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Effect of Zinc Supplementation on Oxidative Stress status in Male Wistar Rats

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ABSTRACT

The study was conducted on 72 weaned Wistar male rats which were divided into 3 groups *viz.* control, T1 and T2. Control rats were given diet without zinc supplementation while the rats of T1 and T2 groups were given diet containing zinc sulphate @ 50 mg and 100 mg/kg body weight/day, respectively for 8 weeks from 4 to 12 weeks of age. Blood samples were collected on 6, 8, 10 and 12 weeks of zinc supplementation and oxidative parameters were studied. Oxidative indices *viz* super-oxide dismutase (SOD), glutathione peroxidise (GPx), glutathione-s-transferase (G-s-T), catalyse (CAT) and lipid peroxidise (LPO) showed decreasing trend in T1 group, whereas increasing trend was observed in T2 group as age advanced; no such increasing or decreasing trend observed in control, except LPO activities, which showed increasing trend with advancing age. Zinc @ 50 mg/kg body weight/day (T1) proved better choice than zinc @ 100 mg/kg body weight/day (T2) in reducing oxidative stress in growing rats.

Keywords: oxidative stress, zinc supplementation, male Wistar rats

Nutritional factors play vital roles in the various physiological events which occur in the attainment of sexual maturity and in the course of the reproductive process (Kadir *et al*., 2010). Zinc is the second most abundant transition metal after iron and it is the only metal which appears in all enzyme classes (Broadley *et al.*, 2007). Thus, it is required for 300 different biological processes including DNA transcription, protein translation, cell proliferation and differentiation and apoptosis (Wu and Wu, 1987; Endicott *et al*., 1995). Moreover, it is an intracellular signalling molecule which plays an important role in antioxidant mechanism through number of proposed mechanisms (Powell, 2000). Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their functions (Halstead *et al*., 1972). Oxidative stress is the set of intracellular or extracellular conditions that leads to the chemical or metabolic generation of the reactive oxygen species (ROS), which are highly reactive intermediates free radicals, such as superoxide anion (O^{-2}), hydrogen peroxide (H_2O_2), hydroxyl free radical (OH), lipid peroxides (Saltman, 1989).

The activities of Cu and Zn containing antioxidant enzymes including superoxide dismutase (SOD), cytochrome-C-oxidase (CCO), catalase (CAT) and glutathione peroxidase (GPx) have been reported to diminish during Zn or Cu deficiency or due to alteration in Cu-Zn ratio (Prohaska, 1991). The present investigation was taken up to study the effect of two different doses of zinc supplementation on oxidative parameters durin the active phase of growth in male Wistar rats.

MATERIALS AND METHODS

The study was conducted in Division of Veterinary Physiology and Biochemistry, Faculty of Veterinary Sciences and Animal Husbandry, Jammu on 72 weaned healthy Wistar male rats for a period of 8 weeks from 4 to 12 weeks of age. The rats were procured from Indian Institute of Integrative Medicine, CSIR Laboratory,

Jammu. They were provided standard pelleted ration and clean drinking water *ad libitum* and maintained under standard managemental conditions. Prior to experiment, the rats were acclimatized in the laboratory conditions for a period of one week. The rats were divided into 3 groups *viz*. Control, T1 and T2. Control rats were given diet without zinc supplementation while rats of T1 and T2 groups were given diet containing zinc sulphate @ 50 mg and 100mg/ Kg body weight/day, respectively for 8 weeks from 4 to 12 weeks of age. All the experimental animals were kept under constant observation during entire period of study. The experimental protocols were duly approved by the Institutional Ethical Committee.

Blood collection and preparation of hemolysate

Total 5 ml of blood samples were collected from retro-orbital sinus of rats from each group on 6, 8, 10 and 12 weeks of experiment using heparinized capillary tubes. Blood was collected in aliquots containing anticoagulant heparin @ 10 IU/ml of blood and the samples were centrifuged at 3000 rpm for 15 minutes. The erythrocyte sediment obtained after separation of plasma was diluted by gently pouring of normal saline solution in the ratio of 1:1 on v/v basis and thoroughly mixed with erythrocyte sediment. The diluted erythrocytes were centrifuged for 10 minutes at 3000 rpm. After centrifugation, the supernatant was discarded along with buffy coat and normal saline was again added to the RBC pellet on v/v basis, by mixing gently and centrifuged and the process was repeated three times. After final washing, phosphate buffer saline (pH 7.4) was taken as diluent to make 1% hemolysate (100µl washed RBC + 9.9 ml 0.1M PBS) and 33% hemolysate (330µl washed RBC + 670µl 0.1M PBS). For the estimation of superoxide-dismutase, glutathione-peroxidase, glutathione-s-transferase and catalase, 1% hemolysate was used and for estimation of lipid peroxidation, 33% hemolysate was used.

