2012 4th International Conference on Chemical, Biological and Environmental Engineering
IPCBEE vol.43 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V43. 35

Supplementation of Excessive Zn in the Diet in Long Term Basis Induced Obesity Associated Oxidative Stress in Wistar Rats.

Kshetrimayum Birla Singh[1] and Shatish Kumar Taneja[2]

[1] Department of Zoology, Pachhunga University College, Mizoram University, Aizawl-796001, India.
[2] Department of Zoology, Panjab University Chandigarh-160014, India

Abstract. Our previous studies revealed that excessive Zn supplementation in the diet trigger the onset of obesity in rats. Since oxidative stress is highly correlated with the metabolic states including obesity and related diseases, the need to study arises to confirm whether the onset of obesity by excess Zn in diet is associated with oxidative stress or not**.** In this study, obesity was induced by feeding the rats on semi-synthetic diet containing increase amount of Zn *viz.* 20 mg (control, group-I), 40mg (group-II) and 80mg Zn /kg (group-III) diet respectively for a period of 180 days. The data of this study revealed that the gain in body weight increased in rats in Zn-concentration dependent manner (P <0.001). The urine examined on weekly basis showed glucosuria in group-II on week 10 and in group-III on week 8 and thereafter**.** Their arterial blood pressure and heart rates were significantly higher in group-II and III than their control counter parts on monthly basis (P < 0.001). The blood profile after 180 days of dietary treatment, displayed a significant rise in serum glucose, total lipids, cholesterol, triglycerides, LDL-cl, VLDL-c whereas HDL-c showed a reduction in their levels in group-II and III than their control counterparts (P < 0.001). The lipid peroxidation products were higher and the enzyme activities of superoxide dismutase, catalase, glutathione reductase, glutathione (reduced) and glucose -6 phosphate dehydrogenase were significantly lowered in liver and kidney of group-II and group-III (P < 0.001). Their mineral status revealed a higher Zn concentration and lower Cu, Mg and Mn both in liver and kidney (P < 0.001). This data suggest that Zn in excess in diet when fed for longer periods of time induces obesity associated oxidative stress.

Keywords: Zn, oxidative stress, obesity, minerals

1. Introduction

Oxidative stress is highly correlated with a wide variety of inflammatory and metabolic disease states, including obesity [1]. Evidence suggests that a clustering of sources of oxidative stress in obesity, hyperglycemia, increased tissue lipid levels and inadequate antioxidant defences [2]. In mammalian cells, there are several mechanisms in which organisms defend itself against oxidative stress. Among them, there are antioxidant scavenging enzymes such as cellular Cu/Zn SOD, CAT, GPx, Glu-s- T, GRD, Glu-6-PD and GSH [3].

It has been reported that deficiency and excess of metals also promotes oxidative stress and lipid peroxidation [4]. The activities of Cu and Zn containing antioxidant enzymes including SOD, CCO, CAT and GPx have been reported to diminish under Zn or Cu-deficiency or due to alteration in Cu-Zn ratio [5].Moreover, there are increasing evidences that excessive Zn in diet induces obesity, diabetes, dyslipidemia and hypertension in experimental animals [6]. Higher concentration of Zn and lower concentration of Cu, Mg and Mn in the tissues of human and some populations have been reported to link the ionic imbalance of nutritionally important elements to the etiology of obesity related diseases [7]. During the past two decades, there has been a rise in the consumption of higher Zn either from Zn fortified foods as in the USA [8] or from vegetables and meat foodstuffs as in some States of India [9]. Inspite of the fact that Zn is an essential micronutrient, the used of Zn supplement has been discourage by some health professional because excessive intake of Zn has been reported to induce Cu, Mg, Mn and Fe and Se

deficiencies due their antagonistic interactions [10]. It is believed that human individuals susceptible to many diseases are genetically predisposed to absorb or retain more Zn than their control counterparts like those of db/db mice and Zucker rats and on spontaneous development of diseases they start losing Zn in their urine. Our previous study has revealed that excessive Zn supplementation in the diet triggers the onset of the observed state of obesity. Therefore, the present study was designed to investigate if the obesity in such state is associated with oxidative stress and its consequence on enzymes of antioxidant defense system in rats which are not genetically predisposed to any diseases.

