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IMPACT OF SODIUM ARSENATE ON SELECTED ENZYMES AND HISTOPATHOLOGICAL STUDIES IN ALBINO MICE

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ABSTRACT

Arsenic is a long-known poison of environmental and industrial origin. Prolonged exposure is associated with vascular disease, skin lesions and cancer. The predominant form of arsenic in the nature is the pentavalent arsenate (AsV), which enters the body mainly *via* contaminated drinking water. Administration of sub lethal dose i.e 4.2 mg/kg/body weight was given to albino mice as single, double and multiple doses. After stipulated period the liver tissue was isolated for enzymes analysis and histopathological studies. The alanine amino transferease (AlAT) activity has shown an increase of 6.03% in single dose, 13.61% in double dose and 27.75% change in multiple dose. Also the activity levels of aspartate amino transferase (AST) has shown an elevation of 9.04% in single, 16.32% in double and 38.08% in multiple dose when compared to control. The glutamate dehydrogenase (GDH) activity has shown an increased level of 9.61% in single, 23.72% in double and 34.62% in multiple doses when compared to control. The liver under sodium arsenate showed necrosis, appearance of vacuoles, nuclear degenerative changes in experimental animals. However, the altered enzymatic and histopathological changes are dose dependent in the present investigation.

KEY WORDS

A/AT, AST and GDH activity, Histopathology, liver, Albino mice.

INTRODUCTION

Transaminases are important enzymes in animal metabolism which are intimately associated with amino acid synthesis. Among these, aspartate and alanine transaminases (AST and A/AT) are widely distributed in the cells of all animals. AST catalyses the inter conversion of aspartic acid and α-ketoglutaric acid to oxaloacetic acid and glutamic acid. While A/AT catalyses the inter conversion of alanine and α -ketoglutaric acid to pyruvic acid and glutamic acid. The enzyme glutamate dehydrogenase (GDH) plays a significant role in the catabolism of amino acids. It catalyses the reversible oxidative deamination of glutamate to α-ketoglutarate and ammonia with pyridine nucleotide (NAD or NADP) as coenzyme. All these three enzymes function as a link between protein and carbohydrate metabolisms and the net outcome is incorporation of keto acids into the TCA cycle. There is much evidence for the alteration in the activities of these enzymes to a variety of environmental and physiological conditions. In the catabolism of amino acids, aminotransferases play a dominant role. They

are the key enzymes of nitrogen metabolism and also an important in energy mobilization.[1] Aspartate and alanine aminotransferases are present both in mitochondria and cytosolic fractions of animal tissues.[2]

Amino acid metabolism is a complex system involving transamination, oxidation, etc. Transamination is an important wing of the amino acid metabolism which is mainly involved in transferring amino group of one amino acid to another keto acid thus forming another amino acid. These enzymes are called aminotransferases.[3] The oxidation of amino acids is mobilized by aminotransferases. Among these, Braunstein[4] suggested that most of NH_3, required for urea synthesis from amino acid nitrogen was liberated via the GDH reaction. AST and A*l*AT were studied in several pathological conditions.[5] The AST and A*l*AT 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.[6,7] Glutamate dehydrogenease (GDH) is a key enzyme in the metabolism of nitrogen.[8] Reversible oxidative deamination of glutamate is catalyzed by GDH using NADP or NADPH as cofactor. GDH provides reducing equivalents to mitochondrial enzyme supplies ketoglutarate to Krebs cycle especially when it is supplied by oxidation of amino acids. Cytoplasmic enzyme recycles ammonia and keeps up glutamate level for mitochondrial transport. Since, GDH allows the incorporation of ammonia into α-ketoglutarate before being transferred by transamination to other α-keto acids.[8] GDH serves to link carbohydrate and amino acid metabolism. GDH can be modified by the energy charge (ADP) and essential effecter in protein synthesis.[9] This suggests in addition to ammonia, GDH may be involved in regulation of energy production and growth.[10] Braunstein[4] suggested that most of NH_3, required for urea synthesis from amino acid nitrogen was liberated via the GDH reaction. Compounds that enter the body *via* the intestinal lymphatic system after oral feeding bypass the liver accordingly. They are not subjected initially either to the detoxifying reactions of the liver or to excrete *via* the biliary system. Compounds transported by oral feeding in effect can be distributed to all parts of the body in their unmetabolised form.[11] If any chemical is taken beyond the safe permissible limits, it could cause pathological damage or injury to cells in an animal. Susceptibility to chemical injury varies greatly among the tissues and cells in an animal and more so among different animal groups. The extent of severity of tissue damage is a function of the concentration and potentiality of toxic compound accumulated in the tissues as it is time dependent.[12] Histopathological changes in different animals by heavy metals have been reported by several workers. [13-15] Histology in a precise sense is the study of the cytoarchitechtural change of the body, which envisages the anatomy and gives the insight into the functioning of tissues and organs. Thus, histology is a structural science and serves to complement the knowledge gained from the anatomy, physiology and pathology. The physiological investigations may not help in the complete understanding of the chemical impact on a tissue. When coupled with cytoarchitechtural studies, the toxicological studies seem to be completed so as to give a picture of the extent of pesticidal effect. The investigations of histopathological effects have not been persuaded with the same vigour compared to bio-chemical aspects. In view of this, an attempt has made to study the effect of sodium arsenate on certain biochemical parameters and histological changes in liver of albino mice.

