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## Histological and Haematological Disturbance Caused by Arsenic Toxicity in Mice Model

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**Abstract:** Histological and hematological disturbance caused by Arsenic containing water were studied in mice model. Animal were divided into four groups. Control group exposed to arsenic free distilled water and 3 treatmental group exposed to the arsenic containing water with 30, 150 and 300 ppb. Blood samples and organs were collected after 40 days. Histopathological results revealed mild to severe type of necrosis and degenerative changes in kidney and liver of arsenic feed animals. Kidney of the 300 ppb group showed severe type of necrosis and degenerative changes in distal and proximal tubules. The renocytes of proximal and distal tubules were showing hydropic and fatty degeneration. Due to degenerative changes cells were showing cytoplasmic vacuolation and cytoplasmic and nuclear blebbing. Glomeruli cells were contracted and increased the bowman's spaces. 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. Spleenocytosis occurred in spleen and the parenchymal and mesenchymal cells were replaced by connective tissue. The lymphocytes were severely damaged by arsenic toxicity. White Blood Cells (WBCs), Red Blood Cells (RBCs) and hemoglobin level in control groups were in normal range where as level were significantly decreased with the increase dose of arsenic in the respective treatmental groups. The data was analyzed statistically and was found that significant was found among the group ( $p < 0.05$ ).

**Key words:** Arsenic toxicity, mice, histopathology, haematology

### INTRODUCTION

Drinking water is derived from a variety of sources depending on local availability. These resources include surface water, ground water and rainwater. The presence of arsenic varies in each source. Higher arsenic contamination is found in ground water as a result of the influence of water-rock interactions or where favorable physical and geochemical conditions are present for arsenic mobilization and accumulation in the aquifer. In natural water arsenic is mostly found in the form of trivalent arsenite As (III) or pentavalent arsenite As (V) (Department of Environmental Quality, 2003).

Pulmonary absorption of soluble forms, such as arsenic trioxide, is rapid while less soluble forms may reside in the lower airways and be absorbed during a prolonged period of time (Marafante *et al.*, 1987). Pentavalent arsenic compounds are almost totally absorbed (till 90%) in most species. The absorption of trivalent arsenic is limited, although the toxicity is greater because of the high lipid solubility (Mahieu *et al.*, 1981).

Experimental studies have indicated that the liver is an important site of arsenic methylation, especially following ingestion, when the absorbed arsenic initially passes through the liver (Marafante *et al.*, 1985). The biomethylation of inorganic arsenic to Dimethylarsinic acid occurs via alternating reduction of pentavalent arsenic to trivalent and addition of methyl groups (Hirata and Tanaka, 1990). Arsenite methyltransferase activity has been detected *in vitro* in liver from rabbit, rat, mouse, hamster, pigeon and rhesus monkeys (Zakharyan *et al.*, 1995; Aposhian, 1997).

Arsenic is difficult to detect while ingesting, as it is tasteless and odorless. The effects are not immediately visible and people can absorb significant quantity of arsenic without any immediate health complications. Local effects in acute poisoning by inhalation are irritation of the respiratory tract, rhinitis, pharyngitis, laryngitis and tracheobronchitis, with cough, pain during inspiration and dyspnoea (Hathaway *et al.*, 1991). Massive inhalation and swallowing of substantial amounts of crude arsenic dust (more than 80% As<sub>2</sub>O<sub>3</sub>) are responsible for the death,

within several hours, of a worker. At autopsy, trachea and main bronchi shows widespread mucosal and submucosal haemorrhages and there is intense visceral congestion. Breathing inorganic arsenic increases the risk of lung cancer (Tahir, 2000).

Patients may develop hepatomegaly, jaundice, portal hypertension or pancreatitis caused by the direct effect of arsenic. However, Labadie *et al.* (1990) suggested that arsenic induced hepatic injury is caused by vascular and not hepatocellular damage. Jaundice, is described after prolonged arsenical medication (Ishinishi *et al.*, 1986). Renal failure is caused by vasodilatation leading to increased glomerular filtration and capillary permeability. The resulting protein leakage causes acute tubular necrosis or diffuse interstitial fibrosis (Cullen *et al.*, 1995). Acute renal tubular necrosis and also cortical necrosis is reported in severe acute poisoning (Agency for Toxic Substances and Disease Registry, 1989).

