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IMPACT OF AMMONIA ON OXIDATIVE METABOLISM IN CERTAIN TISSUES OF ALBINO RAT

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ABSTRACT

Ammonia has received increasing attention over the past few years as a potentially important pollutant. The metabolic changes and potentialities of ammonia in high concentration are reported in all groups of animals as ammonia toxicity and stress. However, at high levels, ammonia is toxic, leading to functional disturbances of the central nervous system that can lead to coma and death. The present study aims to examine the changes in biological oxidative enzymes under sub lethal concentrations of ammonia as a function of chronic stress in Wistar strain albino rat. Lethal doses of ammonium sulphate were determined by the probit method of Finney (1971). Rats were exposed intraperitonially to1/3 of the LD_{50} dose\ i.e., 66.5 mg/kg body weight and vitamin-C 40 mg/kg body weight for one month. Liver, kidney and testes were taken for the present study in control, ammonia exposed and ammonia along with vitamin-C treated rats. The selected biological oxidative enzymes are LDH, SDH and MDH, were estimated by using standard methods. The change in these enzyme levels on ammonia stress is discussed.

KEYWORDS: Ammonia toxicity, oxidative enzymes, vitamin-C.

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INTRODUCTION

Ammonia is a product of the degradation of proteins and other compounds and is present in all living organisms. However, at high levels, ammonia is toxic, leading to functional disturbances of the central nervous system that can lead to coma and death. To avoid the deleterious effects of ammonia, ureotelic animals have developed the urea cycle, located in the liver, which detoxifies ammonia by incorporating it into urea, which is eliminated in urine. When the liver fails, or when blood is shunted past the liver, blood ammonia levels increase and brain function deteriorate: a disorder known as hepatic encephalopathy. However, when the liver fails, ammonia detoxification does not occur properly and the levels of ammonia in the blood and tissues increase, leading to hyperammonemia. There are a number of human illnesses associated with hyperammonemia, the most frequent being liver cirrhosis. The mechanism by which liver failure or hyperammonemia per se leads to disturbances in brain function remains unclear.Acute administration of large doses of ammonium salts leads to the rapid death of the animal. Ammonia toxicity was reported one century ago[1].The metabolic changes and potentialities of ammonia in high concentration are reported in all groups of animals as ammonia toxicity and stress. The major toxic effects of ammonia likely involve changes in cellular pH and the depletion of certain citric acid cycle intermediates in particular α–ketoglutarate. Ammonia has also been a major pathogenic factor associated with inborn error of urea cycle, Reyes syndrome, and disorders of fatty acid oxidation[2]. In the liver, ammonia is removed either in the form of urea in periportal hepatocytes / or as glutamine in perivenous hepatocytes[3].The human body is constantly exposed to a large variety of reactive species (free radicals), which under certain conditions exceed the antioxidant capacity of the body, resulting in oxidative stress. Oxidative stress has been implicated in various pathophysiological conditions, including atherosclerosis, cancer, neurological disorders, diabetes, ischemia/reperfusion, and aging[4]. However, to protect cells and organs against free radicals, biological systems has evolved a highly sophisticated and complex antioxidant system [5]. These antioxidants constitute the body's first line of defense against free radical damage.Vitamin-C and E play a central and complementary roles in quenching free radicals in and around the cell. For example, glutathione and vitamin C are complementary water-soluble antioxidants that scavenge reactive oxygen species (ROS) in the fluid outside and within the cell. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids, capable of neutralizing ROS in aqueous phase to prevent their entry into cells [6].Lactate dehydrogenase is a key enzyme of anaerobic glycolysis and catalysis the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalyzed by LDH interlinks anaerobic and aerobic oxidation of glycose[7].Succinate dehydrogenase is a marker enzyme of mitochondria in the tissues. The oxidation of succinate and malate, and their interplay in metabolic process have been widely established in biological oxidants[7].MDH is a mitochondrial enzyme and play an important role in TCA cycle. MDH is present in both intra and extra mitochondrial compartments of the cell[8] .The enzymes from these two sources in their electrophoretic mobility, molecular weight, amino acid composition and kinetic properties indicating, a differential protein nature[7]. These enzymes have an important role in metabolic oxidation and these enzyme levels have been estimated in the present study to understand the effect of ammonia stress an oxidative metabolism of the animal.

