Effect of Zinc on Serum Amino Transferases in Albino Rats under Ammonium Stress

B. Purushotham and P. Neeraja*

*Division of Molecular Physiology
Department of Zoology,
Sri Venkateswara University,
Tirupati – 517502, A.P. India

Summary:
Ammonia is an excretory product formed by the degradation of proteins in animals. The intention of present study is to assort the mismanaged physiological reactions caused when this ammonia is retained in the body by using Zinc in a beneficial role. Zinc is a nutritional trace element for physiological actions. In this study the mismanaging physiological reactions were induced by administering ammonium sulphate to the rats. The aspartate aminotransferase (AST) and alanine aminotransferase (ALAT) levels in the serum of control, ammonium sulphate, zinc chloride; ammonium sulphate along with zinc chloride maintained rats. The effect of the Zinc on these enzymes under ammonia stress is discussed.

Key words: Ammonium sulphate, Zinc Chloride, AST, ALAT

INTRODUCTION
Proteins are indispensable to the animals. Amino acids are building blocks for proteins. By the degradation of proteins, ammonia is formed in the body. Ammonia is also a principal excretory product. Ammonia is converted as mainly to urea and uric acid according the water availability. In animals, certain amount of the ammonia is requisite for maintaining the acid - base balance in the body. There are normal protective mechanisms in the body from ammonia. Ammonia is converted to glutamine from glutamate in presence of glutamine synthetase. Again Glutamine results in glutamate and ammonia formation. This ammonia is liberated into environment as such or in modified forms as urea or uric acid. This mechanism is failure in Urea cycle disorders and nutrition deficiency. Therefore accumulation of ammonia in the body may be deleterious to the animals. Excess ammonia hinders the oxidative metabolism of the neurons. With this ATP production will decreases, and also ammonia exhilarates the production of free radicals. This will disturb the Krebs cycle chain. This stress leads to oxidative damage of cell lipids, carbohydrates and proteins. Zinc is integral component of an estimated 10% of all proteins in which it contributes to tertiary structure or catalytic activity covering all enzyme classes. Zinc is found in all tissues of human and animals. The biological functions of zinc are numerous and diverse and include glucose and lipid metabolism, cell proliferation, embryogenesis and those related with nervous and immune systems. Zinc is essential for the function of a large number of metallo enzymes. Zinc is involved in cell membrane counterbalance and counteract for oxidative destruction caused by free radicals. Zinc is also known for inducing metallothionein (MT) synthesis, a protein that is able to bind heavy metals and to scavenge hydroxyl radicals. The main objective of this study is to see whether zinc reduces the altered ammonia effects. Here we are studying the effects of transaminase enzymes ALAT (EC 2.6.1.2), AST (EC 2.6.1.1) in serum. ALAT is called as SGPT (Serum glutamate Pyruvate transferase), AST is called as SGOT. These are biomarker enzymes for liver health. These enzymes are generous in plasma of various tissues.

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°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 L-Aspartate aminotransferase (AST) and DL – Alanine aminotransferase (ALAT) in plasma is assayed by Lippi, and Guidi reported colorimetric ultra micro method. The protein levels were measured using Lowry et al., method (1951). The changes in the level of AST, ALAT levels in serum of rats treated with ammonia, zinc chloride, Ammonia+ Zinc chloride treated rats were represented as µ moles /mg protein/ hr.

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 level of AST, ALAT levels in serum of rats treated with ammonia, zinc chloride, Ammonia+ Zinc chloride treated rats were represented in the Table. There was increment in the levels of both the transaminases on ammonia treatment. Greater treatment was observed in ALAT than AST. But the treatment of ammonia stressed animals with zinc chloride gave decreased increment over the ammonia stressed animals. The activity levels in the zinc chloride administered animals were similar to the control animals suggesting that zinc chloride by itself did not have any effect.

DISCUSSION

The above results showed that AST& ALAT levels are increased in ammonium sulphate treated group when compared with control group. Liver is the major site for xenobiotic metabolism. Xenobiotics are potential hepatotoxicant substances. In this study ammonium sulphate is a xenobiotic. Zinc can protect against oxidative damage caused by certain xenobiotics. The protection against the xenobiotics “enzyme induction” is one of the mechanisms. In liver ammonia is detoxified by the urea cycle. In this cycle N-acetyl glutamate synthetase, Carbamoyl phosphate synthetase might be activated by Zinc. In our study also zinc along with ammonium sulphate treated group gave decrement in the activity levels when compared with ammonium sulphate treated rats.

CONCLUSION

In conclusion these AST& ALAT levels in Ammonium sulphate treated group gave increment in the activity levels of the enzymes probably due to hepatocytes membrane damage resulting in increased release and leakage out of these enzymes from the liver cytosol into the blood stream. Because ammonia might have induced free radical generation by the activation of NADPH oxidases. These NADPH oxidases are a group of plasma membrane associated enzymes. These can catalyze the production of ROS (Reactive Oxygen Species). These ROS is able to annihilate the biological structures like lipids, proteins, DNA because it triggers the induction of MPT (Mitochondria Permeability Transition). So there is an increased permeability to protons, ions and other solutes< 1500 Da, which leads to collapse of the mitochondrial inner membrane potential. This leads to osmotic swelling of the mitochondria matrix. Zinc chloride treated group had reduced the levels of AST& ALAT levels. Zinc can impede NADPH oxidases and also instigate the production of Metallothionein proteins which are scavengers for the ROS (Reactive Oxygen Species) and also believing that Zinc can stabilize membranes and protect them against free radical injury. Thus AST and ALAT levels have shown decrement in zinc treated rats when compared with ammonia treated rats.

Table: The changes in the activity levels of AST and ALAT in serum of albino rats treated for 7days with ammonium sulphate, Zinc chloride and Zinc chloride along with Ammonium sulphate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ammonium sulphate</th>
<th>Zinc chloride</th>
<th>Ammonium sulphate + Zinc chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AST</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean SD</td>
<td>64.16</td>
<td>95.66*</td>
<td>66NS</td>
<td>81**</td>
</tr>
<tr>
<td>% change over control</td>
<td>±0.7527</td>
<td>±1.0327 (49.11)</td>
<td>±0.6324 (2.86)</td>
<td>±0.8944 (26.25)</td>
</tr>
<tr>
<td><strong>ALAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean SD</td>
<td>29</td>
<td>45*</td>
<td>31#</td>
<td>33**</td>
</tr>
<tr>
<td>% change over control</td>
<td>±0.4472</td>
<td>±0.7071 (56.09)</td>
<td>±0.8366 (6.93)</td>
<td>±0.7071 (14.47)</td>
</tr>
</tbody>
</table>

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<0.03, * - Values are significantly over control at P<0.05, ** - Values are significantly over Ammonium sulphate at P<0.001.
REFERENCES