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By

Bonita Gupte, Vanita Gupta, Sangeeta Gupta and Vikrant Singh

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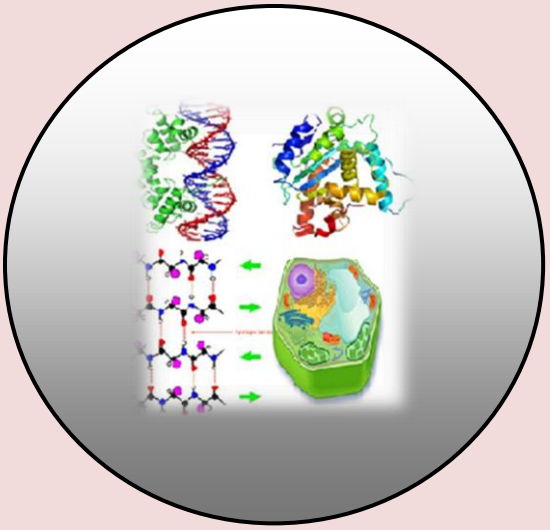

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Dr. Bonita Gupte Dr. Vikrant Singh
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jbiolchemres@gmail.com

RESEARCH PAPER
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Histopathological Effect of Arsenic in Drinking Water on Liver of Albino Rats

Bonita Gupte, Vanita Gupta, *Sangeeta Gupta and **Vikrant Singh
Department of Anatomy, Acharya Shri Chander College of Medical Sciences, Jammu, Jammu and Kashmir
***Department of Anatomy, Government Medical College, Jammu, Jammu and Kashmir**
****Department of Surgical Gastroenterology, Government Medical College, Jammu, Jammu and Kashmir**

ABSTRACT

Arsenic is a common pollutant of water in developing countries and leads to major health problems and clinical conditions. It affects all organs of the body and can be lethal in higher doses. To study the histopathological effects of arsenic in the liver of albino rat and compare it with rats that are not exposed. The study was done on 18 rats, divided in three groups of 6 rats each. Group A acted as control group and received only distilled water, Group B received 50ppm of arsenic in the form of sodium arsenite in drinking water and Group C, received 100ppm of sodium arsenite in drinking water as daily oral dose, each day for 4 weeks. Liver of rats exposed to low dose showed mild central venous dilatation and congestion, portal haemorrhage, dilated portal vein with normal portal architecture. However, liver of rats exposed to higher doses revealed distortion of tissue architecture, haemorrhagic foci, necrosis, vacuolated cytoplasm, mononuclear infiltrate. These changes were much higher than in low dosage exposed rat's liver and rats in the control group showed normal tissue architecture. We conclude from this experimental work that administration of Arsenic to rats in drinking water led to hepatotoxicity.
Keywords: Histopathology, Rat, Sodium Arsenite and Liver.

INTRODUCTION

Arsenic exposure occurs from inhalation, absorption through the skin and, primarily, by ingestion, for example, contaminated drinking water. Arsenic in food occurs as relatively non-toxic organic compounds (arsenobentaine and arsenocholine).

Seafood, fish, and algae are the richest organic sources (Edmonds and Francesconi, 1987). Around 200 million people (NRC 2001) worldwide are at a risk from health effects associated with high concentration of arsenic in their drinking water. Arsenic contamination in drinking water has become a significant concern in Bangladesh, West Bengal, India, China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, DPR Korea, and Pakistan (Mukherjee et al., 2006). The provision of safe drinking water is priority. A variety of methods of diverse complexity are available to remove arsenic from drinking water (Sutherland et al., 2002). The methodology especially in developing countries that is urgently required should be affordable, sustainable by the population and cost effective. Process of precipitation by iron exchange using iron treated gel beads and iron oxide coated sand, is used for removing arsenic from water (McLellan, 2002). Long term arsenic toxicity leads to multisystem disease and the most serious consequence is malignancy. The clinical features of arsenic toxicity vary between individuals, population groups and geographical areas. It is unclear what factors determine the occurrence of a particular clinical manifestation or which body system is targeted (Smith et al., 2004). In chronic arsenic ingestion, arsenic accumulates in the liver, heart, kidney, heart and lungs and smaller amounts in the muscles, nervous system, gastrointestinal tracts and spleen (Benramdane et al., 1999).

