Int J Pharm Bio Sci 2015 July; 6(3): (B) 544 - 548

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PROTECTIVE EFFECT OF ZINC AGAINST THE AMMONIA INDUCED OXIDATIVE STRESS IN THE RAT TESTES

T. SIVA KUMAR AND P.NEERAJA*

Department of Zoology, Sri Venkateswara University, Tirupati-517502

ABSTRACT

The aim of the present study was to assess the effects of ammonium sulphate exposure on the antioxidant defence system and lipid peroxide concentration in the testes of wistar rats, as well as the examine possible protective role of zinc from the adverse effects of ammonia .The LD_{50} of Ammonium Sulphate and Zinc Chloride were determined, after toxicity testing evaluation. It is 91.5 mg/kg body weight for ammonium sulphate and 58 mg/kg body weight for Zinc Chloride. The selected sub lethal concentrations of ammonium sulphate and Zinc Chloride are 18.3/kg body weight 11.6mg/kg body weight respectively. These are administered through intraperitonial method to rats for one week. After one week treatment, antioxidative enzymes like Superoxide dismutase (SOD), Catalase (CAT), and Lipid peroxidation activities were estimated in testes in albino rats by using standard methods. The impact of Zinc Chloride in rat under ammonia stress is discussed.

KEYWORDS: **Ammonia toxicity, Zinc Chloride, Testes, SOD, CAT, Lipid peroxidation**

***Corresponding author**

P.NEERAJA
Department of Zoology, Sri Venkateswara University, Tirupati-517502

INTRODUCTION

Animals excrete three main nitrogen products, ammonia, urea and uric acid, as well as some minor nitrogen excretory products, including tri methylamine oxide, guanine, creatine, creatinine and amino acids. The term ammonia will be used to indicate the total ammonia, whereas NH_3 and NH_4^+ will refer to non-ionic ammonia and ammonium ion, respectively. Whether an animal excretes predominantly ammonia, urea or uric acid depends upon a number of factors in the animal's environment. But one major problem that all animals face is the relatively toxicity of ammonia when it is concentrated in body tissues (Wright., 1995)[1]. Ammonia is toxic when present in high concentrations. Endogenous ammonia intoxication can occur when there is impaired capacity of the body to excrete nitrogenous waste, as seen with congenital enzymatic deficiencies (Auron *et al.,* 2012)[2]. Zinc is a trace element essential for living organisms. More than 300 enzymes require Zn for their catalytic activity. It also plays an important role in the DNA replication, transcription, and protein synthesis, influencing cell division and differentiation (Frederickson., 1989)[3]. It has been noted that Zn has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant system (Powell., 2000; Ozturk *et al.,* 2003; Ozdemir *et al.,* 2005)[4-6]. Zinc is an essential component of the oxidant defense system and functions at many levels (Sato *et al.,* 1993)[7]. One study has shown that Zn deficiency in the diet paves the way for cell damage in the rat testis (Oteiza. 1999)[8]. Furthermore, Zn deficiency increases lipid peroxidation in various rat tissues, whereas the Zn supplementation corrects the impairment (Shaheen., 1995; Ozdemir *et al.,* 2005; Osredkar *et al.,* 2011)[9, 6, 10]. So the objective of the present study was to assess the effect of ammonium sulphate stress on the antioxidants, lipid peroxidation and to investigate the possible shielding role of zinc supplementation in testes of rat.

MATERIALS AND METHODS

Healthy male Wistar strain albino rats (250 ±10 g) purchased from Indian institute of science, Bangalore were maintained in polypropylene cages under laboratory condition (temperature 34 $\pm 2^0$ C light: dark=12:12h humidity 75%) and fed with standard laboratory chow (Hindustan lever limited, Bombay) and water was provided ad libitum. The rats were acclimatized to the laboratory conditions for 7 days. To ascertain LD_{50}, four groups of albino rats, each group comprising of 10 animals were injected intraperitonially with varying doses of ammonium sulphate and Zinc Chloride. After toxicity testing, evaluation, the LD_{50} is determined and is found to be 91.5 mg/kg body weight for ammonium sulphate, 58mg/kg body weight for Zinc Chloride. After determination of LD_{50} dose, 1/5 of the LD_{50} (18.3mg of ammonium sulphate/Kg body weight) was selected, as sub lethal concentrations of Ammonium sulphate. This concentration was selected, so as to keep the animals in ammonia stress, but will not result in mortality. Similar studies using Zinc Chloride were performed to understand the effect of Zinc and 11.6mg of Zinc Chloride /Kg body weight is selected as the test dose. Healthy adult animals were allocated into four groups containing six animals each. The group 1 served as control, the 2nd group of animals treated with ammonium sulphate, 3rd group animals treated with zinc chloride and 4th group treated with ammonium sulphate along with zinc chloride were considered as experimental groups. The control and experimental animals were sacrificed by cervical disarticulation at the end of the treatment i.e., 7 days and rat testes were collected and kept in a deep freezer at -20^0C and used for biochemical analysis. Lipid peroxidation (LPO) was estimated by method of Hiroshi *et al.,(*1979)[11], Superoxide dismutase (SOD) estimated by Misra and Fridovich (1972)[12] method, and Catalase (CAT) was estimated by Aebi (1984)[13] method, Total proteins were estimate by Lowry *et al .,*(1951)[14].The results were subjected to statistical analysis.

