Trop J Nat Prod Res, March 2020; 4(3):105-112 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant Effect of Ex-maradi Okra Fruit Variety (*Abelmuscus esculentus*) on Alloxan-Induced Diabetic Rats

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ARTICLE INFO

Article history:
Received 28 February 2020
Revised 21 March 2020
Accepted 28 March 2020
Published online 30 March 2020

ABSTRACT

Oxidative stress is associated with the development of diabetic complications and supplementation with antioxidants might delay the development of these complications in diabetic patients. The aim of the present study was to investigate the antioxidant potentials of different parts (Whole Okra, 'WO' Okra Peel 'OP' and Okra Seed 'OS') of Ex-maradi Okra fruit variety on Alloxan-induced diabetic rats. Vitamins A, C and E were determined by spectrophotometric method. Catalase activity was assayed using chemical reactivity method of Beers and Sizer and glutathione by the method described by Petterson and Lazarow. Malonaldehyde (MDA) was measured by the method of Shah and Walker. For antioxidant minerals, Atomic absorption spectroscopy (AAS) was used. Significant ($P < 0.05$) reduction in serum levels of vitamins A, C and E, Catalase activity, Reduced Glutathione, Zn, Cr and Mn and significant ($P < 0.05$) increase in serum levels of Cu, Fe and lipid peroxidation product (MDA) were observed in the diabetic untreated rats compared to the normal control rats. Oral administration of the different parts of the Okra fruit each at doses of 100, 200 and 300 mg/kg to the diabetic rats for 21 days resulted in significant ($P < 0.05$) reversal of the observed altered aforementioned biochemical parameters in a dose-dependent manner compared to the normal control rats. The results indicated that the Whole Okra fruit and Okra Seeds of Ex-maradi Okra fruit variety exhibited significant antioxidant effect in Alloxan-induced diabetic rats.

Keywords: Diabetes Mellitus, Oxidative stress, Antioxidants, *Abelmuscus escuientus.*

Introduction

Diabetes mellitus is associated with chronic hyperglycemia that produces multiple biochemical sequelae including diabetes-induced oxidative stress that could play a role in the symptoms and progression of the disease.[1] Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and/or from decreases in antioxidant defense potentials.[2] Several hypotheses have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequent increased formation of glucose-derived advanced glycation end products (AGEs), and enhanced glucose flux through the polyol pathway.[3,4] Elevated generation of free radicals resulting in the utilization of antioxidant defense components including vitamins A, C and E; alteration in the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in conjunction with decreased levels of essential minerals may lead to disruption of cellular functions and oxidative damage to membranes and may also enhance susceptibility to lipid peroxidation.[5,6]

Trace-element deficiencies or problems with its absorption are frequently associated to chronic diseases. Chronic hyperglycemia may cause significant alterations in the status of some micronutrients, and on the other hand, some of these nutrients can directly modulate glucose homeostasis.[7,8] The early imbalances of some specific metals may play a role in upsetting normal glucose and insulin metabolism.[9] Despite the great strides made in the understanding and management of diabetes, the disease and disease related complications remains unabated. Phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics.[10] Epidemiological studies demonstrated that many phytonutrients from fruits might protect the human body against oxidative stress. It is a known fact that nutrition and health care are interrelated. Thus the consumption of natural antioxidant phytochemicals was noted to have potential health benefits.[11]

Abelmoschus esculentus, 'AE' (Okra or lady's finger) is a flowering plant in the mallow family. It is one of the most important vegetables widely cultivated throughout the tropical and temperate regions of the world for its tender fruits.[12] This plant is popular with its mucilaginous properties and has been acclaimed to have various health benefits which include anti-diabetic properties.[13] Okra is also known for being high in antioxidants activity as it is rich in phenolic compounds with important biological properties like quarcetin and flavonol derivatives, catechin oligomers and hydroxycinnamic derivatives.[14] The fruit of this particular plant is rich in nutrients like protein, niacin, riboflavin, thiamine, folate, vitamins A, B6, C and K, Phosphorus, Zinc, Copper, Potassium, Magnesium, Calcium, and Manganese all of which plays vital role in human diet and health.

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Citation: Abbas AY, Muhammad I, AbdulRahman MB, Bilbis LS. Antioxidant Effect of Ex-maradi Okra Fruit Variety *(Abelmuscus esculentus*) on Alloxan-Induced Diabetic Rats. Trop J Nat Prod Res. 2020; 4(3):105-112. doi.org/10.26538/tjnpr/v4i3.8

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Okra Sample Collection

Ex-maradi, (a commercially available dry-Okra fruits variety from vegetable growers/sellers at Maradi, Niger) was obtained from Maggi market at Sokoto State, Nigeria. The sample was botanically identified and authenticated by Mal. A. Umar a taxonomist in the Botany unit of the Department of Biological Sciences at Usmanu Danfodiyo University, Sokoto. A voucher specimen number (UDUH/ANS/0066) was assigned to the sample while the specimen sample was deposited in the Herbarium of the same department.