MATERIALS AND METHODS

Healthy male Wistar strain albino rat (250 ± 20 g) obtained from Indian Institute of Science, Bangalore,were maintained in polypropylene cage under laboratory conditions (temperature 34 ± 20^0 C light: dark=12:12h humidity 75%) and fed with standard laboratory chow (Hindustan lever limited, Bombay) and water

was provided ad libitum. Toxicity of ammonium sulphate was evaluated according to Finney's method[9] and was found to be 199.5 mg/Kg body weight. The sub lethal dose i.e, 1/3 of LD_{50}, which is 66.5 mg ammonium sulphate /Kg body weight, and vitamin-C 40mg/kg body weight, was injected intraperitonially to rats for one month treatment. The sub lethal dose was selected to keep the animal under ammonia stress but does not cause mortality.Healthy adult animals were divided into three groups containing six animals each. The first group of animals was considered as control, the second group of animals which received the ammonium sulphate was considered as experimental. Third group of animals received ammonium along with vitamin-C. The control and experimental animals were sacrificed by cervical dislocation at the end of the treatment and liver, kidney and testis tissues were collected and stored in deep freezer at - 20^0C and used for biochemical analysis.Lactate dehydrogenase (LDH) was estimated by Srikanthan and Krishnamoothy[10], Succinate Dehydrogenase (SDH) was estimated by Nachalas[11] *et al.,* Malate dehydrogenase (MDH) was estimated by Nachalas[11] *et al*., The results were subjected to statistical analysis.The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India (CPCSEA, 2003). The experiments were also reviewed and approved by the Institutional Animal Ethical Committee at S.V.University, Tirupati, India (vide No. IAEC/No (Resolution No: 04/2012-2013/ (i)/ (a)/CPCSEA/IAEC/SVU/PN- JPD/dt. 1.2.2012).

RESULTS

In the present study the liver, kidney and testis tissues of male albino rats was selected and all the enzymes were estimated in control, ammonium sulphate, and ammonium sulphate. along with vitamin-c treated rats. In control rats the LDH levels was found to be highest in liver and the lowest in testes tissues. A significant increase in the LDH content in ammonium sulphate treated rats when compared to control was observed. A significant decrease in LDH levels in ammonium sulphate treated rats with vitamin-c was observed when compared to ammonium sulphate treated rats. (Shown in table.1).The activities of SDH in the kidney and the activities of MDH in the testes of ammonium sulphate administered rats show significant decrease compared to control rats. Treatment with vitamin-C in ammonium sulphate administered rat significantly elevated the SDH and MDH activities, as compared to those animals in ammonium sulphate treatment alone. (Shown in table 2&3).

Table 1

Changes in the Lactate Dehydrogenase levels in different tissues of control and ammonium sulphate &vitamin-c along with ammonium sulphate treated albino rats. (μ moles of formazon formed/mg protein/hour

Tissues	Control	Ammonium sulphate	Ammonium sulphate +Vitamin-c
LIVER			
Mean ±S.D%	1.612±0.0144	2.157±0.0114*	1.720±0.0063**
Change over control		+33.80	+6.69
% change over ammonia sulphate			-20.25
t-test			P<0.001
KIDNEY			
Mean ±S.D%	2.178±0.032	2.818±0.0112*	2.305±0.018**
Change over control		+29.3	+5.83
%change over ammonia sulphate			-18.20
t-test			P<0.001
TESTIS			
Mean ±S.D%	1.061±0.0132	1.401±0.012*	1.101±0.037**
Change over control		+32.04	+3.77
%change over ammonia sulphate			-21.41
t-test			P<0.001