2. Materials and Method

Induction of obesity in experimental rat: The obesity in the rats was induced by increasing Zn concentration in semi-synthetic diet rich in fat and refined sugar. Accordingly, isocaloric semi-synthetic basal diet for inducing obesity in rats was used following Taneja *et al. 2006*[11]. The semi-synthetic basal diet contained (g/100 of diet) : Casein, 30; Agar, 2.0; Corn oil, 5; Cellulose, 8; Sucrose, 51; Vitamin mixture 0.5 [Vitamin mixture (mg/Kg) ; Ascorbic acid, 500; Biotin,4; Calcium pentothenate, 320; Choline chloride, 2500; Folic acid ,10; Inositol ,1000; Nicotinic acid ,300; Pyridoxine HCl ,180; Riboflavin ,120; Thiamin HCl 200; α-tocopherol acetate (E),60; Cyanocobalamin,0.40; Retinol, 0.30; Ergacalciferol, 0.0031] and mineral mixture, 3.5 [Mineral mixture (gm/Kg) : CaH_2PO_4, 25.30; $COCl_3$, 0.04; $CuCl_2$,0.10; $FeSO_4.7H_2O$, 0.60; $MnSO_4.H_2O$,0.30; $MgSO_4.H_2O$, 4.05; KCl, 3.43; KI, 0.004; Na_2C0_3, 1.15; NaF,0.008; $ZnSO_4.7H_2O$,0.088g.

The basal diet was further divided into 3 parts: control diet consisting of basal diet containing 20 mg Zn/kg diet (per-se) for Group- I and obesity inducing-diet (Diet-II-ZS-OB) containing 40 mg Zn/Kg diet for Group – II and obesity inducing diet ((Diet-III-ZS-OB) containing 80mg/Zn diet for Group-III by accordingly increasing $ZnSO_4.7H_2O$ in basal diet.

Experimental Design: Male wistar rats (30), aged 6 weeks, weighing 60-70g was procured from Central Animal House, Panjab University, Chandigarh. They were fed on standard pellet rat feed for one week to acclimatize. Thereafter, the rats were divided into 3 groups – I, II and II and in such a way that their mean initial body weights remained almost similar in each group. The animals were fed on their respective diets *per se* ad libitium and triple distilled deionised water was made freely available to them for 180 days. The body weights were recorded at the beginning of the dietary treatment and thereafter every week. After the end of the dietary treatment of 180 days, the male rats of each group were sacrificed using diethyl ether as anesthesia. The blood samples were collected by puncturing the heart and blood serum was prepared by centrifuging blood at 2500 rpm for 15 minutes. The freshly prepared serum was analyzed for cholesterol [12] triglycerides [13], HDL-cholesterol [14] (all by using commercially available kits- Reckon Diagnostic Pvt, Ltd, Baroda, India and ERBA diagnostic Mannheim GmbH, Mannheim, Germany, supplied through Transasia Bio- Medicals LTD, Daman) and total lipids[15]. The LDL and VLDL-cholesterol was calculated by Friedwald's equation [16]. The liver and kidney of three groups of rats were removed for the study of enzyme activities. The levels of lipid peroxidation (LPO) products were evaluated by the method of Beuge and Aust [17] and glutathione (reduced) (GSH) by the method of Ellman [18] in their PMS fraction. Activities of superoxide dismutase (SOD) [19] catalase (CAT) [20], glutathione reductase (GRD) [21] and glucose-6-phosphate dehydrogenase (G-6-PD) [22] were estimated in PMS of liver and kidney. Protein was evaluated as per of Lowry *et al* [23].Zn, Cu, Mg and Mn were estimated on atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad- AAS 4139). The results were subjected to stastical analysis applying one way ANOV**A.**

