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Antioxidant Role of Sodium Selenite on Ammonium Sulphate Induced Oxidative Stress in Rats

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Abstract | Ammonia is an important source of nitrogen metabolism and it is necessary for synthesis of protein and amino acids. An excessive level of ammonia leads to disturbance in the physiological functions of the body. High concentrations of ammonia enter into the body, because of environmental pollution, urea cycle disorders, liver failure and ingestion of ammonium salts cause physiological disturbance and damage of organs. The present study is to investigate the possibilities of the protective role of Selenium in Ammonium Sulphate (AS)-induced stress in the rat brain and liver. Rats were divided into four groups (six animals in each group). Group I (GI) is served as control, Group II (AS) rats received 18.3 mg/kg b.w of ammonium sulphate via intraperitonially (i.p) injection, Group III (Ss) rats administered with Sodium selenite (0.3 mg/kg b.wi.p) and Group IV (AS + Ss) treated with both of AS (18.3 mg/kg bwi.p) plus Ss (0.3 mg/kg b.wi.p). Acute intoxication of AS treated rats has shown that significantly decreased levels of antioxidant enzymes; namely Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and increased levels of Xanthine oxidase (XOD) levels in brain and liver tissues. Treatments with Ss reversed the AS-induced alteration of antioxidant defence enzyme levels.Selenium administration might be scavenging the excess of ammonium ions and significantly prevent the oxidative stress in liver and brain.

Keywords | Ammonium sulphate, Sodium selenite, Antioxidant enzymes, Liver, Brain

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INTRODUCTION

Ammonia is a nitrogen enriched compound produced during metabolic reactions as a byproduct. However, ammonia is toxic at elevated concentrations and need to elimination from the body fluids (Prakash and Mullen, 2010). Liver is the main detoxification centre for regulation of excessive ammonia levels in the body, eliminate in the form of urea by the kidneys. Extrahepatic organs such as muscle and brain (astrocytes) also remove the excessive ammonia levels through the amidation of glutamate to glutamine via the enzyme glutamine synthetase (GS) (Dasarathy et al., 2017). The main source of the entering the excessive ammonium salts is uncontrolled utilization of the nitrogen substrates in the food and beverage industries and fertilizer industries (ammonium sulphate, ammonium nitrate, ammonium acetate, and ammonium chloride). Ammonium sulphate is a popular agricultural fertilizer and used in house gardens. These compounds have been shown to be harmful to health in epidemiological studies (Lena and Subramanian, 2004; Thenmozhi and Subramanian, 2011; Messaadia et al., 2013; Siva Kumar et al., 2017; Acar et al., 2018).

Excessive levels of ammonia in the body fluids results in generation of the free radicals that induce the oxidative stress as well as tissue damage. An elevated level of ammonia entry is results to primarily effect on brain functions and cause of neurological abnormalities (Back et al., 2011). CNS abnormalities associated with hyperammonemia condition; such as hepatic encephalopathy, Reye's syndrome, several other metabolic disorders, and other toxic encepha-

lopathy's (Bleibel and Al-Osaimi, 2012). If a 5 to- 10- fold ammonia levels increase in the blood; it causes to stimulate the N-methyl-D-aspartate (NMDA) receptor. Stimulated NMDA receptors in the brain mediated the toxic effects by abnormal functioning of ammonia detoxification cycle (urea cycle) and leads to over synthesis of ammonia in the liver by deamination of glutamine and glutamate. Excessive levels of glutamate in the brain leads to function effect on astrocytes probably swelling and inflammation and finally leads neurological disorders, may cause irritability, somnolence, vomiting, seizures, and derangement of cerebral function, coma and death. However, in severe cases of hyperammonemia, as acute liver failure, the normal regulation of cerebral blood flow is also impaired leading to cerebral hypoxia and/or hyperaemia depending on cerebral perfusion pressure (Upadhyay et al., 2016).

