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RESEARCH PAPER

Efficacy of Lime in Ameliorating Arsenic-Induced Toxicity in Swiss Albino Mice

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ABSTRACT

This study investigates the hepatotoxic effects of arsenic and the potential ameliorative role of lime in mice. The experiment involved three groups: a non-treated control group, an arsenic-treated group that received arsenic (5 mg/kg body weight/day for 14 days), and an arsenic with lime treated group which received both arsenic and lime supplementation (25 mg/kg body weight/day for 14 days). Post-treatment, liver function was assessed through enzyme assays measuring AST, ALT and ALP levels and additionally liver, kidney and testes tissues were examined histologically to evaluate configurational modifications. Results indicated significant elevation of liver enzyme levels in the arsenic group compared to the control, signifying hepatic injury, with histological findings showing severe hepatocyte necrosis, inflammation, and early fibrosis. Conversely, the arsenic with lime treated group showed markedly lower enzyme levels and improved histological features, with reduced necrosis, inflammation and fibrosis, suggesting a protective effect of lime against arsenic-induced liver damage. Arsenic exposure caused structural alterations in the kidneys, changes in periglomerular space, eosinophilic casts, and mononuclear infiltration and causes adverse effects on testicular cells, increase Reactive Oxygen Species (ROS), Leydig cell loss, decreased sperm quality, potential leading to infertility. These findings underscore the severe hepatotoxicity induced by arsenic and suggest that lime's antioxidant properties may reduce oxidative stress and inflammation, thereby protecting liver and kidney function and improving sperm quality. The study implies that the antioxidant properties of lime might contribute to its effectiveness in mitigating arsenic-induced toxicity.

Keywords: Arsenic toxicity, Liver enzymes, Inflammation, Histopathology, Lime supplementation

Heavy metals, integral to the earth's crust, persist indefinitely due to their indestructible nature. Their toxicity and accumulation in organisms and the food chain pose severe biological risks (Tewari *et al*. 2019). Among heavy metals, arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are known for their severe toxicity. Once introduced into ecosystems, they persist for long durations and pose significant

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health risks to humans and wildlife due to their non-degradable nature. Arsenic is the most abundant pollutant and a potential human carcinogen with a complex metabolism (Roy and Saha, 2002). Chronic exposure to arsenic, primarily through contaminated water and food, is associated with numerous serious health conditions, including cancers, cardiovascular diseases, and significant liver damage (Rahman *et al*. 2009; Maity *et al.* 2017). Arsenic exists in both organic and inorganic forms, with the inorganic forms, particularly arsenite (As^{3+}) and arsenate (As^{5+}), being more toxic. These inorganic forms are highly reactive and disrupt cellular functions by generating reactive oxygen species (ROS), which lead to oxidative stress, impairing enzymatic activities, and causing DNA damage (Mukherjee *et al.* 2019).

Arsenic was selected for this study due to its well-documented toxicity in humans and animals, particularly affecting the liver, kidneys, and testes. The liver, as the central organ for detoxification, is especially susceptible to arsenic-induced toxicity. It is responsible for processing harmful substances and converting them into less toxic forms that can be excreted through bile or urine. Given the liver's crucial role in metabolism and detoxification, it is particularly prone to the detrimental effects of arsenic-induced oxidative stress (Flora *et al*. 2017). Prolonged exposure to arsenic can result in hepatomegaly, hepatic fibrosis, and even cirrhosis, highlighting the liver's vulnerability (Mazumder *et al*. 2001; Santra *et al*. 2000). Additionally, arsenic interferes with the liver's ability to regulate enzymatic activities, which can lead to elevated levels of liver enzymes such as AST, ALT, ALP - key biomarkers indicating liver injury. Other serious health problems include diabetes, liver, kidney, and CNS disorders (Vahidnia A *et al*. 2007). The kidney, responsible for filtering waste products and toxins from the bloodstream, also suffers from arsenic toxicity, resulting in nephrotoxicity, impaired renal function, and even chronic kidney disease (CKD) (Liu *et al*. 2019). Arsenic concentrates in the kidney during its urinary elimination, affect the function of proximal convoluted tubules. Robust correlations exist between indicators of renal damage and the advancement of chronic kidney disease due to arsenic exposure (Moon *et al*. 2008, Yamauchi and H, 1994; Parrish AR *et al*. 1999). Arsenic acquaintance in experimental rats has revealed to yield steroidogenic disfunction leading to damage of spermatogenesis (Sarkar M *et al*. 2003). The testes, as the male reproductive organ, are affected by arsenic through the disruption of spermatogenesis, hormonal imbalances, and increased oxidative stress, contributing to reproductive toxicity (Wang *et al*. 2016). These three organs are crucial in maintaining overall physiological homeostasis, and their dysfunction due to arsenic exposure can lead to systemic health problems.

