Research article

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Short Term Effect of Zinc Administration on Some Biochemical Parameters and Antioxidant Enzymes in Albino Rats

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ABSTRACT

Zinc is an essential trace element used as a supplement in the treatment of many diseases such as diarrhoea. This study was conceived to evaluate the short term effect of zinc administration on some biochemical parameters in albino rats. Sixteen albino rats (both sexes) were allocated randomly into four groups of four rats each. Group 1 served as the control and were given distilled water, groups II-IV were administered 10, 20 and 40 mg/kg body weight of Zn respectively for 14 consecutive days. The animals were sacrificed on the 15th day and blood was collected for liver and kidney function parameters, antioxidant enzymes activities and malondialdehyde concentration using standard procedures. The concentrations of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) significantly increased ($p < 0.05$) while aspartate aminotransferase (AST) significantly decreased in a dose dependent manner when compared with the control group. There was a significant decrease ($p < 0.05$) in creatinine, a significant increase in potassium and no significant difference in serum urea level when compared with the control group. The activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase significantly increased while MDA significantly decreased when compared with the control group. Increased potassium level is an indication of kidney dysfunction. Increased antioxidant enzymes and decreased MDA indicates that zinc improved antioxidant status and decreased free radical generation. Increased serum ALT activity infers improvement in its activity rather than hepatocellular injury. Further studies on the effect of zinc at larger doses and long term should be carried out.

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Introduction

The element zinc is an essential element which exerts important anti-inflammatory, apoptotic and antioxidants effects [1]. It plays important roles in cellular metabolism[2]. Zinc additionally plays extensive roles in both specific (adaptive) and non specific (innate) immunity at multiple levels of cell differentiation, natural phenomenon and development of immune cells [3]. The extent of harmful or beneficial effects of xenobiotic compounds on

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blood and other body fluids are often determined by assessing the biochemical parameters levels [4]. Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, blood urea nitrogen (BUN) and creatinine are serum biochemical parameters of great significance in assessing liver and kidney function [5]. Blood-related functions of chemical compounds can likewise be explained by assessing the blood level of these liver enzymes which are biomarkers for infection[6]. The body comprises of enzymatic and non-enzymatic antioxidant systems, which include superoxide dimutase (SOD), catalase (CAT), glutathione peroxidase (GPX), e.t.c, that regulate the balance between antioxidants and reactive oxygen species (ROS) [7]. Malondialdehyde (MDA) is an end product of lipid peroxidation. Nielsen *et al*.[8] reported that MDA may be a vital marker of lipid peroxidation and oxidative damage caused by reactive oxygen species. Talpur *et al*.[9] reported that zinc administration decreased the activity of liver enzymes (AST, ALT and ALP) in an induced hepatotoxicity. Mohamed *et al*.[10] also reported a significant decrease in the levels of these liver markers including some renal function parameters like urea, in an induced renal toxicity upon zinc administration. There is little information on the effect of zinc in normal rats and the implication of prolonged usage in normal subjects with no disease. Studies have reported that zinc increased the activities of antioxidant enzymes in subjects with diseases, but little information is available on its effect on normal subjects. This study therefore evaluated the effect of zinc administration on liver, kidney and antioxidant enzyme activity in normal albino rats.

Materials and Methods

Experimental Animals

Albino rats of both sexes were obtained from the animal breeding unit Adamawa State University Mubi, Nigeria. All procedures involving the animals were duly conducted with strict adherence to guidelines and procedures. The experiments were carried out according to the rules and guidelines of the Animal Ethical Committee of Adamawa State University, Mubi.

Source of Zinc

The zincsulphate was obtained from Gombi pharmacy, Adamawa State, Nigeria produced by Archy with NAFDAC registration number A4-2156.

Experimental Design

Sixteen (16) albino rats (both sexes) were allocated randomly into four experimental groups of four rats each. The rats were given distilled water and standard pelletized feed. The rats were fasted overnight. One group served as the control and were given distilled water. The three experimental groups were orally administered with 10 mg/kg body weight, 20 mg/kg body weight and 40 mg/kg body weight of zincsulphate (dissolved in distilled water) respectively. The administration of the zinc sulphate in all groups continued for 14 consecutive days.