3. Results and Discussion

The data suggest that the increase of Zn concentration to 40 mg/kg and 80 mg/kg in diet of group-II and III rats resulted in an increase in the body weight, blood pressure, heart rate and glucosuria, hyperglycemia and dyslipidaemia (Table 1, 2 & 3). These disorders have been reported in our previous studies [11, 24, 25] and the contribution of Zn in induction of obesity, diabetes mellitus, dyslipidaemia and hypertension in wistar rats fed a similar diet containing either 40 mg or 80 mg Zn/kg diet as used in the present investigations have been discussed at length. The lipid peroxidation (LPO) products was found to be higher in the liver and

kidney of group-II and III rats than those of the control group-I showing weak antioxidant defense system in the former Zn supplemented groups in the present study (Table-4 & 5). The production of LPO products depends on activities of antioxidant enzymes such as SOD, CAT, GRD, GSH and Glu-6-PD which registered a significant reduction in liver and kidney of group-II and group-III rats (Table-4 & 5). Since the group-II and group-III rats are displaying alteration in blood lipid metabolisms, as expected the lipid peroxidation products were higher in them than those of control rats indicating that the rats are under the oxidative stress. Oxidative stress is the consequence of a reduction in the antioxidant systems and/or an increase in the production of free radicals and ROS. The damage induced by oxidative stress only occurs when the antioxidant defenses are unable to counteract the production of ROS. In conformity with our study, the alteration of the antioxidant mechanisms has been shown in both obese humans and in experimental obese animal models [26]. Such decrease of antioxidant enzyme may be due to rapid consumption and exhaustion of storage of this enzyme in fighting free radicals generated during development of obesity. Olusi *et al.* [27] also found that SOD and GSH-Px activity were lower in obese persons as compared with nonobese persons and rat models of diet-induced obesity suggesting that in obesity the organism is unable to provide adequate levels of antioxidants to compensate for the production of radicals, thereby generating malonyldialdehyde as an oxidant [27].The assessment of mineral status revealed an ionic imbalance in group II and III rats wherein the Zn concentrations in liver and kidney were higher and that of Cu, Mg and Mn were lower compared to their control counter parts in spite of the fact that these metals are adequate in diet (Table-6). This ionic imbalance may be attributed to the over expression of Zn metallotheionein gene during Zn treatment and continued to exist during their growth phase. As a result of this, they absorbed and retained greater amount of Zn than their control counterpart leading to deficiencies of Cu, Mg, and Mn due to their interactions. This ionic imbalance resulted in hypertension, dyslipidaemia and hyperglycemia in the group II and III rats and consistent with mineral status in the obese, NIDDM and hypertension as reported in some human populations [28] and some obese diabetic animals [29]. Excessive Zn supplementation may influence antioxidant enzymes activities through disturbances in micronutrient status as the metallic ions being an integral component of these enzymes per se, the deficiencies of some of the metals such as Cu, Mg and Mn have been reported to result in the reduction in enzyme activities of antioxidant defense system and increase in peroxidation products in rats [30, 31]. SOD requires Mn, Cu and Zn, GPx needs selenium and CAT contains haem as cofactor. Moreover, several reports underlie the alteration of antioxidant micronutrient status in subjects of high Zn supplementation diet [32, 33]. Thus, the deficiencies/imbalance of these elements are link to subsequent development of dyslipidemia and oxidative stress in these obese rats. The results of the present study thus provide strong evidences that excessive Zn in diet even in pharmacological doses induced obesity related oxidative stress.

4. Acknowledgement

Thanks are due to Professor S.K. Taneja, UGC professor Emeritus, Department of Zoology, Panjab University Chandigarh, India for his critical guidance during this project work and at the time of the preparation of this manuscript.