Okra Sample Preparation

The procured Okra sample was thoroughly sorted to remove any unwanted matter. Five hundred grammes (500 g) of the Okra fruit selected was weighed and grounded to fine powder (500 g) using a domestic grinder. Another 1000 g of the Okra fruit sample was also weighed and broken to separate the seeds from the pods. The two portions of the samples (Okra peels and the seeds) were separately grounded to fine powdered form. The powdered samples were sieved with a fine mesh. The fine powdered samples (200 g each for peel and seed) were placed in a labeled sealed container and stored at normal laboratory conditions until when required for reconstitution and administration.

Experimental Animals

Seventy two (72) apparently healthy young Wistar rats of both sexes weighing between 100-120 g were purchased from the animal house, Department of Biochemistry, Usmanu Danfodiyo University Sokoto and were used for this study. The rats were kept at the animal house under normal environmental conditions and maintained with free access to pelletized growers feed from Grand Cereals Limited, and allowed access to water *ad libitum*. The animals were allowed to acclimatize for two weeks. Ethical approval was obtained from the University Research and Ethics Committee (UREC) with reference number UDUS/UREC/2018/001/8.

Induction of Diabetes Mellitus

The Alloxan diabetic rat models were prepared by adopting the method of Kandur and Goyal.[15] All rats, except the Normal Control Group were intraperitoneally injected with 120 mg/kg body weight of the prepared Alloxan. After 6 h of alloxan administration, rats in their cages were then allowed 10% glucose solution for the next 24 hours in other to prevent alloxan-induced hypoglycemia. The animals were observed for polydipsia, polyuria, polyphagia as well as general reduction of body weight. Seventy two hours after the alloxan administration, the animals were fasted overnight and diabetes was confirmed from the rats by measuring their fasting blood glucose level with the aid of a single touch glucometer. Only rats that have fasting blood glucose level >7.0 mmol/L (126 mg/dL) were considered and included in the study.[15]

Grouping and Administration of Okra Fruit Samples to Rats

Simple random sampling technique was used in grouping the rats for this study. The rats were divided into four (4) large groups: Whole Okra (WO), Okra Peel (OP), Okra Seed (OS) and Control group (C). Each of the large group was further sub-divided into three sub-groups (WO_1, WO_2, WO_3; OP_1, OP_2, OP_3; OS_1 OS_2, OS_3) and the control groups were Metformin, Normal and Diabetic control group (MC, NC and DC) each containing six (6) rats. Each group of rats were housed in a labeled cage, feed with pelletized growers feed and allowed access to water *ad libitum* throughout the period of the study. Okra sample (WO, OP and OS) were administered orally every morning by intubation using intravenous cannula tube at doses of 100, 200 and 300 mg/kg body weight to the respective rats in their respective groups by single forced oral feeding once per day for a period of 21 days. The rats in the Metformin control group (MC) were administered with 500 mg/kg body weight Metformin using the same procedure as mentioned above while the DC and NC (Diabetic control and Normal control groups) were not given any treatment but allowed only normal diet and water.

Collection of Blood Samples

Twenty four hours after the last treatment, the animals were subjected to 12 hours fasting after which the animals were anaesthetized by dropping individual animal in a plastic jar saturated with chloroform vapor.[12] The animals were then remove from the jar and blood samples collected from them through cardiac puncture into labeled plastic sample bottles, the blood were allowed to clot, then centrifuged at 4000 g for ten minutes. The sera obtained were pipetted into labeled specimen test tubes for estimation of serum antioxidant markers.

Determination of Serum Vitamin A

Serum Vitamin A was assayed by spectrophotometric method (Using spectrophotometer; Model: SP300 Optima Germany) as described by Rutkowski *et al.*[16] and a modification of Bessey *et al.*[17] One milliliter (1 mL) of the serum was placed into a centrifuge test tube and 1 mL of KOH solution was added. The tube was shaken vigorously for 1 minute. The tube was heated in a water bath (60^{o}C, 20 minutes), then cooled down in cold water. One millilitre (1 mL) of xylene was added and the test tube was shaken vigorously again for 1 minute. The mixture was centrifuged for 10 minutes at 4000 rpm, the supernatant was transferred to a glass test tube and the absorbance of the solution was read at 335 nm against xylene. This gave the absorbance of retinol and any other material soluble in xylene (A_1). The xylene extract was irradiated to UV light for 30 minutes, until a steady absorbance (A_2) is obtained at 335 nm. This gave the absorbance of the unwanted material only as the entire retinol was bleached. The absorbance of retinol was obtained by subtracting the second absorbance (A_2) from the initial absorbance (A_1). The concentration (C_x) of serum vitamin A (µmol/L) in the sample was calculated using the formula:

$$C_X = (A_1 - A_2).22.23$$

Where: 22.23 is the multiplier on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette about thickness = 1 cm.