The world health organization (WHO) guideline value for arsenic in drinking water is  $10 \mu\text{g}^{-1}$  that is 10 ppb (WHO, 1993), although this level leads to increase lifetime skin cancer risk. WHO has mentioned that a concentration of  $0.05 \text{ mg L}^{-1}$  of arsenic in drinking water is not associated with any adverse health effects. Recommended limit of Pakistan Standards Quality Control Authority (PSQCA) for arsenic in drinking water is 50 ppb.

In Bahawalpur District water of 5 tehsils were found to be unsafe due to high arsenic concentration of 10 ppb (WHO, 1993), however 2.63% were declared as unfit if we followed PSQCA standard in Bahawalpur District. In Rahim Yar Khan District 19.12% water samples exceeded the limit of 10 ppb where as 3.02% samples exceeded the arsenic concentration limit of 50 ppb in Multan city 37.61% of water samples exceeded the limit of 10 ppb and 2.87% water samples exceeded the arsenic contamination limit of 50 ppb. In Pakistan the highest concentration of arsenic is present in village Bhind Waas of Multan district and the concentration is 328 ppb which is very high value and it is toxic for people living there (Kahlown *et al.*, 2003). In different areas of Pakistan the level of arsenic in water is more than the safety guidelines of WHO and also exceed from Pakistan Standards Quality Control Authority guidelines. Arsenic is very hazardous to the health that's why this present study has been conducted by using mice as experimental animal.

## MATERIALS AND METHODS

Present study were designed in 2007 to found out histological effects of arsenic toxicity in different organs and hematological disturbances in mice model, for this study 20 mice, 30 g of weight were used at animal house

in National Veterinary Laboratories (NVL) Islamabad. The mice were further divided into four groups i.e., group 1, group 2, group 3 and group 4 and each group containing 5 mice. The experimental group was provided with the arsenic free water to control group and treated group were given arsenic water in concentration of 30, 150 and 300 ppb, respectively.

The mice were anesthetized by ether after 40 days. Blood was collected by cardiac puncture before sacrifice for complete blood picture and for this purpose Auto-Hematology Analyzer (Backmann, USA) was used. The Liver, kidneys, spleens were removed and were fixed in 10% buffered formalin for histological examination. These were processed, microtomed at  $5 \mu$  and stained with haemotoxylin and eosin (H and E) stain.

## RESULTS AND DISCUSSION

**Histopathological changes in kidney:** The kidneys of the control group were showing no histopathological changes, the bowman's capsular or renal spaces were remained normal (Fig. 1). The kidney showed varied degree of the gross lesion of hemorrhages and milder to dark plum color appearance. The renocytes of the proximal and distal tubules were showing mild hydropic and fatty degeneration of the cells in the group that is exposed to 30 ppb arsenic containing water (Fig. 2). Moderate degenerated cells were showing cytoplasmic vacuolation and cytoplasmic blebbing. Necroses of the convoluted, distal and proximal tubules were in abundance. The necrosed cells were exposed to coagulated and liquifactive types of necrosis. The bowman's capsular spaces were expanded due to the shrinkage of the glomeruli cells in group exposed to spiked 150 ppb arsenic water (Fig. 3). Cytoplasm has granular appearance due to the hydropic and fatty degeneration showing vacuoles. The group that is exposed to water spiked with 300 ppb of arsenic showing severe hydropic and fatty degenerative changes. Due to the hydropic and fatty degenerative changes the cells of the tubules were turgid and occluding the lumen of the tubules. The renocytes of the proximal and distal tubules were showing severe hydropic and fatty degeneration of the cells. Due to severe degenerative changes cells were showing cytoplasmic vacuolation and cytoplasmic blabbing and nuclear blabbing were also observed (Fig. 4). The brush borders of the convoluting tubules were deciliated in varied degree of necrosis. Necrosed patches were in abundance. Glomeruli cells were contracted in the center and increased the bowman or renal spaces.

The kidneys showed necrosis, hydropic and fatty degenerative changes in the distal and proximal tubules.

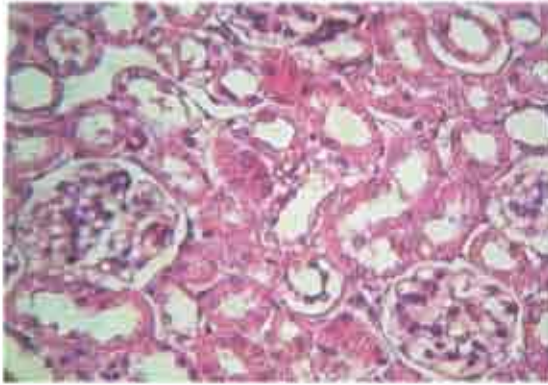


Fig. 1: Kidney of mice of control group showing no necrotic and degenerative changes (1600X H and E)

