TOXICITY OF ARSENIC ON LIVER GLYCOGEN RESERVES IN ALBINO RATS

Kshama Dwivedi*

Abstract

Arsenic (As) poisoning has become a worldwide public health issue. Although, it is an essential trace element in nutrition but exposure to high concentrations of arsenic causes debilitating and often fatal illness that affects most organ systems. Chronic exposure appears to cause cancer, particularly of skin and lungs. The aim of the present study was to determine the dose- dependent effect of oral exposure to sodium arsenite ($NaAsO_2$) on the liver glycogen of albino rats for 15, 30, and 60 days. Animals exposed for different doses showed significant dose- dependent depletion of liver glycogen reserve. The high doses of arsenic showed more adverse effect as compared to low dose. The decrease in liver glycogen under low dose for 15, 30 and 60 days were found to be 4.06%, 21.90% and 29.94% respectively. The decrease in liver glycogen under highest dose of arsenic for 15, 30, and 60 days was found to be 11.16%, 31.44%, and 37.84% respectively. Exposure to 60 days duration, showed maximum percentage fall. The effect of arsenic in albino rats was found to be dependent on the period of exposure and concentration of metal.

Key Words: Sodium arsenite, hepatic toxicity, glycogen, diabetes.

* **Department of Zoology, Bareilly College, Bareilly. 243005**

Introduction

Toxic properties of arsenic have been known for centuries. It is widely distributed in the environment so that all humans are exposed to low level of arsenic. The anthropogenic contribution to the environmental arsenic originates mostly from mixing, smelting (Welch *et al.*, 1982) and in the refining of certain ores and also from burning of coal. In most population worldwide, the principal source of non-occupational arsenic intake (at 25 to 50µg/d) is food with drinking water while air mostly minor source (Boppal *et al.*, 1995).

Being carcinogenic for skin, bladder, liver and kidney (WHO 1971), arsenic is placed among top hazardous substances by ATSDR and UPEPA (ATSDR, 1999). It also causes reproductional dysfunction and can damage the respiratory, gastrointestinal, cardiovascular and haemopoietic system (WHO, 1971, Engel and Smith, 1994; Chen *et al*., 1995) and skin (Burnstam and Nriagu, 2000).

Inhibitory effect on cellular carbohydrate metabolism, have been studied by a number of investigators (Berry and Smyth, 1959; Havu 1969). Acute arsenic poisoning is reported to cause carbohydrate depletion (glucose and glycogen) and death in rats (Szinicz and Forth, 1988). In acute arsenic (AS_2O_3) poisoning for 52 hours, all died mice showed significant decrease in liver glucose and glycogen content. In liver of survivors with glucose supply in diet, the glucose and glycogen content was not different when compared from control group (Reichl *et al.*, 1990 and 1998). Few data also suggest that mice are less susceptible than humans to diabetogenic effect of chronic exposure to inorganic arsenic (iAs) due to more efficient clearance of arsenic or its metabolites from target tissues (Paul *et al*., 2007).

Toxic effect of arsenic are attributed to its ability to induce toxic oxidative stress leading to enhanced production of reactive oxygen species (ROS), that results in altered antioxidant system, increased oxidative stress and cell death (Lantz and Hays, 2006). Arsenic is associated with alteration of glucose homeostasis (Pal and Chatterjee, 2005) and is also reported to be associated with diabetes mellitus in wistar rats (Patel and Kalia, 2013) and humans (Pan *et al*., 2013). Other heavy metals like lead also cause glycogen depletion in liver tissues of rats (Ahmad *et al*., 2014). Reports are there that arsenic exposure leads to glucose intolerance in female mice by disturbing the hormonal disturbance (Huang *et al*., 2015).

In the view of above, present investigation was undertaken to elucidate the impact of subchronic arsenic exposure on liver glycogen reserves in albino rats for different durations.

Materials and Methods

Experimental animals

Albino rats of 6 to 10 weeks old weighing approx. 150- 160 grams were purchased from the Laboratory Animal Resource Section, Indian Vertinary Research Institute (IVRI) Izatnagar Bareilly, U. P. and maintained in experimental animal shed of the division. Animals were kept for a week to be conditioned to the new environment prior to the start of experiments. Animals were kept under conventional condition (6 rats per steel cage, 12 hr. light to dark cycle). The animals were made available to standard rat food and tap water ad libitum. All the chemicals used were from Sigma Chemicals Co., Merk and Qualigens.

