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Effect of zinc on total protein, proteases, free amino acids content in ammonia induced stress rats

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Abstract
Ammonia is generated by the degradation of protein content. This ammonia is modified as urea in mammals. When this ammonia didn't modified into urea ammonia content will increase. This condition is lethal to animals. Zinc is a trace element which have more beneficial role in the physiological activities after the iron (Vasak and Hasler 2000) [1]. When zinc injected in ammonia induced stress the total protein, proteases, and free amino acids content retain to normal levels.

Keywords: Ammonium sulphate, zinc chloride, total proteins, proteases, free amino acids

Introduction
Proteins have multifaceted role in the cells. This is why called when it is as apart in nucleic acids act as information molecules, when it is present in membranes act as structural items, transportation molecules, and also act as catalysts in the biochemical reactions. For normal activities of proteins conformation and configuration is important. Why because proteins need to maintain a constant composition in the face of constantly changing surroundings.

In cells small molecules, macromolecules, and supra molecular complexes are regularly harmonized and then broken down in chemical reactions that involve a constant flux of mass and energy through the system and maintain proper equilibrium and establish steady state. All biological macromolecules are much less thermodynamically stable than their monomeric subunits, yet they are kinetically stable their uncatalyzed breakdown occurs so slowly (over years rather than seconds) that, on a time scale that matters for the organism, these molecules are stable. When water molecules surrounded the hydrophobic molecules and form solvation layer. These weak bonds also increase the conformation of the proteins (Nelson & Cock 2005) [2].

Proteases can chunk the proteins and act as molecular knives. Proteases have key role in the destabilizing the bonds among the proteins. Protease act as enzymes and performs action by catalytic actions. These proteases have diversity and specificity. By the activity of proteases on proteins free amino acids will generate.

In body free amino acid pool is there. This pool is maintained by 3 ways. 1) Formation of free amino acids by the action of proteases the protein content in the body degrades. 2) From dietary protein digestion free amino acids will generate 3) Synthesis of non essential amino acids. The first one is harmful to the body.

Materials and methods
Experimental design
Healthy Wister strain male albino rats weighing 300±50gm procured from Indian institute of science, Bangalore were housed in polypropylene cages under hygienic conditions. The rats were fed with standard pellet diet supplied by Sai Durga feeds and foods, Bangalore and water *ad libitum* in laboratory conditioned environment (34±2 ^{0}C) with a 12-hour light and 12-hour dark cycle. The rats were acclimatized to the laboratory environment for 7 days.

Rats were allocated into four groups containing six animals in each. The group 1 served as control, the 2nd group of animals treated with ammonium sulphate, 3rd group animals treated with zinc chloride for comparing with the control group and 4th group treated with ammonium sulphate along with zinc chloride, for the identification of zinc preventive role. These doses are given by Intra peritoneal method for one week duration with 24 hrs time interval. The selected doses were 18.3 mg/kg for ammonium sulphate and 5mg/kg for zinc chloride after toxicity evaluation.

The control and experimental animals were sacrificed by cervical dislocation at the end of the treatment, i.e. 7 days and blood was collected into tubes and centrifuged at 3000 rpm and then serum collected is utilized for the evaluation of liver marker enzymes. All the experiments were conducted by the consonance of S.V university institutional ethical committee Tirupati. (Resolution No 07/2012-2013(i)/a/CPCSEA/IAEC/SVU/PN-BP/dt1.2.2012).

Analytical Methods

The change of values in Total proteins, Proteases, Free amino acids is assayed in order Lowry *et al*., method (1951), Davis and Smith, (1955), Moore and Stein, (1954). The changes in the level of Total proteins, Proteases, Free amino acids levels in liver and brain tissue of rats treated with ammonia, zinc chloride, Ammonia+ Zinc chloride treated rats were represented as µ moles of Tyrosine / gm wet weight of the tissue.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) using SPSS software package 16.0. The results were expressed as mean ± SD from six rats in each group, and P-values of <0.05 were considered as significant.

Results:

The changes in the levels of Total proteins, proteases and Free Amino acids in liver and brain tissue of rats treated with ammonia, zinc chloride, Ammonia+ Zinc chloride treated rats were represented in the Tables 1,2,3.

The total proteins content in liver is more than in brain. When treated with ammonium sulphate the total proteins content is decreased in both tissues. The total protein content retains in zinc sulphate and ammonia sulphate treated group. The proteases levels also increase in ammonium sulphate treated rats in both tissues. The proteases amount keep back in zinc sulphate and ammonia sulphate treated group. The free amino acids content increased in ammonium sulphate treated rats in both tissues. The free amino acids content maintains normal in zinc sulphate and ammonia sulphate treated group.

Discussion

Protein turnover means the total protein content how much degraded in body is resynthesized this leads the protein content in normal. In oxidation stress free radicals will generate in the body. These free radicals are capable to effect the confirmation of proteins. In the animals, ammonia induced stress also leads to generation of free radicals, ROS, (Kosenko *et al*., 1997) [3].

