produced in the Fe (II) induced lipid peroxidation in the isolated rats pancreas homogenates in a dose dependent manner. The reason for the inhibition of the lipid peroxidation by the extracts cannot be categorically stated, however it will not be farfetched from the possibility that the phytochemicals could have scavenged the $OH^{\cdot}$ produced from the Fenton reaction (Oboh et al., 2012a).

Sodium nitroprusside (SNP) is a component of antihypertensive drugs; it causes cytotoxicity through the release of cyanide or $NO^{\cdot}$, which acts in cooperation with other ROS to cause neuronal damage (Manian et al., 2008). The extracts inhibited SNP-induced lipid peroxidation in a dose-dependent manner, although there was no significant change in their inhibitory ability from the species assessed. It was obvious from the study there was agreement between the total phenol content, reducing power, vitamin C and Fe (II) induced and SNP-lipid peroxidation, and this goes a long way to confirm the possible correlation between the antioxidant activities of these extracts.

Fe (II) chelating ability and $OH^{\cdot}$ scavenging ability of the extract were also determined. Both extracts chelated Fe (II) in a dose-dependent manner. The ability of the solanum extracts to form complexes with Fe (II) may have caused a significant reduction in the available Fe (II) that will be required to catalyze the production of free radicals in order to initiate lipid peroxidation in the tissues. Furthermore, the Fe (II) chelating ability may have contributed immensely to the ability of the extract to prevent Fe (II) induced lipid peroxidation in the isolated rat's pancreas homogenates. The ability of the aqueous extracts to inhibit Fe (II)/H_2O_2-induced decomposition of deoxyribose is an index for hydroxyl radical ($OH^{\cdot}$) scavenging ability as shown from the EC_{50}. EC_{50} values represent eggplant concentrations required to scavenge 50% radicals as presented; a low EC_{50} translates to a higher antioxidant activity.

Results revealed that there was no change in $OH^{\cdot}$ scavenging ability ribose decomposition. Moreover, it was observed that aqueous extracts of the eggplant had better inhibitory properties on SNP-induced lipid peroxidation than Fe (II) induced lipid peroxidation. This result is in the same trend with studies on polyphenols of aqueous extract of plantain and ginger (Shodehinde and Oboh 2012; Oboh et al., 2012b).

Conclusion

The antioxidant properties of the a~~Therefore,a~~queous extracts from the eggplant species (*S. macrocarpon* and *S. melongena*) and their strong inhibition of SNP and Fe^{2+}-induced lipid peroxidation suggest that they could be a source of natural antioxidants in the management

of degenerative diseases. However, it is worth knowing that *S. macrocarpon* showed a high antioxidant properties just like the commonly known *S. melongena* and could be promoted.

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REFERENCES

Ademosun AO, Oboh G (2012). Inhibition of Acetylcholinesterase Activity and Fe^{2+}-Induced Lipid Peroxidation in Rat Brain In Vitro by Some Citrus Fruit Juices. J. Med. Food 15(5):428–434.

Akanitapichat P, Kallayanee P, Kwunchai N, Suparichart P (2010). Antioxidant and hepatoprotective activities of five eggplant varieties. Food Chem. Toxicol. 48(10):3017-3021.

Anosike C, Abonyi O, Ubaka C (2011). Does the African garden egg offer protection against experimentally induced ulcers? Asian Pac. J. Trop. Med. pp.163-166.

Arrigoni O, De Tullio M C (2002). Ascorbic acid: much more than just an antioxidant. Biochimica et Biophysica Acta.1569: 1-9.

Belle NAV, Dalmolin GD, Fonini G, Rubim MA, Rocha JBT (2004). Polyamines reduces lipid peroxidation induced by different prooxidant agents. Brain Res. 108:245-251.

Benderitter M, Maupoil V, Vergely C, Dalloz F, Briot F, Rochette L (1998). Studies by electron paramagnetic resonance of the importance of iron in hydroxyl scavenging properties of ascorbic acid in plasma:effects of iron chelators. Fundament. Clin. Pharmacol. 12:510-516.

Chinedu SN, Olasumbo AC, Eboji OK,Emiloju OC, Arinola OK, Dania DI (2011). Proximate and Phytochemical Analyses of *Solanum aethiopicum* L. and *Solanum macrocarpon* L. Fruits. J. Chem. Sci. 1(3).2231-606X.

Chua MT, Tung YT, Chang ST (2008). Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. Bioresour Technol. 99: 1918–1925.