Sample Collection for Biochemical and Antioxidant Assays

This was done according to the method described by Mahmoud *et al*.[11] . Briefly, on the 14th day, all the animals were sacrificed 24 hours after the administration of the final dose and blood samples from each animal was collected in to plain bottles, and was centrifuged at 5000rev/min for 10minutes to obtain the serum for biochemical analysis and antioxidant assay.

Biochemical Analysis

The method for biochemical analysis was adapted from Olorunfemi *et al*.[12]. Appropriate commercial kits (Randox laboratory) were used to determine the concentrations of ALT, ALP, AST, Serum urea, creatinine and potassium using a spectrophotometer.

Antioxidant parameters

The method of Hosseini-Vashan *et al*.[13] as modified by Fathi *et al*.[14] was adopted. In this method, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determind in 2 mL blood sample, washed and centrifuged (748 g for 10 min) several times with 0.9% NaCl. The washed-centrifuged erythrocytes volume was made up to 2.0 mL with cold redistilled water. At this point, the lysate was prepared using the procedures in the packs manual RANSEL and RANDOX (Ransel, RANDOX/RS-504 provided by Randox Laboratories) to decide the movement of GPx and SOD, individually. The absorbance was determined spectrophotometrically at 340 and 505 nm for GPx and SOD,

respectively. The activities of catalase was determined using the method as described by Luck,[15] where the reaction mixture contained 50 mmol/L potassium phosphate support (pH 7.0), 1.25 × 10 −2 mol/L H_2O_2, and the sample. Each of the sample was analyzed with appropriate blanks without H_2O_2. The change in absorbance was determined at 240 nm, and the activities of the enzyme were expressed as nanomoles of H_2O_2 decomposed per minute per milligram protein. Lipid peroxidation (MDA) assay was determined using the method as described by Ohkawa *et al*.[16] where a mixture of 8% dissolvable sodium dodecyl sulfate (0.2 mL), 0.9% TBA (thiobarbituric corrosive; 0.2 mL), and 20% acetic corrosive (1.5 mL) was prepared, in which 0.2 mL of hemolysate was added and the volume was made up to 4 mL by adding distilled water. After boiling for 60 minutes, the mixture was allowed to cooled, and 5 mL of solution of n-butanol + pyridine (vol/vol 15:1) was added and centrifuged at 1000g for 15 minutes, and the absorbance in the supernatant was mesured at 532 nm using spectrophotometer.

Statistical Analysis

All results were expressed as mean ± standard error of the mean (SEM). Data was analyzed using one-way ANOVA followed by Duncans multiple range test. $p < 0.05$ was considered as statistically significant, using SPSS software version 20.

Results

Biochemical Parameters Analysis

Table 1 shows the result of zinc administration on liver biochemical parameters in albino rats treated with zinc for 14 days. The concentrations of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) significantly increased ($p < 0.05$) when compared with the control while aspartate aminotransferase (AST) shows no significant difference ($p > 0.05$) with the control group. The serum creatinine concentration significantly decreased ($p < 0.05$) when compared with the normal control group while there was a significant increase in serum potassium concentration. There was no significant difference in serum urea concentration at all test doses when compared with the control group.

Table 1 Effect of Zinc administration on biochemical parameters

Parameters	Control	10 mg/kg b.w of zinc	20 mg/kg b.w of zinc	40 mg/kg b.w of zinc
ALP (U/L)	16.92±1.96[a]	40.48±1.43[b]	43.24±1.61[b]	45.89±1.02[b]
ALT (U/L)	59.33±2.91[a]	77.00±1.00[b]	83.33±1.76[c]	84.33±0.67[c]
AST (U/L)	53.67±2.18[a]	52.67±2.73[a]	53.00±1.53[a]	52.33±1.85[a]
Creatinine (μmol/L)	64.65±1.53[b]	14.23±0.19[a]	13.92±0.44[a]	13.96±0.04[a]
Urea (mmol/L)	4.54±0.21[a]	3.94±0.42[a]	4.42±0.15[a]	4.58±0.11[a]
Potassium (mEq/L)	6.00±0.22[a]	11.89±0.64[b]	21.50±0.50[c]	22.49±0.73[c]

Values are expressed as mean ± S.E.M, (n=4).Values across the same row with same superscripts are not significantly different. $p < 0.05$ was considered significant.

The data summarized in table 2 shows the effect of zinc on antioxidant enzymes. There was a significant increase in GPx, SOD and CAT activities after 14 days zinc administration when compared with the control group.