A free radical is any species that contains one or more unpaired electrons (Pryor WA 1966) [4]. Ammonia contains one lone pair of electrons. By this potentiality it is able to induce the oxidative stress. Mitochondrial permeability transition (MPT) means increased the permeability of the mitochondria membrane for < 1500 Da (Zoratti and Szabo, 1995) [5]. Ammonia was recently shown to induce the MPT in cultured astrocytes (Bai *et al*., 2001) [6].

For several cells if zinc supplementation provide it protect such cells from oxidative stress (Chung, M. J *et al.* 2005) [7]. Zinc has ability to protect the cells from oxidative stress by its antioxidant and membrane stabilizing activity. (Beattie, J. H.; Kwun, I. S 2004) [8]. Zinc has ability induce function more like anti oxidant enzymes like GSH (Ha, K. –N *et al.* 2006)[9] and also it is contributing for the formation of SOD (Bray TM, Bettger WJ. 1990) [10]. Zinc actively participated in tubulin polymerization and forms actin filament. This may stabilize the free radical reactions (Avery RA, Bettger WJ 1992) [11].

Along with this chemically Zinc had a filled d – orbital. With this it didn't participated in redox reactions and receive the electron pairs and act as Lewis acid (Williams 1987) [12]. Ammonia by donating the lone pair of electrons it act as Lewis base (David d. nelson, jr., Gerald t. fraser, William klemperer *et al.* 2012) [13]. Zinc ions have hydrophilic nature (Haim Tapiero, Kenneth D. Tew 2003) [14]. In the cell zinc react with water and formation of zinc hydrate ion ($Zn(H_2O)_4^{2+}$). With this ammonia react and forms $[Zn(NH_3)_4]^2$ then liberate the water. For formation of $[Zn(NH_3)_4]^2$ compare with ($Zn(H_2O)_4^{2+}$) requires less bond energy (Douglas B. Kitchen and L. C. Allen 1987) [15].

Conclusion

Finally it is concluded that ammonia is able to induce the oxidative stress. Zinc have protective role from that ammonia induced oxidative stress. When ammonia content in the body increases it disturb the spatial arrangement and stability of the protein molecules this leads to the increase of the entropy (disorder energy). This entropy is called as conformational entropy. This leads to the detriment for the protein conformation in the cell physiology. By the supplementation of zinc more secretions of antioxidant enzymes like GSH, SOD generate and reestablish the protein confirmation.

Table 1: Changes in the Total Protein levels in Brain and Liver tissues of Albino rat in control, Ammonium Sulphate, Zinc Chloride and Ammonium sulphate along with Zinc chloride treatment. (mg / gm wet weight of the tissue)

Name of the Tissue	Control	Ammonia	Zinc	Ammonia + Zinc
Liver Mean SD % Change over control % Change over ammonium sulphate	97.142 0.198	76.147* 0.260 -21.612	97.243NS 0.179 +0.103	91.764** 0.755 -5.536 +20.50
Brain Mean SD % Change over control % Change over ammonium sulphate	77.2272 0.370	60.163* 0.796 -22.095	78.3595NS 0.229 +1.466	73.007** 0.225 -5.463 +21.35

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

Table 2: Changes in the Free Amino acid levels in Brain and Liver tissues of Albino rat in control, Ammonium Sulphate, zinc chloride and Ammonium sulphate along with Zinc chloride treatment. (µ moles of Tyrosine / gm wet weight of the tissue)

Name of the Tissue	Control	Ammonia	Zinc	Zinc + Ammonia
Liver Mean SD % Change over control % change over ammonium sulphate	73.273 ±0.062	110.488* ±0.0426 +50.787	74.857NS ±0.0432 +2.16	84.122** ±0.0255 +14.80 -23.57
Brain Mean SD % Change over control % change over ammonium sulphate	68.383 ±0.341	101.067* ±0.0791 +47.794	67.924NS ±0.127 -0.671	77.248** ±0.0507 +12.962 -23.57

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

Table 3: Changes in the activity levels of Protease enzyme in Brain and Liver tissues of Albino rat in control, Ammonium Sulphate, Zinc chloride and Ammonium sulphate along with Zinc Chloride treatment. (µ moles of tyrosine / gm wet weight of the tissue)

Name of the tissue	Control	Ammonia	Zinc	Zinc + Ammonia
Liver Mean Standard Deviation % change over control % change over ammonium sulphate	1.350 0.057	1.949* 0.002 +44.28	1.016NS 0.00017 -24.76	1.481** 0.0002 +9.66 -24.01
Brain Mean Standard Deviation % change over control % change over ammonium sulphate	1.215 0.00081	1.6748* 0.0289 +37.768	0.9989NS 0.0002 -17.82	1.342** 0.001 +10.454 -19.83

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

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