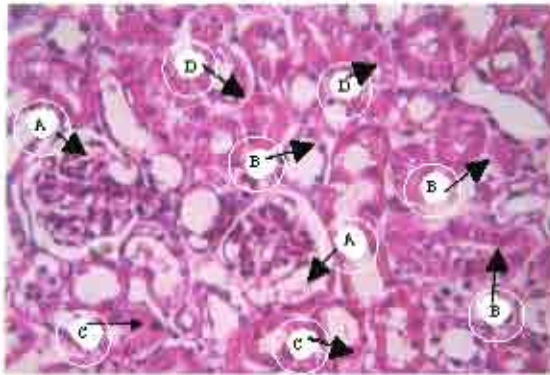


Fig. 2: Kidney of mice showing varied degree of necrotic and degenerative changes exposed to 30 ppb of arsenic through daily water. Shrinkage of glomeruli and mild expansion of bowman's capsule (A) coagulative and mild ligifactive necrosis (B) hyotopic and fatty degenerative changes in distal tubules (C) hydropic and fatty degenerative changes in proximal tubules (D) mild cytoplasmic blabbing (G) (1600 X H and E)

It could be due to increase glomerular filtration and capillary permeability by arsenic toxicity as a result of which leakage of proteins occurs that cause tubular necrosis as also observed by Cullen *et al.* (1995). In this study severe degenerated cells were observed that showed cytoplasmic vacoulation or blebbing. It might be due to degradation of cytoplasmic material specially denaturation of proteins components which produce vacuoles in the cytoplasm. In the kidney shrinkage of glomerulus and increase in Bowman's spaces were observed it is due to infiltration of liquid material from glomeruli to Bowman's spaces as also evident by Roy and Bhattacharya (2006).

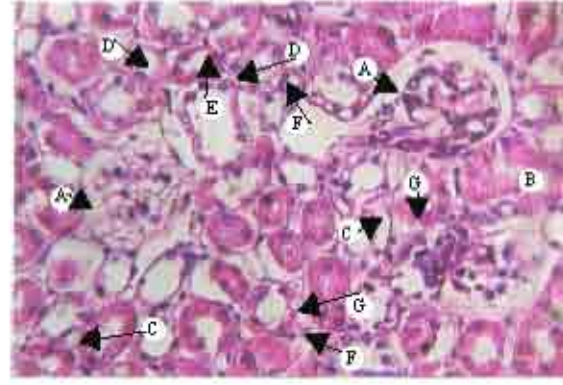


Fig. 3: Kidney of mice showing necrotic and degenerative changes exposed to 150 ppb of arsenic through daily water. Shrinkage of glomeruli and moderate expansion of bowman's capsule (A), coagulative and moderate liquifactive necrosis (B), moderate necrosis, hydropic and fatty degenerative changes in distal tubules (C), hydropic and fatty degenerative changes in proximal tubules (D), nuclear blabbing (F), moderate cytoplasmic blabbing (G), (1600X H and E)

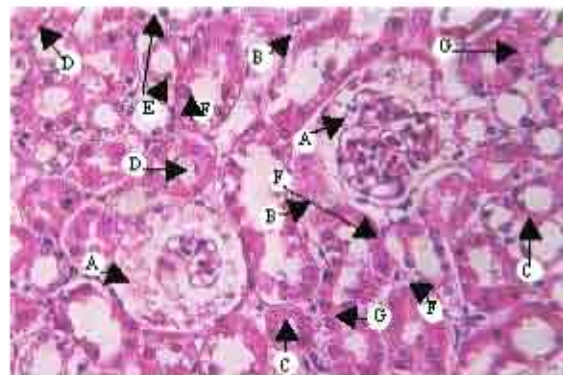


Fig. 4: Kidney of mice exposed to 300 ppb of arsenic through daily water. Severe shrinkage of glomeruli and expansion of bowman's capsule (A), severe coagulative and liquifactive necrosis (B), severe hydropic and fatty degenerative changes in distal tubules (C), severe type of necrosis, hydropic and fatty degenerative changes in proximal tubules (D), severe nuclear blabbing (F), severe cytoplasmic blabbing (G), (1600X H and E)

**Histopathological changes in liver :** Liver were showing main gross lesions of hemorrhages. The liver was showing varied degree of necrosis and degenerative changes in the hepatocytes and central vein. The cells of the liver were showing mild hydropic and fatty degeneration of the cells (Fig. 5). The degenerated cells