MATERIALS AND METHODS

Healthy adult albino mice of same age group 60 ± 2 days and weight (30 ± 5g) were taken from veterinary college, Bangalore and maintained in laboratory conditions (26 ± 2 º C; 12hr light and 12 hr darkness). Toxicity of sodium arsenate was evaluated according to Finney[16] and was found to be 42 mg/kg body weight. Ten fold lower concentration of LD_{50} *i.e.* 4.2 mg/kg body weight is taken as sublethal dose. Adult animals were divided into four groups. The first group of animals was considered as controls. To the second group of animals, single dose single was given. To the third and forth group of animals, double and multiple doses were given. After stipulated time (single dose on 3rd , double dose on 5th multiple on 9th days) the mice were

sacrificed, then tissues isolated for histopathological and enzymes analysis.

RESULTS

AlAT and AST activities were enhanced in all the experimental animal tissues. The GDH activity levels also increased in experimental tissues. The GDH activity showed more elevation in multiple dose treated liver (23.72%) showed in table1.

Normal structure of Mice liver

The liver of normal mice comprises continuous mass of hepatic cells with cord like formation. The cells are large in size with more or less centrally placed nucleus and homogenous cytoplasm. There is no clear division of the hepatic cells in to lobules. The hepatic cells are hexagonal in their nature (Figs. A & B).

Mice liver under the impact of different doses of sodium arsenate

The mice liver under single dose did not show any pathological changes. But, double and multiple doses exposed animals have exhibited peripancreatic necrosis, necrosis in hepatocytes (Fig. C), nuclear degeneration and appearance of vacuoles (Fig. D). In the case of, multiple dose of sodium arsenate treated mice more severe changes were observed. They include nuclear degeneration, cytoplasmic degeneration, emptied portal vein, binucleated condition and appearance of vacuoles in hepatocytes (Figs. E & F).

DISCUSSION

Severe alterations in serum total protein and aspartate aminotransferase were observed in Lead and Selenium administered broiler chickens.[17] Protein content decreased in Channa *punctatus* with simultaneous increase in amino acid content following exposure to Cadmium was observed. [18]When Mercury was given to fresh water mussel, it showed increased levels of AST and AlAT.[19] Under the effect of mercury chloride in fresh water grass carp, depletion of total protein content and increased free amino acid content was observed.[20] The administration of sodium arsenate showed an increase in the aminotransferase (AST & AlAT) activities. These changes could lead to the alterations in the associated enzyme activities involved in intermediary metabolism. Treatment of animals with toxic agents is known to produce pathological lesions being associated with increased proteolysis. [21] As GDH occurs with high activity in the mitochondrial matrix it is commonly used as a marker for matrix space.[22] It has a great importance in neurotransmitter balance in brain tissue and maintenance of nitrogen in liver tissue. As GDH plays an important role in detoxification of ammonia, increased glutamate dehydrogenase activity was observed in the present investigation. [23]When mercuric chloride was given to fresh water mussel, Parreysia *rugosa*, GDH was increased.[19] GDH may have helped in deaminating the amino acids and incorporated the carbon skeleton into the energy cycles. GDH may act as regulatory enzyme for controlling the levels of amino acids.[24] Increased protein catabolism observed in the tissues resulted in elevated amino acid level which should normally lead to increased transamination or deamination.[25-26] Large amount of ammonia accumulates in the tissues when amino acids are deaminated.[27] An elevation in the levels of ammonia during sodium arsenate treatment may be attributed to an increased deamination of elevated free amino acid levels. In the present study an elevation in ammonia level indicates its increased fixation through Keto acids leading to glutamate formation by the action of GDH. Increased synthesis of urea (Table 1.3 & Fig 1.3) was observed in the present investigation to divert excessive ammonia and avert its toxic effects. Thus, the sodium arsenate in the present investigation has altered the protein metabolism in experimental mice. The degree of change is directly proportional to the amount of sodium arsenate in experimental mice. However, all tissues have showed highly significant changes in all the parameters investigated under multiple dose Sodium arsenate.