Determination of Vitamin C

Serum vitamin C was assayed by spectrophotometric method (Using spectrophotometer; Model:SP300 Optima Germany) as described by Omaye *et al.*[18] To 0.5 mL of serum, 2 mL of freshly prepared 6% metaphosphoric acid was added, mixed thoroughly and centrifuged for 10 minutes at 3500 rpm. Similarly, blank and standard were prepared but instead of serum, 0.5 mL of distilled water and standard were added. To 1.2 mL of supernatant, 0.4 mL of DTC reagent (5 mL of 5% thiourea solution + 5 mL of 0.6% copper sulphate + 10 mL DNPH reagent) was added, mixed and incubated in a water bath at 37^{o}C for 3 hours. The test tubes were transferred into ice bath for 10 minutes. Two millilitres (2 mL) of 12 M sulphuric acid was added and mixed. The absorbance was read at 520 nm after zeroing the instrument with blank.

$$Concentration\ of\ vit\ C = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times Concentration\ of\ standard$$

Determination of Serum Vitamin E

Serum vitamin E concentrations were estimated using the method of Hashim and Schuttringer.[19] The major principle of this method is based on the reduction of ferric ions to ferrous ions, which form red colour with α, α-dipyridyl. About 1.5 mL of standard vitamin E and 1.5 mL of distilled water were dispensed into test tubes labeled as test, standard, and blank, respectively. 1.5 mL of ethanol was added to test and blank. 1.0 mL of xylene was added into all the tubes. The contents were mixed and centrifuged at 2500 rpm for 10 minutes, 1 mL of xylene layer was pipetted into another set of identically labeled test tubes and1 mL of α, α-dipyridyl reagent was added to the test tubes. The contents of the test tubes were mixed and the absorbance of sample and standard were read against blank at 460 nm. After 3 minutes 0.33 mL of ferric chloride was added to the test tubes and another absorbance was taken at 520 nm.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

$$Conc.\,of\,Vit.E = \frac{Abs.of\ sample\ at\ 520nm - Abs.of\ sample\ at\ 460nm \times Conc.of\ std.}{Abs.of\ std.at\ 520nm}.$$

Estimation of Catalase Activity
Catalase activity was assayed using chemical reactivity method Beers and Sizer.[20] The principle is based on disappearance of peroxide which was followed spectrophotometrically at 240 nm. One unit enzyme decomposes one mole H_2O_2 per minute at 25^0C and pH 7.0 under the specified conditions.

Estimation of Reduced Glutathione
Estimation of reduced glutathione was as described by Petterson and Lazarow.[21] The principle is based on the fact that Glutathione reacts with an excess of Alloxan to produce a substance (dialluric acid) with an absorbance peak at 305 nm.

Estimation of Lipid Peroxidation product
Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured by the method of Shah and Walker.[22] The assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid, forming an MDA-TBA_2 adduct that absorbs strongly at 535 nm. The protein in serum were precipitated by TCA and then removed by centrifugation. Two (2) test tubes were labeled Test and Blank, and to the Test test tube 50 µL of serum, 450 µL of distilled water, 250 µL of 1.3 TBA and 250 µL of 40% TCA were added. For Blank test tubes 500 µL of distilled water, 250 µL of 1.3 TBA and 250 µL of 40% TCA were added. The tubes were mixed and incubated in water bath at 90^oC for 30 minutes. The tubes were allowed to cool at room temperature for 20 minutes. Then the content of the tubes was centrifuged at 200 rpm for 15 minutes, the supernatant was read spectrophotometrically at 535 nm.

Calculation: The concentration of malondialdehyde was calculated as follows:

$$\text{Malondialdehyde: (nmol/L)} = \frac{\text{Absorbance of test} - \text{Absorbance of Blank}}{1.56 \times 100}$$

Estimation of Antioxidant Minerals
The antioxidant minerals (Cu, Fe, Zn, Mn and Cr) were determined by the use of Atomic absorption spectroscopy.

Preparation of Samples
Wet digestion was carried out on the serum samples in accordance with the method of Bhatti *et al.*,[23] 0.5 mL serum was taken in 50 mL conical flask, 5 mL Nitric acid (HNO_3) were added slowly and heated for 20 minutes until yellow fumes disappear. The flask was allowed to cool for 30 minutes and then 2.5 mL of perchloric acid ($HClO_4$) was added and the mixture heated until it becomes colourless. The heated samples was diluted by adding 20 mL distilled water then filtered into plastic bottle and stored until when needed for the spectroscopic measurements. The concentrations of metals were determined using Atomic Absorption Spectrophotometer (BUCK Scientific; Model 210 VGP).

Statistical Analysis
The data obtained were presented as mean ± standard error of the mean. Results were analyzed statistically by one way analysis of variance (ANOVA) followed by postHoc, Duncan test using the statistical package for Social Sciences (SPSS) software, version 20. A *p*-value < 0.05 was considered statistically significant.

