

Ameliorative Effects of Vitamin C and Zinc in Alloxan-induced Diabetes and Oxidative Stress in Wistar Rats

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Abstract: This study was undertaken to evaluate the ameliorative effects of vitamin C and zinc on blood glucose levels and lipid peroxidation in Alloxan-induced diabetic Wistar rats. Diabetes was induced in animals by intraperitoneal injection of Alloxan monohydrate (150 mg/kg b w). Diabetic rats were randomly divided into three groups (n = 5): Rats in group I were given 1ml of distilled water and served as the control. Rats in group II and group III were administered 100 50 mg/kg b w of vitamin C and zinc respectively. The regimens were given once daily for seven days. Blood samples collected from the animals at the end of the treatment period and assayed for malonaldehyde (MDA) as index of lipid peroxidation. The study showed that there was a significant (p<0.05) decrease in the blood glucose levels of 206.40±33.71, 115.80±14.75, 204.20±55.93 and 125.80±25.44, 118.0±9.55, 123.60±31.71, with Vitamin C 100 mg/kg and zinc 50 mg/kg respectively when compared to control group. Alloxan induced group showed an increased concentration of Malondialdehyde (MDA) of 3.16±0.98. However, there was a significant reduction (p<0.05) in Malondialdehyde concentration in the group that received 50 mg/kg b w of zinc, while no significant change (p>0.05) was observed in the group that were administered 100 mg/kg b w of vitamin C when compared to the diabetic control group. The present study has shown that vitamin C and zinc had a beneficial effect on Alloxan induced hyperglycemia and oxidative stress as evidenced by decreased malondialdehyde (MDA) concentration.

Key words: Alloxan, diabetes mellitus, malondialdehyde, oxidative stress, vitamin C, zinc

INTRODUCTION

Diabetes Mellitus (DM) is a major health problem worldwide in recent time, and Asia and Africa are the most viable area where the disease is feared to rise 2-3 folds (Jamkhande *et al.*, 2010) it is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin (Maritim *et al.*, 2002) the sustained hyperglycemia attacks both microvessels throughout the body and leads to various complications like blindness, neuropathy, end stage kidney disease, liver damage and cardiovascular events (Tea and Henrik, 2009). Increased oxidative stress is a widely accepted participant in the development and progression of diabetic tissue damage and induced changes in the activities of antioxidant enzymes in various tissues (Ceriello, 2000; Ahmed, 2005a, b). In diabetes mellitus, oxidative stress seems mainly to be due to an increased production of free radicals and/or a sharp reduction of antioxidant defenses (Low *et al.*, 1997; Giugliano *et al.*, 1996). Free radical production caused by hyperglycemia may occur via at least four different routes:

- Increased glycolysis
- Intercellular activation of sorbitol pathway

- Auto-oxidation of glucose nonenzymatic protein glycation (Vaag *et al.*, 1992; Williamson *et al.*, 1993; Wolff *et al.*, 1991; Ceriello *et al.*, 1992).

Vitamin C (ascorbic acid) is a water-soluble micronutrient required for multiple biological functions (Halliwell, 2001). It is found intra- and extracellularly as ascorbate, and is well absorbed from the gastrointestinal tract (Chihuilaf *et al.*, 2002; Woollard *et al.*, 2002; Asiley *et al.*, 2004). Vitamin C is a natural antioxidant that prevents the increase production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Sies *et al.*, 1992). It has been shown to react directly with superoxide hydroxyl radicals, and singlet oxygen (Hemila *et al.*, 1985; Bielski, 1982; Bodannes and Chan, 1999). The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may particularly prevent certain types of hepatic cellular damage (McDowell, 1989; Parola *et al.*, 1992; Sies *et al.*, 1992; Burtis and Ashwood, 1994)

