

## Preliminary Studies of Effects of Vitamin C and Zinc on Some Liver Enzymes in Alloxan-induced Diabetic Wistar Rats

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**Abstract:** Oxidative stress has been implicated in the pathogenesis of diabetes mellitus through increased generation of reactive oxygen species and consequent decline in antioxidant defenses. Diabetes is known to alter the levels of liver enzymes due to production of free radicals. The free radicals may cause hepatic injury. The current study was aimed at evaluating the effects of vitamin C and Zinc on the levels of some liver enzymes in alloxan-induced diabetic Wistar rats. Diabetes was induced in animals by intraperitoneal injection of Alloxan (150 mg/kg). Diabetic rats were randomly divided into four groups (n = 5): Group I (Normal control) received distilled water, Group II (Diabetic control) received distilled water, while Group III and IV were orally administered 100 and 50 mg/kg b w of vitamin C and Zinc respectively for seven days. Blood samples collected from the animals were assayed for liver enzymes viz: serum Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT) and Alkaline phosphatase (ALP). The result showed that the activities of liver enzymes such as AST, ALT and ALP were significantly increased in the diabetic control. Oral treatment with 100 mg/kg of vitamin C and 50 mg/kg of zinc significantly decreased ( $p < 0.05$ ) the concentration of serum of AST and ALT, while no significant change ( $p > 0.05$ ) was observed on the serum levels of ALP. The results obtained from this study may suggest that vitamin C and zinc may play an important role in the prevention of hepatocellular injury that occurs in diabetes.

**Key words:** Alloxan, diabetes mellitus, liver enzymes, oxidative stress, vitamin C, zinc

### INTRODUCTION

Diabetes Mellitus (DM) is a heterogeneous metabolic disorder characterized by hyperglycaemia resulting from defective insulin secretion, resistance to insulin action or both (Gavin *et al.*, 1997). Type 1 diabetes is the consequence of an autoimmune-mediated destruction of pancreatic  $\beta$ -cells, leading to insulin deficiency. Type 2 diabetes is characterized by insulin resistance and relative, rather than absolute, insulin deficiency. The capacity of nutrients to stimulate insulin release from the pancreatic  $\beta$ -cell reflects their capacity to augment oxidative fluxes in the islet cells. Also, oxidant stress associated with insulin resistance and non-insulin-dependent diabetes mellitus (Gopaul *et al.*, 1995; Nourooz-Zadeh *et al.*, 1995) contributes to poor insulin action (Paolisso *et al.*, 1994; Rudich *et al.*, 1997). 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 (Cross *et al.*, 1987; Oberley, 1988; Hunt *et al.*, 1992). Oxygen-derived free radicals have been implicated in the pathophysiology of various disease states, including diabetes mellitus (Giugliano *et al.*, 1996). Also, Jang *et al.* (2000) found that increased oxidative stress has been suggested to be involved in the pathogenesis and

progression of diabetic tissue damage. On the other hand, there is evidence that diabetes induces changes in the activities of antioxidant enzymes in various tissues (Oberley, 1988). The role of liver in the pathogenesis of diabetes is increasingly being recognized. Both directly determined liver fat content and circulating levels of alanine aminotransferase (ALT) have been used in assessment of liver status during diabetes. Liver enzyme abnormalities in people with type 2 diabetes in randomized trials have been reported. This has also been predicted in new-onset type 2 diabetes and in experimental diabetes (Vijan and Hayward, 2004; Vozarova *et al.*, 2002).

Fruits, vegetables and grains are rich sources of antioxidant because they contain ascorbate, tocopherols, tocotrienols, flavonoids, other phenols and carotenoids (Stangeland *et al.*, 2008). An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfer electron from a substance to an oxidizing agent. Oxidation reaction can produce free radicals. Antioxidant terminates the chain of the reaction by removing free radicals and inhibits other oxidation reaction by oxidizing themselves (Jenkinson *et al.*, 1999). Antioxidant supplements or food rich in antioxidants may be used in reducing oxidative

damage by free radicals and active oxygen, and can protect the body cells against lipid peroxidation (Gulcin *et al.*, 2002). The present work was aimed at evaluating the effects of vitamin C and zinc on some liver enzymes in Alloxan-induced diabetic Wistar rats.

