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Original Article

HEPATOCELLULAR INDICES OF DIABETIC RATS SUPPLEMENTED WITH ANTIOXIDANT MICRO NUTRIENTS

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ABSTRACT

Complications associated with diabetes mellitus can be moderated by supplementation with antioxidant micro nutrients. In the present study, manganese, copper and zinc were supplemented in alloxan-induced diabetic rats for a period of 4 weeks. The mean serum activity (ui) of AST, ALT and ALP were 19.14±1.56, 36.43±1.74 and 48.29±2.64 in the supplemented group against 25.0±0.95, 37.57±1.70 and 54.43±1.72 observed in the unsupplemented group respectively. Mean serum levels of total protein (g/dl) and albumin (g/dl) in the supplemented group were 6.34±1.38 and 3.98±1.53 against 6.33±1.51 and 3.50±0.76 observed in the unsupplemented group respectively. Superoxide dismutase, glutathione peroxidase and catalase activities in the supplemented group was 1.69±0.59U/mg protein, 44.86±4.81U/mg protein and 57.14±6.56U/mg protein respectively, against 1.61±0.50U/mg protein, 36.86±5.00U/mg protein and 52.14±5.84U/mg protein recorded in the unsupplemented group respectively. The concentration of malondialdehyde in the supplemented group was 1.91±0.36nmol/ml as against 2.39±0.39nmol/ml seen in the unsupplemented group. The results suggest that antioxidant micronutrients supplementation might be beneficial in strengthening the antioxidant defense enzymes with resultant possible decrease in lipid peroxidation and improved hepatic indices of alloxan-induced diabetic rats.

Keywords: *Diabetics, Liver and Antioxidant defences.*

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder with micro and macro vascular complication that result in significant morbidity and mortality. In Nigeria the prevalence of diabetes is about 2.7%. In 2005 it is estimated that the country lost 400 million dollars in national income due to diabetes and cardiovascular diseases, and it was projected that the loss will increase to 8 billion in coming decades[1]. As diabetes complications mostly affect individuals in their economically productive years, the disease has enormous socio-economic impact. It is estimated that societal cost associated with diabetes mellitus exceeds 20 billion dollars per year[2]. Diabetic human and experiment animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia. This might deplete the activity of antioxidant defence system, and thus promote *denovo* free radicals generation[3]. Lipid peroxidation is a free radical mediated propagation of oxidative insult to polyunsaturated fatty acids, and termination only occurs through enzymatic antioxidant means or by free radical scavenging micronutrients[4]. The aim of this research is to study the effect of supplementing some selected antioxidant micronutrients, on antioxidant defence enzymes lipid peroxidation, and hepatic functions of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals

Male albino wistar rats (120180g) were purchased from Animal House, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. The animals were housed under similar conditions in standard cages environmentally controlled room at 25 ± 2°C, with light/dark cycle. The animals were fed with laboratory chaw (commercial feeds) and water *ad libitum*.

Chemicals

Alloxan was purchased from Sigma Aldrich Chemical Co. (UK), kits for the assay of

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malondialdehyde (MDA) and Catalase (CAT), Superoxide dismutase (SOD) and glutathione peroxidase (GPX) were purchased from North-West Life Science Specialties, Vancouver Canada. All reagents used for the study were of analytical grade.

Induction of Diabetes

Experimental diabetes was induced by a single intra-peritonial injection of freshly dissolved alloxan (150mg/kg) in normal saline maintained at 37°C, to rats fasted for 12 hours. Control rats received a similar volume of normal saline alone. After 72 hours of alloxan injection, the animals were fasted overnight and their fasting blood glucoses were estimated using a glucometer. Only rats with fasting blood glucose level greater than 126mg/dl (7.0 mmol/l) were included in the study.

Experimental Design

The rats were divided into 3 groups of 7 rats in each group as follows:- Group i: Normal control ii: Diabetic + metformin (250 mg/kgbw); Group iii: Diabetic + metformin (250 mg/kgbw) + copper (2mg/kgbw) + manganese (10mg/kgbw) + zinc (15mg/kgbw). The supplementation lasted for one month and after which the animals were fasted over night and sacrificed under anaesthesae. Blood sample was collected after the animal has been sacrificed and divided into plain and EDTA containing centrifuge tubes. Humane procedure was adopted throughout the experiment.

Measurement of Biochemical Analytes

Blood glucose was assayed using glucose oxidase method[5]. MDA level was assayed based on MDA reaction with thiobarbituric acid (TBA) and activities expressed as nmol/ml[6]. CAT activities were measured using H_2O_2 as substrate, and the unit is expressed as U/mg protein[7]. GPX activities were measured by NADPH oxidation, and the activities are expressed as U/mg protein[8]. SOD activity was assayed using the auto-oxidation of hematoxylin and the unit of activity is expressed as U/mg protein[9].

Total protein and albumin was determined using Biuret method[10] and Bromocresol Green (BCG) binding method[11] respectively. Serum AST and ALT activities were determined by the method of Reitman-Frankel[10]. Serum ALP activities was determined by the Nitrophenol method[10].

Statistical Analysis: All data were expressed as mean ± standard error of mean (SEM). Data was subjected to Instat3 Statistical Test. Differences in mean were considered to be significant when $p<0.05$.