Estimation of oxidative stress parameter

The indices of superoxide dismutase (SOD), lipid peroxidation (LPO), glutathione peroxidase (GPx), glutathione-s-transferase (G-s-T) and catalase (CAT) were determined by methods given by Marklund and Marklund (1974), Shafiq-ur-Rehman (1984), Hafeman *et al.* (1974), Habig *et al.* (1974) and Aebi (1983), respectively in erythrocyte lysate.

Statistical analysis

For all the observed data in the experiment, the standard statistical procedures recommended by Snedecor and Cochran (2004) have been followed. The data were presented by showing mean and standard error. The significant differences of means for different parameters studied were assessed by the test of two-way analysis of variance. The significant difference between groups and weeks were calculated by Tukey's test. All the above calculations were carried out using SPSS software version 16.0.

RESULTS AND DISCUSSION

It is known that zinc is one of the essential components of the body's most important natural antioxidant system (Chian *et al*., 2000). In addition, zinc has been reported to have a membrane stabilizing antioxidant activity and maintains sperm viability by inhibiting DNAase activity (Aitken *et al.*, 1987). It is also an integral part of many metallo-enzymes, which is believed to stabilize membranes and protect them against free radical injury. The study (Table 1) revealed that SOD activity showed a decreasing trend from 8 to 12 weeks of age in T1 group; whereas, in T2 group, enzymes activity showed an increasing trend with advancement of age. Similar trend was also observed in other enzymes like GPx, G-s-T, CAT and LPO in both the groups. LPO is a free radical mediated chain reaction, is treated as best marker of oxidative stress and its concentration increased during oxidative stress (Saygili *et al*., 2003). Increased oxidative enzymes in T2 group thus provided strong evidence that excessive zinc in diet even in higher doses induced oxidative stress. Decreased enzyme levels as in T1 group with advancement of age indicated less oxidative stress with zinc supplementation @ 50 mg/kg body weight/day. Nagalakshmi *et al*. (2013) also reported that lipid peroxidation was significantly higher ($P<0.01$) in rats fed diet without zinc supplementation and decreased in a dose dependent manner up to dose rate of 36 ppm while increased at 48 ppm.Similarly, they also reported that the activity of GPx was also significantly increased ($P<0.05$) at 48 ppm zinc supplemented rats followed by 36, 24 and 12 ppm supplementation in a dose dependent manner. Zinc has antioxidative nature and inhibits production of reactive oxygen species and hence improved fertility (Bray *et al*., 1997). Zinc prevents lipid

Table 1: Oxidative stress indices following zinc supplementation in male Wistar rats