Antioxidants and thiols are vital for metabolizing and excreting heavy metals, assisting in arsenic methylation and binding to metallothionein. Arsenic-induced oxidative stress is a key factor in tissue damage and organ degeneration. Given the health risks of arsenic exposure, natural antioxidants have gained attention as potential protective agents to reduce arsenic-related oxidative damage. Among these, lime (*Citrus aurantiifolia*) has been recognized for its hepatoprotective properties, largely due to its high content of vitamin C and flavonoids. Vitamin C is a potent antioxidant known for neutralizing ROS and enhancing the body's overall antioxidant defense system (Carr *et al*. 2017). Flavonoids, which possess anti-inflammatory and antioxidant activities, have been shown to protect against hepatic injury by modulating cellular pathways and reducing oxidative stress (Middleton *et al*., 2000; Sharma *et al.* 2017). Its antioxidant properties make it a viable candidate for ameliorating arsenic-induced toxicity in critical organs (Khan *et al*. 2021).

The research focuses on assessing liver function through enzyme assays, including AST, ALT, and ALP levels, which are critical biomarkers of liver injury (Manna *et al*. 2018). Histopathological analyses were performed to assess organ damage and recovery, offering a detailed view of arsenic's health effects and lime's protective potential. This research provides crucial insights for developing dietary interventions against arsenic toxicity, particularly in contaminated areas. The findings have public health implications, emphasizing the need for strategies to counter heavy metal exposure, especially in developing regions.

MATERIALS AND METHODS

Chemicals and kits

Sodium arsenite ($NaAsO_2$), anaesthetic ether, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) kits were used for this study.

Experimental design

All the experimental procedures and protocols used in this study are reviewed and approved by the institutional animal ethical committee of Serampore College, Serampore, Hooghly, West Bengal. Healthy Swiss albino male mice aged 6-8 weeks and average body weight of 20-25 g were purchased from a licenced organisation (Saha Enterprise, Birati, Kolkata – 700051, Registration No.- 1828/PO/Bt/S/15/CPCSEA). Mice are housed in a controlled environment with a 12-hour light and dark cycle, maintaining a temperature of 22 ± 2°C for 7 days prior to the start of the treatment. The animals were housed in polypropylene cages with steel wire tops and provided diet and water ad libitum. 12 mice were randomly selected and divided into 3 groups – control group, arsenic group (sodium arsenite was given at a dose of 5 mg/kg body weight) and arsenic with lime treated group (simultaneously treated with both arsenic and lime, lime at a dose of 25 mg/kg body weight) and kept in 3 different cages, 4 mice in each cage. The experiments were conducted for 14 days.

Sample collection and biochemical assay

Blood sample were collected from directly puncturing the heart of mice after anesthetized using anesthetic ether. Blood was kept at room temperature about 30 min for coagulation followed by centrifugation at 3000 rpm for 10 minutes to separate the serum or plasma from the blood cells and stored at -20°C until further analysis. For the measurement of liver enzyme activity commercially available MBK kits were used according to the respective manufacturer's protocol.

Study on histopathology of liver, kidney and testes

Each organ were collected from the sacrificed mice and fixed in Carnoy's solution for a minimum of 48 hours to ensure optimal preservation of cellular structures. After dehydration and clearing, tissues were embedded in paraffin wax. Thin sections (usually 5-7 micrometers thick) were cut from the tissue blocks using microtome and stained with hematoxylin and eosin. Tissue sections were examined under a light microscope.