Table 2.3

Changes in the Succinate Dehydrogenase Levels in Different Tissues of Control and Ammonium Sulphate & Vitamin-C along with Ammonium Sulphate Treated Albino Rats. (μ moles of formazon formed/mg protein/hour)

Tissues	Control	Ammonium sulphate	Ammonium sulphate +Vitamin-c
LIVER			
Mean ± S.D %	0.9306±0.014	0.711±0.0102*	0.884±0.0104**
Change over control		-23.5	-5.00
%change over ammonium sulphate			+24.36
t-test			P<0.001
KIDNEY			
Mean ± S.D%	0.4706±0.0102	0.36±0.0106*	0.44±0.0105**
Change over control		-23.5	-6.50
%change over ammonium sulphate			+22.22
t-test			P<0.001
TESTIS			
Mean ± S.D%	0.56±0.012	0.496±0.014*	0.527±0.011**
Change over control		-11.42	-7.14
%change over ammonium sulphate			+6.25
t-test			P<0.001

TABLE 2.5

Changes in the Malate Dehydrogenase levels in different tissues of control and ammonium sulphate & vitamin-c along with ammonium sulphate treated albino rats. (μ moles of formazone formed/mg protein/hour).

Tissues	Control	Ammonium sulphate	Ammonium sulphate+ Vitamin-c
LIVER			
Mean ±S.D%	0.612±0.012	0.493±0.0157*	0.589±0.0109**
Change over control		-19.44	-3.75
%change over ammonium sulphate			+19.47
t-test			P<0.001
KIDNEY			
Mean ±S.D%	0.455±0.0105	0.352±0.0081*	0.428±0.0075**
Change over control		-22.63	-5.93
%change over ammonium sulphate			+21.59
t-test			P<0.001
TESTIS			
Mean ±S.D%	0.427±0.0159	0.314±0.016*	0.405±0.047**
Change over control		-26.43	-5.15
%change over ammonium sulphate			+28.98
t-test			P<0.001

DISCUSSION

Increased activity of LDH is a characteristic feature of a shift from aerobic to anaerobic metabolism leading to an elevated rate of pyruvate conversion into lactate, resulting in lactic acidosis. The LDH activity increases during conditions favoring anaerobic respiration to meet energy demands, when aerobic respiration is lowered.[12] Increased LDH activity was supported in fish treated with ammonium sulphate[13]. Similar increase in LDH activity in Albino rat treated with sodium selenete[14], with arsenite treatment in fresh water fishes[15], in albino mice with sodium fluoride[16] and also in albino mice treated with aluminum acetate[17] was reported.Ganguly[18] *et al.,* reported that significant increase of LDH was observed in Benzene induced rats.' Previous reports have suggested a close relation between Oxidative damage and Benzene induction. ROS produced during Benzene metabolism responsible for oxidative cellular damage[19].The antioxidants involved both intracellular and extra cellular defense factors where vitamins like Vitamin E, Vitamin A, Vitamin C and some trace elements like Selenium, Zinc etc play major role[20]. In our present study, the levels of LDH showed significant alterations. Shalan[21] *etal.,* reported that increased LDH activities in response to alcohol administration. Elevated serum levels of LDH were also observed[22].