5. References

[1]. Sonta T, Inoguchi T, Tsubouchi H, Sekiguchi N, Kobayashi K, Matsumoto S, Utsumi H, Nawata H: Evidence for contribution of vascular NAD(P)H oxidase to increased oxidative stress in animal models of diabetes and obesity. *Free Radic Biol Med* 2004, **37**:115-115.

[2]. Vincent HK, Taylor AG: Biomarkers and potential mechanisms of obesity induced oxidant stress in humans. *Int J Obes* 2006, **30**:400-400.

[3]. Kasapoglu M, Ozben T: Alteration of antioxidant enzymes and oxidative stress makers in aging. *Exp Gerontol* 2001; **36**:209-220.

[4]. Hirayam K, Yasutake A: Free radicals and trace elements. *J Trace Elem Exp Med* 1998; **11:** 209-217.

[5]. Prohaska JR: Changes in Cu, Zn- superoxide dismutase, cytochrome-c oxidase, glutathione peroxidise and glutathione transferase activities in copper deficient mice and rats. *J Nutr* 1991; **121**: 355-363.

[6]. Pomp D, Oberbauer AM, Murray JD: Growth and body composition of OMT-la-OGH transgenic male mice with differing periods of transgenic activation. *J Anim Sc* 1992; **70(1):** 198-201.

[7]. Taneja SK, Mandal R: Assessment of minerals in obesity related diseases in Chandigarh (India) population. Bio Trace Elem Res 2008; **121**: 106-123**.**

[8]. Arsenault JE, Brown KH: Zn intake of US preschool children exceeds new dietary references intakes. Am J Clin Nutr 2003; **8**:1011-1016.

[9]. Singh KB, Taneja SK: 2010. Concentration of Zn, Cu and Mn in vegetables and meat foodstuffs commonly available in Manipur: a North Eastern State of India. *Elec J Env Agric Food Chem* 2010; **9 (3)**:610- 616.

[10]. Aaseth J, Haugen M, Forre OC: Rheumatoid arthritis and metal components – perspective on the role of oxygen radical detoxification. *Analyst* 1998; **123**:3-6.

[11]. Taneja SK, Mandal R, Girhotra S: Long term excessive Zn supplementation promotes metabolic syndrome-X in wistar rats fed sucrose and fat rich semi-synthetic diet. *Ind J Exp Biol* 2006; **44:** 705-718.

[12]. Allain CC, Poon LS, Chand CSG, Richmond W, Fu P: Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; **20:** 470-475.

[13]. McGowan MW, Artiss JD, Stranberg DR, Zak BA: Peroxide coupled method for colorimetric determination of serum triglycerides. *Clin Chem* 1983; **29:** 438-542.

[14]. Burstein M, Schonick HR, Morfin R: Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipids Res*, 1970; **11:**583-595.

[15]. Frings CS, Dunn RT: A calorimetric method for determination of total serum lipids based on sulpha-phosphovanillin reaction. *Am J Clin Path* 1970; **53**: 89-91.

[16]. Friedewald WT, Levy RI, and Fredrickson DS: Estimation of concentration of LDL-cholesterol in plasma without use of preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499-502.

[17]. Beuge JA, Aust SD: Microsomal lipid peroxidation. *Method Enzymology* 1978; **52:** 302-310

[18]. Ellman GL: Tissue sulphydryl groups. *Arch Biochem Biophy* 1959; **82**: 70-77.

[19]. Nishikimi M, Rao NA, Yagi K: The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophys Res Commun* 1972; **46**: 849-859.

[20]. Aebi HE: Catalase in: Methods of enzymatic analysis (Bergnmeyer H V ed) 3rd Edn Vol Academic Press New York pp 1983; 273-386.

