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# Effect of ground water arsenic on the liver of albino rat

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***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 dimethyl arsinic 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, aklaline 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

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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 bilary 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 novegicus*) 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 ± 2ºC), 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.

| Treatment time (in days) | Biological Parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Body weight | | Organ weight | | SGOT | | SGPT | | ALP | | ACP | |
| | Control mean ± S.Em | Treated mean ± S.Em | Control mean ± S.Em | Treated mean ± S.Em | Control mean ± S.Em | Treated mean ± S.Em | Control mean ± S.Em | Treated mean ± S.Em | Control mean ± S.Em | Treated mean ± S.Em | Control mean ± S.Em | Treated mean ± S.Em |
| **7** | 141.4 ± 1.17 | 128 ± 1.41*** | 4.89 ± 0.11 | 3.96 ± 0.071*** | 60.26 ± 2.11 | 77.24 ± 2.68** | 34.048 ± 2.09 | 44.628 ± 1.42** | 140.432 ± 1.922 | 151.584 ± 2.37** | 27.026 ± 0.998 | 36.442 ± 2.005* |
| **14** | 141.4 ± 1.71 | 119.6 ± 0.753*** | 4.89 ± 0.11 | 3.76 ± 0.017*** | 60.26 ± 2.11 | 89.902 ± 2.112*** | 34.048 ± 2.09 | 63.232 ± 2.454*** | 140.432 ± 1.922 | 162.068 ±3.697** | 27.026 ± 0.998 | 52.152 ± 2.22* |
| **21** | 141.4 ± 1.17 | 113.2 ± 1.71*** | 4.89 ± 0.11 | 3.55 ± 0.018*** | 60.26 ± 2.11 | 98.07 ± 2.54*** | 34.048 ± 2.09 | 110.906± 3.552*** | 140.432 ± 1.922 | 190.896 ±3.118*** | 27.026 ± 0.998 | 69.612 ±2.713** |

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

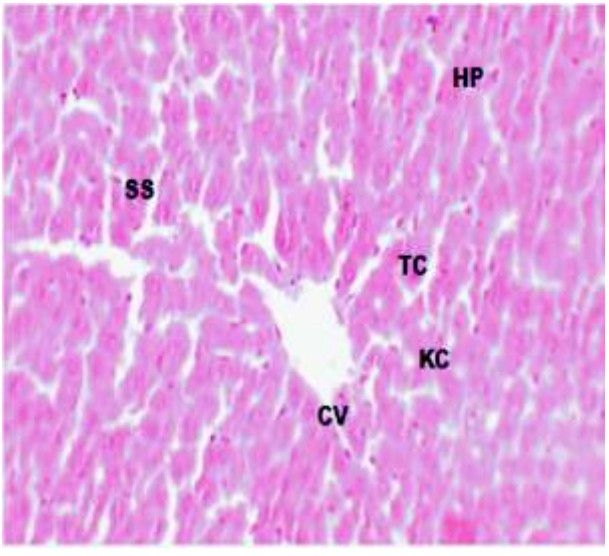


**Fig. 1 -** Photomicrograph of liver of control rat showing uniform hepatic parenchyma (HP), round nuclei with centrally placed nucleolus, normal sinusoid (SS), tubular canal (TC), central vein (CV) and kuffer cells (KC) [H/E-400X]

nucleus and homogenous cytoplasm. There is not clear division of the hepatic cells in to lobules. The hepatic cells are hexagonal in their nature .

The histophathological 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 pseudulobules 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

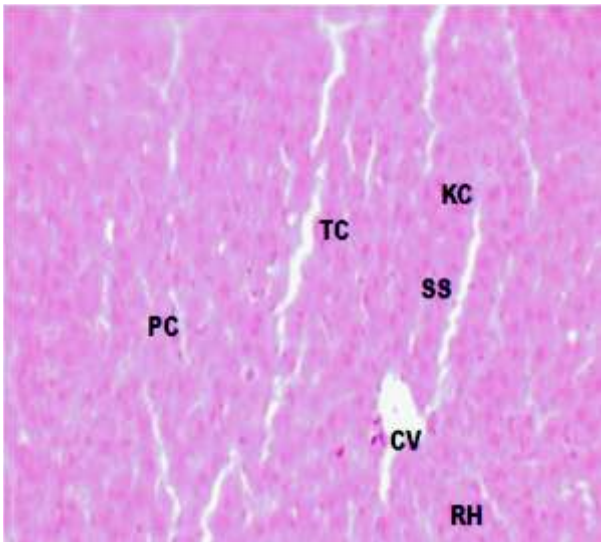


**Fig. 2 -** Photomicrograph of liver after 7 days treatment with ground water arsenic showing portal cirrhosis (PC), sinusoidal spaces (SS), ruptured hepatocyte (RH), central vein (CV), normal tubular canal (TC) [H/E-400X]