Davey MW, Montagu MV,Inzé D,Sanmartin M, Kanellis A,Smirnoff N, Benzie IJ, Strain JJ, Favell D,Fletcher J (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 80,825-860.

Degl'innocenti E, Pardossi A, Tattini M. Guidi L (2008). Phenolic compounds and antioxidant power in minimally processed salad. J. Food Biochem. 32:642–653.

Dougnon TV, Bankolé HS, Johnson RS, Klotoé JR, Fernand GD, Assogba GF (2012). Phytochemical Screening, Nutritional and Toxicological Analyses of Leaves and Fruits of *Solanum macrocarpon* Linn (Solanaceae) in Cotonou (Benin). Food Nutr. Sci. 3:1595-1603.

Eun-Ju J, Myung-Suk B, Eun-Kyung J, Young-Hong J, Seung-Cheol L (2011). Antioxidant activity of different parts of eggplant. J. Med. Plants Res. 5**(**18):4610-4615.

Fan JB, Wu LP, Chen LS, Mao XY, Ren FZ, (2009). Antioxidant activities of silk sericin from silkworm *Bombyx mori*. J. Food Biochem. 33:74–88.

Fasuyi AO (2006). Nutritional potentials of some tropical vegetable leaf meals:chemical characterization and functional properties. Afr. J. Biotechnol. 5:49–53.

Ghasemi K, Ghasemi Y, Ebrahimzadeh MA (2009). Antioxidant activity,phenol and flavonoid contents of 13 citrus species peels and tissues. Pak. J. Pharm. Sci. 3:277–281.

Gyamfi MA, Yonamine M, Aniya Y (1999). Free radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally induced liver injuries.Gen. Pharmacol. 32:661-667.

Halliwell B, Gutteridge JMC (1981). Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. Febs Lett. 128:347-352.

Huang WY, Zhang HC, Liu WX, Li CY (2012). Survey of antioxidant capacity and phenolic composition of blueberry,blackberry,and strawberry in Nanjing. J Zhejiang Univ Sci B (Biomed. Biotechnol. 13(2):94-102.

Hui G, Ni C, Juan Z, Bini W, JianJun D, Wei C (2011). Antioxidant activities and phenolic compounds of date plum persimmon (*Diospyros lotus* L.) fruits. J. Food Sci. Technol. DOI 10.1007/s13197-011-0591.

Ijarotimi OS, Ekeh O, Ajayi OP (2010). Nutrient composition of selected medicinal leafy vegetables in western Nigeria. J. Med. Food 13(2):476-479.

Kensara OA (2013). Protective effect of vitamin C supplememtation on oxonate-induced hyperuricemia and renal injury in rats. Int. J. Nutr. Metabol. 5(4)pp.61-68.

Khizar H, Shabbar A, Chengsheng J, Shuqin X, Xiaoming Z (2011). Comparative study on phenolic compounds and antioxidant activity of feutrell's early and kinnow peel extracts. J. Food Biochem. 35: 454–471.

Klein BP, Kurilich PAC (2000).Processing effects on dietary antioxidants from plant foods. HortScience, 35(4), 580-584.

Kwon YI, Apostolidis EK, Shetty D (2008). In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresour. Technol. 9:2981–2988.

Manian R, Anusuya N, Siddhuraju P, Manian S (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz,*Ficus bengalensis* L. and *Ficus racemosa* L. Food Chem. 107:1000–1007.

Meda A, Lamien CE, Romito M, Millogo J Nacoulma OG (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. Food Chem. 9:571-577.

Minotti G, Aust SD (1987). An investigation into the mechanism of citrate-Fe^{2+}-dependent lipid peroxidation. Free Radic. Biol. Med. 3:379-387.

Oboh G, Akinyemi AJ, Ademiluyi AO, Adefegha SA (2010). Inhibitory effect of aqueous extracts of two varieties of ginger on some key enzymes linked to type 2-diabetes *In-vitro*. J. Food Nutr. Res. 49(1):14-20.

Oboh G, Akinyemi AJ, Ademiluyi AO (2012a). Antioxidant and inhibitory effect of red ginger (Ziingiber officinale Var.Rubra) and white ginger (Zinguber officinale Roscoe) on Fe^{2+}-induced lipid peroxidation in rat brain *In vitro*. Exp.Toxicol. Pathol. 64:31-36.

Oboh G, Ekperigin MM, Kazeem MI (2005). Nutritional and haemolytic properties of eggplants (*Solanum macrocarpon*) leaves. J. Food Comp. Anal. 18:153-160.