Selenium is an essential trace element for humans and animals, involving diverse physiological actions. Selenium is an integral part of the catalytic site of several enzymes, including glutathione peroxidase (GPx) and thioredoxin reductase (TRR); the former catalyzes the reduction of hydro peroxides and hydrogen peroxide by reduced glutathione, the latter catalyses the NADPH dependent reduction of the redox protein thioredoxin, and both functions to protect cells from oxidative damage (Yan and Johnson, 2011). Nevertheless, the narrow range between its therapeutic dosage and tolerable upper intake level severely compromises wide applications. It is well known that the supplemental selenium may be acquired through the diet, but selenium bioavailability depends on the source (Finley, 2006).

The biological importance of selenium is at least 3-fold. First, it forms the prosthetic group of some critical selenocysteine-containing enzymes, such as glutathione peroxidase, iodothyronine 5`-deiodinase, and thioredoxin reductase (Stadtman, 1996). Second, sodium selenite is protective against a number of toxicants. Third, selenium excessive intake cause toxic potential (Combs and Gray, 1998). The aim of present study is to evaluate the protective role of selenium against the ammonia induced oxidative stress in liver and brain tissues. Using an experimental model, the present study describes the liver and brain tissue enzymes SOD and Catalase, GPx and XOD in order to analyze antioxidant status in ammonia treated rats and after pre-treatment with Sodium selenite.

MATERIAL AND METHODS

Animal's Acclimatization and Maintains

Male wistar strain rats were purchased from a certified dealer (Raghavendra Enterprises, Bangalore, India) and used in the present study as an experimental model. Rats were housed in polypropylene cages lined with sterilized paddy husk as bed linen material, renewed every 24 h with *ad libitum* access to tap water and rat chew (purchased from Sai Durga Agencies, Bangalore, India). The animals were maintained in well a controlled environment (25± 2°C) with a 12-h light and 12-h dark cycle. The experiments were carried out in accordance with the guidelines of the Institutional Animal Ethical Committee, (Resolution Number: 06/2012-2013/ (I) / (a) CPCSEA/ IAEC/ SVU/PN-ASR/ dt. 01.02.2012) Sri Venkateswara University, Tirupati, India.

Experimental Design

The total 24 healthy adult Wistar rats were used for the present study and they were divided into four groups containing six animals in each. The Group I: served as control; Group II: animals treated with ammonium sulphate (AS) (18.3 mg/ kg bw; i.p); Group III: animals treated with Sodium selenite (Ss) (0.3 mg/kg bw; i.p) (Zafar et al., 2003; Atalay et al., 2011) for comparing with the control group and Group IV: animals treated with ammonium sulphate (As) along with Sodium selenite (Ss), for 7 days within 24 hr time interval. The test chemicals dosage confirmed by the determination of lethal dose of 50 percent mortality (LD_{50}) in the rats and 1/5th LD_{50} of As (91.5/5) was selected. The control and experimental animals were fasted overnight at the end of the 7th day and sacrificed by cervical dislocation. Liver and brain tissues were excised immediately and rinsed in ice- chilled normal saline and kept in deep freezer at – 20°C and used for biochemical analysis.

Biochemical Analysis

Superoxide dismutase (SOD) was estimated by Misra and Fridovich (1972) method, Catalase (CAT) was estimated by Aebi (1984) method, Glutathione peroxidase (GPx) was estimated by Flohe and Gunzler (1984) method, Xanthine oxidase activity was assayed by the dye reduction method of Srikanthan and Krishnamurthy (1955). Total proteins were assayed by the method of Lowry et al. (1951). The changes in the level of SOD, CAT, GPx and XOD levels in Liver and Brain tissues of rats treated with As, Ss, As+ Ss 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 values $p<0.05$ were considered statistically significant.

RESULTS

Antioxidant enzymes levels such as SOD, CAT, GPx and XOD levels were estimated in the liver and brain tissues of male albino rat. The obtained results were represented in t-

Table 1: The changes in the antioxidant activity levels of SOD, CAT, GPx and XOD in Liver tissue of albino rats treated for 7 days with Ammonium sulphate (As), Sodium selenite (Ss) and Ss along with As.