RESULTS

Summary of the results is shown in table 1 and 2. The lowest mean serum MDA concentration was obtained in the control group (1.83±0.16nmol/ml). Diabetic rats treated only had mean MDA concentration of 2.39±0.15nmol/ml, while diabetic rats treated and supplemented had mean MDA concentration of 1.9±0.14nmol/ml.

The mean value of MDA in the unsupplemented diabetic rats was significantly higher ($p<0.05$) than similar value in the control group. However, no statistically significant different was observed between the control and the diabetic treated and supplemented groups ($p>0.05$).

Serum SOD activities was 2.0±0.17U/mg protein in the control group, 1.61±0.18U/mg protein in the diabetic treated group and 1.67±0.22U/mg protein in the diabetic treated and supplemented group.

The difference between the groups however, was not significant statistically ($p>0.05$). The GPX activities were 46.43±3.25U/mg protein in the control group, 36.86±3.91U/mg protein in diabetic treated group and 44.86±1.82U/mg/protein in the treated and supplemented group. There is statistically significant difference between the control and the unsupplemented group ($p>0.05$). However, no statistically significance difference exists between the control and the supplemented group ($p>0.05$).

The CAT activities were 59.86±1.08U/mg protein in the control group 39.86±3.04U/mg protein in the treated group and 57.14±2.48U/mg protein in the treated and supplemented group. There is statistically significance difference between the control and unsupplemented group ($p<0.001$) and between the supplemented and unsupplemented group ($p<0.001$).

Serum activities of AST were 17.71±0.89U/I in control group 25.00±0.95U/I in the unsupplemented group and 19.14±1.57U/I in the supplemented group. There was a statistically significant difference between the control and the unsupplemented group, as well as between the supplemented and the unsupplemented group. The ALT activities were 17.71±0.89U/I in the control, 37.57±1.70U/I in the unsupplemented group. There was statistically significance difference between the control and unsupplemented group. So also between supplemented and control group.

ALP activities was 38.43±2.17U/I in the control, 52.43±1.72U/I in the unsupplemented group and the activities of 48.29±2.04U/I was measured in the supplemented group. There was statistically significant difference between ALP activities seen in the control and unsupplemented groups ($p<0.001$). There was also statistically significant difference between supplemented and control group ($p<0.001$).

Mean serum total protein value of 6.90±0.18g/dl was detected in the control group, 6.33±0.15g/dl in the unsupplemented group, and a concentration of 6.34±0.14g/dl was found in the supplemented group. Serum albumin level of 3.70±0.20g/dl was found in the control, 3.50±0.76g/dl in the unsupplemented group and 3.98±0.15g/dl in the supplemented group. The difference between total protein and albumin levels, observed in all the groups are statistically not significant ($p>0.05$).

DISCUSSION

All the enzymes studied showed decreased activities in the supplemented group, when compared to the activities recorded in the unsupplemented group. The lipid components of cellular membrane are the principal target of free radical activities which might result in compromised protective functions, with subsequent leakage of cellular contents[12].

Elevated levels of antioxidant enzymes observed in the supplemented group, when compared with those seen in the unsupplemented group, might be responsible for the observed protective effects. Antioxidant enzymes works in synergy to quench the destructive tendencies of free radicals generated in the body, thereby sparing the body cells against lipid peroxidation[13].

Our findings also show decreased concentration of malondialdehyde in the supplemented group of diabetic rats, as against the concentration seen in the unsupplemented group of rats. Malondialdehyde is widely accepted as a marker of lipid peroxidation and itself, is capable of reacting with lipid component of the cells thereby causing pathology[14]. Therefore, the present study showed the ability of the micronutrient antioxidants supplements to reduce possible lipid peroxidation activity in the supplemented diabetic rats.

Administration of antioxidants might actually stimulate cell survival through strengthening of the antioxidant defense system. The principal aim of the current communication is the consideration of antioxidants micronutrients as adjunct therapy in the management of patients with diabetes mellitus.

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Table 1 **Mean Serum Concentration of Certain Liver Analyses of Alloxan -Induced Diabetic Rats After 4 Weeks of Antioxidant Micronutrient Supplementation**

Group	AST (U/l)	ALT (U/l)	ALP (U/l)	T. Prot. (g/dl)	ALB (g/dl)
Control (n=7)	17.71±0.89	18.74±0.89	38.43±2.17	6.90±0.18	3.70±0.20
Diabetic Treated only (n=7)	25.00±0.95*	37.57±1.70±	52.43±1.72±	6.3.3a±0.15	3.50±0.76
Diabetic treated and supplemented (n=7)	19.14±57**	36.43±1.74*	48.29±2.64*	6.34±0.14	3.98±0.15

* = Values differ sign ificantly from control
** = Values differ significantly from unsupplemented

Table 2 **Mean Concentration of Serum Malandoaldehyde (MDA), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and Catalase (CAT) in control, Diabetic Treated and Diabetic Treated and Diabetic Treated and Supplemented Whistar Rats.**

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)	2.37±0.159*	1.61±0.18	36.86±3.91*	39.86±3.03*
Diabetic treated and supplemented (n=7)	1.91±0.14	1.67±0.22	44.86±1.82	57.14±2.48**

* = Values differ significantly from control
** = Values differ significantly from unsupplemented