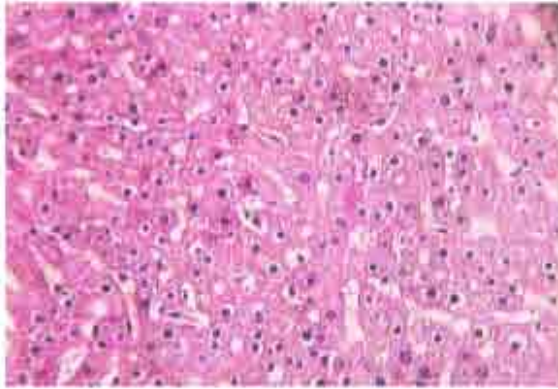


Fig. 5: Liver of mice of control group showing no necrotic and degenerative changes. Histology is almost normal (1600 X H and E)

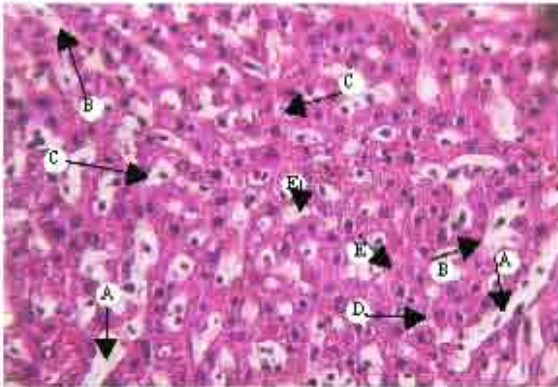


Fig. 6: Liver of mice showing mild degree of necrotic and degenerative changes exposed to 30 ppb of arsenic through daily water. Expansion of sinusoidal spaces (collecting ducts) (A), mild necrosis of hepatocytes (B), free nuclei (C), mild degree of nucleus blabbing (D), mild cytoplasmic blabbing due to hydropic and fatty degeneration (E), (1600X, H and E)

were showing mild cytoplasmic vacuolation and cytoplasmic blabbing. Necrosis of the hepatocytes and lymphatic cells were in small number. The necrosed cells were exposed to low level of coagulative and liquifactive type of necrosis. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatic cells (Fig. 6). Necrosed patches were in small number, hemorrhages were not frequent through out the organ. Cytoplasm has granular appearance due to the hydropic and fatty degeneration, showing vacuoles.

The group exposed to water containing 150 and 300 ppb of arsenic revealed moderate and severe type of histopathological changes. Degenerated cells were

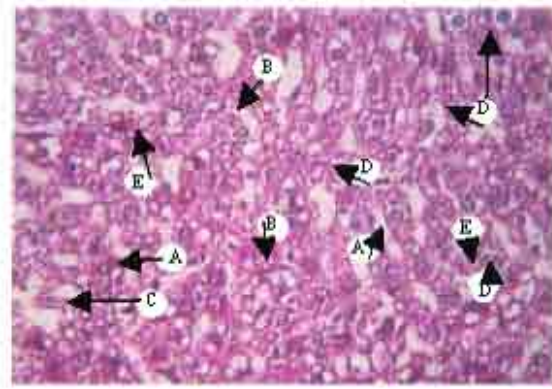


Fig. 7: Liver of mice showing moderate degree of necrotic and degenerative changes exposed to 150 ppb of arsenic through daily water. Moderate expansion of sinusoidal spaces (collecting ducts) (A), moderate necrosis of hepatocytes (B), free nuclei (C), Moderate degree of nucleus blabbing (D), moderate cytoplasmic blabbing due to hydropic and fatty degeneration (E), (1600X, H and E)

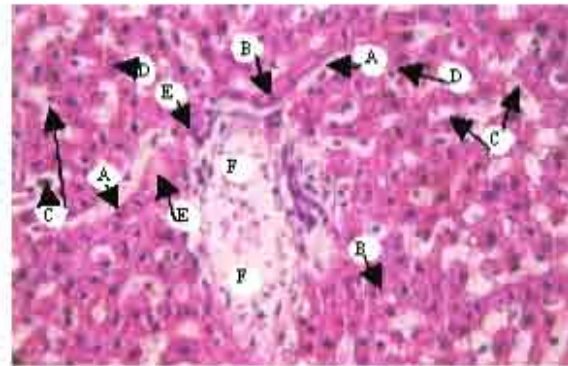


Fig. 8: Liver of mice exposed to 300 ppb of arsenic in daily water. Expansion of sinusoidal spaces (collecting ducts) are severe and in abundance (A), severe necrosis of hepatocytes (B), free nuclei (C), nucleus vacuolation or blabbing (D), severe cytoplasmic vacuolation (blabbing) due to hydropic and fatty degeneration (E), central vein in showing necrosis of epithelium (1600X, H and E)

showing severe cytoplasmic vacuolation and cytoplasmic blabbing. Necrosis of the hepatocytes and lymphatic cells were in large numbers. The necrosed cells were exposed to moderate and high level of coagulative and liquifactive types of necrosis. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatic cells. Necrosed patches were in small number, hemorrhages were frequent through out the organ (Fig. 7, 8).