[21]. Horn HD: Glutathione reductase. *In:* Methods of enzymatic analysis (Bergmeyer H.V. ed.) Academic Press, New York, London. 1971; 875-881.

[22]. Lohr GW, Waller HD: Glucose - 6 – phosphate dehydrogenase. *In:* Methods of enzymatic analysis (Bergmeyer HU ed) Academic Press New York. 1963; pp.744.

[23]. Lowry OH, Rosebrough NJ, Farr AL**,** Randall RJ: Protein measurement with the folin phenol reagent. *J Bio Chem* 1951; **193:** 265-275.

[24]. Singh KB, Taneja SK: Effect of long term excessive Zn supplementation on blood lipid profile and tissue minerals status in wistar rat. *J Exp Sc* 2010; **1(3):** 4-9.

[25]. Taneja SK, Mahajan M Arya P: Excess bioavailability of Zn may cause obesity in humans. *Experientia* 1996; **52:** 31-33.

[26]. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond)* 2006; **30**:400–418.

[27]. Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans. *Int J Obes Relat Metab Disord* 2002; **26:**1159–1164.

[28]. Anderson R A, Roussel A M, Zouari N, Mahjoub S, Matheau J M & Kerkeni A, Potential antioxidant effects of zinc and chromium supplementation in people with Type-II diabetes mellitus, *J Am Coll Nutr*, 2001; **20:** 212-218 .

[29]. Prohaska J R, Changes in Cu-Zn Superoxide dismutase, Cytochrome oxidase, Glutathione peroxidase and glutathione transferase activities in copper deficient mice and rats, *J Nutr,* 1991; *121:* 355-363.

[30]. Failla ML, Gardel CRY: Influence of spontaneous diabetes on tissue status of zinc, copper, manganese in the wistar rats. *Proc Soc Exp Bio Med*, 1985; **180:** 317-322

[31]. Evans P, Halliwell B: Micronutrients: oxidant/antioxidant status, *Br J Nutr*, 2001; **85(2):** 67-74.

[32]. Strain JJ: Disturbance of micronutrient and antioxidant status in diabetes, Proc Nutr Soc, 1991; 50: 591-597.

[33]. Walter RM, Uriu hare JY, Olin KL, Oster MH, Anwalt BD, Critchfield JW, Keen CL, Copper, zinc, manganese and magnesium status and complications of diabetes mellitus, *Diabetes Care,* 1991,14: 1051-1054.

Table 1: Month wise body weight of male rats of Group-I [(fed on basal diet(Control)], Group-II [(fed on obesity inducing-diet (Diet-II-ZS-OB)] and Group- III [(fed on obesity inducing-diet (Diet-III-ZS-OB)] during 180 days of dietary treatment. [Values are mean ± SE of 6 observation each].

Time duration(in days)	Group-I (Control)	Group-II	Group-III
0	68.0 ± 0.68	67.0 ± 0.67	67.2 ± 0.79
30	165.5 ± 1.12	190.5 ± 1.13[a]	230.6 ± 1.67[a]
60	195.17 ± 1.53	270.67 ± 1.67[a]	*295.5 ± 1.14[a]
90	*260.6 ± 1.97	325.33 ± 1.70[a]	*360.3 ± 0.860[a]
120	*280.50 ± 1.70	345.4 ± 1.60[a]	*380.5 ± 1.39[a]
150	*320.67 ± 1.13	365.57 ± 1.78[a]	*410.5 ± 1.67[a]
180	*370.5 ± 0.82	330.83 ± 1.10[a]	*355.67 ± 1.67[a]

Units: gram; *P* values: [a] < 0.001(values of group- II and group-III were compared with group-I).
*: Onset of glucosuria as revealed by the Benedict's test.

Table 2: Month wise blood pressure and heart rates in male rats of Group-I [(fed on basal diet(Control)], Group-II [(fed on obesity inducing-diet (Diet-II-ZS-OB)] and Group- III [(fed on obesity inducing-diet (Diet-III-ZS-OB)] during 180 days of dietary treatment. [Values are mean ± SE of 6 observation each].