RESULTS AND DISCUSSION

From the results it is clear that there is a significant decrease in SOD,CAT activity levels in ammonium sulphate treated rats when compared to control rats, where as SOD,CAT activities were neutralized significantly in testes of rats exposed to ammonium sulphate along with Zinc chloride when compared with ammonium sulphate treated rats (Table). The data presented in Table shows that significant change in the concentrations of LPO levels during the treatment of rats with ammonium sulphate and zinc chloride alone or in combination. The results showed that LPO concentration significantly increased in testes of rats treated with ammonium sulphate in comparison to control. Pretreatment with zinc chloride was very effective in the prevention of oxidative damage induced by ammonium sulphate, which resulted in significantly lower LPO concentration. Zinc chloride treatment alone had shown no significant effect. A testis, being rich in polyunsaturated fatty acids, is highly susceptible to reactive oxygen species (ROS) attack. To negate the harmful effects of ROS, testis is equipped with a powerful antioxidant defense system involving enzymes like SOD and Catalase (Vernet *et al.,* 2004)[15]. SOD is considered the first line of defense against deleterious effects of oxyradicals in the cell by catalyzing the dismutation of superoxide anion radicals to H_2O_2, which is readily degraded by catalase. In the biological system, the antioxidant enzymes catalase protects SOD inactivation by H_2O_2, while the SOD reciprocally protects catalase against inhibition by superoxide anion. Thus, balance of this enzyme system may be essential to eliminate superoxide and peroxide radicals generated in the tissues. In the present study, exposure to ammonia decreased the activities of SOD and catalase, and concomitantly increased the levels of lipid peroxidation in the testis. The reduction in activities of antioxidant enzymes shows the failure of primary antioxidant system to act against free radicals. Increased lipid peroxidation may indicate an increased generation of ROS, which can cause damage to sperm and other cytoplasmic organelle membrane structures through peroxidation of lipids, proteins and nucleotides, thereby altering sperm motility (Aitken *et al.,* 1989)[16]. The mechanism of ROS induced altered sperm motility is still unclear. However, it is hypothesized that H_2O_2 one of the lipid peroxidation products, might diffuse across the membrane and affect the vital enzymes in the sperms thereby resulting in decreased sperm motility (Makker *et al.,* 2009)[17]. It is well recognized that Zinc has ability to cross cell membrane and the role of Zinc in preventing lipid peroxidation is well known (Amara *et al.,* 2008)[18]. Several studies have shown that zinc acts as a free radical scavenger, quenching hydroxyl radicals, superoxide ion radicals, singlet oxygen, and peroxide and that it seemed to also boost the activities of antioxidant enzymes and it significantly neutralizes the lipid peroxidation in the testes and blood in cadmium exposed rats (Jemai., *et al.,* 2005 and Amara *et al.,* 2008)[19,18]. The present data also demonstrate that co-administration of Zinc significantly mitigated the effects of ammonia-induced oxidative stress in testis of rat by increasing antioxidant enzymes and decreasing lipid peroxidation.