Results and Discussion

Diabetes Mellitus is characterized by elevated levels of oxidative stress indices, decreased levels of antioxidants and abnormalities due to lipid peroxidation.[24] In the current study, it was observed that there was significant increase in oxidative stress markers in alloxan-induced diabetic rats compared with the normal control rats.

Effect of Different Parts of Okra Fruit on Serum Levels of Antioxidant Vitamins in alloxan diabetic rats
The effect of different parts (WO, OP and OS) of the Okra fruit on serum levels of antioxidant vitamins (Vitamin A, C and E) in alloxan-induced diabetic rats are presented in Table 1. The results indicated that the Alloxan-induced diabetic untreated group (DC) of rats have significantly ($p < 0.05$) lower levels of vitamin A, C and E as compared with the normal control group (NC). However administration of different parts of okra fruit to the treatment groups after three weeks showed significant ($p < 0.05$) increase in the levels of serum vitamin A, C and E in comparison with the diabetic untreated group (DC). It was also observed that there were significant ($p < 0.05$) decreases in the serum vitamin A, C and E levels in the Metformin control group than that of the normal control group. This finding is in agreement with that of Aliyu *et al.*[25] who reported significantly low levels of antioxidant vitamins in diabetes. Some studies indicated that these deficiencies have been correlated with the presence of diabetic complications.[26] Hyperglycemia generates reactive oxygen species (ROS) and also attenuate antioxidant mechanism, creating a state of oxidative stress. Results of the effect of treatments with different parts of the Okra fruit in the different experimental groups after three weeks indicated significant ($p < 0.05$) increase in the serum levels of these vitamins in the entire Okra treated groups in comparison with the diabetic control (DC) group. This increment might be obvious because Okra fruit have been reported to be a good source of such vitamins, and other components that may be required for the synthesis of such vitamins.[27] Okra fruit may therefore serve as supplements of those vitamins and their precursors. Although, the effect of treatments with the different parts of the Okra fruit showed significant increment in the serum levels of vitamins A, C and E; However, when all the treatments with the different parts (WO, OP, and OS) of the Okra fruit were compared with one another, the result indicated that serum vitamins A, C and E levels were found to be higher in WO_3 group followed by OP_3 where the least was in OS_3 group. Hence, Okra fruit (most importantly, WO) may therefore serve as a good supplements that may restore and balance the loss antioxidant vitamins in diabetes.

Effect of Administration of Different Parts of Okra Fruit on Serum Catalase activity, reduced Glutathione (GSH) and MDA Levels
The effect of different parts (WO, OP and OS) of the Okra fruit on antioxidant indices (Catalase activity, Reduced Glutathione and MDA levels) are presented in Table 2. The results indicated significant decrease ($p < 0.05$) in serum levels of reduced glutathione (GSH) and Catalase activity in the diabetic untreated group (DC) as compared with the normal control group (NC). It was also observed that there was significantly increased serum MDA level in the diabetic untreated group (DC) as compared with that of the normal control group (NC). Administration of different parts (WO, OP, and OS) of the Okra fruit after three weeks resulted in significant ($p < 0.05$) increase in serum levels of reduced glutathione (GSH) and Catalase activity as well as significant ($p < 0.05$) decrease in serum level of MDA when compared with the diabetic untreated group (DC). The result also showed that, there was no significant difference in serum Catalase activity, reduced Glutathione and MDA levels when the Normal and Metformin control groups are were compared. Diabetes mellitus (DM) is characterized by elevated levels of oxidative stress markers and decreased level of antioxidants leading to increased lipid peroxidation.[24] It has been suggested that oxidative stress plays an important role in many diseases, including DM, since hyperglycemia alone could not be exclusively responsible for the complications associated with DM.[28] In the current study, the results indicated that the alloxan-induced diabetic untreated rats (DC) have significantly ($p < 0.05$) lower levels of serum reduced glutathione (GSH) and Catalase activity but significantly ($p < 0.05$) higher level of serum MDA, (a marker of lipid peroxidation) as compared with the normal control group (NC) (Table 2). The significant increase in lipid peroxidation as revealed by the high level of MDA formed in the alloxan-induced diabetic rats compared to the normal control rat suggest that the natural antioxidant

