Effect of ground water arsenic on the liver of albino rat

*P.K. Singh, Sheeba Hussain and Ajay Pratap Singh
Department of Zoology, School of Life Sciences, Khandari Campus,
Dr. B.R. Ambedkar University, Agra – 282 002

Abstract: Arsenic is a known poison of environmental and industrial origin. Prolonged exposure is associated with vascular diseases, 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. The arsenic contaminated water samples were collected from different areas of Agra district and the arsenic concentration was estimated. Four groups of five albino rats each were administered 0.02 mg/litre arsenic present in drinking water for 7, 14 and 21 days respectively. After stipulated period the liver tissue was isolated for histopathological studies. The body weight, organ weight and their ratio decreased significantly (p<0.001). However, the SGOT, SGPT, ACP and ALP has shown an increase significantly (p<0.001) as compared to control groups. The histopathology of liver shows necrosis, appearance of vacuoles, nuclear degeneration changes after arsenic toxication. However, the alteration in enzymes and histopathological changes are dose dependent in the present investigation.

Key words: SGPT, SGOT, ACP, ALP, Arsenic, Albino rat, Hepatopathology.

Introduction
Arsenic, a toxicant of natural occurrence in mineral deposit, is used in many human activities such as manufacturing, agriculture, and medicine (Nabi et al. 2005). Arsenical compounds are transported into the environment mainly by water from wells drilled into the arsenic rich geologic strata or by ambient air during smelting and burning of coal (Thornton and Farago, 1997). The main route of arsenic exposure for the general population is via drinking water. After absorption, inorganic arsenic is accumulated in the liver, spleen, kidneys, lungs and gastrointestinal tract. During metabolism, most of the inorganic arsenic such as As (III) and As (V) are metabolized to dimethylarsinic acid and monomethylarsonic acid and then rapidly cleared from the tissues through urine (Chris et al. 2000). However, this biomethylation process can easily become saturated and lead to the excess inorganic arsenic being deposited in the skin, hair and nails, where it binds tightly to keratin (Baldwin and Marshall, 1999).

Arsenic is used as herbicides, fungicide and rodenticides. Arsenic is a great environmental concern due to extensive contamination of ground water (Rana et al. 2008). Drinking polluted water is a common cause of arsenic poisoning (Ahmad et al. 2008). Exposure to arsenic is associated with various metabolic disorders, hypertrophy of adrenal gland (Biswas et al. 1994), and anemia (Sarkar et al. 1992). A number of proteins and enzyme systems containing sulfhydral group have been found to be altered by arsenic (Robert and Jud, 1986). Arsenic effects mitochondrial enzymes and impairs tissue respiration, which seems to be related to the cellular toxicity (Brown et al. 1976).

Transaminases are important enzymes in animal metabolism which are intimately associate with amino acid synthesis. Among these aspartate and alanine transaminases, alkaliene and acid phosphatases are widely distributed in the cells of all animals. All these enzymes functions as a link between protein and carbohydrate metabolism. There is much
evidence for the alteration in the activities of these enzymes to a variety of environmental and physiological conditions (Devaraju et al. 2010).

The trivalent form of arsenic is able to bind to sulfhydryl groups of enzymes in the pyruvate dehydrogenase system and glyceraldehyde – 3 – phosphate dehydrogenase. Arsenic is able to bind to enzymes specially bound with lipoic acid in the tricarboxylic acid cycle and therefore can interfere with oxidative phosphorylation in cells (Maiti and Chatterjee, 2001). In the pentavalent form, arsenic can also exert toxicity by competitively substituting its ions for the body’s phosphate ions. This can lead to breaking down by hydrolysis of high energy bonds in compounds such as ATP resulting in a marked depletion of cellular ATP and eventually death of the metabolizing cells (Tseng et al. 2002).

Compounds that enter the body via the intestinal lymphatic system after oral feeding by pass 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 (Turner and Shanks, 1980). It could causes pathological damage or injury to cells in an animal. The extent of severity of tissue damage is a function of the concentration and potentiality of toxic compound accumulated and potentiality of toxic compound accumulated in the tissues as it is time dependent (Jayantha Rao, 1984). In view of this, an attempt has been made to study the effect of ground water arsenic on the enzymes and histological changes in liver of albino rats.

**Materials and Methods**

Twenty male albino rats (Rattus norvegicus) of wistar strain weighing 140 to 170 ± 25 gm and eight weeks old were randomly divided in to four groups of 5 rats each. Each group was kept in a separate polypropylene cages and maintained in controlled temperature (25 ± 2°C), humidity (65 ± 10%) and proper circadian rhythm. The animals were acclimatized for 20 days before starting the experiment. During this period animal had free access to normal diet and the water given ad libitum.

The arsenic water was collected from Sikandra area of Agra region from as usual water sources like hand pumps in poly propylene bottles. The concentration of arsenic in drinking water was found to be 0.102 mg/l the and concentration of arsenic in water sample was measured by the method of Aggett and Aspell (1976).