Table

The changes in the levels of SOD, CAT, and LPO activities in testes of albino rats treated for 7days with ammonium sulphate & Zinc Chloride treated albino rats and effect of Ammonium sulphate along with Zinc Chloride treated albino rats

Parameter	Control	Zinc Chloride	Ammonium sulphate	Ammonium sulphate + Zinc Chloride
SOD				
Mean	1.4696	1.4797^{NS}	0.9041*	1.3244**
SD	±0.0731	±0.0296	±0.0867	±0.0706
% Change over control		(0.68)	(-38.47)	(-9.88)
% Change over ammonium sulphate				(46.48)
CAT				
Mean	0.4912	0.5112^{NS}	0.3316*	0.4406**
SD	±0.0284	±0.0259	±0.0342	±0.0137
% Change over control		(4.08)	(-32.65)	(-10.20)
% Change over ammonium sulphate				(32.87)
LPO				
Mean	6.4916	6.6102^{NS}	9.8801*	7.4206**
SD	±0.2354	±0.3463	±0.4183	±0.2855
% Change over control		(1.84)	(52.23)	(14.32)
% Change over ammonium sulphate				(-24.89)

All the values are mean of six individual observations % - Percent change over control, SD – Standard deviation, NS – Not significant over control, * - Values are significantly over control at P<0.05, ** - Values are significantly over Ammonium sulphate at P<0.05.

Units

SOD-units of Superoxide anion reduced/ /mg protein/min

CAT-µ moles of H_2O_2 degraded/mg protein/min

LPO- µ moles of MDA formed / gm wet weight of the tissue

CONCLUSION

It can be concluded that from the present results exposure to ammonia generate ROS by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation thereby causing oxidative stress in testis of rat. This may leads to interruption in the functional integrity of cell organelles. Thus it is suggested that the impairment of spermatogenesis in ammonia exposed rat could me mediated through the induction of oxidative stress in addition to the direct effect on the germinal compartment. The present study also reveals that co-administration of Zinc reduces ammonia-induced oxidative stress by decreasing lipid peroxidation and activating antioxidant enzymes in the testes, thereby ameliorating ammonia induced suppressed reproduction in male rat.

REFERENCES

1. Wright P.A. Nitrogen excretion: three end products, many physiological roles. J Exp Biol , 198(Pt 2):273-81, (1995)
2. Auron A., Brophy PD. Hyperammonemia in review: Pathophysiology, diagnosis, and treatment. Pediatr Nephrol, 27(2):207-22, (2012)
3. Frederickson CJ. Neurobiology of zinc and zinc containing neurons. Int Rev Neurobiol , 31:145–238, (1989)
4. Powell SR. Antioxidant properties of zinc. J Nutr, 130(5S Suppl):1447S-54S, (2000)
5. Ozturk A., Baltaci AK., Mogulkoc R et al. Effects of zinc deficiency and supplementation on malondialdehyde and glutathionelevels in blood and tissue of rats performing swimming exercise. Biol Trace Elem Res, 94(2):157-66, (2003)
6. Ozdemir G., Inanc F. Zinc may protect remote ocular injury caused by intestinal ischemia reperfusion in rats. Tohoku J Exp Med, 206(3):247-51,(2005)
7. Sato M, Bremner I. Oxygen free radicals and metalothionein. Free Radical Biol Med, 14(3):325-37, (1993)
8. Oteiza PI., Adonaylo VN., Keen CL. Cadmium-induced testes oxidative damage in rats can be influenced by dietary zinc intake. Toxicology, 137(1):13-22, (1999)
9. Shaheen AA., el-Fattah AA. Effect of dietary zinc on lipid peroxidation, glutathione, protein levels and superoxide dismutase activity in rat tissues. Int J Biochem Cell Biol, 27(1):89-95, (1995)
10. Osredkar J., Sustar N. Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. J Clinic Toxicol, S3:001, (2011)
11. Hiroshi O., Nabuko O., Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95:351-358, (1979)
12. Misra HP., Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem, 247(10):3170-5, (1972)
13. Aebi H. Catalase in vitro. Methods Enzymol, 105:121-6, (1984)
14. Lowery D.H., N.J. Rosen brough., A.L.Farr and R.S. Randall. Protein measurement with folin- phenol reagent. Journal of Biological chemistry, 193:265-273, (1951).
15. Vernet P., Aitken RJ., Drevet JR. Antioxidant strategies in the epididymis. Mol. Cell Endocrinol, 216(1–2):31–39, (2004)
16. Aitken RJ., Clarkson JS., Fishel S. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol. Reprod, 40:183-197, (1989)
17. Makker K., Agarwal A., Sharma R. Oxidative stress and male infertility. Indian J Med Res, 129(4):357-67, (2009)
18. Amara S., Abdelmelek H., Garrel C et al. Preventive Effect of Zinc Against Cadmium-induced Oxidative Stress in the Rat Testis. J Reprod Dev, 54(2):129-34, (2008)
19. Jemai H., Messaoudi., Chaouch A., Kerkeni A. Protective effect of zinc supplementation on blood antioxidant defense system in rats exposed to cadmium. Journal of Trace Elements in Medicine and Biology, 21:269–273, (2007).