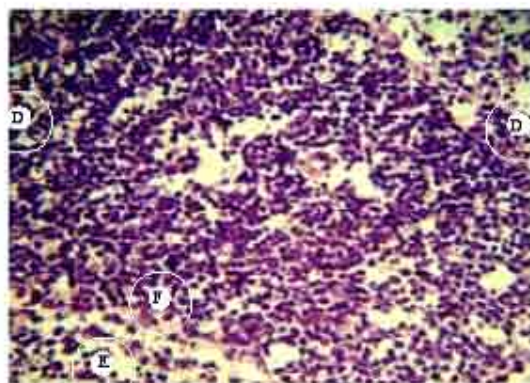


Fig. 9: Spleen of mice of control group showing no necrosis and no connective tissues proliferation (1600X, H and E)

Hepatic necrosis may be due to oxystress induced by arsenic that further involved in the cellular protein degradation. The sinusoidal spaces were expanded due to shrinkage and necrosis of hepatic cells because arsenic increases the permeability from which infiltration of cellular material take place. Santra *et al.* (2007) conducted a study on arsenicosis and their results are parallel to this study and they concluded that gross lesions and apoptosis of liver cells were due to oxidative stress in mitochondria which plays important role in the pathogenesis of arsenic induced apoptotic liver cell injury. Arsenic increases the level of oxygen reactive species which induced apoptosis of the cells as also evident by the study of Somia *et al.* (2006) and Gupta *et al.* (2007). It was observed that nuclear blabbing both in liver and kidney cells which might be due to cleavage of nuclear material by arsenic as also evident by the research of Somia *et al.* (2006).

**Histopathological changes in spleen:** Mice exposed to water spiked with 30 ppb. The spleen was showing mild degree of necrosis and degenerative changes in the spleenocytes and lymphocytes (Fig. 9). The cells of the spleen showed mild hydropic and fatty degeneration. The degenerated cells showed mild cytoplasmic vacuolation and cytoplasmic blebbing. Necrosis of the spleenocytes and lymphatic cells were mild. The necrosed cells were exposed to mild coagulative and liquifactive type of necrosis. There were mild numbers of free nuclei due to cell necrosis. Cells of spleen were showing mild hydropic and fatty degenerative changes. Parenchymal cells and mesenchymal cells were replaced by connective tissues but the condition is mild there was indication of connective tissues proliferation (Fig. 10).

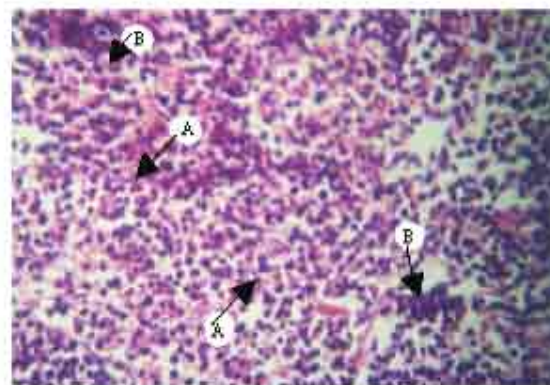


Fig. 10: Spleen of mice exposed to 30 ppb of arsenic showing varied degree of necrosis, initial or very mild degree of connective tissues proliferation (A) mild necrosis of spleenocytes (B) (1600X, H and E)

In experimental animals exposed to spiked water with 150 ppb of arsenic the spleen was showing moderate degree of necrosis and degenerative changes in the spleenocytes and lymphocytes. The cells of the spleen showed moderate hydropic and fatty degeneration. The degenerated cells showed moderate cytoplasmic vacuolation and cytoplasmic blebbing. Necrosis of the spleenocytes and lymphatic cells were moderate. The necrosed cells were exposed to moderate coagulative and liquifactive types of necrosis. Free nuclei due to cell necrosis were also observed. Parenchymal cells and mesenchymal cells were replaced by connective tissues the condition was moderate there was varied degree of connective tissues proliferation and lymphocytosis (Fig. 11).

