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## ANTIOXIDANT MICRONUTRIENT POTENTIALS IN STRENGTHENING THE ANTIOXIDANT DEFENSE IN ALLOXAN- INDUCED DIABETIC RATS

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### ABSTRACT

Diabetes mellitus is associated with elevated oxidative stress via increased generation of reactive oxygen species (ROS), and decline in antioxidant defences. Increased oxidative stress is thought to play a role in the development of diabetic complications. Such damages may be prevented or moderated by supplementation with antioxidant micronutrients that serve as cofactors and are thought to strengthen the denovo synthesis of antioxidant enzymes. In the current study, manganese, copper and zinc were supplemented in alloxan-induced diabetic rats for a period of four weeks. Antioxidant enzymes superoxide dismutase (SOD) activities was found to be  $2.00 \pm 0.17\text{U/mg}$  protein in the control group, as against  $1.67 \pm 0.22\text{U/mg}$  protein in the supplemented group and  $1.61 \pm 0.18\text{U/mg}$  protein in the unsupplemented group. The activities of glutathione peroxidase (GPX) was found to be  $46.43 \pm 4.25\text{U/mg}$  protein in the control group as against  $44.86 \pm 1.82\text{U/mg}$  protein in the supplemented group and  $36.86 \pm 3.91\text{U/mg}$  protein in the unsupplemented group. The activities of catalase (CAT) was found to be  $59.86 \pm 1.08\text{U/mg}$  protein in the control group, as against  $57.14 \pm 2.48\text{U/mg}$  protein in the supplemented group and  $39.86 \pm 3.03\text{U/mg}$  protein in the unsupplemented group. The concentration of malondialdehyde (MDA) was found to be  $1.83 \pm 0.16\text{nmol/ml}$  in the control group, as against  $1.91 \pm 0.14\text{nmol/ml}$  in the supplemented group and  $2.37 \pm 0.19\text{nmol/ml}$  in the unsupplemented group. There is no statistically significant difference between controls and supplemented group in all the parameters studied ( $P>0.05$ ) but the difference between control and unsupplemented group was statistically significant in MDA concentration and catalase activities. It is concluded that in an effort to properly manage diabetes mellitus, supplementation with antioxidant micronutrient might be beneficial in strengthening the antioxidant defence enzymes and decreasing lipid peroxidation.

**Keywords:** *Diabetes Mellitus; Micronutrients; Lipid Peroxidation*

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### INTRODUCTION

Diabetes mellitus is one of the oldest diseases known to man. The first chemical description of the disease was

made by Causes in about 1000 AD, and the name diabetes was introduced by Aratacus at about the same time. It is a debilitating and often life threatening

disease which is associated with impaired nutrients homeostasis and has increasing incidence throughout the world (WHO, 1980; Gill, 1990; Manes and Farnsworth, 1995).

Diabetes increase oxidative stress in tissues of both humans and animals, and increase oxidative stress might play a role in the development of diabetic complications (Amstrong *et al.*, 1992; Baynes, 1991; Kowluru *et al.*, 2000). Possible sources of oxidative stress in diabetes include increase generation of reactive oxygen species by auto-oxidation of glucose, decreased tissue concentration of low molecular weight antioxidants and impaired activities of antioxidant defence enzymes (Kowluru, 2000; Tesfamariam and Coten, Kern *et al.*, 1994).

Akinosun and Bolajoko (2007) reported decreased total antioxidant status (TAS) in type 2 Nigerian diabetics and proposed that reduction in free radical activity may probably minimize the chronic complications in diabetic patients.

Free radicals when generated in large quantities are believed to be etiogenesis of several disorders which includes atherosclerosis, carcinogenesis, neurodegenerative diseases and many pathobiological effects (Frei, 1994). The first line of body defence against free radical damage are the antioxidant enzymes, superoxide dismutate (SOD), glutathione peroxidase (GPX) and catalase (CAT).

Oxidative stress contributes to impairment of islet cell function (Haskin *et al.*, 2004; Coriello, 1999) insulin resistance, micro and macro vascular disease (Rema *et al.*, 1994; Valko *et al.*, 1986), and may increase their requirement for micronutrients with antioxidant effects (Ametaj *et al.*, 2000;

Dagenais *et al.*, 2000).