Arsenic is considered as a toxic metal, which reflects on human health. Various workers have observed systemic disorders. [28-30] In the present investigation by exposing the mice to the sodium arsenate several pathological changes were observed in liver of mice at the different doses (A-F). Severe histopathological lesions are observed by exposing to the sodium arsenate at

the multiple dose. In the present investigation, various pathological changes have been observed in experimental mice liver like peripancreatic necrosis, necrosis in hepatocytes, nuclear degeneration and vacuoles under the impact of sodium arsenate. On the other hand, multiple dose animals have possessed and showed more pronounced changes comparatively with other experimental animals. Though the liver is major metabolic center to detoxify toxic pollutants is also badly affected by the sodium arsenate. In multiple dose mice liver several changes were occurred like nuclear degeneration, cytoplasmic degeneration, emptied portal vein, binucleated condition and also exhibition of vacuoles in hepatocytes (Figs. E & F). Earlier workers have also mentioned histological disturbance caused by Arsenic containing water by the study in mice model and histopathological results revealed mild to severe type of necrosis and degenerative changes in kidney and liver of arsenic feed animals. [31] Human arsenic exposure is related to severe health problems such as skin cancer, diabetes, liver, kidney and CNS disorders.[32] It also causes many other toxic effects. [33-35.] The liver is the major target organ of inorganic arsenic, which is explained by the affinity of As (III) toward vicinal dithiol in hepatic cytosolic proteins. The binding of arsenic in target organs was postulated as a first step of arsenic detoxification. Nevertheless, this binding is also responsible for the major intoxication symptoms: hepatic and renal failure and cardiovascular and neurological effects. Marafante *et al*., [36] performed an *in vivo* study in rabbits to determine the arsenic distribution in tissues. Varied degrees of changes were also observed in 30 and 150 ppb exposed group. Necrosis of hepatocytes and cytoplasmic blebbing were also observed. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatocytes.[37] These changes may alter the physiological functions of the treated mice with sodium arsenate. In the present investigation severity of histopathological lesions have been observed in multiple dose sodium arsenate when compared to that of single and double dose which clearly indicates that the repeated exposure to low doses or concentrations cause deleterious effects and making them less fit for better survival. Sodium arsenate in the present investigation could cause cytoarchetechtural changes in liver of double and multiple dose animals but not single dose animals. Thus, repeated exposure to sodium arsenate will definitely cause histopathological changes in non-target animals which inturn result several biochemical changes.

Table 1

Alterations in Alanine & Aspartate aminotransferase and Glutamate dehydragenase activity levels in liver tissue of control and sodium arsenate treated albino mice.

Name of the Enzyme	Single Dose		Double Dose		Multiple Dose	
	Control	Experimental	Control	Experimental	Control	Experimental
AlAT (µmoles of pyruvate formed/mg protein/hr)	1.492 ±0.033	1.582 ±0.044 (6.03)	1.510 ±0.032	1.695 ±0.023 (13.61)	1.498 ±0.033	1.906 ±0.029 (27.75)
AST (µmoles of pyruvate formed/mg protein/hr)	1.305 ±0.027	1.423 ±0.044 (9.04)	1.312 ±0.026	1.52 ±0.029 (16.32)	1.308 ±0.025	1.802 ±0.023 (38.08)
GDH (µmoles of formazon formed/mg protein/hr)	0.156 ±0.020	0.171 ±0.031 (9.61)	0.158 ±0.025	0.193 ±0.024 (23.72)	1.160 ±0.022	0.210 ±0.013 (34.62)

Values in parentheses indicate the percent change over control.
Values are mean ± SD of six individual observations.

