Research Paper

Volume : 3 | Issue : 1 | January 2014 • ISSN No 2277 - 8179

Effect of Sodium Selenite on Selected Enzymes in Liver and Brain Tissues of Albino Rat Under Ammonia Stress

Zoology

KEYWORDS : Selenium, Ammonia and Albino Rat

K. Sujatha	Department of Zoology, Sri Venkateswara University, Tirupati – 517 502
M. Siva Sankar	Department of Zoology, Sri Venkateswara University, Tirupati – 517 502
P. NEERAJA	Department of Zoology, Sri Venkateswara University, Tirupati – 517 502

ABSTRACT

Ammonia is a major by product of protein and nucleic acid catabolism, and its nitrogen can be incorporated into urea, amino acids, nucleic acids and many other nitrogenous compounds. Selenium plays a vital role in the body being an integral component in certain enzymes, protecting cell membranes. The present study aims to examine whether selenium alleviates ammonia stress conditions. The LD50 of Ammonium Sulphate and Sodium Selenite were determined. After toxicity testing evaluation are 91.5mg/kg body weight for ammonium sulphate and 1.5mg/kg body weight for sodium selenite through intraperitoneal method to rats for one week. After one week treatment, biochemical parameters like free amino acids, aspartate aminotransferase (AST) and alanine aminotransferase (AlAT) were estimated in liver and brain tissues of albino rats by using standard methods. The impact of sodium selenite in rat under ammonia stress will be discussed.

INTRODUCTION

Ammonia is an important nitrogen substrate in several reactions and plays an important role in nitrogen homeostasis of mammalian cells. Ammonia is produced by amino acid and protein catabolism and is toxic to brain and muscles. Ammonia toxicity results in free radical generation that leads to oxidative stress and tissue damage **(Lena and Subramanian, 2004)**. Excess of ammonia in the body leads to ammonia stress. Randomly body detoxifies ammonia by various methods in different tissues like liver and brain in the body.

Selenium has a special role in the normal function of the liver and tissues throughout the body. Found directly below sulphur on the periodic table, selenium thus shares many of the chemical properties of sulphur **(Jacob et al., 2003).** In fact, most selenium in the body is found in selenoproteins, which contain the amino acid selenocysteine (Sec), a modification in which selenium is found in place of the sulphur atom of cysteine. Failure to produce selenoproteins in the liver causes liver necrosis in mice, attesting to the importance of selenium in normal liver function **(Carlson et al., 2004).** The present study aims to examine whether selenium alleviate ammonia stress conditions through the estimation of the enzymes namely aspartate aminotransferase, alanine aminotransferase and free amino acids in the tissues like liver and brain of the albino rat.

MATERIAL AND METHODS

Healthy Male pathogen free Wistar strain albino rat of the same group (100 ± 10days) and weight 150±20g obtained from Indian Institute of Science, Bangalore was maintained in polypropylene cages under standard laboratory conditions. Toxicity evaluation for ammonium sulphate and sodium Selenite was performed. To ascertain LD_{50}, Six groups of albino rats, each group comprising of 10 animals were injected intraperitoneally with varying doses of Ammonium Sulphate and Sodium selenite. After toxicity testing evaluation, the LD_{50} is determined and is found to be 91.5 mg/ kg body weight for ammonium sulphate and 1.5 mg/ kg body weight for Sodium Selenite. After determination of LD_{50} dose, 1/5 of the LD_{50} (18.3 mg/ 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 sodium selenite was performed to understand the effect of Selenium (0.3 mg of selenium/ kg body weight).

Healthy adult animals were divided randomly into four groups, six animals each. The first group of animals was used as control. The second groups of animals were used as experimental. The animals of third group were used to find out the selenium effect. The fourth groups of animals were used for the effect of Ammonium sulphate and Sodium selenite. The control and experimental animals were sacrificed by cervical dislocation at the end of the treatment i.e., 7 days and tissues were stored in deep freezer at 20^0c and used for biochemical analysis.

Free amino acids level was estimated by the method of Moore and Stein (1954) as given by Colowick and Kaplan (1957), L – Aspartate aminotransferase (AST) and DL – Alanine aminotransferase (ALAT) were assayed by the colorimetric method of Reitman and Frankel (1957) as described by Bergmayer (1965). The results were subjected to statistical analysis. The experimental protocol was approved by institutional animal ethics committee (IAEC). (Resolution Number: 05/2012-2013/(i)/(a)CPCSEA/IAEC/SVU/PN-KSJ/dt. 1.2.2012).