Organ specific histological evaluation is currently the gold standard to determine the degree of organ injury during chronic metal exposure. The microscopic structure of liver of albino rats is quite similar to that of human beings. This study is designed to evaluate the histological changes by chronic arsenic exposure on liver architecture in adult Wistar rat.

MATERIALS AND METHODS

In the present study, Albino rats served as experimental animals. Healthy Wistar rats, 18 in number of either sex weighing 125-160 grams were taken for the study. The rats were procured from the Central Animal House of Government Medical College, Jammu. The investigation was conducted upon getting clearance from Institutional Animal Ethics Committee (IAEC).

After two weeks of acclimatization, the rats were randomly divided into three groups a shown in table no 1. Identification number was given to rats of each group. Laboratory conditions (temperature 25 ± 2ºC with 12 hours' light/ 12 hours' dark cycle) with free access to standard pellet diet and water throughout the study. The cages were made of solid plastic sides and base and stainless steel grid top. Rice husk was used as bedding material. The animals were observed for abnormal physical or behavioural changes throughout experimental period.

Chemical used is the study was Sodium Arsenite. It was procured from the AVI Chemicals laboratory. Sodium Arsenite was dissolved in distilled water to obtain desired concentration. Fresh dosing solution was made every time. Experimental animals were given oral Sodium Arsenite, using a 2 cc syringe, fitted with a long hypodermal needle.

Group A-served as control and were administered distilled water orally via a 2cc syringe daily. Group B-rats were administered 50ppm (50mg/kg) of sodium arsenite in distilled water for 4 weeks. First dose was administered on 14/04/2019 and followed for 28 days, daily. Group C-rats were administered 100ppm (100mg/kg) of sodium arsenite in distilled water for 4 weeks. After the first dose was administered on 14/04/2019 and followed for 28 days, daily.

At the end of 4 weeks, the rats were sacrificed and the liver was treated with 10% buffered Formalin solution, passed through ascending grades of ethanol, cleared in toluidine and embedded in paraffin. Tissues were sectioned at 4µm and stained with Haematoxylin and Eosin (H&E). The sections were examined under Light microscope.

Observations

Group A

Light microscopic examination of liver sections of group A rats revealed the normal basic architecture of the liver, showing the hexagonal classic hepatic lobules with central veins located in the centre of the lobule (Fig: 1) and portal areas containing portal triad formed by portal vein, hepatic arteriole and bile ductule surrounded by connective tissue at 3 to 5 corners of the lobule (Fig: 2).

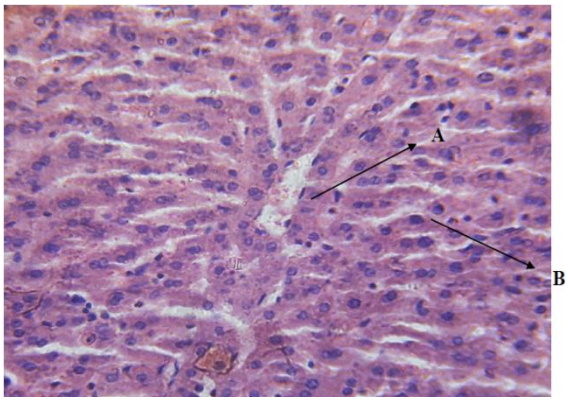

Figure 1. Photograph of slide of Control Liver showing normal architecture with architecture Central Vein (A) and hepatic sinusoids (B). (H and E Stain 400 x)