Experimental design

The experimental albino rats were divided into three groups A, B and C each comprising of 6 animals. Group A (control) received plain tap water while group B with low dose of sodium arsenite (4.3 mg/kg.b.wt.) and group C with high dose of sodium arsenite (8.6 mg/ kg.b.wt.). Albino rats were exposed to two test doses of sodium arsenite ($NaOAs_2$) for varying exposure periods of 15, 30 and 60 days. The compound was given in tap water per os by gavage. Mortality rate, food consumption, clinical signs and symptoms were recorded daily. Bodyweight gain was calculated weekly.

After termination of the experimental period, animals were sacrificed under chloroform anaesthesia, dissected and the organs along with liver were removed and washed in ice-cold saline solution. Liver tissue was weighted and homogenised in potassium phosphate buffer, pH 7.4 in mortar and pastel. The glycogen levels were determined by Anthrone Method of Van Der (1954).

Results

The results of the effect of sodium arsenite on liver glycogen reserve in albino rats are given in table 1 while the percentage changes in table 2.

Table 1: Effect of sodium arsenite ($NaAsO_2$) on the liver glycogen reserve of albino rats (mg/g of wet tissue).

Groups	Durations of exposure		
	15 days	30 days	60 days
Group A (Control)	1.028±0.063	0.954±0.042	0.949±0.02
Group B (L)	0.986±0.015	0.745±0.022	0.665±0.03
Group C (H)	0.913±0.029	0.654±0.032	0.590±0.03

- As (L) = Sodium Arsenite Low Dose
- As (H) = Sodium Arsenite High Dose

Table 2: Percentage change in the liver glycogen reserve of albino rats exposed to sodium arsenite ($NaAsO_2$).

Groups	Durations of exposure		
	15 days	30 days	60 days
Group B (L)	-4.06	-21.90	-29.94
Group C (H)	-11.16	-31.44	-37.80

- As (L) = Sodium Arsenite Low Dose
- As (H) = Sodium Arsenite High Dose

Animals treated for 15 days shows fall in the liver glycogen reserve in both low and high intoxicated groups i.e. B and C respectively. Group C receiving high dose of arsenic shows significant depletion of glycogen reserve when compared to group B. In group B percentage fall of 4.04% while in group C percentage fall of 11.16% was observed.

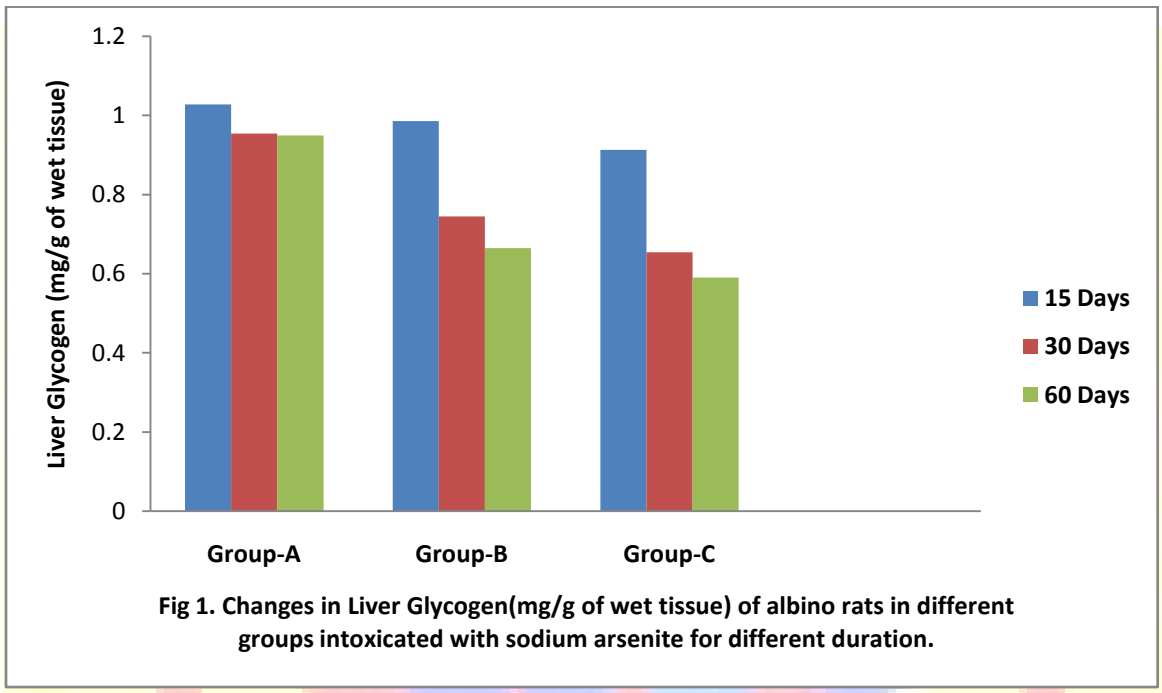

Fig 1. Changes in Liver Glycogen(mg/g of wet tissue) of albino rats in different groups intoxicated with sodium arsenite for different duration.