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

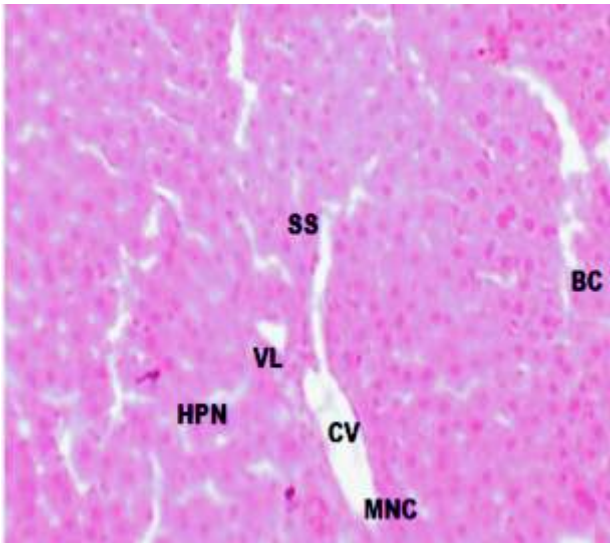


**Fig. 3 -** Photomicrograph of liver after treatment with ground water arsenic showing hypertonic nucleus (HPN), massive necrosis (MNC), vascular lesions (VL) and balloon cells (BC) [H/E-400X]

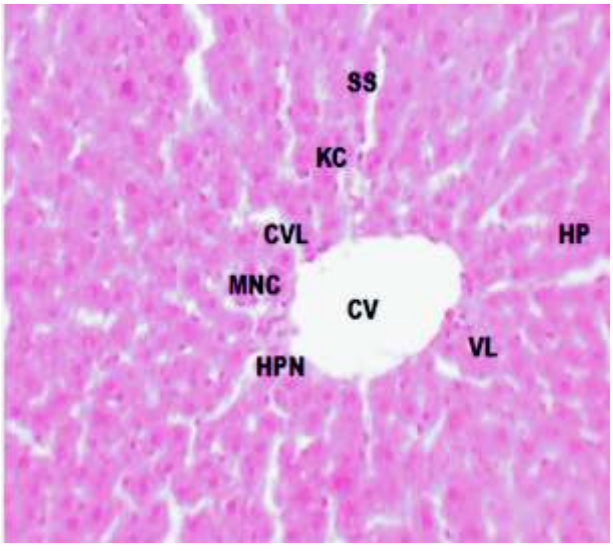


**Fig. 4 -** Photomicrograph of liver alter 21 days treatment with ground water arsenic showing vascular lesions (VL), central vein lesions (CVL), portal cirrhosis (PC), hypertonic nucleus (HPN) and massive necrosis (MNC) [HIE-400X]

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 corelated 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

Ahmad, I., Hussain, T. and Mahmood, A.K. (2008) Arsenic induced microscopic changes in rats testis. *Prokssional Med*., **15,** 287-291.

Baldwin, D.R., and Marshall, W.I. (1999) Heavy metal poisoning and its laboratory investigation. *Annals Clin. Biochem*. **36**, 267-300.

Biswas, N.M., Chowdhury, Roy G., and Sarkar, M. (1994) Effect of sodium arsenite on adreno cortical activities of male rates, does-duration dependent responses, *Med. Sci. Res.* **23**, 153-154.

Biswas, U., Sarkar, S., Bhowmik, M.K., Samanta, A.K. and Biswas, S. (2000) Chronic toxicity of arsenic in goats: clinicobiochemical changes, pathomorphology and tissue residues. *Small Ruminant Res*., **38,** 229-235.

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

cells. *J. Toxicol. Environ. Health* **1,** 504-514.

Chris, Le, X., Mingesheng, Ma, Xiufen, Lu., William, R., Cullen, H., Aposhian, 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.

Ferzand, R., Gadhi, A., Saleha S. and Ali. Q. (2008) Histological and haematological disturbance caused by arsenic toxicity in mice model. *Pak. Biol. Sci*., **11,** 1405-1413.

Ghosh, A., Sarkar, S. Pramanik, A.K. Palchowdhary S. and Ghosh. S. (1993) Selenium toxicosis in grazing buffaloes and its relationship with soil and plants of west Bengal. *Ind. J. of Animal Sci.*, **63,** 557-560.

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.

Jayantha Rao, K. (1984) Effect of systemic Pesticide, Phosphomidon on some aspects of metabolism in fresh water fish, *Tilapia mossambia* (Peters). Ph.D. Thesis. Sri Venkateshwara University, Tirupathi, India.

Kind, P.R.N. and Kings. E.J. (1954) Estimation of alkaline phosphatase in serum. *J. Clin. Path*., **7,** 322-329.

King, E.J. and Jagatheesan, K.A. (1959) Estimation of acid phosphatase in serum. *J. Clin. Path*., **12,** 85-92.

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.

Nabi, A.H.M., Mahfuzur Rahman, M. and Laila Islam,. N. (2005) Evaluation of biochemical changes in chronic arsenic poisoning among Bangladeshi patients. *Int. J. Enviorn. Res. Public Hlth.* **2**, 385-393.

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.

Rietman, S. and Frankel. S. (1957) Estimation of SGOT and SGPT in serum. *Ann. Clin. Path*., **28,** 56-62.

Robert, E.M., and Jud, O.N. (1986) Water and soil pollutant. *New York Macmillan Publishing Company*, pp825.

Sarkar, M, Ghosh, D., and Biswas, H.M. (1992) Effect of sodium arsenite on haematology in male albino rats. *Ind. J. Physiol. Allied. SC.* **46**, 116-120.

Thornton, I., and Farago, M. (1997) The geochemistry of arsenic. 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 diabetogenic effect of arsenic. *Toxicol. Lett.* **133**, 69–76.

Turner, J. C. and Shanks, V. (1980) Absorption of some organochlorine compounds by rat small intestine *in vivo, Bull. Environ., Contam. Toxicol.,* **24** 625-631.