Rats of group A were treated as control group and were given distilled water, while rest three groups B, C and D were treated with ground water arsenic (10.2 mg/l) of above area daily for 7, 14 and 21 days respectively.

Body weight was measured before and after the experimental period. At the end of each experimental period, the animals were sacrificed and liver were dissected out and weighed individually.

The liver was fixed in Bouin's fixative, embedded in paraffin and 5 ml (µ) thick section were stained with routine hematoxylin and eosin. Histopathological changes in the liver were examined under optical microscope.

The alkaline phosphatase (ALP) in serum was estimated by the method of Kind and King's (1954), and acid phosphatase (ACP) in the serum was estimated by the King and Jagatheesan (1959).

Serum glutamate oxaloacetic transaminase (SGOT) or (AST) and serum glutomate pyruvic transaminase (SGPT) or (ALT) was estimated by the method of Reitman and Frankel (1957).

The data were expressed as mean ± SEM and were evaluated for statistical significance with the student "t" test.

**Results and Discussion**

In the present investigation the effect of ground water arsenic for 7, 14 and 21 days have been carefully studied on liver histopathology and serum enzymes in albino rats (Rattus norvegicus). A decrease in organ weight, body weight and organ weight and body weight ratio...
### Table 1 – Effect of ground water arsenic on the liver of albino rats.

<table>
<thead>
<tr>
<th>Treatment time (in days)</th>
<th>Biological Parameters</th>
<th>Body weight</th>
<th>Organ weight</th>
<th>SGOT</th>
<th>SGPT</th>
<th>ALP</th>
<th>ACP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control mean ± S.Em</td>
<td>Treated mean ± S.Em</td>
<td>Control mean ± S.Em</td>
<td>Treated mean ± S.Em</td>
<td>Control mean ± S.Em</td>
<td>Treated mean ± S.Em</td>
<td>Control mean ± S.Em</td>
</tr>
<tr>
<td>7</td>
<td>141.4 ± 1.17</td>
<td>128 ± 0.11</td>
<td>4.89 ± 0.11</td>
<td>60.26 ± 2.11</td>
<td>77.24 ± 2.68***</td>
<td>44.628 ± 2.09**</td>
<td>140.432 ± 1.922</td>
</tr>
<tr>
<td>14</td>
<td>141.4 ± 1.71</td>
<td>119.6 ± 0.11</td>
<td>4.89 ± 0.11</td>
<td>60.26 ± 2.11</td>
<td>89.902 ± 2.112***</td>
<td>63.232 ± 2.09</td>
<td>140.432 ± 1.922</td>
</tr>
<tr>
<td>21</td>
<td>141.4 ± 1.17</td>
<td>113.2 ± 0.11</td>
<td>4.89 ± 0.11</td>
<td>60.26 ± 2.11</td>
<td>98.07 ± 2.54***</td>
<td>110.906 ± 2.09</td>
<td>140.432 ± 1.922</td>
</tr>
</tbody>
</table>

N = 6
No. of Observations – 12 [NS – non significant (p > 0.05)* - Significant (p<0.05), **-Highly Significant (p<0.01), ***-Very Highly Significant (p<0.001)]
is very highly significant \((p < 0.001)\) after 7, 14 and 21 days of ground water arsenic treatment. While serum AST and ALT, SGPT and SGOT level were increased, very highly significant \((p < 0.001)\) after 7, 14 and 21 days of ground water arsenic treatment.

The microscopic observations of liver in control group rats showed continuous mass of hepatic cell with cord like formation. The cells are large in size with more or less centrally placed nucleus and homogenous cytoplasm. There is not clear division of the hepatic cells into lobules. The hepatic cells are hexagonal in their nature.

The histopathological observations of liver after 7, 14 and 21 days of ground water arsenic treatment of rat showed some predominant recovery in centrilobules degeneration, fibrosis and clumping of nuclei. The balloon cells indicated hypertonic degenerations. The portal cirrhosis also showed significant lesions. However, no pseudolobules could be observed and the hepatocytes were radial from central vein at places. Increased sinusoidal spaces were also seen. Some of the hepatocytes are bi

**Fig. 1** - Photomicrograph of liver of control rat showing uniform hepatic parenchyma (HP), round nuclei with centrally placed nucleolus, normal sinusoid (SS), tubular canal (TC), central vein (CV) and kuffer cells (KC) [H/E-400X]

**Fig. 2** - Photomicrograph of liver after 7 days treatment with ground water arsenic showing portal cirrhosis (PC), sinusoidal spaces (SS), ruptured hepatocyte (RH), central vein (CV), normal tubular canal (TC) [H/E-400X]

and tri nucleated and connective tissues have started to rapture.

After 14 days of ground water arsenic treated rats showed disappearance of centrilobular necrosis like massive necrosis. Cirrhosis lesions in central vein were congered and

**Fig. 3** - Photomicrograph of liver after treatment with ground water arsenic showing hypertonic nucleus (HPN), massive necrosis (MNC), vascular lesions (VL) and balloon cells (BC) [H/E-400X]
gained hepatic cells with large nucleus regenerated nodules in hepatic nodules by thick fibrous septa. In the case of 21 days arsenic treated rats showed severe changes like nuclear degeneration, cytoplasmic degeneration, emptied portal vein, binucleated condition and appearance of vacuoles in hepatocytes.