RESULTS

The changes in the levels of Free Amino acids (FAA), Aspartate aminotransferase and Alanine aminotransferase in brain and liver tissues of Control and Ammonium Sulphate & Sodium selenite treated albino rats and effect of Sodium selenite along with Ammonium sulphate treated albino rats are shown in Table 1 to 3.

In the present study, the free amino acid content of liver and brain tissues of control, ammonium sulphate treated albino rats along with sodium selenite is represented in Table 1. In control rats the FAA levels was found to be highest in liver and the lowest in brain tissues. A significant increase in the free amino acid content in ammonium sulphate treated rats when compared to control was observed. Sodium selenite treated rats gave no change when compared to control and a significant decreased Free amino acid levels in ammonium sulphate treated rats with sodium selenite when compared to ammonium sulphate treated rats in both tissues was observed.

The specific activities of AST and AlAT were estimated in the tissues of control and experimental treated rats along with sodium selenite are represented in Table.2 & 3. The administration of ammonium sulphate showed an increase in activities of aminotransferases (AST & AlAT) activities when compared to control rats. In Sodium selenite treated rats no change was observed when compared to control and a significant decrease in aminotransferase levels in ammonium sulphate treated rats along with sodium selenite when compared to ammonium sulphate treated rats in both tissues was observed. In control rats the aminotransferases was found to increase in brain and decrease in the liver tissue.

Table 1: Changes in the **Free Amino acid** levels in different tissues of Control and Ammonium Sulphate & Sodium selenite treated albino rats and effect of Sodium selenite along with Am-

monium sulphate treated albino rats.

(µ moles of Tyrosine / gm wet weight of the tissue)

Name of the tissue	Control	Ammonium sulphate	Sodium selenite	Ammonium sulphate + Sodium selenite
BRAIN Mean SD % Change over control	68.7850 ±0.2026	78.1417 ±0.1988 (+13.6)	68.717 ±0.434 (-0.09)	72.2167 ±0.5368 (+4.9)
LIVER Mean SD % Change over control	75.2000 ±0.3304	87.4750 ±0.4340 (+16.3)	75.2956 ±0.4554 (+0.12)	78.9250 ±0.6609 (+4.9)

Table 2: Changes in the Alanine aminotransferase (AlAT) activity levels in different tissues of Control and Ammonium Sulphate & Sodium selenite treated albino rats and effect of Sodium selenite along with Ammonium sulphate treated albino rats.
(µ moles of Pyruvate formed / mg of protein / hr)

Tissues	Control	Ammonium sulphate	Sodium selenite	Ammonium sulphate + Sodium selenite
BRAIN Mean SD % Change over control	1.6217 ±0.0491	2.3250 ±0.0568 (43.3)	1.6117 ±0.0688 - (0.61)	1.7467 ±0.0206 (7.7)
LIVER Mean SD % Change over control	1.0917 ±0.0331	1.6267 ±0.0413 (48.9)	1.0850 ±0.0187 - (0.63)	1.1983 ±0.0116 (9.7)

Table 3: Changes in the Aspartate aminotransferase (AAT) activity levels in different tissues of Control and Ammonium Sulphate & Sodium selenite treated albino rats and effect of Sodium selenite along with Ammonium sulphate treated albino rats.
(µ moles of Pyruvate formed / mg of protein / hr)

Tissues	Control	Ammonium sulphate	Sodium selenite	Ammonium sulphate + Sodium selenite
BRAIN Mean SD % Change over control	1.7583 ±0.1026	2.3700 ±0.04858 (+34.78)	1.7517 ±0.09496 (-0.37)	1.8650 ±0.0592 (+6.06)
LIVER Mean SD % Change over control	1.3200 ±0.0357	1.9150 ±0.0339 (+45.07)	1.3150 ±0.0535 (-0.3)	1.4233 ±0.0524 (+7.82)

All the values are mean of six individual observations; %- Percent change over control, SD- Standard deviation

DISCUSSION

The oxidation pathway starts with the removal of amino group by the transaminase and the amino group is then fed into the urea cycle (Brosnan, 2000). The product of transamidations is that a keto acid enters the citric acid cycle. Glucogenic amino acid can also be converted into glucose through gluconeogenesis (Young and Ajami, 2001). The amino acids released during protein degradation due to activation of proteolysis will once again return to the amino acid pool and thus the free amino acids are the currency through which protein metabolism operates showing interdependence of both amino acids and proteins (Muray *et al.,* 2007). The sub lethal dose of ammonium sulphate gave an increment of free amino acids levels probably due to ammonia stress.