## MATERIALS AND METHODS

**Chemicals used:** 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.

**Drugs used:** Each tablet of ascorbic acid (100 mg; Med Vit C<sup>®</sup>, 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 prior to daily administration.

**Experimental animals:** A total of twenty (20) 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 and were allowed to acclimatize to the laboratory environment for a period of 2 weeks before the commencement of the experiment. They were maintained on standard animal feeds and drinking water *ad libitum*.

**Experimental induction of diabetes mellitus:** The experimental animals were fasted overnight and were allowed free access to water before the induction of diabetes. Diabetes was induced by single intraperitoneal injection of Alloxan monohydrate (Sigma St. Louis, M.S., U.S.A.) at a dose of 150 mg/kg body weight dissolved in 0.9% cold normal saline solution (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 hypoglycemia (Dhandapani *et al.*, 2002).

**Experimental protocol:** After 72 h of Alloxan treatment, blood was collected from tail vein of the rats and blood glucose measured using glucose oxidase method (Beach and Turner, 1958) using a digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany). 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 control rats and received 1ml of distilled water orally daily.

**Group 2:** Diabetic control rats and received 1ml of distilled water orally daily

**Group 3:** Diabetic treated with 100 mg/kg body weight of Vitamin C orally daily

**Group 4:** Diabetic and received 50 mg/kg body weight of Zinc orally daily

The animals were subjected to daily oral doses of vitamin C and zinc for seven days.

**Collection and preparation of sera samples for liver enzymes analysis:** Blood samples were drawn from the heart of animals via cardiac puncture after they have been fasted for 16-18 h. Blood samples were collected in plain 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 were then subjected to liver enzyme assay.

**Estimation of serum liver enzymes:** The serum enzymes alanine aminotransferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were determined, using Agape Diagnostic kits, India.

**Determination of serum aspartate aminotransferase (AST):** This was estimated by method as described by, Bergmeyer and Walefeld (1978). Briefly, 1000  $\mu$ L of the reagent was added to 100  $\mu$ L of the samples and then mixed and incubated at 37°C for 1 min. The change in absorbance of the sample was measured per minute spectrophotometrically at the wavelength of 590 nm as follows:

$$\text{AST activity (U/L)} = \Delta \text{AB/min} \times 1768$$

**Determination of serum alanine aminotransferase (ALT):** This was estimated by method as described by, Bergmeyer and Walefeld (1978). Briefly, 1000  $\mu$ L of the reagent was added to 100  $\mu$ L of the samples and then mixed and incubated at 37°C for 1 min. The change in absorbance of the sample was measured per minute as follows:

$$\text{ALT activity (U/L)} = \Delta \text{AB/min} \times 1768$$

**Determination of serum alkaline phosphatase (ALP):** This was estimated by method as described by, Bowers and Mc Comb (1966). Briefly, 0.5 mL of the reagent was added to 0.05 mL (50  $\mu$ L) of the samples and then mixed and incubated at 37°C for 10 min. The change in absorbance of the sample was measured per minute

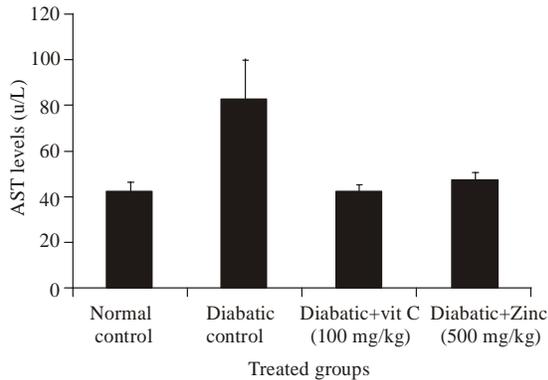


Fig. 1: Effect of Vitamin C and Zinc on Serum Aspartate aminotransferase level in Alloxan-induced Diabetic Wistar Rats. (Bars represent mean±SEM) (n = 5) for each group. Values are statistically significant compared to control group at <sup>a</sup>p<0.05, while ns = not significant