In animals treated for the duration of 30 days same trend was observed. Group C with high dose of sodium arsenite shows more depletion in liver glycogen in comparison to group B when compared to control (Group A). Group B and C shows percentage change of 29.94% and 37.8% respectively.

The trends in 60 days exposure period is not much different from 15 days and 30 days exposure period. Group B and C showed significant depletion of liver glycogen reserve. The fall of 21.45% and 31.43% was observed in group B and C respectively when compared to control. Percentage fall of sodium arsenite in 60 days treated rats is high compared to 15 days and 30 days treated rats.

Discussion

The major glycogen reserve in animals occurs in liver. Present study shows that sub acute administration of sodium arsenite to albino rats results in marked depletion of liver glycogen reserves. Liver being the organ for inter-conversion and storage of food stuff and centre of all oxidative detoxification mechanisms shows maximum alteration in its tissue composition. Depletion in glycogen reserve in experimental rats was duration and dose dependent.

Although, short term exposure to single acute dose of arsenic causes hyperglycaemia and marked glucose intolerance (Ghafghazi *et al.*, 1980), arsenical disturbance of in vivo carbohydrate metabolism is a complex phenomenon which involves a number of organ systems and their functional interrelationships. It is difficult to assess the cause of depressed liver tissue glycogen but reduction in glycogen amount may reflect a stress response in arsenic treated rats. This finding is in close agreement with the data of Berry and Smythe (1959) and Szinicz and Froth (1988) in mice and rats. These authors proposed that trivalent arsenic causes complete carbohydrate depletion in animals which die and a higher susceptibility to its toxicity in starving animals where the glycogen stores are depleted. Substitution of glucose slows down carbohydrate depletion in acute experimental poisoning with As_2O_3 (Reichl *et al.*, 1990). Authors say surviving animals on acute arsenic poisoning when treated with glucose did not show a marked decrease in glycogen content.

Pal and Chatterjee (2005) reported, depletion of liver glycogen and pyruvic acid contents in wister rats exposed to arsenic for 30 days leading to glycogenolytic as well as glycolytic activities of liver. Arsenic induces hepatotoxicity which was manifested by an increase in serum ALT, AST and ALP (Patel and Kalia, 2013).

The result of the present study indicates that chronic exposure to arsenic results in depletion of glycogen content in liver. Many authors have reported that pancreatic β- cells are affected by apoptosis leading to less insulin production resulting to hyperglycaemic condition (Pax *et al.* 2014). It is also possible that arsenic toxicity also leads to stimulation of α- cells leading to increased production of glucagon which leads to glycogen breakdown (glycogenolysis).Toxic metals can cause structural and functional changes in adrenal glands and it has been reported that

thyroid hormone kinetics are affected by a number of metallic compounds (Rana, 2014). Adrenalin (Epinephrine) secreted by adrenal medulla is a potent glycogenolysis enzyme. Possibly, the nephrotoxic arsenic may induce abnormally high production of adrenalin hormone leading to glycogen depletion in liver. Arsenic toxicity stress might induce the hyperactivity of adrenalin leading to increase rate of glycolysis together with glycogenolysis in liver resulting to impaired glucose metabolism.

According to Pimpar and Bhave (2010) exposure to high levels of arsenic causes diabetes and increased levels of glucose in blood. Decrease in glycogen level in fish due to pesticide effect has been reported (Vijayavel *et al*., 2006; Crestani *et al*., 2005). Chandra Mouli (2008) also reported a fall in glycogen levels in fish *Heteropneustes fossilis* exposed to cypermethrin. Arsenic accumulation was more pronounced in liver than pancreatic tissue (Patel and Kalia, 2013).

Decrease in glycogen level was reported by some other authors in fluoride treated mice liver (Jayasankar, 2007; Ravi Sekhar *et al*. 2009) in albino rat exposed to cypermethrin. Madhava Rao (2007) also found decreased glycogen levels in amphibian model, *Rana hexadactyla* exposed to azadirachtin. Thus, more catabolic rate of glycogen could be another possible reason for decrease of glycogen and increase of blood glucose in the present investigation. The effect becomes more significant when the dose and exposure duration is increased. Thus, effect of arsenic in albino rats was found to be dependent on the period of exposure and concentration of metal.

Conclusion

Thus, the present investigation shows that on exposure of albino rats to sub acute dose of arsenic for 15 days, 30 days and 60 days duration results in fall of liver glycogen. Probably the metal exposure stimulates hormones that accelerate glycogen breakdown or inhibition of those enzymes or intermedins involved in glycogen synthesis.

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