Oboh, G, Akinyemi AJ, Ademiluyi AO (2012b). Inhibition of Acetylcholinesterase activities and some pro-oxidant induced lipid peroxidation in rat brain by two varieties of ginger (*Zingiber officinale*). Exp. Toxicol. Pathol. 64:315-319.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351-358.

Oyaizu M (1986). Studies on products of browning reaction:antioxidative activity of products of browning reaction prepared from glucosamine. Jpn. J. Nutri. 44:307–315.

Pan Y, Wang K, Huang S, Wang H, Mu X, He C (2008). Antioxidant activity of microwave assisted extract of longan (*Dimocarpus Longan* Lour.) peel. Food Chem. 106:1264–1270.

Pham-Huy LA, He H, Pham-Huy C (2008). Free Radicals, Antioxidants in Disease and Health. Int. J. Biomed. Sci. 4:89-96.

Prashant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K (2011). Phytochemical screening and Extraction: A Review: Int. Pharm. Sci. 1:98-106.

Puntel RL, Nogueira CW, Rocha JBT (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *in vitro*. Neurochem. Res. 30:225-235.

Rawat S, Jugran A, Giri L, Indra DB, Ranbeer SR (2011). Assessment of Antioxidant Properties in Fruits of *Myrica esculenta*: A Popular Wild Edible Species in Indian Himalayan Region. Evidence-Based Complem. Altern. Med. ID 512787.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans CA (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26:1231-1237.

Sanaa MMS, Soha SMM, Emad AS, Ghada IM (2012). Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. Asian Pac J. Trop. Biomed. 608-615.

Saniburu E, Saliha S, Ahin CD, Cihat TU,Vildan U (2010). Phenolic content and antioxidant-activity of raspberry and blackberry cultivars. J. Food Sci. 75:328-334.

Scalzo LR, Fibianl M, Mennella G, Giuseppe L, Rotino S, Sasso M (2010).Thermal treatment of eggplant (*Solanum melongena* L.) increases the antioxidant content and the inhibitory effect on human neutrophil burst. J. Agric. Food Chem. 58:(6):3371-3379.

Shodehinde SA, Oboh G (2012). Assessment of antioxidant capacity, proximate composition and inhibitory activity of unripe plantain (*Musa paradisiaca*) products on Fe^{2+} and sodium nitroprusside -induced oxidative stress *in vitro.* J. Toxicol. Environ. Health Sci. 4(3):46-56.

Singleton VL, Orthofer R, Lamuela RRM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin– Ciocalteu reagent. In: Packer L (Ed.): Methods in enzymology. Vol. 299 : Oxidants and antioxidants, Part A. San Diego, California : Academic Press:152-178.

Sodipo OA, Abdulrahman FI, Sandabe UK, Wampana B (2013). Effects of the Aqueous Fruit Extract of *Solanum macrocarpum* L., α-solanidine and Standard Lipid Lowering Agents on Biochemical Kidney Function of Hyperlipidaemic Rats Administered Triton-X Orally for 7 Days. Int. Res. J. Plant 4(1):25-32.

Zar JH (1984). Biostatistical Analysis. Prentice-Hall, Upper Saddle River NJ.

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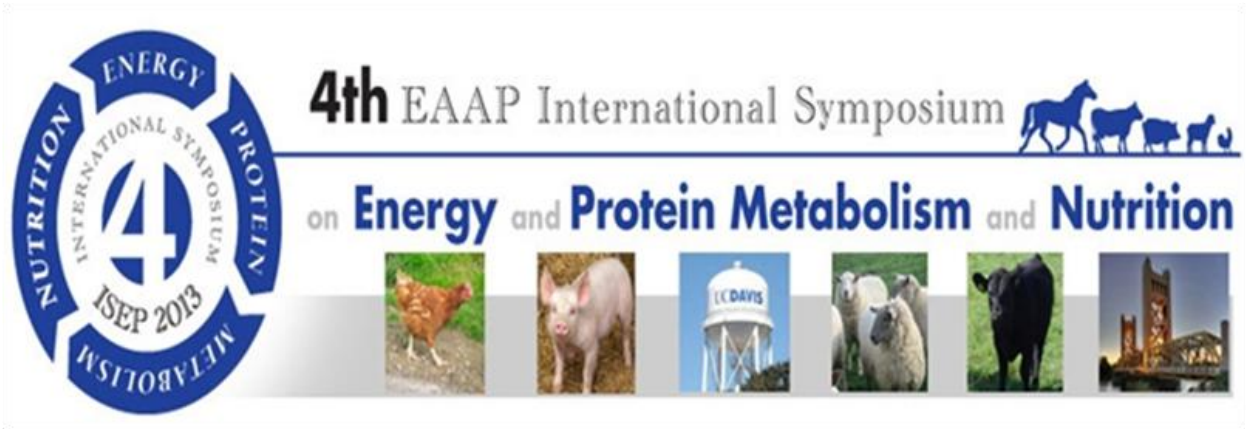