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 current study was designed to assess the effect of 4 weeks of oral supplementation of antioxidant micronutrients in alloxan-induced diabetic rat.

## MATERIALS AND METHODS

**Experimental Animals:** Male albino Wistar rats (120-180 g) were purchased from Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were housed under similar conditions in standard cages at  $25 \pm 2^{\circ}\text{C}$ , with 12-h light/dark cycle in the Animal House, Department of Human Physiology, Ahmadu Bello University, Zaria. The animals were maintained on poultry feed (Vital Feeds, Jos) *ad libitum*.

**Chemicals:** All the reagents used for the study were of analytical grade. Alloxan was purchased from Sigma Aldrich Chemical Co. (U.K), Kits for the assay of MDA, CAT, SOD and GPX were purchased from North-West Life Science Specialties, Vancouver, Canada.

**Induction of Diabetes:** Experimental diabetes was induced by a single intraperitoneal injection of freshly dissolved Alloxan (150 mg/kg) in normal saline maintain at  $37^{\circ}\text{C}$ , to rats fasted for 12 hour. Control rats received a similar volume of normal saline alone. After 72 hour of Alloxan injection, the animals were fasted overnight and their fasting blood glucose estimated using a commercial glucose kit. Only rats that had fasting blood glucose level of 126

mg/dl ( $>7.00\text{mmol/l}$ ) and partial destruction of pancreas tested with positive response to metformin were included in the study.

**Experimental Design:** The rats were divided into 3 groups of 7 rats each:- Group i: Normal control; Group ii Diabetic+ metformin (250 mg/kg); Group iii Diabetic + metformin (250 mg/kg) + Copper (2 mg/kg) + Manganese (10 mg/kg) + Zinc (15 mg/kg). The supplementation lasted for one month and after the last day; the animals were fasted over night and anaesthetized by dropping each in a transparent plastic jar saturated with chloroform vapour. Incision was made on the abdomen, and some vital organs collected for histological studies (not reported). Blood sample was collected through cardiac puncture and divided into plain and EDTA containing centrifuge tubes. Humane procedure was adopted throughout the experiment.

**Measurement of Biochemical Analytes:-** Blood glucose concentration was assayed using glucose Oxidase method (Trinder, 1969). MDA level was assayed based on MDA reaction with thiobarbituric acid (TBA) and activities expressed as nmol/ml (Botsoglou, 1954). CAT activities were measured using  $\text{H}_2\text{O}_2$  as substrate, and the unit expressed as U/mg protein (Beers and Sizer, 1952). GPX activities were measured by NADPH oxidation, and the activities expressed as U/mg protein (Paglia and Valentine, 1967). SOD activity was assayed using the autooxidation of hematoxylin and the unit of activity expressed as U/mg protein (Martin *et al.*, 1987).

**Statistical Analysis:** All data were

expressed as the mean  $\pm$  Standard Error of Mean (S.E.M). Data was analyzed using analysis of variance (ANOVA) by InStat3 software. Differences in mean were considered to be significant when  $p < 0.05$ .

## RESULTS

The results of the current work are presented in table 1. Mean serum concentration of malandialdehyde (MDA) was  $1.83 \pm 0.16$  nmol/ml in the control group, diabetic treated not supplemented had a concentration of  $2.39 \pm 0.15$  nmol/ml, while in the diabetic treated and supplemented, a concentration of  $1.91 \pm 0.14$  nmol/ml was recorded. The difference between the controls and diabetic treated and not supplemented was statistically significant ( $P < 0.05$ ), while the difference between the controls and diabetics treated and supplemented was statistically not significant ( $P > 0.05$ ). Mean serum activities of superoxide dismutase (SOD) was  $2.0 \pm 0.17$  U/mg protein in the controls,  $1.61 \pm 0.18$  U/mg protein in the diabetic treated not supplemented, and  $1.69 \pm 0.22$  U/mg protein in the diabetic treated and supplemented group. There was however, no statistically significance difference ( $P > 0.05$ ) between the controls and the two classes of the diabetics. The mean serum activities of glutathione peroxidase (GPX) was  $46.43 \pm 3.25$  U/mg protein in the controls,  $36.86 \pm 3.91$  U/mg protein in the diabetic treated not supplemented and  $44.86 \pm 1.82$  U/mg protein in the diabetic treated and supplemented group. There is no statistically significant difference between the groups ( $P > 0.05$ ).