Zinc is the second most abundant trace element in the body (Zhou *et al.*, 2007). It is contained in hundreds of enzymes and is involved in numerous aspect of cellular metabolism. It is required for the catalytic activity of

approximately hundred enzymes (Sandstead, 1994). Numerous aspect of cellular metabolism are zinc dependent (Institute of Medicine, Food and Nutrition Board, 2001), and in even more protein domains, particularly in a number of cellular processes, including cellular proliferation and differentiation (Franco *et al.*, 2009). Zinc plays an important role in the structure and function of biological membranes (Bettger and O'Dell, 1993). Zinc has been shown to have an antioxidant potential through the non-enzymatic stabilization of biomembrane and biostructures. The protective effects of zinc could be attributed to its ability to reduce collagen accumulation in liver and also it exert critical physiological role in regulating the structure and function of cells (Sidhu *et al.*, 2004). Apart from being an essential component of the antioxidant enzyme, superoxide dismutase, zinc also antagonizes the catalytic properties of the redox activity transition metals iron and copper in promoting the formation of hydroxyl from hydrogen peroxide and superoxide in Fenton reactions (Powell, 2000). Zinc also induces the expression of cystein rich antioxidant protein metallothionein (Dhawan and Goel, 1995). And metallothionein plays a role in the detoxification of heavy metals and stabilize membrane (Vallee and Falchuk, 1993). Zinc is an important component of the body's antioxidant system and play a role in retarding the oxidative processes particularly related to diabetes mellitus. Specifically, Zinc is required for the adequate formation and function of the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), and various metallothioneins (Disilvestro, 2000).

Zinc is suspected as having a significant role in normal insulin metabolism. This includes the ability to regulate insulin receptor intracellular events that determine glucose tolerance and the ability to support normal pancreatic reaction to a glucose load (Chausmer, 1998; Faure *et al.*, 1995). Since the complications of diabetes may be mediated, at least in part, through oxidative stress, which potentially affect the heart, vascular system, kidney, retina and peripheral nerves; zinc play a key role in the cellular antioxidative defense (Arthur, 1998; Bonnefont-Rousselot, 2004). Therefore, this study was aimed at evaluating the ameliorative effects of vitamin C and zinc on blood glucose levels and oxidative stress in Alloxan-induced diabetic Wistar rats.

MATERIALS AND METHODS

Chemicals and drugs used: This study was conducted in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University Zaria, Kaduna state in Northern Nigeria in the month of August, 2011. All chemicals and drugs used were of analytical grade. Alloxan was purchased from (Sigma chemical Company St. Louis U.S.A.). A digital glucometer (Accu-Chek

Advantage, Roche Diagnostic, Germany) was used for the determination of the blood glucose levels of the animals. Each tablet of ascorbic acid (100 mg; Med Vit C[®], Dol-Med Laboratories Limited, Lagos, Nigeria was reconstituted to 100 mg/mL suspension, just prior to its daily administration. Zinc gluconate tablet (50 mg/tablet, Nature field U.S.A) was obtained from a pharmaceutical store in Zaria, Nigeria. They were reconstituted in distilled water prior to daily administration.

Animal care and management: A total of thirty (30) apparently healthy Wistar albino rats of both sexes between the ages of 8-10 weeks old and weighing between 150-200 g were used for the study. The animals were kept in well aerated laboratory cages in the Department of Human physiology animal house and were allowed to acclimatize to the laboratory environment for a period of 2weeks before the commencement of the experiment. They were maintained on standard animal feeds and drinking water *ad libitum*.

Induction of experimental diabetes mellitus: The animals were fasted for 16-18 h with free access to water prior to the induction of diabetes. Diabetes was induced by single intraperitoneal injection of Alloxan monohydrate (Sigma St. Louis, U.S.A.) at a dose of 150 mg/kg b w dissolved in 0.9% cold normal saline solution into 16-18 h fasted rats (Katsumata *et al.*, 1999). Since Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemic (Dhandapani *et al.*, 2002)..