RESULTS AND DISCUSSION

Over 14 days, distinct behavioral and physical differences were observed among the three mice groups. The control group showed normal activity, healthy fur, regular eating, and stable weight. Conversely, the arsenic-exposed group exhibited lethargy, rough fur, reduced appetite, weight loss. The arsenic with lime treated group showed intermediate improvements, with better activity, smoother fur, and higher food intake than the arsenic group.

Biochemical Changes

In order to confirm the hepatocellular degeneration of hepatic tissue, activities of AST, ALT, ALP were then estimated. The leakage of above enzymes in the blood reflects liver injury indirectly due to Arsenic -induced hepatotoxicity. These enzyme activities were significantly increased in arsenic-treated mice group when compared to the normal control group. A significant lower levels of these altered enzymatic activities were observed in arsenic-treated mice supplemented with lime. AST levels in the arsenic group reached 111.34 IU/L, significantly higher than the control group's 39.41 IU/L, while the arsenic with lime group recorded 86.29 IU/L (Fig. 1 A). This pronounced rise in AST activity indicates severe hepatic damage and impaired liver function, aligning with established evidence of arsenic-induced hepatotoxicity. ALT levels followed this pattern, with the arsenic group rising to 121.50 IU/L compared to 33.83 IU/L in the control and 65.76 IU/L in the lime-treated group (Fig. 1 B). This sharp increase in ALT suggests extensive hepatocellular injury and inflammation, common markers of liver toxicity. Additionally, ALP levels were also significantly raised in the arsenic-treated group, reaching 146.54 IU/L, compared to 73.15

IU/L in the control group and 110.52 IU/L in the arsenic with lime-treated group, further reinforcing the evidence of arsenic-induced hepatic dysfunction (Fig. 1 B). These elevated enzyme levels highlight the protective role of lime in reducing arsenic-related liver damage.

Histological Changes in Liver

Histological analysis of liver tissues showed clear differences among the groups. The control group had normal liver architecture (Fig. 2 A), while the arsenic-exposed group exhibited severe damage, including disrupted structure, widespread cell degeneration, and necrosis (Fig. 2 B). The arsenic with lime group showed partial preservation, with reduced degeneration and necrosis compared to the arsenic-only group (Fig. 2 C).

Histological Changes in Kidney

Histological analysis of kidney tissues revealed distinct structural differences across the groups. The control group exhibited normal kidney architecture (Fig. 3 A), while the arsenic-treated group showed significant damage, including inflammatory infiltration and hemorrhage (Fig. 3 B). In the arsenic with lime group, lemon juice partially reversed the damage, reducing abnormalities and suggesting a protective effect against nephrotoxicity (Fig. 3 C).