In the present study the body weight and liver weight and their ratio decreased as compared to the control group due to side effect of arsenic intoxication, while it can also be correlated with certain histopathological changes in the liver. Similar findings have been reported by Ahmad et al. (2008) in rats. The SGOT, SGPT, ALP and ACP were increased significantly due to side effect of ground water arsenic and can also be correlated with histological changes in the liver. Similar findings have also been supported by Biswas et al. (2000) in goats due to arsenic toxicity, while Ghosh et al. (1993) showed in rats due to arsenic intoxication, Nabi et al. (2005) in human due to chronic arsenic poisoning causes significant elevation of inorganic phosphatase in serum. An increased in the activities of ALP, ACP in serum due to arsenic poisoning were also observed Devaraju et al. (2010) observed similar findings in albino mice due to impact of sodium arsenate. They have reported an increase in the SGOT, SGPT, ALP and ACP activities due to toxic effect of arsenic.

In the present study severe histopathological lesions are observed in the liver after ground water arsenic treatment like parapancreatic necrosis, necrosis in hepatocytes, nuclear degeneration and vacuoles under the impact of arsenic. Though the liver is major metabolic center to detoxify toxic pollutants but it is also badly affected by the arsenic. Devaraju et al. (2010) reported several changes in the liver occurred such as nuclear degeneration, cytoplasmic degeneration and emptied portal vein, binucleated condition and also exhibition of vacuoles in hepatocytes. Ferzand et al. (2008) have also mentioned histological disturbance caused by arsenic containing water in mice and revealed mild to severe type of necrosis and degenerative changes in the kidney and liver of mice, while Javaid et al. (2008) in mice due to arsenic toxicity observed necrosis of hepatocytes, cytoplasmic blebbing, sinusoidal spaces were expanded due to shrinkage and necrosis of hepatocytes. These changes may alter the physiological changes in the treated mice with the sodium arsenate. Thus, in the present investigation it was observed that histopathological changes in albino rat resulted in several biochemical changes.

**References**

Aggett, J. and Aspell, A.C. (1976) Determination of arsenic (III) and total arsenic by the silver diethyldithiocarbonate method. Analyst. 101, 912-916


Brown, M.M., Rhyne, B.C., Bcyes, R.A. (1976) Intracellular effects of chronic arsenite administration on renal proximal tubule...
Effect of ground water arsenic

Chris, Le, X., Mingeshe, Ma, Xiufen, Lu., William, R., Cullen, H.,
Aposhan, V., and Booshan, Z. (2000) Determination of
monomethyl arsonous acid, a key arsenic methylation
intermediate, in human urine. Environ. Hlth. Perspec. 108,
1015-1018.
Devaraju, T., Sujatha, K. Madhava Rao S. and Jayantha Rao K.
(2010) Impact of sodium arsenate on selected enzymes
and histopathological studies in albino mice. Int J. Pharm.
Biose., 1, 1-6.
and haematological disturbance caused by arsenic toxicity
Ghosh, A., Sarkar, S., Pramanik, A.K. Palchowdhary S. and
and its relationship with soil and plants of west Bengal. Ind.
Javaid, Ali Gadahi, Qurban Ali, Rubina Ferzard and Samim Saleha
(2008) Histological and haematological disturbance
caused by arsenic toxicity in mice model. Pakistan J. Biol.
Sci. 11, 1405-1413.
Phosphomidon on some aspects of metabolism in fresh
Venkateshwar University, Tirupathi, India.
Kind, P.R.N. and Kings. E.J. (1954) Estimation of alkaline
King, E.J. and Jagatheesan, K.A. (1959) Estimation of acid
Maiti, S., and Chatterjee, A.K. (2001) Effects on levels of
glutathione and some related enzymes in tissues after an
acute arsenic exposure in rats and their relationship to
dietary protein deficiency. Arch. toxicol. 75. 531–537.
Evaluation of biochemical changes in chronic arsenic
poisoning among Bangladeshi patients. Int. J. Enviorn.
Rana, T., Sarkar S., Mandal T. and Batabyal S. (2008)
Haematobiochemical profiles of affected cattle at arsenic
prone zone in haringhata block of Nadia district of West
Bengal in India. The int. J. Haematol., 4, 1-5.
arsenite on haematology in male albino rats. Ind. J.
Physiol. Allied SC. 46, 116-120.
In : Arsenic exposure and health effects. Chapman and
Hall, London, pp. 1-16.
Tseng, C.H., Tseng C.P., Chiou, H.Y., Hsueh, Y.M., Chong, C.K.,
and Chen, C.J. (2002) Epidemiologic evidence of
Turner, J. C. and Shanks, V. (1980) Absorption of some
organochlorine compounds by rat small intestine in vivo,