Several authors reported increased free amino acids in different animals. Increased free amino acids in ammonia stress conditions in fish (Hari, 2010) and Cockroach (Kishor *et al.,* 2010) have been reported. Sodium Selenite, selenium compound with its antioxidant properties might have been used to scavenge the present ammonia toxicity.

Aminotransferases play an important role in the utilization of amino acids for the oxidation and/or for gluconeogenesis. Increased AST and AlAT activities might be also due to disruption of mitochondrial integrity or increased synthesis of enzymes. The AST and AlAT are two important enzymes working as an important link between carbohydrates and protein metabolism. They provide much needed keto acids for the functioning of Krebs's cycle. The activities of these aminotransferases were shown to be altered in tissues under several pathological conditions.

Several authors reported increased AST and AlAT activities in different animal models under pesticidal toxicity, such as in frogs treated with azadirachtin (Madhava Rao, 2007), Cypermethrin in rat (Jacob Doss et al., 2007).

The administration of ammonium sulphate showed increase in the free amino acid content and activity levels of aminotransferases (AST & AlAT). These changes could lead to the alterations in the associated enzyme activities involved in intermediary metabolism. Treatment of animals with toxic agents might produce pathological lesions being associated with increased proteolysis. Selenium seems to decrease ammonia stress. Further work is required to confirm the above statement.

REFERENCE

1. P. J. Lena and P. Subramanian, (2004). "Effects of melatonin on the levels of antioxidants and lipid peroxidation products in rats treated with ammonium acetate," Pharmazie, vol. 59, no. 8, pp. 636-639. | 2. Jacob C, Giles GI, Giles NM et al. (2003) Sulfur and selenium: the role of oxidation state in protein structure and function. Angewandte Chem Int Edn in English 42, 4742-4758. | 3. Carlson BA, Xu X-M, Kryukov GV et al. (2004) Identification and characterization of phosphoseryl-tRNA kinase. Proc Natl Acad Sci USA 101, 12848-12853. | 4. Colowick, S.P. and Kaplan, N.O (1957) In: "methods in enzymology". Acadamic Press, New York. Vol.II, PP.501. | 5. Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of Serum Glutamic Oxaloacetate and glutamic pyruvic transaminase Am.J.Clin. Pathol., 28: 56-63. | 6. Bergmeyer, HV (1965). In: Methods of enzymatic analysis. Ed. H.V.Bergmeyer, Academic Press, New York, 401. | 7. Brosnan J.T (2000). Glutamate, at the interface between amino acid carbohydrate metabolism. The Journal of Nutrition. 130.998s -90s. | 8. Young V.R. and A.M. Ajami (2001) Glutamine: the emperor (or) his clothes the Journal of Nutrition. 131: 24495: discussion 24865 – 75. | 9. Murray R.K., Daryl K., Granner, Peter A.Mayes, Victor W. and Rodwell (2007). In : Harper's Illustrated Biochemistry. International 26th edition. The Mc Graw-Hill Companies, Inc, PP.46-47. | 10. Jacob doss, P., S. Ramanaiah, A. Nagarjuna, N. Suhasini, Y. Savithri and S. Rajendra Prasad (2007). Toxicity of cypermethrin on brain and liver tissues of fresh water edible fish Labeo rohita with special reference to selected biochemical parameters. Indian journal of Environmental sciences, 11(1): 23-27. | 11. Madhava Rao, S (2007). Ph.D Thesis, Sri Venkateswara University, Tirupati. | 12. Hari, P. (2010). Ambient ammonia stress on certain metabolic aspects of fish, Cyprinus carpio, Ph.D Thesis, Sri Venkateswara University, Tirupati. | 13. Kishore S., Ravikanth S.V., and P. Neeraja (2010). Acute ammonia stress on certain metabolic aspects of brain tissue of Cockroach Periplaneta Americana. The Bioscan Journal, Special volume, 2:343-347. | 14. Moore, S and W.H. Stein (1954). Modified ninhydrin reagent for the photometric determination of amino acids and related compounds. Journal of Biological Chemistry, 221: 907-913. |