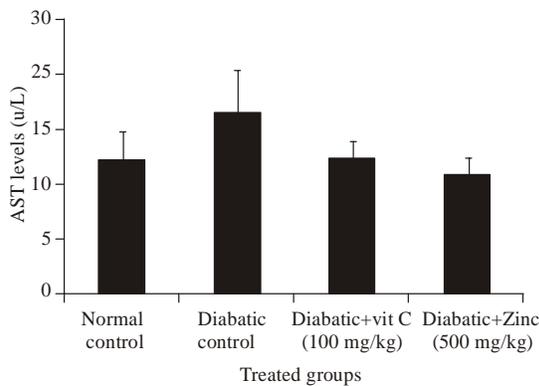


Fig. 2: Effect of Vitamin C and Zinc on Serum Alanine aminotransferase level in Alloxan-induced Diabetic Wistar Rats. (Bars represent mean±SEM) (n = 5) for each group. Values are statistically significant compared to control group at <sup>a</sup>p<0.05, while ns = not significant

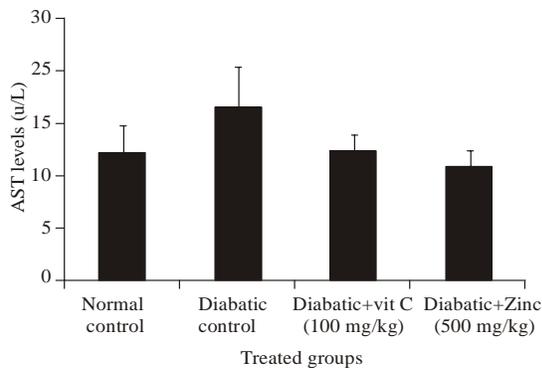


Fig. 3: Effect of Vitamin C and Zinc on Serum Alkaline phosphatase level in Alloxan-induced Diabetic Wistar Rats. (Bars represent mean±SEM) (n = 5) for each group. Values are statistically significant compared to control group at <sup>a</sup>p<0.05, while ns = not significant

spectrophotometrically at wavelength of 590nm as follows:

$$\text{Absorbance of sample} / \text{Absorbance standard} \times \text{Value of standard (U/L)}$$

**Statistical analysis:** Serum liver enzymes levels were expressed in U/L as mean±SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group (Duncan *et al.*, 1997). The values of p<0.05 were considered significant.

## RESULTS AND DISCUSSION

**Effect of vitamin C and Zinc on serum level of aspartate aminotransaminase:** The study showed that oral administration of Vitamin C (100 mg/kg b w) and zinc (50 mg/kg b w) recorded a statistically significant decrease (p<0.05) on the serum concentration of Aspartate aminotransaminase (AST) when compared to the diabetic control group as shown in Fig. 1.

**Effect of vitamin C and Zinc on serum level of alanine aminotransaminase:** There was a significant reduction (p<0.05) on the serum level of Alanine aminotransaminase (ALT) in the groups administered with 100 mg/kg b w of Vitamin C and 50 mg/kg b w of zinc when compared to the control group as shown in Fig. 2.

**Effect of vitamin C and Zinc on serum level of alkaline phosphatase:** However, there was no statistically significant change (p<0.05) on the serum concentration of alkaline phosphatase (ALP) when compared to the control group as represented in Fig. 3.

## DISCUSSION

The results of the present study showed that after three days of injection of Alloxan, there were significant increased activities of serum aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatase (ALP) in the diabetic animals as compared to the control group. General analysis of the activities of some basic liver function enzymes such as AST, ALT and ALP in the plasma or serum can be used to indirectly assess the integrity of liver tissue and extent of damage after being exposed to certain pharmacological agent such as Alloxan. These enzymes are usually liver makers whose plasma concentration above homeostatic limit could be associated with various forms of disorders which affect the functional integrity of the liver tissue (Ravikumar *et al.*, 2010; Uboh *et al.*, 2010). In addition the measurement of enzymatic activities of phosphatase such as acid phosphatase (ACP) and alkaline phosphatase (ALP) during diabetes is of clinical and toxicological importance as changes in their activities are indicative of