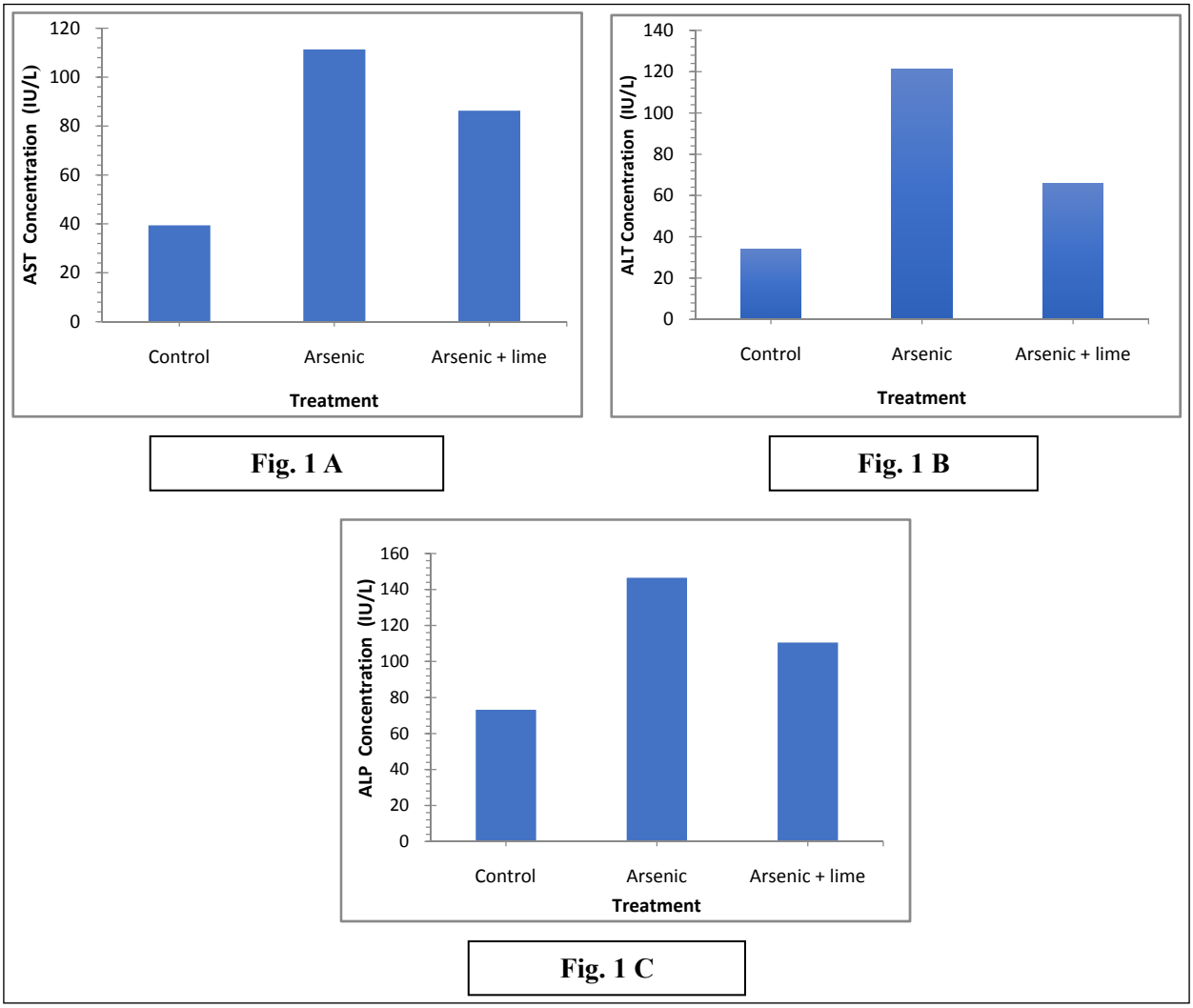

Fig. 1: The effect of arsenic and lime supplementation on liver enzymes AST **(A)**, ALT **(B)** and ALP **(C)**