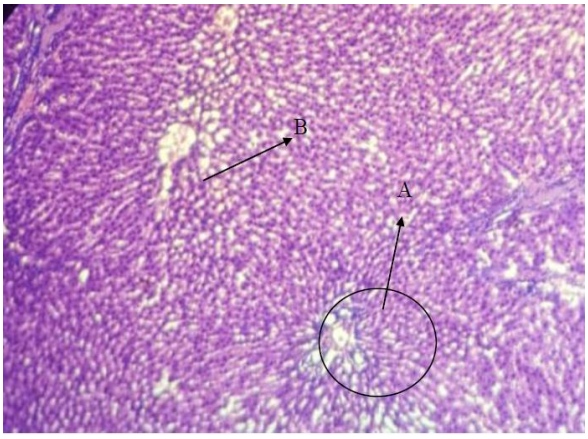

Figure 2. Photograph of slide of Liver of Control group showing normal with portal triad (A) and sinusoids (B). (H and E Stain 100 x).

Within the Classical hepatic lobule, the central veins had a thin connective tissue wall lined internally by endothelial cells and were present in the centre of the lobules. The cords of the hepatocytes which were one cell thick at most of the places were found to be radiating from the central veins (Fig: 1) at the periphery of the lobule which contained the portal areas.

The sinusoids (Fig: 1) were lined mostly by endothelial cells and contained a few kupffer cells. Sinusoids present in the lacunae between the cords of hepatocytes were found to be of normal calibre and contained a few blood cells. The portal areas at the corners of the hepatic lobule contained connective tissue, fibroblasts and stained light pink in colour. Embedded in the connective tissue were seen structures such as portal venule, hepatic arteriole and bile ductule (Fig: 2). The portal vein was thin walled and lined by endothelial cells, the hepatic arteriole was thick walled containing smooth muscle in its wall and lined by endothelial cells and bile duct, lined by cuboidal cell. Hepatocytes were polygonal, stained pink in colour and had centrally placed spheroidal, euchromatic nucleus which stained light blue in colour and contained one nucleolus. Occasionally, hepatocytes containing two nuclei were also seen. Endothelial cells lining the lumen of sinusoids had scanty, pink cytoplasm with a centrally placed flattened nucleus.

Group B

On examination of liver of group B of rats, the basic architecture of liver was found to be preserved; however, various histopathological changes were observed. Focal areas of haemorrhage were seen in the parenchyma. Also there was central venous dilatation and congestion (Fig: 3.4). Dilated and congested portal venules were present in the portal areas (Fig: 5, 6). Severe inflammatory cells were also seen in portal areas and also around the central vein. (Fig: 5, 6). The cord of hepatocytes was one cell thick, as in the control group and had normal radiating pattern. Normal sinusoidal arrangement was seen. Hepatocytes were polygonal, stained pink in colour and had euchromatic nucleus which stained blue. Few hepatocytes were bi-nucleated.

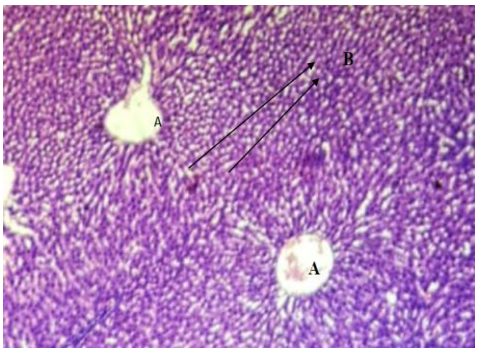

Figure 3. Photograph showing the slide of slide liver of Group B showing Central Vein Dilatation (A) and Mild Dilatation of sinusoids (B). (H and E Stain 100x).

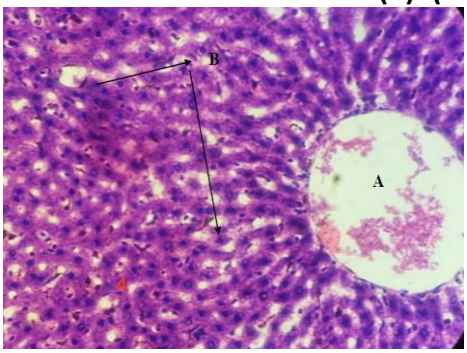

Figure 4. Photograph showing the liver of Group B showing Central Vein Dilatation (A) and Mild Sinusoidal Dilatation (H and E stain 400x).