Table 2 Effect of zinc supplementation on antioxidant enzymes

Treatment	SOD (U/L)	GPX (U/L)	Catalase (U/L)
Control	15.33±1.37[a]	15.50±0.51[a]	16.00±1.00[a]
10 mg/kg b.w of zinc	51.67±1.33[b]	14.67±0.67[a]	25.53±1.23[b]
20 mg/kg b.w of zinc	53.67±0.84[b]	35.33±1.05[b]	31.27±1.20[c]
40 mg/kg b.w of zinc	53.33±0.78[b]	40.00±1.00[c]	39.83±0.39[d]

Values are expressed as mean ± standard error of mean (S.E.M), n=4.Values along the same column with same superscripts are not significantly different. $P < 0.05$ was considered significant.

Table 3 shows the result of zinc administration on malondialdehyde (MDA) concentration. There was a significant decrease ($p < 0.05$) in MDA concentration in the zinc treated groups when compared with the control.

Table 3 Effect of zinc supplementation on Malonaldehyde (MDA)

Treatment	MDA (mg/dl)
Control	34.83 ± 1.09^{c}
10 mg/kg b.w of zinc	17.20 ± 0.98^{b}
20 mg/kg b.w of zinc	14.67 ± 1.16^{b}
40 mg/kg b.w of zinc	10.07 ± 1.03^{a}

Values are expressed as mean ± standard error of mean (S.E.M), n=4.Values along the same column with same superscripts are not significantly different. $P < 0.05$ was considered significant.

Discussion

Biochemical Parameters

The concentration of alanine aminotransferase is said to be higher in the liver than in the kidney, heart, skeletal muscle, pancreas, spleen, and lung tissue [17]. The increased levels of ALT and ALP from this study may indicates an improvement in the activities of this liver enzymes or hepatocellular damage. ALT is a cytosolic enzyme found predominantly in the liver. An increase in plasma concentration may be due to damage to the cell membrane of the liver [18]. This is in line with the studies from Seyyed *et al*.[19] who reported that zinc pretreatment has hepatoprotective effect by increasing the serum liver enzymes concentration of ALT and ALP. There was no significant difference in the concentration of AST.

Renal function parameters/indices are important for the diagnosis and treatment of kidney disease. The observed result from this study shows a decrease in serum creatinine level which may suggest kidney damage. Serum urea as observed from the study shows no significant difference among the treated groups and the control group. The observed significant increased level of potassium in serum from this study is associated with glomerular filteration rate in the kidney, where K^+ is freely filtered by glomerulus [20]. According to Palmer, [20] he reported that the bulk of K^+ is reabsorbed in the proximal tubule of Henle so that only 10% of the filtered load reaches the distal nephrone and the reabsorbed K^+ are transported in the blood and this absorption is approximately proportional to Na^+ and water absorption. Therefore, the significant increase in serum potassium concentration indicates dysfunction in reabsorption of potassium by the kidney indicating possible kidney damage.

Antioxidant Enzymes

The observed significant increased ($p < 0.05$) in the antioxidant enzymes superoxidedismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), when compared with the control group is an indication that zinc increased the activities of these enzymes to suppresses the generation of reactive oxygen species and this is in line with the studies from Prasad,[21] and Ozturk and Gumuslu,[22] who reported that zinc is capable of scavenging free radicals and can prevent lipid membrane damage. This is evidenced by the decreased concentration of MDA, which is an index biomarker of lipid peroxidation. Thus, Low concentration of MDA may suggest that zinc can prevent membrane damage and thus, decreased in lipid peroxidation, this is in line with the study of Zhao *et al*. [23] who reported that appropriate concentration of zinc can reduce the concentration of malondialdehyde (MDA) and enhance antioxidant activities to suppress the generation of ROS. This further indicates that the increased ALT and ALP observed above is not due to liver cell membrane damage since zinc is able to prevent lipid peroxidation that can cause cell damage. Results from this study has shown that zinc is able to prevent oxidative damage of cell membrane due to lipid peroxidation, thus the increased ALT observed in this study is due to reasons (such as improvement of the enzyme activity) rather than oxidative damage.

Conclusion

Fourteen (14) days administration of zinc improved liver function biomakers, antioxidant status and prevented lipid peroxidation in normal albino rats. It had toxic effect on the kidney. Zinc supplementation should therefore be used with caution.

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