Parameter/Group	Control	As	Ss	As + Ss
SOD				
Mean	6.2617	4.6467*	6.5167NS	5.9633**
SD	±0.2705	±0.2399	±0.2686	±0.2707
% change over to control		(-25.79)	(+4.07)	(-4.76)
% change over to As				(28.33)
CAT				
Mean	0.2550	0.1650*	0.2617NS	0.2324**
SD	±0.0176	±0.0054	±0.0160	±0.0074
% change over to control		(-35.29)	(+2.62)	(-8.3)
% change over to As				(40.84)
GPx				
Mean	0.4397	0.2683*	0.4597NS	0.3980**
SD	±0.0343	±0.0337	±0.0251	±0.0389
% change over to control		(-38.9)	(+4.54)	(-9.48)
% change over to As				(48.34)
XOD				
Mean	0.1762	0.2422*	0.1835NS	0.1912**
SD	±0.0129	±0.0241	±0.0070	±0.0181
% change over to control		(+37.45)	(+4.14)	(+8.51)
% change over to As				(-21.05)

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

Table 2: The changes in the activity levels of SOD, CAT, GPx and XOD in Brain tissue of albino rats treated for 7 days with Ammonium sulphate (As), Sodium selenite (Ss) and Ss along with As.

Parameter/Group	Control	As	Ss	As + Ss
SOD				
Mean	14.5217	11.9100*	14.9483NS	13.8367**
SD	0.4941	±0.2720	±0.3331	±0.4209
% change over to control		(-18.00)	(+2.93)	(-4.71)
% change over to As				(16.17)
CAT				
Mean	0.6144	0.4467*	0.6290NS	0.5917**
SD	±0.0094	±0.0163	±0.0095	±0.0075
% change over to control		(-27.3)	(+2.37)	(-3.69)
% change over to As				(32.46)
GPx				
Mean	0.8112	0.6300*	0.8450NS	0.7650**
SD	±0.0211	±0.0189	±0.025	±0.0327
% change over to control		(-22.3)	(+4.16)	(-5.69)
% change over to As				(21.42)
XOD				
Mean	0.5850	0.7700*	0.6062NS	0.6283**
SD	±0.0137	±0.0178	±0.0294	±0.0231
% change over to control		(+31.6)	(+3.70)	(+7.40)
% change over to As				(-18.40)

All the values are mean of six individual observations % - Percent change over control, % - Percentage change over As, 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.

Unites:

SOD: Superoxide anion reduced /mg protein/min; CAT: µ moles of H_2O_2 degraded/mg protein/min; GPx: µ moles of NADPH Oxidized /mg protein/minl; XOD: µ moles of formazon formed/mg protein/hour

he Table 1 & Table 2. As treated animals showed that decreased antioxidant levels in the liver tissue (Table 1), such as SOD (4.6467 ±0.2399), CAT (0.1650 ±0.0054), GPx (0.2683 ±0.0337) and significant increased levels of XOD (0.2422 ± 0.0241) were observed, when compared with control animals (6.2617 ±0.2705, 0.2550 ±0.0176, 0.4397 ±0.0343 & 0.1762 ±0.0129). Brain antioxidants, SOD (11.9100±0.2720), CAT (0.4467±0.0163) GPx (0.6300±0.0189) decreased and elevated levels of XOD (0.7700±0.0178) (Table 2) were observed in the As administration rats when compared with control group animals (14.5217±0.4941, 0.6144±0.0094, 0.8112±0.0211 & 0.5850±0.0137). The decreased levels of these antioxidant enzymes and increased levels of stress marker (XOD) were stabilized to normal range in the parallel administration of AS + Ss group, when compare with As treated group. Whereas Sodium selenite (Ss) alone treated group showed to some extent alteration in the antioxidants enzymes (increased somewhat), however these changes are not significant compared with control rats.