defense mechanism to scavenge excessive free radicals have been compromised in rats induced with diabetes.[29] Decrease in antioxidant enzyme activity as well as increased MDA as observed in diabetes mellitus might be due to an altered intracellular ratio between free radicals and antioxidant capacity because the reactive oxygen species (ROS), which are excessively produced in diabetes, are able to overwhelm the endogenous defense systems, then a state of oxidative stress originates.[26] Results of the effect of treatments with different parts (WO, OP and OS) of the Okra fruit after three weeks of treatment showed significant ($p < 0.05$) elevation in the serum levels of GSH and CAT activity in comparison with that of the diabetic control (DC) group. It was observed that the OS treated groups have statistically similar CAT activity and GSH levels with the normal and Metformin (NC and MC) control groups (Table 2). Also, the result indicated that MDA levels was significantly ($p < 0.05$) reduced in the OS treated groups when compared with the diabetic untreated group. The decreased concentration of MDA and increased levels of GSH and Catalase activity as observed in the diabetic rats treated with WO, OP and most importantly OS might be connected with their antioxidant activity where the WO, OP and most importantly OS exerts their positive ability by restoring the levels of GSH, antioxidant vitamins and some minerals; hence, reactivating the antioxidant system.[30] The different parts (WO, OP and most importantly OS) of the Okra fruit may also exert their effect by inhibiting the formation of lipid peroxides and restores the levels of fatty acids.[31] Okra fruit have been reported to possess antioxidant substances like iso-quercetin and quercetin-3-O-beta-glucopyranosyl- (1"→6")-glucoside.[32] These are thought to be effective in increasing the activities of antioxidant defense enzymes, scavenging free radicals, preventing oxidative damage and thereby sparing lipid components of the cells against lipid peroxidation.[33] This finding is in agreement with the finding of Holeckek *et al*.[34] who reported an increased activity of antioxidant enzymes and decrease concentration of MDA in diabetic patients supplemented with mixture of antioxidant vitamins and trace elements. Hunker *et al*.[35] also reported an increase in the activity of GPX and CAT in STZ-induced diabetic rats supplemented with cod liver oil. Therefore, the different parts (WO, OP and most importantly, OS) of the Ex-maradi Okra fruit variety as tested in alloxan-induced diabetic rats were able to correct lipid homeostasis and restore redox status in alloxan-induced diabetic rats, effects that are probably due to their antioxidant and free radical scavenging properties.

Effect of Administration of Different Parts of Okra Fruit on Serum Levels of Antioxidant Minerals

The effect of different parts (WO, OP and OS) of the Okra fruit on serum antioxidant and pro-oxidant minerals (Cu, Cr, Zn, Mn and Fe) are presented in Table 3. The results indicated significant ($p < 0.05$) elevation in the levels of serum Cu, and Fe but significantly ($p < 0.05$) decreased levels of serum Zn, Mn and Cr in the diabetic untreated group (DC) as compared with the normal control group (NC). Treatments with the different parts (WO, OP, and OS) of the Okra fruit after three weeks showed significant ($p < 0.05$) increase in the serum levels of Zn, Cr and Mn as compared with that of the diabetic untreated group (DC) while that of Fe and Cu were observed to significantly ($p < 0.05$) decrease (Table 3). Although, treatments with all the different parts of the Okra fruit (WO, OP, and OS) showed significant increment ($p < 0.05$) in the serum levels of Zn, Mn and Cr when compared with the diabetic control (DC) group; however, there were no significant ($p > 0.05$) differences in serum Zn, Mn and Cr levels when the entire Okra treated groups, the Metformin treated group and the normal control (NC) group were compared with one another. The data also indicated that there was no significant ($p > 0.05$) difference in the serum levels of Cu and Fe in the normal and Metformin (NC and MC) control group; However, the serum levels of Fe in all the groups treated with the different parts of the Okra fruit were observed to be statistically ($p > 0.05$) the same while the serum levels of Cu varies in most of the groups (Table 3). Again, when the effect of treatments with all the different parts (WO, OP, and OS) of the Okra fruit were compared with one another, the result indicated that serum levels of Fe, Zn, Mn and Cr were found to be statistically similar in the WO, OP, and OS groups but that of Cu varies significantly in some of the groups. Imbalances of specific metals may play a role in upsetting normal glucose and insulin metabolism.[36] The data obtained from the current investigation indicated that the alloxan-induced diabetic untreated (DC) group of rats had significantly ($p < 0.05$) elevated levels of serum Cu, and Fe as compared to that of the normal control group (NC) while serum Zn, Cr and Mn concentrations were found to be significantly ($p < 0.05$) low in the diabetic untreated (DC) rats as compared to that of the normal control (NC) group (Table 3). This contradicted the findings of Giovanni *et al*.[37] where their report did not show significant difference in serum Fe content in diabetic patients compared to the non-diabetic counterpart and thus, they suggested that the Fe metabolism was not altered in diabetes. Similar results have also been reported by (Kazi *et al*.,[38] Serdar *et al*.,[39] and Basaki *et al*.[40] However, in other researches, increase in plasma Fe levels and in lymphocytes have been reported in diabetic population.[41,42] The Increased concentration of serum Iron in diabetes could be explained based on the fact that Iron (Fe) is a pro-oxidant molecule.[43] Since the state of oxidative stress could be originated in diabetic condition where powerful oxidizing species can bind with normal cellular components and results in cellular oxidative damages,[44] hence, ruptured red blood cells could lead to high concentration of free Fe in diabetic condition.[45] It has been reported that high concentration of free Fe is involved in the pathogenesis of diabetic retinopathy.[43] Excess plasma Fe has been implicated in the pathogenesis of diabetes and its complications.[46] Furthermore, free iron (Fe) serves as a catalyst for lipid and protein oxidation and the formation of reactive oxygen species.[47] Indeed, In the presence of hyperglycemia and inflammation, Iron may contribute to the development and progression of oxidative injury.[47] As regards to copper (Cu), diabetes-related complications seemed to arise both with decreased and increased levels of serum Cu.[37] Serum Cu accumulation is well correlated with the macroangiopathy and microangiopathy, hypercholesterolemia, and hypertension in diabetics.[48] Also, the Cu deficiency might be a contributor to the glucose intolerance in diabetic patients Many authors have observed an accumulation of Cu in diabetes[38,39,42,49,50] while some observed a decrement[40] where as others observed the absence of any relationship between Cu level and diabetes onset or diabetes complications.[41] In the present study, it was observed that there was increase in serum level of Cu in the diabetic untreated group than the normal control group. This finding is supported by the findings of[51] where they reported that increase in Cu concentration has been linked to disorders in the structure of the arterial walls, stress, infection, and diabetes mellitus.[42] Also, the relationship between increase in Cu concentration and the oxidation of low-density lipoproteins have been reported by Zbronska *et al*.[52] and Tan *et al*.[53] Failla and Kiser[54] reported the markedly elevated concentrations of Cu in the liver and kidney of insulin deficient diabetic rats. The elevated level of Cu was later normalized by insulin treatment and they suggested that hormonal imbalance may be related to altered tissue Cu content.[54] Moreover, intestinal Cu absorption in diabetic rats was shown to be markedly enhanced; this may also contribute to increased tissue Cu content.[55] Treatment with different parts (WO, OP and OS) of the Okra fruit resulted in significant ($p < 0.05$) reduction in the serum Fe and Cu concentration when compared with the diabetic untreated control group (DC).