In experimental animals exposed to water spiked with 300 ppb of arsenic showing severe type of necrosis and degenerative changes in the spleenocytes and lymphocytes. The cells of the spleen showed severe hydropic and fatty degeneration. The degenerated cells showed severe cytoplasmic vacuolation and cytoplasmic blebbing. Necrosis of the spleenocytes and lymphatic cells were severe. The necrosed cells were exposed to severe coagulative and liquifactive types of necrosis. There were severe numbers of free nuclei due to high number of cell necrosis. Cells of spleen were showed severe hydropic and fatty degenerative changes. Parenchymal cells and mesenchymal cells were severely replaced by connective tissues the condition was very severe. There was varied degree of connective tissues proliferation, which was shown by fiber like appearance (Fig. 12). Cytoplasm due to hydropic and fatty



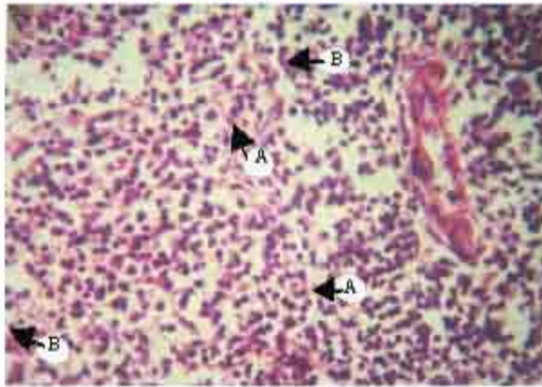


Fig. 11: Mice exposed to 150 ppb of arsenic showing varied degree of necrosis and other degenerative changes, moderate level of connective tissues proliferation (A) moderate necrosis of splenocytes (B) (1600X, H and E)

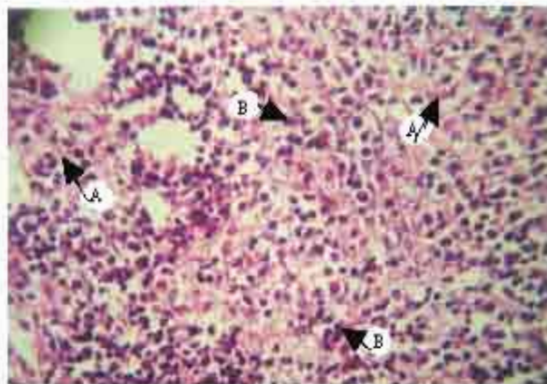


Fig. 12: Mice exposed to 300 ppb of arsenic showing necrosis and other degenerative changes, in splenocytes frequently, severe degree of connective tissues proliferation (A) severe necrosis of splenocytes (B) (1600X, H and E)

degeneration, showing vacuoles which gave the cytoplasm granular appearance. Due to this entire disturbance there was severe disturbance in spleen functional activity. The lymphocytes were severely damaged by the arsenic toxicity.

Sakurai *et al.* (2004) studied the effects of arsenic on spleen and their results are related to this study and both studies confirmed that spleen showed splenocytosis and proliferation of connective tissues. The effect of arsenic was not pronounced on spleen as compared to other organs like liver and kidney.

**Hematological disturbance due to arsenic toxicity:** Blood were also analyzed for the complete picture (CP) of control

Table 1: Hematological studies of control and arsenic exposed group

Parameter	Experimental value				Normal values
	Control	30 ppb	150 ppb	300 ppb	
WBC $10^9/L$	05.50	04.95	03.35	03.10	06.40-26.20
RBC $10^{12}/L$	06.91	05.51	04.03	02.75	07.00-10.00
Hgb (g $dL^{-1}$ )	09.10	08.94	06.82	04.70	10.00-17.50
Hct (%)	27.86	25.02	21.05	16.20	35.00-51.00
MCV (fl)	59.00	58.44	52.97	51.87	57.00-65.00
MCH (pg)	17.00	16.78	16.01	16.00	15.00-22.00
MCHC (g $dL^{-1}$ )	32.01	31.10	29.30	27.01	30.00-35.00
Plt $10^9/L$	462.40	717.50	372.00	359.75	190.0-1000