DISCUSSION

Antioxidant defence system plays a prominent role in protection of biological system from toxic pollutants. Several processes enhance the production of reactive oxygen species (ROS) or deplete the antioxidant defence system. Oxidative stress is such one of the cause to generation of ROS. Oxidative stress in cells or tissues results in the enhanced generation of reactive oxygen species and/or depletion of the antioxidants in the defence system, thereby causing an imbalance between the peroxidants and antioxidants. In the present investigation ammonium sulphate induced rats exhibited a significant decreased level of SOD, CAT, GPx and increased levels of XOD in liver and brain tissues. SOD is the first line defence enzyme, it protects the cells from oxidative damage by conversion of superoxide radicals into H_2O_2 (hydrogen peroxide)n (Birben et al., 2012). The H_2O_2 is further metabolized by another enzyme catalase (CAT) into molecular oxygen and water (Day, 2009). The reduced levels of antioxidant enzyme levels in both tissues (liver & brain) might be that AS induction leads to generation of oxidative stress and lead to damage of tissues. Previous studies also reported that exposure of ammonium acetate, ammonium chloride, ammonium nitrite and ammonium sulphate etc., rats exhibited the decreased levels of antioxidant enzymes and elevated levels of stress markers like, lipidperodaion (LPO) and NO (nitric oxide) (Lena and Subramanian, 2004; Thenmozhi and Subramanian, 2011; Messaadia et al., 2013; Tekuri and Pabbaraju, 2015). Elevated levels of LPO in the body fluids is responsible for the creation of the oxygen tension in the cells and tissues, thereby probably leads to oxidative stress and tissue damage. Sodium selenite (Ss) administration significantly normalized the activities of SOD and CAT in liver and brain of As induced rats and it was also reported that selenium possesses superoxide scavenging and most antioxidant activities during stressful conditions, which agree with our present investigation.

A significant decreased level of GPx levels and increased levels of XOD in liver and tissues of As administered rats compared with control was noticed. Glutathione peroxide (GPx) is the first line defence enzyme as correlate with CAT and SOD for removal of free radicals (superoxide radicals) in the cells, tissue through conversion of H_2O_2 in to water and oxygen. GPx can also terminate the chain reaction of lipid peroxidation by removing lipid hydro peroxides from the cell membrane (Ighodaro and Akinloye, 2018). Selenite supplemented rats showed stabilizing levels GPx in both tissues, revealed that selenium inhibits the toxicity of As by the scavenging of oxidative species in liver and brain tissues and thus selenium has the anti-hyperammonemia effect.

Xanthine oxidoreductases exists in two forms, as Xanthine dehydrogenase (XDH) and Xanthine oxidase (XOD), which is formed through post translational modifications of Xanthine dehydrogenase (XDH), both forms, particularly with Xanthine oxidase form numerous ROS and RNS (Chung et al., 1997). In the present investigation increased levels of XOD in As treated rats was observed. The elevated levels of Xanthine oxidase indicates the over production of Superoxide anion (O_2^-) in the liver and brain tissues of male albino rat. The increased activity might be due to conversion of Xanthine dehydrogenase to Xanthine oxidase. Xanthine oxidase is produced when the native form of Xanthine dehydrogenase is altered either by sulphydryl oxidation or by limited proteolysis. The increased activity of XOD in our study was in agreement with Hari et al. (2012) has reported increased XOD levels in adult *Cyprinus carpio* exposed to ammonia. Ss treated rats along with As administered rats showed significant increased levels of XOD were maximally normalized. These findings suggest that selenite supplementation have a positive role to reduce the ammonium salt induced stress in rats.

CONCLUSION

In conclusions, the results of the present study provide evidence that Sodium selenite enhances the functional recovery of liver and brain antioxidant enzyme levels and reduce the damage in ammonium sulphate induced stress rats.This could be due to the inhibition or reduction of oxidative stress marker (XOD) by its antioxidant, maintained cellular integrity neuroprotective and hepatoprotective role.

CONFLICT OF INTERESTS

All authors declared that they have no conflict of interest.

AUTHORS CONTRIBUTION

ASR: participated in the study design, carryout the experimental work and drafted the manuscript. SK: helped in biochemical estimation, statically analysis, graphs and interpretation of manuscript and editing. PN: supervision the study, coordination of the study and wrote part of the manuscript and typos correction.

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