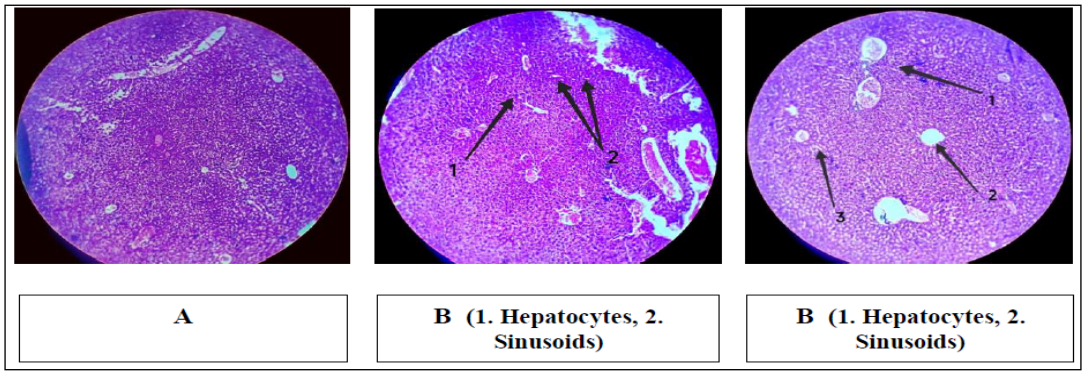

Fig. 2: (A) The photomicrograph of liver tissue section of control group, which received normal food, displayed a well-preserved hepatic architecture, with hepatocytes arranged in orderly plates around a clearly defined central vein. Cellular borders were intact, and no signs of degeneration, necrosis, or inflammation were present. The sinusoids appeared normal, without congestion or dilation, and minimal inflammatory infiltrates were observed, indicating healthy liver tissue. **(B)** In stark contrast, the photomicrograph of liver tissue of arsenic-exposed group exhibited severe disruptions in hepatic architecture, characterized by widespread cellular degeneration and necrosis. The hepatocytes showed pale staining, indicative of significant damage, while the central vein was obscured by the surrounding tissue destruction. Extensive necrosis, increased connective tissue indicating fibrosis, and congested sinusoids were prominent, along with a substantial inflammatory response marked by infiltrates of lymphocytes and macrophages. These changes reflect a profound toxic effect of arsenic on liver tissue. **(C)** The photomicrograph of liver tissue in the arsenic with lime-treated group showed that liver architecture was partially preserved compared to the arsenic-only group. While some cellular degeneration and pale areas remained, the central vein was more discernible, and necrosis was less extensive. Inflammatory infiltrates were reduced, indicating a moderated immune response. Although sinusoids were still dilated or congested, the overall damage was less severe, suggesting that lime had a mitigating effect on arsenic-induced hepatotoxicity. These results indicate that lime offers a protective role, reducing the extent of liver damage caused by chronic arsenic exposure.

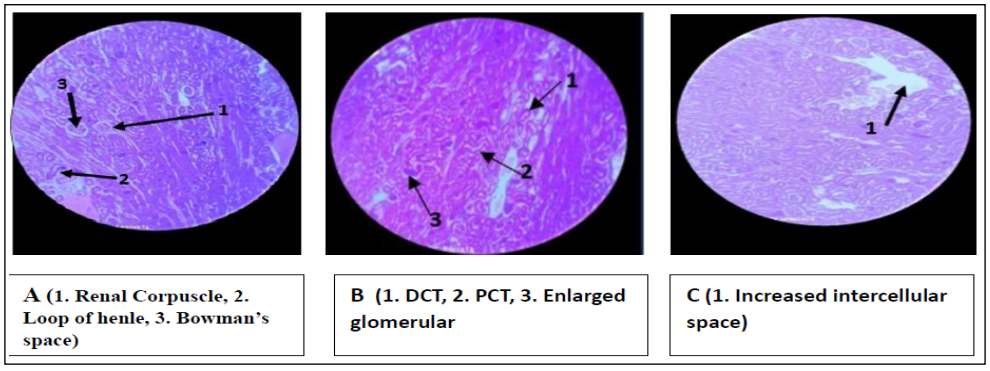

Fig. 3: (A) The control group exhibited normal kidney architecture, with well-defined cortical and medullary regions. Glomeruli were enclosed within Bowman's capsules, and proximal convoluted tubules (PCT) were more prominent than distal convoluted tubules (DCT). The renal corpuscles showed no signs of damage, and the blood vessels were intact, indicating healthy renal function. **(B)** In contrast, the arsenic-treated group displayed marked pathological changes. Diffuse inflammatory cell infiltration and interstitial hemorrhage were evident, highlighting significant renal tissue damage. Additionally, there was a reduction in glomerular size, with increased peri-glomerular space, and glomerular tuft shrinkage, leading to an enlarged Bowman's space. These alterations disrupted normal kidney architecture, with some renal corpuscles showing severe structural damage, while others exhibited less pronounced changes. **(C)** The group treated with arsenic and lime showed partial recovery, with reduced structural abnormalities compared to the arsenic-only group. Although the epithelial lining of the PCT and DCT remained mildly disrupted, the extent of fibrosis, inflammation, and nucleus clumping was noticeably diminished. Lime treatment appeared to mitigate arsenic-induced nephrotoxicity, as kidney tissue showed signs of repair, and the overall architecture was more preserved. These findings suggest that lime offers a protective effect against arsenic toxicity, helping to restore kidney structure and function while reducing the severity of arsenic-induced damage.