and arsenic exposed mice and results showed blood composition were significantly disturbed in arsenic feed groups. White blood cells (WBCs) level in control groups were in normal range 05.50 ( $10^9/L$ ) where as level were significantly decreased as 04.95, 03.35 and 03.10 ( $10^9/L$ ) in Group 2, 3 and 4, respectively. RBCs level were recorded as 06.91, 05.51, 04.03 and 02.75 ( $10^{12}/L$ ) in group 1, 2, 3 and 4, respectively, same kind of results were also recorded for hemoglobin (Hgb) as 08.94, 09.10, 06.82 and 04.70 g  $dL^{-1}$  in group 1, 2, 3 and 4, respectively. Hematocrit percentage was recorded as 27.86, 25.02, 21.05 and 16.20% in group 1, 2, 3 and 4, respectively, platelets level were recorded as 462.4, 717.50, 372.00 and 359.75 ( $10^9/L$ ) (Table 1).

In this study the effect of arsenic on blood was studied and it was observed that complete picture (CP) of blood was significantly disturbed by arsenic toxicity. The White blood cells (WBCs) level was decreased in arsenic feed groups. It might be due to apoptotic effect of arsenic on plasma cells as also studied by Rousselot *et al.* (2004). In the same type of studies carried by Breton *et al.* (2006) and Gupta and Flora (2006) and they examined the hematological alteration due to arsenic and their results are correlated with our study that red blood cells (RBCs) and hemoglobin level is decreased with increase concentration of arsenic. This could be due to binding ability of arsenic to hemoglobin that led to inhibition of hem synthesis pathway. It was observed during the study that RBCs, WBCs and hemoglobin level were disturbed then automatically other parameters like hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration were also be disturbed because these are dependent on the former parameters. Only platelets were not disturbed by arsenic exposure. The preferential effect of arsenic on B cells and monocytes could account for the immunotoxic effects of these metals on the humoral immune system same effects have been found by Fuente *et al.* (2002).

The data was statistically analyzed by ANOVA through which it was observed that mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration have no significant difference ( $p > 0.05$ ) while white blood cells, red blood cells, hemoglobin,