Time duration (in days)	Group- I(Control)		Group- II		Group- III	
	Blood pressure*	Heart rates**	Blood pressure*	Heart rates**	Blood pressure*	Heart Rates**
30	92.67 ± 1.04	179.17 ± 1.54	130.17 ± 0.49[a]	280.67 ± 1.36[a]	147.3 ± 0.95[a]	349.50 ± 1.34[a]
60	96.67 ± 0.950	200 ± 1.32	146.50 ± 0.96[a]	306.17 ± 1.29[a]	164.0 ± 1.34[a]	375.50 ± 1.55[a]
90	99.83 ± 0.75	213.3 ± 1.28	162.0 ± 1.29[a]	314.5 ± 1.20[a]	180.6 ± 1.26[a]	400.83 ± 2.39[a]
120	103.83 ± 1.25	235.0 ± 1.83	172.83 ± 0.94[a]	349.67 ± 1.08[a]	196.7 ± 1.54[a]	426.17 ± 2.24[a]
150	103.83 ± 1.30	239.17 ± 1.57	193.3 ± 0.96[a]	380 ± 1.37[a]	212.3 ± 0.94[a]	452.17 ± 0.97[a]
180	110.0 ± 1.83	247.17 ± 1.03	230.50 ± 1.61[a]	404 ± 1.40[a]	288.0 ± 1.26[a]	453.0 ± 0.90[a]

Units: *: mm of Hg; **: beats per minute, *P* values: [a] <0.001(values of group- II and group-III were compared with group-I).

Table 3:Blood lipid profile of male rats of Group-I [(fed on basal diet(Control)], Group-II [(fed on obesity inducing-diet (Diet-II-ZS-OB)] and Group- III [(fed on obesity inducing-diet (Diet-III-ZS-OB)] during 180 days of dietary treatment. [Values are mean ± SE of 6 observation each].

Parameters	Group-I(Control)	Group-II	Group-III
Total Lipids*	200.50 ±2.14	255.17 ± 1.78[a]	320.33 ± 2.86[a]
Cholesterol*	65.67 ± 2.17	85.0 ± 1.02[a]	120.0 ± 1.86[a]
Triglycerides*	65.67 ± 1.56	105.83 ± 1.76[a]	125.67 ± 2.67[a]
VLDL- Cholesterol*	12.7 ± 0.320	20.97 ± 0.360[a]	24.3 ± 0.340[a]
HDL-Cholesterol*	24.67 ± 1.12	14.67 ± 0.710[a]	10.83 ± 0.410[a]
LDL- Cholesterol*	28.27 ± 1.35	45.37 ± 0.760[a]	85.83 ± 1.94[a]

Units: *: mg/dl; *P* values: [a] <0.001(values of group- II and group-III were compared with group-I)

Table 4: Mean lipid peroxidation and enzymes activities in liver of male rats of Group-I [(fed on basal diet(Control)], Group-II [(fed on obesity inducing-diet (Diet-II-ZS-OB)] and Group- III [(fed on obesity inducing-diet (Diet-III-ZS-OB)] during 180 days of dietary treatment. [Values are mean ± SE of 6 observation each].

Parameters	Group-I(Control)	Group-II	Group-III
Lipid peroxidation$	0.75 ± 0.05	0.95 ± 0.04 [a]	1.65 ± 0.19 [a]
Superoxide dismutase*	16.10 ± 0.20	10.4 ± 0.16 [a]	7.50 ± 0.27 [a]
Catalase**	52.77 ± 0.93	43.08 ± 0.68 [a]	32.03 ± 1.25 [a]
Glutathione (reduced) #	8.23 ± 0.16	6.70 ± 0.17 [a]	3.52 ± 0.11 [a]
Gutathione reductase##	7.63 ± 0.24	4.95 ± 0.12 [a]	3.45 ± 0.34 [a]
Glucose-6-phosphate dehydrogenase###	10.20 ± 0.45	6.25 ± 0.24 [a]	3.50 ± 0.13 [a]