tissue damage by toxicants (Ravikumar *et al.*, 2010). Due to the fact that liver tissues are grossly damaged during diabetes, raised levels of liver enzymes in the blood was found in this study which with the findings of others researchers (Vojarova *et al.*, 2002). Furthermore, the levels of liver enzymes have also been reported to alter during streptozotocin induced diabetes (Al-Shamsi *et al.*, 2006). In addition, increased serum AST and ALT levels may be as a result of metabolic changes in the liver following administration of toxin, cirrhosis of the liver, hepatitis and liver cancer have also been reported (Chalasanani *et al.*, 2004). Similarly in this study, it was also observed that the levels of serum AST and ALT in alloxan-induced diabetes rats were elevated. Liver-ALP is mobilized most rapidly into blood and its levels in plasma may increase at early periods of liver damage. High ALP serum level is usually indicative of cholestasis. Cholestasis may also result in a progressive liver disease-biliary cirrhosis (Murray *et al.*, 1988; Burtis and Ashwood, 1994). Increase in the levels of these enzymes in diabetes may be as a result of leaking out of these enzymes from the tissue into the blood stream as a result of the adverse effect of in the liver. In the present study, Vitamin C and Zinc were observed to decrease the raised activities of AST and ALT. However, there was no significant change on the serum levels of ALP. Thus, the observed effect may be as a result of antioxidant properties/activity of vitamin C and zinc. Vitamin C have been shown to possess several antioxidant properties and it is an important water-soluble antioxidant in biological fluids (Anitra and Balz, 1999). Vitamin C may play an important role in physiological reactions such as mixed function oxidation involving incorporation of oxygen into a biochemical substrate. In addition, this vitamin is considered the most important antioxidant in extracellular fluids and its antioxidant function has been shown to efficiently scavenge superoxide, hydrogen peroxide, hydroxyl, peroxy and singlet oxygen radicals. Deficiency in vitamin C causes damage of collagen synthesis in cellular basal membranes, structure of mucosal epithelium and increased capillary fragility (McDowell 1989; Sies and Stahl, 1992; Burtis and Ashwood, 1994) Vitamin C is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent. It is said to have ascorbate oxidant activity (Sedhrouchni *et al.*, 2002). Ascorbate effectively scavenge singlet oxygen, superoxide, hydroxyl and water soluble peroxy radical and hypochlorous acid (Smirnoff and Wheeler, 2000). Vitamin C can efficiently scavenge free radicals before they can initiate lipid peroxidation, and contribute to stability of cellular and basal membranes. The antioxidant vitamin C may suppress the hepatotoxic effects of alloxan by interference with intermediary metabolites in the cytochrome P450 microsomal system (Murray *et al.*, 1988; McDowell, 1989; Sies and Stahl, 1992; Burtis and Ashwood, 1994). Vitamin C may protect lipids and lipoproteins in cellular membranes against

oxidative damage caused by toxic free radicals at early stage. The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may partially prevent certain types of hepatic cellular damage (McDowell, 1989; Parola *et al.*, 1992; Sies and Stahl, 1992; Burtis and Ashwood, 1994; Netke *et al.*, 1997). Similarly, the antioxidant effect of zinc has been well documented (Moustafa, 2009; Zhou *et al.*, 2005). Apart from being an essential component of antioxidant enzymes, superoxide dismutase, zinc also antagonizes the catalytic properties of the redox active transition metals iron and copper promoting the formation of hydroxyl from hydrogen peroxide and superoxide in Fenton reactions (Powell, 2000). Zinc plays an important role in the structure and function of biological membranes (Bettger and O'Dell, 1993). Zinc has also 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). Zinc also induces the expression of cysteine 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).

## CONCLUSION

In conclusion, the present study has demonstrated that Alloxan-induced diabetes could increase the liver enzyme levels. Increase in these enzymes levels may occur due to peroxidation reactions, arising from Alloxan biotransformation during diabetes, and these reactions may inflict oxidative injury oxidative injury to cellular components. In the light of these results, vitamin C may play a role in the prevention of hepatic cellular injury produced by Alloxan. However, there is a need for more detailed studies in order to assess the possible relationships between antioxidants and Alloxan hepatotoxicity.

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