Experimental design: After 72 h of Alloxan treatment, blood was collected from tail vein of the rats. Rats having fasting blood glucose level greater than 200 mg/dL were considered as diabetic (Stanley and Venugopal, 2001). After induction of diabetes the diabetic animals were randomly divided into different group as follows:

- **Group 1:** Normal rats and received distilled water orally
- **Group 2:** Diabetic untreated Wistar rats and were given 1 mL of distilled water orally daily.
- **Group 3:** Diabetic treated 100 mg/kg body weight of Vitamin C orally daily
- **Group 4:** Diabetic and received 50 mg/kg body weight of Zinc orally daily

Determination of blood glucose levels: All blood samples were collected from the tail vein of the rats at intervals of 0, 1, 3, 5 and 7 day. Fasting blood glucose levels were determined by using glucose oxidase method

(Beach and Turner, 1958) using a digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) and the results were expressed in the unit of mg/dL (Rheney and Kirk, 2000).

Evaluation of serum malonaldehyde concentration:

Blood samples were drawn from the heart of the animals via cardiac puncture and collected in centrifuge tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10 min and the supernatant (serum) collected. Lipid peroxidation as evidenced by formation of TBARS was measured by the modified method of Niehaus and Samuelson (1968) and described by Akanji *et al.* (2009). Briefly, The following reagents were mixed; TBA (0.37%, i.e., 0.37 g/100 mL), TCA (15%) and HCL, the working reagent was obtained. The serum (0.15 mL) added to working reagent (0.2 mL) in each centrifuge tube. Tubes were placed in water bath at 90 degree for 60 min (1 hour). They were removed to room temperature and centrifuged at 3000 rpm for 5 min. The supernatant was decanted and absorbance read at 535 nm. Absorbance was read against a blank (containing 0.15 mL distilled water instead of serum).

Statistical analysis: Blood glucose and serum malondialdehyde levels were expressed in mg/dL and mmol/L as mean±SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group (Duncan *et al.*, 1997). The value of $p < 0.05$ was taken as significant.

RESULTS

Effects of vitamin C and zinc on blood glucose levels in alloxan-induced diabetic wistar rats: The Mean blood glucose levels on day 0 indicates the fasting blood glucose before the commencement of treatment. The oral administration of 100 mg/kg body weight of vitamin C showed no statistically significant change ($p > 0.05$) on blood glucose level after day 1, when compared to the diabetic control group. Also, in the group treated with 50 mg/kg body weight of zinc there was also no statistically significant difference ($p > 0.05$) on blood glucose level after day 1 when compared to the control group. While after day 3 there was a significant decrease ($p < 0.05$) on the blood levels in the diabetic group administered with 100 mg/kg body weight of vitamin C and 50 mg/kg body weight of Zinc when compared to the control group. In addition, there was a statistically significant change ($p < 0.05$) on the blood glucose level in the groups treated with 100 mg/kg body weight of vitamin C and 50 mg/kg body weight of zinc after day 5 and day 7 as shown in Fig. 1.

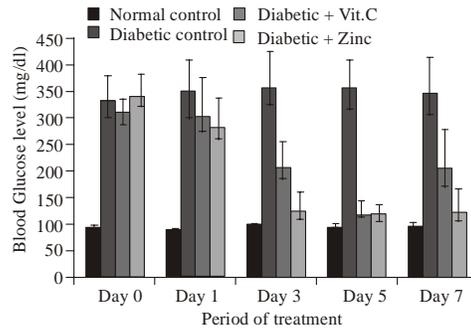


Fig. 1: Effects of vitamin C and zinc on blood glucose levels of alloxan-induced diabetic wistar rats. (Bars represent mean±SEM) (n = 5) for each group. Values are statistically significant compared to control group at ^a: $p < 0.05$; ns: not significant