The mean serum activities of catalase (CAT) was  $59.86 \pm 1.08$  U/mg protein in

the controls,  $39.86 \pm 3.04$  U/mg protein in the diabetic treated not supplemented, and  $57.14 \pm 2.48$  U/mg protein in the diabetic treated and supplemented. There

is statistically significant difference between the control and the unsupplemented groups ( $P < 0.05$ ).

**Table 1: Concentration of Serum Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and Catalase (CAT) Diabetic Whistar Rats Supplemented with Antioxidant Micronutrients**

Group	MDA (nmol/ml)	SOD (U/mg Prot.)	GPX (U/mg Prot.)	CAT (U/mg Prot.)
Control (n=7)	1.83±0.16	2.0±0.17	46.43±4.25	59.86±1.08
Diabetic treated only (n=7)	2.39±0.15 <sup>a</sup>	1.61±0.18	36.86±3.91	39.86±3.03 <sup>a</sup>
Diabetic treated and supplemented (n=7)	1.91±0.14	1.67±0.22	44.86±1.82	57.14±2.48

*P* = Statistical Test of significance, *a* = Statistically significant difference from control, ( $P < 0.05$ ) using InStat3 Statistical Software

## DISCUSSION

Increased oxidative stress has been proposed to be one of the major causes of hyperglycaemia induced diabetic complication, and hyperglycaemia in an organism stimulates reactive oxygen species (ROS) formation from variety of sources (Valko *et al.*, 2007).

In the present study, we showed that 4 weeks supplementation with antioxidation micronutrients Cu, Zn and Mn has an effect on all the studied antioxidant enzymes. This is in agreement with the finding of Irene *et al.*, (2003) who reported and increase in the activities of SOD, when he supplemented his subjects with some micronutrients, believe to have antioxidant potentials. Mahmud *et al.*, (2006) also reported elevated levels of all the studied antioxidant enzymes, after treatment of his subject with an extract from a tree rich in micronutrients. Increased activities of these denovo antioxidant enzymes might be responsible for the scavenging effect, with subsequent protection of cells against lipid peroxidation. The

micronutrients are known co-factors to the studied enzymes, and their increased availability through supplementation might have a direct effect in raising the activities of the enzymes.

MnSOD is a tetramer enzyme with manganese at its active site. Copper is essential for the enzyme catalytic activity, and zinc imparts stability to the protein structure (Fridovich, 1995). Cell lacking adequate activities of Cu, ZnSOD or MnSOD are oxygen intolerant and exhibit a shortened lifespan (Fridovich, 1995).

Our finding also showed decreased concentration of Malondialdehyde (MDA) in the supplemented group, as against the level observed in the unsupplemented group. MDA is a known-marker of lipid peroxidation (Marnett, 1999). Aribal-Kocaturk *et al.*, (2007) reported a decrease in MDA concentration and an increase in the activities of SOD and CAT in streptozocin – induced diabetic rats, pretreated with resveratrol, and concluded that, the observed effect was due to the antioxidant effect of the drug.

MDA can react and damage DNA bases, it is reported to be mutagenic in bacterial and mammalian cells and carcinogenic in rats (Valko *et al.*, 2007). The decrease in MDA concentration observed in the current study might not be unconnected with the increased activities of the antioxidant enzymes, which might have exerted the scavenging effect on the generated free radicals and thereby sparing the cells.

Administration of antioxidants might actually stimulate cell survival through strengthening of the defence systems. The principal aim of the current communication is the consideration of antioxidants micro nutrients in raising the activities of denovo antioxidant enzymes, decreasing MDA concentration and sparing the cells of diabetic against lipid peroxidation.

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