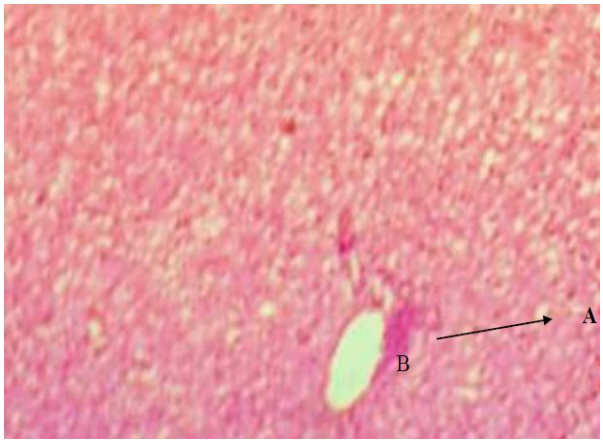

Figure no 5 Photograph of slide of liver of Group of B showing dilated portal vein (A) and Periportal venule (A), Inflammatory cells (B). (H and E Stain 100x).

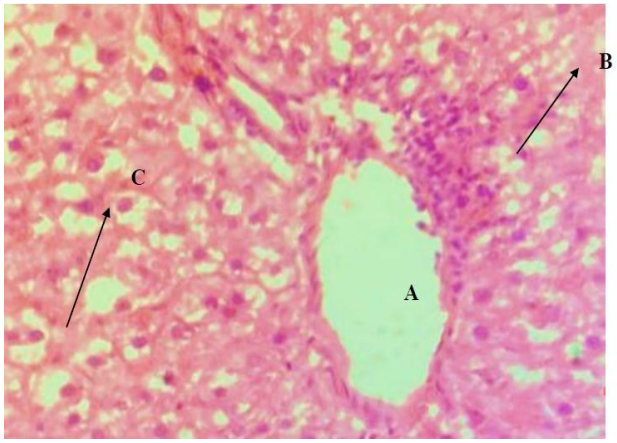

Figure no 6: Photograph of slide of liver Group B showing dilated portal periportal cells of inflammation (B) periportal cells of inflammation (B) and Bi-nucleated hepatocytes (C) (H and E Stain 400x).

Group C

On examination of liver of group C of rats, normal tissue architecture of the liver was disrupted. Markedly dilated central vein and central venous haemorrhage was seen. (Fig: 7) Mononuclear inflammatory cell infiltrate (Fig: 8) was seen, along with the areas of focal haemorrhage.

There were necroinflammatory foci in the liver. Hepatocytes were enlarged, swollen and oedematous, with ill-defined boundaries and irregularly clumped cytoplasm (Fig: 9). Clear spaces known as cloudy swellings which are vacuoles of varying sizes, were seen as small and large empty spaces within the hepatocytes (Fig: 9). Apoptotic cells with hyper eosinophilic cytoplasm, small shrunken, condensed nuclei with increased basophilia (pyknotic nucleus) were also seen at certain places. Some of the hepatocytes showed pleomorphism (varied appearance) and were hyperchromatic. Others displayed karyomegaly (enlarged nuclei), few had karyopyknosis (small condensed nuclei) and others showed karyolysis (dissolution of nuclei).