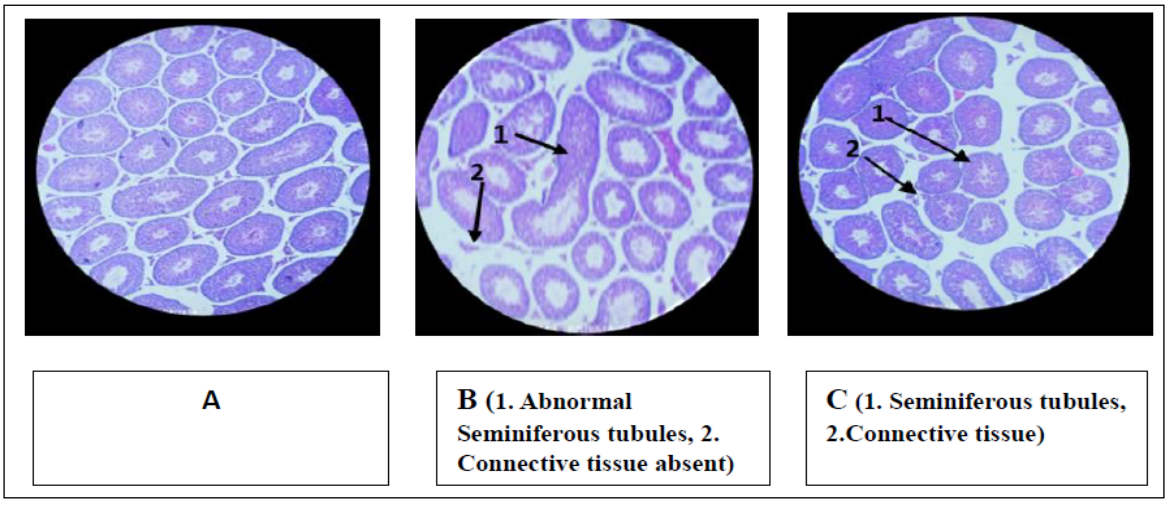

Fig. 4: (A) Histological examination of the testes across the experimental groups revealed significant structural differences. In the control group, the testis displayed normal architecture, characterized by numerous well-organized seminiferous tubules, surrounded by intertubular connective tissue containing distinct Leydig cells. The seminiferous tubules were oval or round, with a thin basement membrane, and cells at various stages of spermatogenesis were clearly observed. Spermatogonia were located along the periphery, while primary and secondary spermatocytes, distinguished by their size and nuclei, maintained their natural organization, reflecting healthy testicular function. **(B)** In contrast, the arsenic-treated group showed severe histopathological damage. There was a significant reduction in the number of mature sperm cells, and Leydig cells were either absent or displayed irregular shapes. The intertubular connective tissue was disorganized or missing, and the nuclei of Leydig cells appeared deformed. Furthermore, the seminiferous tubules exhibited disrupted architecture, with spermatozoa no longer connected to Sertoli cells, indicating a breakdown in the spermatogenic process. Tubular vacuolation and abnormal germinal epithelial cell structure further underscored the extensive damage caused by arsenic toxicity. **(C)** However, the group treated with arsenic and lime demonstrated partial recovery. The number of Leydig cells and connective tissue increased, and the seminiferous tubules regained their normal structure. The epithelial germ cells appeared healthy, and the number of sperm cells improved significantly. The natural stratification within the seminiferous tubules was restored, and Sertoli cells regained their natural nuclear shape. The overall testicular structure and function were partially restored, indicating the protective role of lime in mitigating arsenic-induced testicular damage.

Histological Changes in Testes

In the control group, the testis exhibited normal architecture, with well-organized seminiferous tubules surrounded by connective tissue containing Leydig cells (Fig. 4 A). The arsenic-treated group showed significant damage including a reduced number of mature sperm cells, irregularly shaped and absent Leydig cells, and disorganized connective tissue (Fig. 4 B). However, in the arsenic with lime-treated group, testicular structure showed partial recovery (Fig. 4 C).