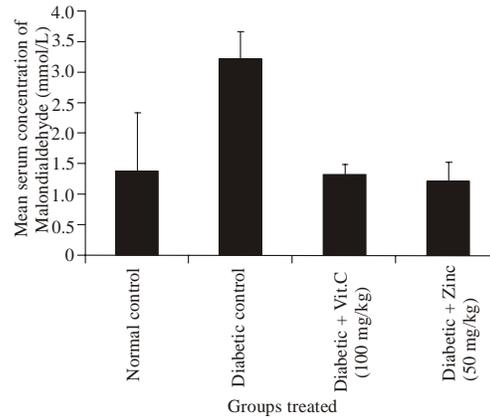


Fig. 2: Effect of vitamin C and zinc on malondialdehyde concentration of alloxan-induced diabetic wistar rats. (Bars represent mean±SEM) (n = 5) for each group. Values are statistically significant compared to control group at ^a: $p < 0.05$; ns: not significant

Effect of vitamin C and zinc on serum malondialdehyde concentration in alloxan-induced diabetic wistar rats:

The study showed that there was no statistically significant change ($p > 0.05$) on the serum concentration of MDA in the group treated with Vitamin C (100 mg/kg b w) as compared to the diabetic control group. However, there was a statistically significant change ($p < 0.05$) on the serum level of MDA in the group that was administered with Zinc (50 mg/kg b w) when compared to diabetic control group as shown in Fig. 2.

DISCUSSION

Diabetes mellitus is a metabolic disorder in the endocrine. This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind (Edwin *et al.*, 2008). It has now become an epidemic with

a worldwide incidence of 5% in the general population. The number of adults with diabetes mellitus in the world will rise from 135 million in 1995 to 300 million in the year 2025 (Torben, 2002). Diabetes is a chronic metabolic disorder involving carbohydrate, proteins and fat characterized by hyperglycemia and insufficiency secretion or action of endogenous insulin (Devlin, 1997; Barar, 2002). Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated (Shewade *et al.*, 2001). Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta-cells of the pancreas (Prince and Menon, 2000; Jelodar *et al.*, 2003). Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with simultaneous massive increase in cystolic calcium concentration, which causes rapid destruction of pancreatic beta-cells of Islets of Langerhans thereby inducing hyperglycemia (Grover *et al.*, 2000; Szudelski, 2001). Insulin deficiency leads to various metabolic alterations in the animals such as increased blood glucose, total cholesterol, alkaline phosphatase and transaminases (Begum and Shanmudnaram, 1978). Therefore, alloxan induced diabetes represent a good model for the study of insulin dependent diabetes mellitus. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes mellitus and its complications (Baynes and Thorpe, 1990; Baynes, 1991; Ceriello, 2000). In diabetes mellitus, oxidative stress seems mainly to be due to increased production of free radicals and/or a sharp reduction of antioxidant defenses (Ahmed, 2005a, b). Oxygen derived free radicals have been implicated in the pathology of various disease states, including diabetes mellitus (Giugliano *et al.*, 1996). Free radical production caused by hyperglycemia occurs at least via four different routes viz: auto-oxidation of glucose, increased glycolysis, intracellular activation of sorbitol (polyol) pathway and non enzymatic protein glycation (Ceriello *et al.*, 1992). Vitamin C is a water soluble antioxidant that was firstly isolated and characterized by (Sato *et al.*, 1979). It is found intra- and extracellular as ascorbate (Chihuilaf *et al.*, 2002). Vitamin C is a natural antioxidant that prevents the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues as found in diabetes mellitus (Sies *et al.*, 1992).