PLATE -1

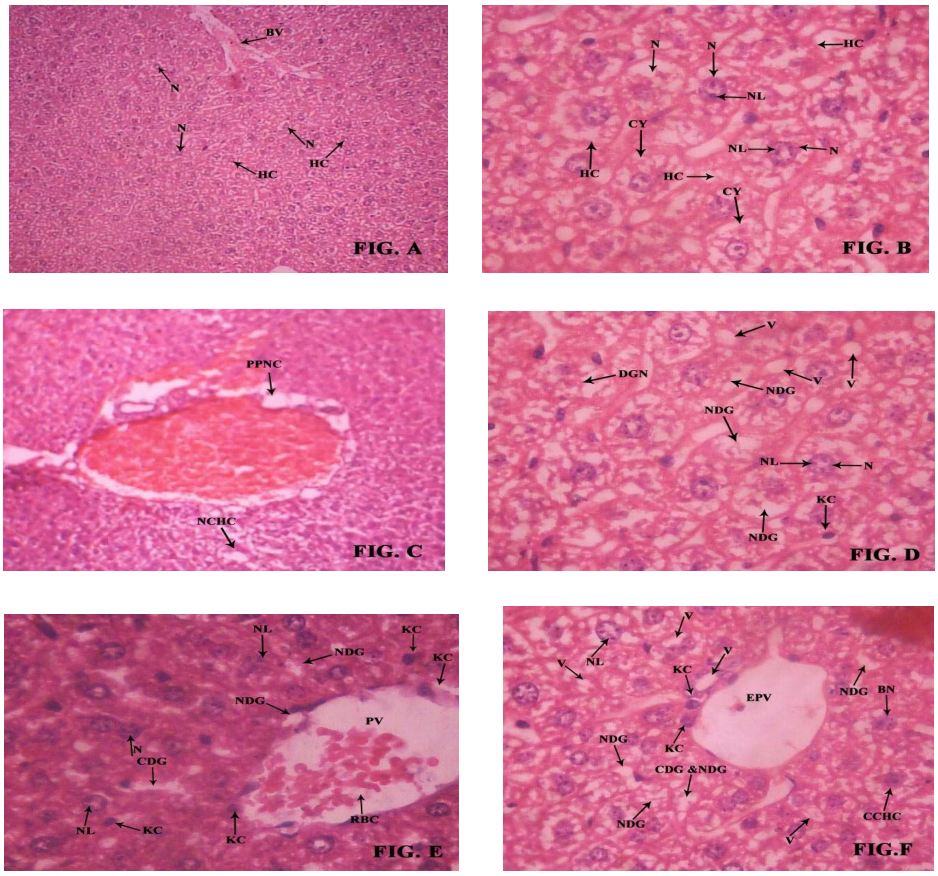

***Fig.A**: Mice Liver showing hepatocytes (HC) with centrally placed nucleus – H & E. 100 X.*
***Fig.B**: Control liver at higher magnification showing hexagonal hepatocytes (HC) – with one or more nucleoli (NL) and cytoplasm (C)-H & E.400 X.*
***Fig.C**: Mice liver under double dose of sodium arsenate – showing peripancreatic necrosis (PPNC) and necrosis in hepatocytes (NCHC) – H & E. 400 X.*
***Fig.D**: Mice liver under double dose of sodium arsenate showing nuclear degeneration (NDG) and appearance of vacuoles (V) in hepatocytes – H & E. 400 X.*
***Figs. E & F**: Mice liver under multiple dose of sodium arsenate showing few RBC in portal vein (PV), nuclear degenerative changes (NDG) and cytoplasmic degenerative changes in cytoplasm (CDG), emptied portal vein (EPV), appearance of severe necrosis in cytoplasm (NCC), clumped chromatin in hepatocytes (CCHC) and appearance of vacuoles (V) in hepatocytes – H & E. 400 X.*

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