The research conducted here provides a comprehensive evaluation of arsenic toxicity in mice and the potential protective effects of lime. The results reveal that lime treatment along with arsenic intoxication markedly ameliorated effects of arsenic toxicity by significantly restoring hematological and serum biochemical parameters toward normal values.

The liver is the most important target organ of arsenic toxicity (Guha Mazumder and D.N. *et al*. 2008; Liu *et al*. 2008). Several enzymes of blood serum are considered as indicators of hepatic dysfunction and damage, and the leakage of hepatic enzymes such as AST, ALT, and ALP into blood are routinely used as a reliable biochemical index for hepatocellular damage (Haldar PK *et al*. 2011). These enzymes are basically located inside hepatocytes, where they participate in different metabolic pathways (Johnston DE, 1999). Any interference in these enzymes leads to impaired liver function which has been described in the literature (Kavitha C *et al*. 2010; Gyasi SF *et al*. 2012; Sarker RSJ *et al*. 2012). In this study, it was found that arsenic administration substantially increased

serum AST, ALT and ALP activities, which are more specific to liver damage. This increase indicates cellular leakage and failure of functional integrity of liver cell membranes (Sharma V and Singh M, 2014). Co-administration of lime as a supplementation significantly reduced arsenic-induced elevation of AST, ALT, and ALP activities. These results indicated that lime had a protective effect on arsenic-induced liver injury.

In the current investigation several histopathological changes were seen in liver, kidney and testis. The liver tissue of control group maintained normal histological features, while the arsenic exposed group displayed severe alterations, including hepatocyte necrosis, inflammation, sinusoidal congestion, and early signs of fibrosis. The necrotic changes occurred as arsenic cause hepatocellular damage by producing increased reactive oxygen species which eventually disrupts the membrane system (Singh AP *et al*. 2011). In the arsenic with lime treated group, histological analysis revealed less severe damage with mild necrosis and inflammation, the overall liver architecture was better preserved. This improvement supports the biochemical data, indicating that lime exerts protective effects at the cellular level, reducing both acute and chronic liver damage.

In this study, the kidneys of mice in the test group exhibited varying glomerular sizes, including both large and small glomeruli. Additionally, there were changes in the glomerular space. These observations align with evidence from previous literature (Mehta M and Hundal S., 2015; McLellan F., 2002; Rosenberg HG., 1974; Sarkar S *et al*. 2014; Anwar-Mohamed *et al*. 2012). Arsenic exposure leads to increased oxidative stress in tissues, affects organ function by altering urinary elimination and impacting the proximal convoluted tubules. Arsenic exposure induces lipid peroxidation in the kidney, leading to functional deterioration. Testicular histology in this study exhibited severe cellular damage in spermatogenic cell. Moreover, the appearance of eosinophilic multinucleated giant cell in the seminiferous tubule indicated cellular degeneration that arsenic exposure severely disrupts the maturation of spermatogonia through the process of meiosis (Sharpe RM *et al*. 1990). The reduction in Leydig cell population, along with their degeneration, likely led to decreased testosterone synthesis affecting spermatogenesis. It's well established that Leydig cells play a crucial role in maintaining the structural and functional integrity of seminiferous tubules and contribute to testosterone production—a key component in regulating the post-meiotic stage of spermatogenesis (Hemalatha P *et al*. 2013; Singh PK. *et al*. 2012; Chowdhury DU *et al*. 2016). Therefore, this study showed the potentiality of lime to reduce arsenic toxicity in mice model. Thus, the results suggested that lime could be useful therapeutically in future to reduce or prevent the toxic effect of arsenic in humans.

CONCLUSION

In conclusion, this study highlights the severe toxic effects of arsenic on vital organs such as the liver, kidney, and testes, demonstrating its potential to cause oxidative stress, cellular degeneration, inflammation, and structural damage. The use of lime, rich in antioxidants like vitamin C and flavonoids, shows great promise in mitigating arsenic-induced toxicity, highlighting its potential as a natural therapeutic agent for organ protection. Further research is essential to validate these findings and explore broader application of lime in detoxification strategies.

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