Zinc is the most abundant trace elements in the body (Zhou *et al.*, 2007). It contained in hundreds of enzymes

and even more protein domains participating in a number of cellular processes, including cellular proliferation, differentiation and apoptosis (Franco *et al.*, 2009). It is ubiquitous in subcellular metabolism and is an essential component of catalytic site(s) of at least one enzyme in every enzyme classification (Coyle *et al.*, 2002). The antioxidant effect of zinc has been well documented (Moustafa, 2004). Apart from being an essential component of the antioxidant enzyme, superoxide dismutase, zinc also antagonizes the catalytic properties of the redox active transition iron and copper in promoting the formation of hydroxyl from hydrogen peroxide and superoxide in Fenton reactions (Powell, 2000). Zinc also induces the expression of cysteine-rich antioxidant protein, metallothionein (Dhawan and Goel, 1995). The liver is an important insulin-dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes mellitus (Seifter and England, 1982).

The present study showed that blood glucose level was increased in alloxan-induced animals, since alloxan causes a massive reduction in insulin release, by the destruction of beta-cells of Islet of Langerhans and hence, inducing hyperglycemia (Ravikumar *et al.*, 2010). And also, there was increased serum concentration of malondialdehyde (aldehydic products of lipid peroxidation) which is a biomarker of intensified lipid peroxidation and also indirect evidence high free radical production in diabetes (Maritim *et al.*, 2003). Oral administration of antioxidant micronutrients and vitamins (Vitamin C 100 mg/kg and zinc 50 mg/kg) resulted in a significant decrease in the levels of blood glucose and serum concentration of MDA. Low levels of plasma vitamin C are known to occur in several conditions of increased oxidative stress such as diabetes mellitus (Evans, 2000; Polidori *et al.*, 2001; Jaruga *et al.*, 2002). Zinc has been reported to play a key role in the regulation of insulin production in pancreatic tissue. Therefore, it seems reasonable that any change in body zinc status could affect production, storage and secretion of insulin. Several reasons have been adduced that abnormal zinc metabolism could play a role in the pathogenesis of diabetes mellitus, which is accompanied by severe oxidative stress (especially lipid peroxidation) as result of an increased oxygen free radical production (Feillet-coudray *et al.*, 1999; Zine kachrid and Naima, 2007). Free radicals have been reported in the pathogenesis of diabetes mellitus as a result of their severe cytotoxic effects, such as lipid peroxidation and protein denaturation of the cell membrane followed by alteration of the membrane receptor and fluidity properties (Gupta and Chari, 2005). Zinc is one of the important micro elements among magnesium, copper, manganese needed for the beta-cells (Mohammed *et al.*, 2006; Edwin *et al.*, 2008). Therefore, dietary supplementation with the antioxidants (vitamin C and Zinc) has been suggested as a possible means of controlling diabetes and its

complications as well as damage to lipids by oxygen radicals (Trancrede *et al.*, 1983; Martinek, 1964). Marvin *et al.* (1985) reported that supplementation of vitamin E might alter insulin receptors in muscle or adipose tissue by increasing membrane motility. In addition, vitamin E may enhance glucose uptake by the diaphragm. This therefore, may be possible mechanism for the reduction of blood glucose vitamin C observed in this present study.

Furthermore, zinc induces the production of metallothionein, an effective scavenger of hydroxyl radicals (Sahin and Kucuk, 2003). While vitamin C is a scavenger of free oxygen radicals which are toxic by-product of many metabolic process in streptozotocin-induced diabetes (Gupta and Chari, 2005; Manea *et al.*, 2004). Therefore, the decrease in the level of blood glucose observed in this present study may be attributed the antioxidant effects of vitamin C and zinc on these free radicals.

CONCLUSION

In conclusion, the present study have revealed that oral administration of vitamin C (100 mg/kg b w) and Zinc (50 mg/kg b w) reduced blood glucose levels and lipoperoxides formation as evidenced by reduction in the serum concentration of malondialdehyde in alloxan-induced hyperglycemia in Wistar rats.

ACKNOWLEDGMENT

The authors of this research study wish to acknowledge the technical assistance of Mallam Bashiru M. of the Department of Biochemistry Ahmadu Bello University, Zaria, Nigeria.

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