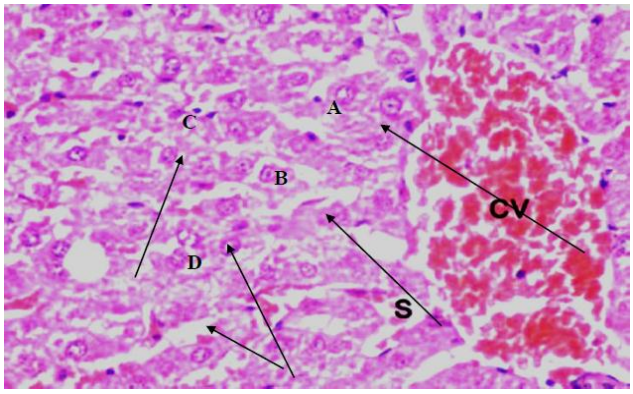

Figure 7. Photograph showing the slide of Liver of Group C, showing markedly dilated and congested central vein (A), dilated and congested sinusoids (B), balloon degeneration of hepatocytes (C) and nucleus with multiple nucleoli (D) (H and E Stain 400x).

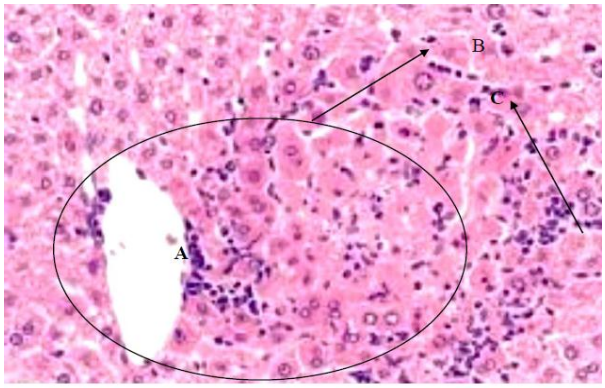

Figure no 8. Photograph of slide of liver of Group C showing central dilated vein (A), loss of radiating hepatocyte cord structure and Mononucleated Inflammatory cells (C) (H and E Stain 400x).

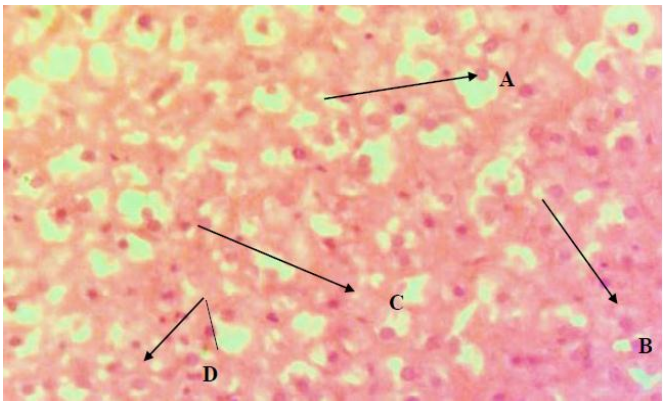

Figure 9. Photograph of slide of liver of Group C, showing Vacuolated cytoplasm (A), hydropic degeneration (B), loss to cell boundary of hepatocyte (C) and small fragment pyknotic nuclei (D) (H and E Stain 400x).

DISCUSSION

Arsenic, one of the high ranking global environmental toxicants, has currently drawn increasing concentration as a major contaminant of food-chain and drinking water.

The present study showed that the exposure to arsenic induced histopathological changes in liver. Liver of Group B displayed mild dilatation of central vein of liver with congestion. These changes are in accordance with the changes seen in the study conducted by Hemalatha P et al, who observed similar changes in the liver, when the rat was exposed to arsenic, as sodium arsenite in the dose of 10mg/kg b.w, every day for 4 weeks. Similar changes are also seen in another study conducted by Singh TS et al, who exposed the rats to sodium arsenite in the dose of 10.2mg/l, daily for 14 days.

In the present study, there is presence of cells of inflammation around the portal venule in the rats of Group B. the changes are in accordance with the study conducted by Chowdhury D.U.S et al, who exposed the rats to sodium arsenite in the dose of 50 ppm for 28 days.