Units: $: n mol MDA produced/ hr/ mg protein; *: unit/mg protein; **: µ mol H_2O_2 decomposed/ min /mg protein; #: n mol GSH/mg/protein; ##: n mol NADPH oxidized/min/mg protein; ###: n mol NADPH formed/min/mg protein; P values: [a]<0.001; [b]<0.05 (values of Group -II and Group -III were compared with Group -I).

Table 5: Mean lipid peroxidation and enzymes activities in kidney of male rats of Group-I [(fed on basal diet(Control)], Group-II [(fed on obesity inducing-diet (Diet-II-ZS-OB)] and Group- III [(fed on obesity inducing-diet (Diet-III-ZS-OB)] during 180 days of dietary treatment. [Values are mean ± SE of 6 observation each].

Parameters	Group-I(Control)	Group-II	Group-III
Lipid peroxidation$	0.65 ± 0.03	0.85 ± 0.02 [a]	1.25 ± 0.03 [a]
Superoxide dismutase*	12.07 ± 0.16	12.12 ± 0.11 [a]	8.42 ± 0.14 [a]
Catalase**	45.80 ± 0.78	35.10 ± 0.47 [a]	35.75 ± 1.60 [a]
Glutathione (reduced) #	7.65 ± 0.22	4.30 ± 0.17 [a]	3.65 ± 0.32 [a]
Glutathione reductase##	6.35 ± 0.16	4.95 ± 0.24 [a]	1.25 ± 0.24 [a]
Glucose-6-phosphate dehydrogenase###	6.85 ± 0.57	4.70 ± 0.19 [a]	2.80 ± 0.31 [a]

Units: $: n mol MDA produced/ hr/ mg protein; *: unit/mg protein; **: µ mol H_2O_2 decomposed/ min /mg protein; #: n mol GSH/mg/protein; ##: n mol NADPH oxidized/min/mg protein; ###: n mol NADPH formed/min/mg protein; P values: [a]<0.001; [b]<0.05 (values of Group -II and Group- III were compared with Group- I).

Table 6: Mean Zinc (Zn), Copper (Cu), Magnesium(Mg) and Manganese (Mn) Concentration in the liver and kidney of male rats of Group-I [(fed on basal diet(Control)], Group-II [(fed on obesity inducing-diet (Diet-II-ZS-OB)] and Group- III [(fed on obesity inducing-diet (Diet-III-ZS-OB)] during 180 days of dietary treatment. [Values are mean ± SE of 12 observation each].

Parameters	Group-I(Control)	Group- II	Group-III
Liver Zn@	35.7 ± 0.88	45.1 ± 0.84 [a]	50.0 ± 0.65 [a]
Liver Cu@	65.3 ± 0.68	47.9 ± 1.13 [a]	35.3 ± 0.77 [a]
Liver Mg@	55.0 ± 1.03	45.0 ± 0.49 [a]	36.0 ± 0.90 [a]
Liver Mn@	35.80 ± 0.59	25.40 ± 0.65 [a]	20.90 ± 0.66 [a]
Kidney Zn@	35.4 ± 0.54	40.7 ± 0.69 [a]	50.1 ± 1.52 [a]
Kidney Cu@	45.5 ± 0.74	32.50 ± 0.68 [a]	30.10 ± 0.63 [a]
Kidney Mg@	50.50 ± 0.84	40.90 ± 0.54 [a]	34.20 ± 0.76 [a]
Kidney Mn@	40.80 ± 1.87	30.90 ± 0.94 [a]	25.20 ± 0.62 [a]

Units: @: µg/g tissue; *P* values: [a] < 0.001(values of group- II and group-III were compared with group-I).