The decreased levels of serum Zn, Cr and Mn as obtained from this findings is in line with many reports where it was confirmed that "Chronic hyperglycemia may cause significant alterations in the status of some micronutrients, and on the other hand, supplementation of some of the nutrients could directly modulate glucose homeostasis".[56] Deficiencies of certain minerals such as Mn, Zn, and Cr have been shown to predispose a person to glucose intolerance and to promote the development of diabetic complications.[49] The level of Zn was low in the diabetic untreated rats as depicted from the data (Table 3). Similar result have been observed by Chung *et al*.[57] where they reported increased urinary excretion of zinc in diabetic patients resulting in lower values in serum Zn concentration, especially in patients with diabetic nephropathy.[58]

With reference to the result of this study, serum Cr level in the diabetic non treated rats was also significantly lower than the normal control group (Table 3). This may reflect a reduction in chromium

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

uptake and increased urinary Cr excretion in diabetic patients as observed by other researchers.[59] Nsonwu *et al.*[60] reported that, urinary Cr excretion of subjects with type I diabetes was more than twice as observed in control subjects. Morris *et al.*[61] reported that plasma Cr was low by 33% in diabetics and that plasma Cr was inversely correlated with plasma glucose during the onset of non-insulin-dependent diabetes mellitus. Also, our result is in good agreement with the investigation reported by Cefalu *et al.*[62] who observed that severe Cr deficiency in diabetics led to fasting hyperglycemia, glucosuria, and impaired growth. The implication of these findings cannot be over emphasized. Chromium has been reported to increase insulin binding to cells, number of insulin receptors and activate insulin receptor kinase leading to increase insulin sensitivity.[63] Anderson[64] elucidated the action of Cr in diabetes and showed that the administration of Cr may have beneficial effects on diabetics. Again, Chromium supplementation was reported to raise plasma HDL:LDL ratio.[65]

Based on the data obtained, it was observed that the diabetic untreated rats had significantly ($p < 0.05$) lower levels of serum Manganese as compared to the normal controls. This may be obvious since Mn in association with SOD forming Mn-SOD could be decreased due to increased oxidative stress as a result of persistent hyperglycemia. This is supported by the data of Adewumi *et al.*[66] and Kazi *et al.*[38] In fact, Lee *et al.*[67] found that the Mn-SOD metallation and activity can be augmented with Mn supplementation in normal mice on normal chow, and Mn treatment can increase insulin synthesis, secretion and improve glucose tolerance under conditions of dietary stress. Moreover, Appropriate Mn levels are required for normal insulin synthesis and secretion.[68] Ekmekcioglu *et al.*[41]showed that insulin-resistant diabetic patients responded well to oral doses of Mn. Indeed, the deficiencies of certain minerals such as Zinc (Zn), Manganase (Mn) and Chromium (Cr) have been shown to predispose a person to glucose intolerance and to promote the development of diabetic complications.[49]

It was reported that Zn is involved in the synthesis, storage, secretion and conformational integrity of insulin, Zn and insulin monomers assemble to a dimeric form for storage and secretion as crystalline insulin.[69] Lower level of Zn in body may affect the ability of the islet cells of the pancreas to produce and secrete insulin, particularly in type-II diabetes.[70] Many epidemiological studies reported decreased plasma Zn and intracellular Zn concentrations, and increased urinary Zn excretion compared to non-diabetic subjects.