Supplementations with vitamins (C, E), selenium and silymarin reduced the effect of alcohol intake on body weight. Supplementations with antioxidants significantly reduced the effect of alcohol intake on serum LDH activities. The reports are similar to our present study in the tissues of Liver, kidney and testis of male albino rats where LDH were estimated.Krishnamoorthy[23] reported that Increase in the marker enzymes such as LDH in sodium nitrite-treated rats indicates hepatic damage in experimental animals due to significant increase of LPO in liver [24, 25]. Earlier reports of Samira Bensoltane[26] *et al.,* also demonstrated the increase in the level of LDH in ammonium nitrate-treated rats due to the formation of free radical from nitric oxide. The decrease in the activities of liver marker enzymes such as LDH in vit- C and sodium nitrite-treated rats compared to nitrite-treated rats may be due to the antioxidant effect of vit-C.SDH is a vital enzyme of citric acid cycle, catalyses the reversible oxidation of succinate to fumarate. This decrease would affect the conversion of succinate to fumarate and might cause a block in the Krebs cycle. The activity of succinate dehydrogenase, an oxidative enzyme involved in the Krebs cycle, was significantly decreased in liver and brain tissues after treatment with ammonium sulphate exposure. Decreased SDH activity was supported by several authors in fish treated with ammonium sulphate[15] in gastrocnemius muscle and liver of mice with combined exposure of calcium fluoride and Aluminum[27] in mice treated with sodium fluoride, in gastocnemius muscle, liver and brain[28] in rat treated with zinc sulphate [29]. This decrease would affect the conversion of succinate to fumarate and might cause a block in the Krebs cycle. Decreased SDH activity of all tissues in the present study clearly indicates depletion in the oxidative metabolism at the level of mitochondria leading to depression of TCA cycle under ammonia exposure Debasish Bandyopadhyay[30] *et al.,* reported that treatment of rats with lead acetate at a dose of 15 mg/kg bw i.p. every consecutive day for a period of 7 days caused oxidative stress-induced damage in the hepatic and renal tissue as reflected from the alterations in the levels of SDH. A significant decrease in the SDH content in ammonium sulphate treated rats when compared to control, while a significant increased SDH levels in ammonium sulphate treated rats with vitamin-c when compared to ammonium sulphate treated rats was observed.This decrease would affect the conversion of succinate to fumarate and might cause a block in the Krebs cycle. Decreased SDH activity of all tissues in the present study clearly indicates depletion in the oxidative metabolism at the level of mitochondria leading to depression of TCA cycle under ammonia exposure. This seems to be reversed with vitamin-C treatment.The drop in MDH activity denotes fluctuations of oxidative metabolism, and also reflects the turnover of carbohydrates and energy output. It is well known that any alteration in mitochondrial structure inhibits the activity of MDH. Reduction in MDH activity might also be due to the inhibition exerted by oxaloacetate, because a decrease in the activity of TCA cycle dehydrogenase is consistent with the disintegration of mitochondria of Co_2 formation from acetate. This results in the accumulation of oxaloacetate, which in turn inhibits NAD-specific MDH. The decrement of MDH suggests that there is a shift in the respiratory metabolism towards anaerobiosis[31]. Similar decreased MDH activity in tissues of albino rats due to sodium selenate intoxication has been reported [32]. In the present study, the activity levels of MDH showed inhibited pattern in selected tissues of rats exposed to ammonia stress. MDH is a NAD-dependent enzyme which converts malate to oxaloacetate, and reversible oxidation of fumarate to malate. Oxaloacetate also plays a significant role in Co_2 fixation and in gluconeogenesis. Rajeswara reddy[33] *et al.,* reported in his study that MDH activity decreased in diabetic rat kidney tissue. The increased production of free radicals in mitochondrial cells in the tissue, also a decrease in oxygen consumption, respiratory ratio was observed in mitochondria *Pimpinella tirupatiensis* has altered the levels of MDH and increased the activity levels. In the present study MDH was estimated in the tissues of Liver, kidney and testis of male albino rats. A

significant decrease in the MDH content in ammonium sulphate treated rats when compared to control, while a significant increased MDH levels in ammonium sulphate treated rats with vitamin-c when compared to ammonium sulphate treated rats was observed.Ammonia stress in rats in the present study revealed inhibition of the activity levels of dehydrogenases suggesting a trend towards anaerobic condition under ammonia stress.

CONCLUSION

The present study indicated a damage of the oxidative metabolism in rats exposed to high concentrations of ammonia or long-term exposure. A decline of oxidative metabolism efficiency and the increase of excess ROS might be a consequence of ammonia stress in the albino rats. The antioxidants are the natural defense system against free radical mediated tissue damage in several organs. This study describes the possible effect of ammonia stress on the involvement of oxidative metabolism in rats. The stress seems to be relieved with vitamin-c treatment.

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