Group	Age in weeks			
	6	8	10	12
Superoxide dismutase (SOD) activity (U/mg of protein)				
Control	$54.37^{bc} \pm 0.30$	$52.74^{aA} \pm 0.24$	$55.15^{cB} \pm 0.19$	$53.40^{abB} \pm 0.64$
Treatment (T1)	$53.40^{d} \pm 0.66$	$51.76^{cA} \pm 0.25$	$49.99^{bA} \pm 0.27$	$48.30^{aA} \pm 0.43$
Treatment (T2)	$54.03^{a} \pm 0.22$	$55.07^{aB} \pm 0.28$	$57.51^{bC} \pm 0.38$	$60.73^{cC} \pm 0.56$
Lipid peroxidation (LPO) activity (n mol MDA/ml)				
Control	$2.22^{a} \pm 0.11$	$2.40^{abAB} \pm 0.11$	$2.51^{abcB} \pm 0.07$	$2.80^{cB} \pm 0.07$
Treatment (T1)	2.20 ± 0.11	$2.11^{A} \pm 0.11$	$2.09^{A} \pm 0.09$	$2.00^{A} \pm 0.07$
Treatment (T2)	$2.26^{a} \pm 0.10$	$2.59^{abB} \pm 0.00$	$2.83^{bcB} \pm 0.08$	$2.99^{cB} \pm 0.09$
Glutathione peroxidase (GPx) activity (U/mg of Hb)				
Control	2.04 ± 0.24	$2.05^{AB} \pm 0.20$	$1.99^{B} \pm 0.07$	$2.05^{B} \pm 0.07$
Treatment (T1)	$2.01^{a} \pm 0.13$	$1.89^{abA} \pm 0.16$	$1.44^{bcA} \pm 0.11$	$1.25^{cA} \pm 0.07$
Treatment (T2)	$2.01^{a} \pm 0.25$	$2.40^{abB} \pm 0.09$	$2.66^{bC} \pm 0.05$	$2.81^{bC} \pm 0.11$
Glutathione-s-transferase (G-s-T) activity (µmol/min/mg of Hb)				
Control	0.35 ± 0.02	0.34 ± 0.01	$0.35^{A} \pm 0.01$	$0.36^{A} \pm 0.02$
Treatment (T1)	0.34 ± 0.01	0.33 ± 0.02	$0.31^{A} \pm 0.01$	$0.30^{A} \pm 0.01$
Treatment (T2)	$0.35^{a} \pm 0.02$	$0.37^{ab} \pm 0.03$	$0.43^{bcB} \pm 0.01$	$0.46^{cB} \pm 0.01$
Catalase (CAT) activity (µmol H_2O_2 utilized/min/mg of protein)				
Control	$62.76^{aB} \pm 0.48$	$64.06^{abB} \pm 0.33$	$63.03^{aB} \pm 0.37$	$63.55^{bB} \pm 0.47$
Treatment (T1)	$60.60^{cA} \pm 0.55$	$59.88^{cA} \pm 0.59$	$57.86^{bA} \pm 0.60$	$55.86^{aA} \pm 0.26$
Treatment (T2)	$64.70^{aC} \pm 0.51$	$66.45^{bC} \pm 0.62$	$67.41^{bC} \pm 0.34$	$69.83^{cC} \pm 0.20$

Means with superscript a,b,c differ significantly in rows; Means with superscript A, B, C differ significantly in column.

peroxidation and stabilizes lysosomal membrane (Kimball *et al*., 1995). Zinc supplementation also increased catalase activity in seminal plasma within tolerable limits can improve fertility but detrimental at higher doses (Alavi-shoushtari *et al.,* 2009; Egwurugwu *et al.*, 2013). Singh (2012) reported that excess zinc in diet fed for longer period of time induces oxidative stress by altering the status of minerals in the body. In another study, Omu *et al*. (2015) reported that zinc deficient rats, Cu-Zn SOD activity was significantly decreased ($P<0.05$) as compared to zinc supplemented group (28 mg/kg b. wt), whereas GPx activity was not significantly different between zinc deficient and zinc supplemented groups. Table 1 suggested that SOD, GPx, G-s-T and CAT activities showed no particular increasing or decreasing trend in control group as age advanced from 6 to 12 weeks of age; whereas, the LPO activity showed an increasing trend. Lipid peroxidation is the oxidative degradation of lipid. It is the process in which free radicals steal electro from the lipids of all membrane, resulting cell damage. LPO measurements increased in an age-limited, tissue specific fashion (Cook and Yu,1998).

CONCLUSION

It can be concluded from the study that zinc @ 50 mg/kg body weight/day proved better choice than zinc @ 100 mg/kg body weight/day in reducing oxidative stress in growing rats.

REFERENCES

Aebi, H.E. 1983. Catalase. In: Bergmeyer, H.U. (eds.). *Methods of Enzymatic Analysis,* pp. 276-286. Academic Press. New York.

Aitken, R.J., Fisher H.M., Fulton, N., Gomez, E., Knox, W., Lewis, B. and Irvine, S. 1987. Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by the flavor protein inhibitors diphenylene iodonium and quinacrine. *Mol. Reprod. Dev.,* **47**: 468-482.

Alavi-Shoushtari, S.M., Rezai, S.A., Ansari, M.H.K.H. and Khaki, A. 2009. Effects of seminal plasma zinc content and catalase activity on semen quality of water buffalo (Bubalus bubalis) bulls. *Pak. J. Biol. Sci.,* **12**(2): 134-139.

Bray, T.M., Levy, M.A., Noseworthy, M.D. and Iles, K. 1997. The role of zinc in free radical mediated diseases. In Fscher, P.W.F., Abbe, M.R., Cockell, K.A. and Gibson, R.S, editors. Trace elements in man and animals. *Proceedings of ninth international symposium on trace elements in man and animals*, pp 3333-3336, Ottawa, ON: NCR Research Press.