Treatment with different parts (WO, OP and OS) of the Okra fruit for three weeks resulted in significant ($p < 0.05$) reduction in the serum Fe and Cu concentration and also, significant ($p < 0.05$) increment in the serum levels of Mn, Cr, and Zn concentration when compared with the diabetic untreated control group. These could be due to the hypoglycemic effect showed by all the different parts of the Ex-maradi Okra fruit. However, based on general observations of the results obtained, the OW_3 treated group showed better results than OP and OS. This might be connected with the fact that the whole Okra fruit contains more concentrations of micronutrients and antioxidant potentials which exert its hypoglycemic and antioxidant effects than the use of Okra peel or Okra seed alone.

Table 1: Effect of Different Parts of Okra Fruit on Serum Levels of Antioxidant Vitamins in alloxan induced diabetic rats

GROUP	VIT. A (µM)	VIT. C (µM)	VIT. E (mg/dl)
[NC]	2.74 ± 0.35^{c}	39.48 ± 1.06^{f}	25.26 ± 1.58^{cd}
[DC]	1.01 ± 0.09^{a}	11.60 ± 0.94^{a}	14.50 ± 1.20^{a}
[MC]	1.75 ± 0.16^{b}	19.98 ± 0.82^{b}	27.32 ± 1.09^{d}
[WO_1]	1.24 ± 0.24^{ab}	28.10 ± 3.42^{d}	21.43 ± 0.92^{b}
[WO_2]	2.66 ± 0.14^{c}	33.43 ± 4.74^{e}	21.89 ± 1.16^{bc}
[WO_3]	2.91 ± 0.21^{c}	34.93 ± 0.93^{f}	22.11 ± 0.94^{bc}
[OP_1]	0.88 ± 0.20^{a}	23.52 ± 1.42^{c}	20.10 ± 0.85^{b}
[OP_2]	1.70 ± 0.37^{b}	26.87 ± 1.63^{d}	19.97 ± 0.73^{b}
[OP_3]	1.82 ± 0.11^{b}	31.51 ± 1.21^{e}	25.37 ± 1.15^{cd}
[OS_1]	0.84 ± 0.10^{a}	17.43 ± 1.13^{b}	18.30 ± 0.98^{b}
[OS_2]	1.03 ± 0.09^{a}	21.27 ± 1.70^{c}	19.49 ± 1.27^{b}
[OS_3]	2.02 ± 0.11^{b}	20.06 ± 1.67^{c}	19.32 ± 2.05^{b}

Values are expressed as mean ± S.E.M., Mean values having different superscript letter in the same column are significantly different at ($p < 0.05$) while those having the same superscript letter in the same column are significantly not different at ($p < 0.05$).

Key: VIT. A: (Vitamin A); VIT. C: (Vitamin C); VIT. E: (Vitamin E); NC: Normal Control, DC: Diabetic Control, MC: Metformin Control (500 mg/kg) body weight of Metformin; WO_1, WO_2, WO_3: (100, 200 & 300 mg/kg) body weight of Whole Okra., OP_1, OP_2, & OP_3: (100, 200 & 300 mg/kg) body weight of Okra Peel and OS_1, OS_2, & OS_3: (100, 200 & 300 mg/kg) body weight of Okra Seed.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Effect of Different Parts of Okra Fruit on Serum Catalase Activity, Reduced Glutathione (GSH) and MDA Levels in alloxan induced diabetes in rats

GROUP	CAT (IU/L)	GSH(mg/dL)	MDA (nmol/L)
[NC]	41.70 ± 1.20^{d}	33.04 ± 3.02^{c}	54.93 ± 4.83^{a}
[DC]	26.85 ± 1.19^{b}	18.62 ± 2.22^{a}	103.42 ± 6.45^{e}
[MC]	35.37 ± 2.09^{cd}	31.53 ± 1.75^{c}	53.47 ± 2.61^{a}
[WO_1]	23.63 ± 0.75^{c}	24.12 ± 4.60^{b}	95.25 ± 3.47^{e}
[WO_2]	28.40 ± 0.69^{c}	22.16 ± 2.24^{b}	91.91 ± 5.10^{c}
[WO_3]	29.37 ± 1.71^{c}	29.40 ± 2.35^{c}	57.35 ± 3.89^{b}
[OP_1]	31.01 ± 2.83^{a}	19.65 ± 1.97^{b}	90.53 ± 4.38^{e}
[OP_2]	31.94 ± 2.10^{c}	19.88 ± 2.21^{b}	81.70 ± 6.75^{d}
[OP_3]	36.31 ± 2.70^{c}	22.64 ± 3.33^{b}	71.93 ± 6.20^{e}
[OS_1]	44.01 ± 3.91^{d}	23.62 ± 3.60^{b}	69.77 ± 4.88^{c}
[OS_2]	46.82 ± 2.21^{d}	29.97 ± 0.89^{c}	71.93 ± 7.11^{c}
[OS_3]	43.75 ± 3.60^{d}	33.38 ± 3.31^{c}	59.50 ± 4.41^{b}