In the present study, there is necrosis of the liver with tissue disruption and loss of hepatic architecture and normal radiating pattern in the rats of Group C. A study conducted by Noman et al (2015) concluded that rats exposed to sodium arsenite in the dose of 10mg/kg b.w for 8 weeks revealed similar results. Another study done by Ibrahim (2007), on rabbits, exposed them to arsenic in the dose of 500nm; daily for 18 weeks also demonstrated the same results.

In the present study, there is marked dilatation of central vein with haemorrhage in rats of Group C. These changes correspond with the changes seen in study conducted by Hemalatha et al (2013) who exposed the rats to sodium arsenite in the dose of 150ppm/day, every day for 4 weeks. These changes are also in accordance with another study conducted by Mohapatra et al (2011), who gave single dose of arsenic trioxide, orally as 30mg/b. w and sacrificed the animal after 96 hours.

In the present study, mononuclear cells of inflammation are seen around portal vein and also elsewhere in the tissue. Similar changes are seen in the study conducted by Hemalatha et al (2012) [7], who gave arsenic orally, dissolved in distilled water as 150ppm/day, daily for 4 weeks. Study done by Al-Forkan et al (2016), also showed similar results in rats exposed to arsenic in the dose of 50ppm, daily for 90 days.

In the present study, liver of group C revealed sinusoidal dilatation. This change is in accordance with the study conducted by Hemalatha et al (2012), which revealed similar results in rats exposed to arsenic in the dose of 150ppm, daily for 28 days. Similar results were seen in the study conducted by Al-Forkan et al (2016), with arsenic exposed rats in the dose of 50ppm, daily for 3 months. These changes also correspond with the changes seen in the study conducted by Mehta et al (2015), in rats of all test groups receiving daily dose of 10, 20 and 50ppb of arsenic in water for 30 days. In the present study, liver of Group C revealed areas of tissue haemorrhage which is in accordance with the study conducted by Tandon et al (2012), who exposed the rats to sodium arsenite in the dose of 20mg/kg b.w for 6 weeks. These observations are also in agreement to those seen in the study conducted by Al-Forkan et al (2016), who exposed the rats to arsenic in the dose of 150 ppm, daily for 90 days. In the present study, liver of Group C revealed vacuolated cytoplasm of hepatocytes with ballooning degeneration and cellular oedema. These changes are in agreement with the changes seen in the experiment done by Mohapatra et al (2011), exposed the rats to single dose of arsenic trioxide as single dose (30 mg/kg b.w).

Also, these changes are in accordance with the study done by Tandon et al (2012), who exposed the rats to arsenic (20mg/kg), daily for 6 weeks. Similar changes were seen in the study conducted by Devaraju et al (2010) [16] who gave a single sub-lethal dose of arsenic (4.2mg/kg b.w) to rats of test group. Another study done by Singh et al (2012), who administered arsenic as 0.02mg/litre for 21 days, revealed similar findings.

In the present study, hepatocytes of liver of Group C have Nuclear Pleomorphism and Pyknotic bodies, which is also seen in the study done by Choudhury et al (2016), who gave arsenic in the dose of 150ppm, daily for 28 days to the rats. These changes are in accordance with the study done by Mohapatra et al (2011), who exposed the rats to a single dose of arsenic trioxide (30mg/kg b.w). Nuclear pleomorphism was also present in the study done by Devaraju et al (2010), who exposed the rats to a single sub-lethal dose of arsenic (4.2mg/kg). In a study done by Hemalatha et al (2011) who gave the same dose of Sodium arsenite in distilled water as in our study. However, this study concluded mild fatty changes along with necrosis, which was not in accordance with our study. Another study done by Amer et al (2016) revealed periportal fibrosis, when the rats were exposed to single sub-lethal dose of arsenic (40mg/kg), which was not seen in our study.

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Corresponding Author: Dr. Bonita Gupte, Department of Anatomy, Acharya Shri Chander College of Medical Sciences, Jammu, Jammu and Kashmir
Email: bonitagupte1@gmail.com