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I., and Lux, A. 2007. Zinc in plants. *New Phytologist,***173**(4): 677-702.

Chian, S.E., Ong, C.N., Chua, L.H., Ho, I.M. and Tay, S.K. 2000. Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. *J. Androl.,* **21**: 53-57.

Cook, C.I. and Yu, B.P. 1998. Iron accumulation in ageing: modulation by dietary restriction. *Mech. Aging Dev.*, **102**(1): 1-13.

Egwurugwu, J.N., Ifedi, C.U., Uchefuna, R.C., Ezeokafor, E.N. and Alagwu, E.A. 2013. Effects of zinc on male sex hormones and semen quality in rats. *Nigerian J. Physiol. Sci.,* **28**: 017-022.

Endicott, J.A., Noble, M.E., Garman, E.F., Brown, N., Rasmussen, B., Nurse, P. and Johnson, L. N. 1995. The crystal structure of p13suc1, a p34cdc2-interacting cell cycle control protein. *EMBO J.*, **14**: 1004-1014.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. 1974. Glutathione-S-Transferase. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.,* **249**: 7130-7139.

Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. 1974. Effect of dietary selenium on erythrocyte and Glutathione Peroxidase in rats. *J. Nutri.,* **104**: 580-587.

Halstead, J.A., Ronaghy, H.A., Abadi, P., Haghshenas, M. and Amerhakemi, G. H. 1972. Zinc deficiency in man. The Shiraz experiment. *Am. J. Med.,* **53**: 277-284.

Kadir, A.O., Kara, C., Topal, E., Arkungoz, E. 2010. Effect of supplementary nutrition in yearling saanen kids on sexual behaviors and reproductive traits. *J. Anim. Vet. Adv.,* **9**: 3098-3103.

Kimball, S.R., Chen, S.J., Risica, R., Jefferson, L.S. and Leuredupree, A.E. 1995. Effects of zinc deficiency on protein synthesis and expression of specific mRNAs in rat liver. *Metabolism,* **44**(1): 126-133.

Marklund, S. and Marklund, M. 1974. Involvement of superoxide anion radical in auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase. *Europ. J. Biochem.,* **47**: 469-474.

Nagalakshmi, D., Parashu Ramulu, S. and Usha Rani, M. 2013. Effect of graded levels of zinc supplementation on growth performance and oxidative defense mechanism in rats. *IOSR J. Pharm.,* **2**(6): 36-41.

Omu, A.E., Al-Azemi, M.K., Al-Maghrebi, M., Mathew, C.T., Omu, F.E., Kehinde, E.O., Anim, J.T., Oriowo, M.A. and Memon, A. 2015. Molecular basis for the effects of zinc deficiency on spermatogenesis: An experimental study in the Sprague-dawley rat model. *Ind. J. Urol.*, **31**: 57-64.

Powell, S.R. 2000. Antioxidant properties of zinc. *J. Nutri.*, **130**: 1447-1454.

Prohaska, J.R. 1991. Changes in Cu, Zn-Superoxide Dismutase, Cytochrome-C Oxidase, Glutathione Peroxidase and Glutathione Transferase Activities in Copper Deficient Mice and Rat. *J. Nutri.,* **121(**3): 355-363.

Saltman, P. 1989. "Oxidative Sress: A Radical View," *Seminars in Hematol.,* **26:** 249-256.

Saygili, E.I., Konukoglu, D., Papila, S. and Aksay, T. 2003. Levels of plasma Vit E, Vit C, TBARS and Cholesterol in male patients with colorectal tumors. *Biochem. (Moscow),* **68**(3): 317.

Shafiq-ur-Rehman. 1984. Lead induced regional lipid peroxidation in brain. *Toxico. Lett.,* **21**(3)**:** 333-337.

Singh, K.B. 2012. Long term excessive zinc supplementation induced oxidative stress in wistar rats fed on semi-synthetic diet. *Food Nutri. Sci.,* **3**: 724-731.

Snedecor, G.W. and Cochran, W.G. 2004. Statistical Methods, 8th Edn., Oxford and IBH Pub. Co., Kolkata.

Wu, F.X.H. and Wu, C.W. 1987. Zinc in DNA replication and transcription. *Annual Rev. Nutri.,* **7**: 251-271.