Values are expressed as mean ± S.E.M., Mean values having different superscript letter in the same column are significantly different at ($p < 0.05$).
Key: CAT: Catalase, GSH: reduced glutathione, MDA: malondialdehide, NC: Normal Control, DC: Diabetic Control, MC: Metformin Control (500 mg/kg) body weight of Metformin; WO_1, WO_2, WO_3: (100, 200 & 300 mg/kg) body weight of Whole Okra., OP_1, OP_2, & OP_3: (100, 200 & 300 mg/kg) body weight of Okra Peel and OS_1, OS_2, & OS_3: (100, 200 & 300 mg/kg) body weight of Okra Seed.

Table 3: Effect of Different Parts of Okra Fruit on Serum Levels of Antioxidant Minerals in alloxan induced diabetic rats

GROUP	Cu (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cr (ppm)
[NC]	0.96 ± 0.04^{c}	0.33 ± 0.07^{ab}	0.76 ± 0.09^{c}	1.21 ± 0.16^{c}	0.59 ± 0.06^{b}
[DC]	1.72 ± 0.10^{d}	1.46 ± 0.04^{d}	0.14 ± 0.03^{a}	0.04 ± 0.01^{a}	0.10 ± 0.01^{a}
[MC]	0.92 ± 0.10^{c}	0.28 ± 0.03^{a}	0.98 ± 0.09^{e}	1.01 ± 0.03^{bc}	0.89 ± 0.06^{c}
[Wo_1]	0.68 ± 0.07^{b}	0.50 ± 0.05^{bc}	0.51 ± 0.13^{c}	0.82 ± 0.10^{b}	0.49 ± 0.06^{b}
[Wo_2]	0.99 ± 0.17^{c}	0.59 ± 0.06^{c}	0.80 ± 0.16^{dc}	0.88 ± 0.10^{bc}	0.58 ± 0.06^{b}
[Wo_3]	0.75 ± 0.03^{c}	0.54 ± 0.04^{c}	0.98 ± 0.03^{e}	0.96 ± 0.12^{bc}	0.61 ± 0.08^{b}
[Op_1]	0.74 ± 0.12^{c}	0.67 ± 0.07^{c}	0.36 ± 0.11^{b}	0.68 ± 0.12^{b}	0.43 ± 0.05^{b}
[Op_2]	0.69 ± 0.04^{b}	0.63 ± 0.07^{c}	0.68 ± 0.27^{c}	0.79 ± 0.04^{b}	0.40 ± 0.04^{b}
[Op_3]	0.66 ± 0.05^{b}	0.60 ± 0.05^{c}	0.78 ± 0.11^{c}	0.88 ± 0.15^{bc}	0.42 ± 0.04^{b}
[Os_1]	0.60 ± 0.04^{a}	0.60 ± 0.12^{c}	0.82 ± 0.07^{dc}	0.99 ± 0.12^{bc}	0.47 ± 0.02^{b}
[Os_2]	1.06 ± 0.22^{c}	0.51 ± 0.05^{bc}	0.77 ± 0.07^{c}	0.93 ± 0.04^{bc}	0.60 ± 0.05^{b}
[Os_3]	0.91 ± 0.08^{c}	0.63 ± 0.06^{c}	1.08 ± 0.12^{e}	1.20 ± 0.07^{c}	1.03 ± 0.11^{c}

Values are expressed as mean ± S.E.M., Mean values having different superscript letter in the same column are significantly different at ($p<0.05$) while those having the same superscript letter in the same column are significantly not different at ($p<0.05$).
Key: GRP: Group, Cu: Cupper, Fe: Iron, Zn: Zinc, Mn: Manganese, Cr: Chromium, NC: Normal Control, DC: Diabetic Control, MC: Metformin Control (500 mg/kg) body weight of Metformin; WO_1, WO_2, WO_3: (100, 200 & 300 mg/kg) body weight of Whole Okra., OP_1, OP_2, & OP_3: (100, 200 & 300 mg/kg) body weight of Okra Peel and OS_1, OS_2, & OS_3: (100, 200 & 300 mg/kg) body weight of Okra Seed.

Conclusion

Based on the biochemical analysis conducted, the findings of this study led us to conclude that the biochemical abnormalities induced by alloxan in rat model include; oxidative stress, reduced level of antioxidants vitamins, enzymes and minerals. All of these were markedly restored to near normal levels by treatment with Ex-maradi Okra fruit (WO). However, the Okra seed showed better results in the antioxidant enzymes than the Okra peel. Whole Okra fruits contain the combination of nutrients in addition to minerals and antioxidants which could be the significant parts of the biochemical components needed in exerting and facilitating the reduction of oxidative stress markers which may help in the prevention or delaying diabetic complications.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to acknowledge the contribution of Tertiary Education Trust Fund (TET Fund) via Umaru Musa Yaradua University Board.

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