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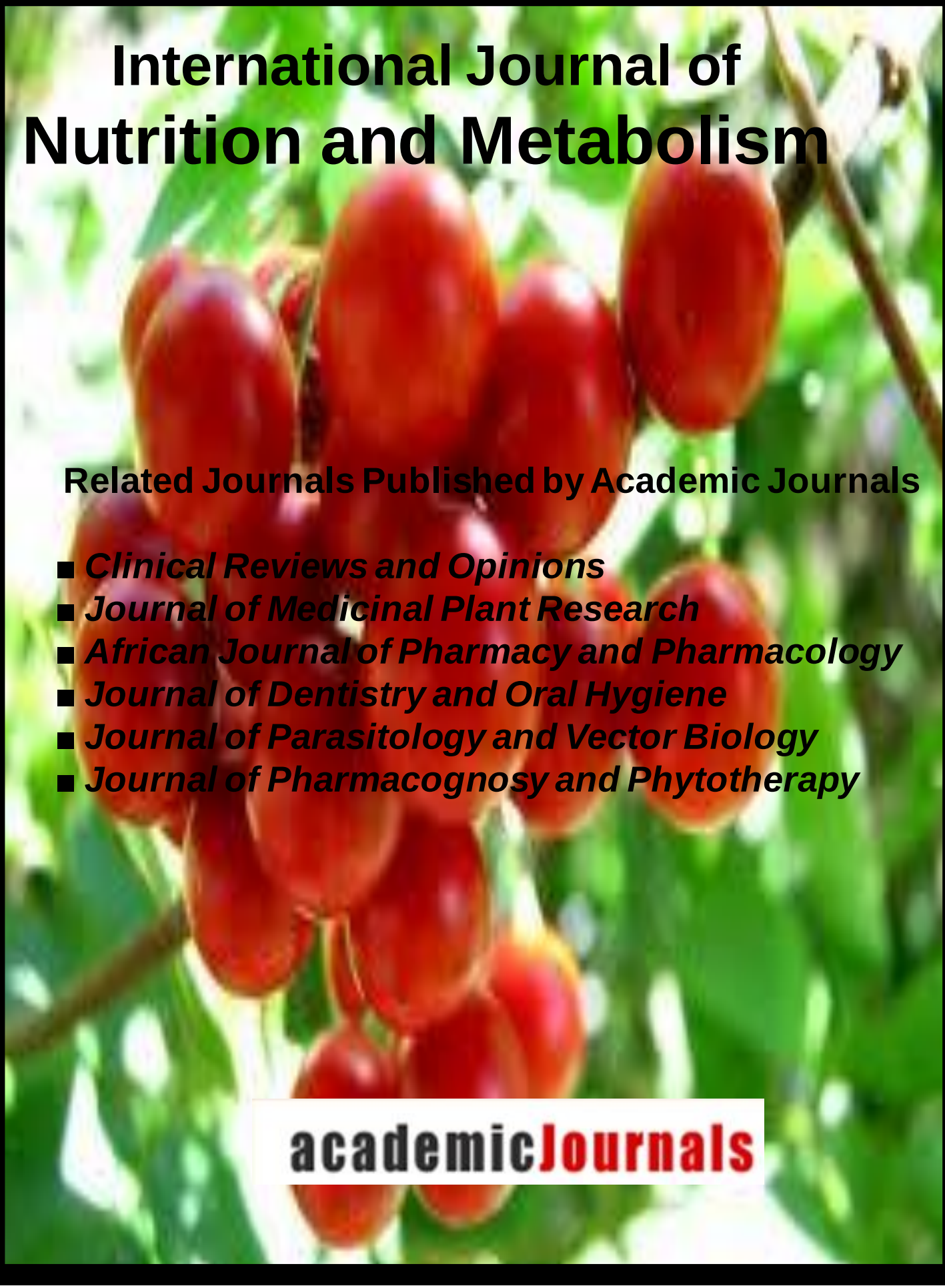

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