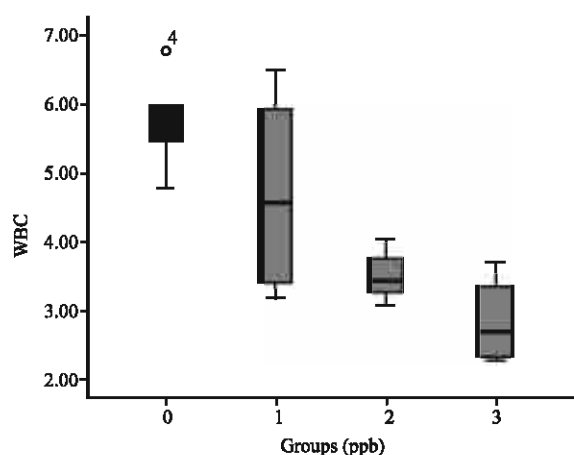


Fig. 13: Box-plot of statistical data of WBC's

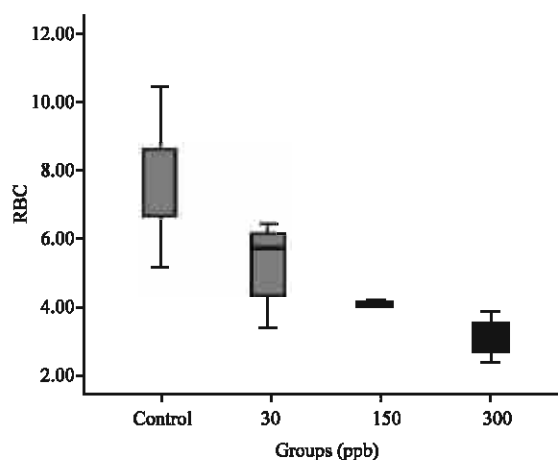


Fig. 14: Box-plot of statistical data of RBC's

hematocrit and platelets have ( $p < 0.05$ ) significantly different values so for these LSD Test was further applied to calculate the significant difference at 0.05 level among different groups. In case of WBC's the control group is only significantly different from 150 ppb and 300 ppb groups ( $p < 0.05$ ). Similarly 30 ppb group is only significantly different from 300 ppb group ( $p < 0.05$ ). There was no significant difference ( $p > 0.05$ ) between control and 30 ppb group. The 30 ppb group is non significant to 150 ppb, while there is no significant difference between 150 and 300 ppb groups ( $p > 0.05$ ) (Fig. 13). The italic and bold words show significant difference at  $p = 0.05$  level. The data was represented by BOX plot. Similar procedure was applied for RBC's, hemoglobin, Hematocrit and platelets. In case of RBC's that 30 ppb is significantly different from control and 300 ppb group ( $p < 0.05$ ). There is significant difference between 150 ppb and control group while 300 ppb is significantly different from control and 30 ppb group ( $p < 0.05$ ) (Fig. 14). For hemoglobin 30 ppb group is significantly different from 150 and 300 ppb group ( $p < 0.05$ ). 150 and 300 ppb are significantly different from control and 30 ppb group ( $p < 0.05$ ) (Fig. 15). In case of Hematocrit control and group of 30 ppb both were significantly different from 50 and 300 ppb group ( $p < 0.05$ ). While 50 ppb group had significant different values from control and 30 ppb groups. 300 ppb is significant different from control and 30 ppb group while no significant difference was found with 150-ppb group ( $p > 0.05$ ) (Fig. 16). Statistical analysis of platelets readings showed that 30 ppb group was significantly different from rest of the groups, while 150 and 300 ppb group were only significantly different from control and 30 ppb group ( $p < 0.05$ ) (Fig. 17).

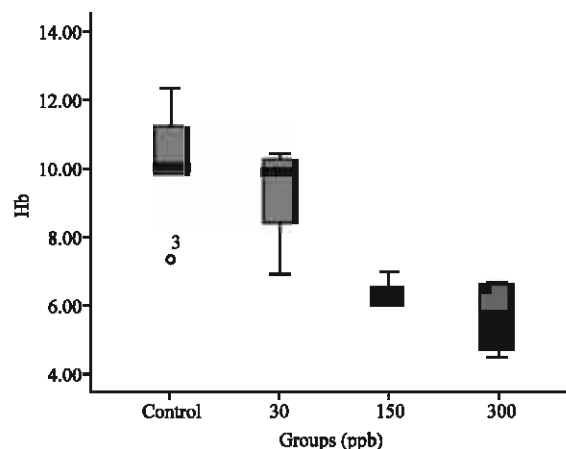


Fig. 15: Box-plot of statistical data of Hb

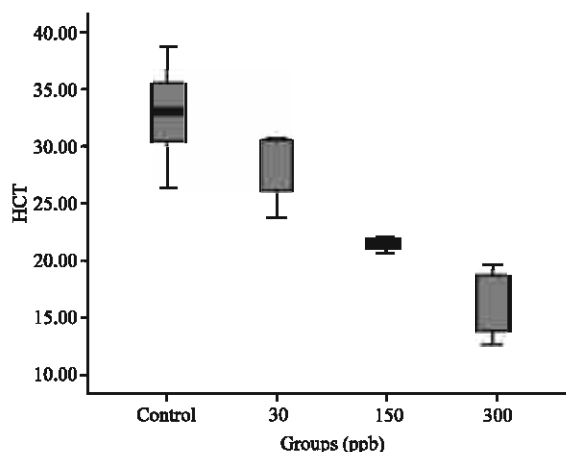


Fig. 16: Box-plot of statistical data of HCT



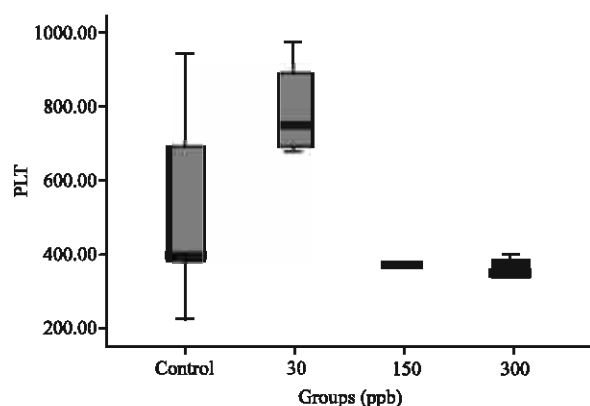


Fig. 17: Box-plot of statistical data of PLT

## CONCLUSION

This study concluded that arsenic contaminated water is very hazardous to the health of human as well as to livestock. The drinking water must not contain the toxic level of arsenic. Significant intervention and work has required detecting the level of arsenic in drinking water in all over Pakistan, according to PCRWR-UNICEF report arsenic contamination in ground water of various parts of southern Punjab is greater than guideline values. Finally it is concluded that survey of other areas of Pakistan especially the areas where deep well water is using for drinking purpose should be performed. There should be Arsenic Level Directory and data base of different areas of Pakistan, which would help to make design for water supply so that the people and livestock are provided with almost arsenic free water and necessary information on arsenic contamination.

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