In vivo exposure to sodium arsenite produce hepato-nephro alterations, enzymatic inhibition, and neurobehavioral expression in Charles Foster rats

Prabhat Shankar[1]
Rana Vikram Singh[1]
Arun Kumar[2]
Mohammad Ali[2]
Abhinav[2]
Mini Tiwari[3]
Ashok Kumar Ghosh[2]

Exposição in vivo *ao arsenito de sódio produz hepato-nefro alterações, inibição enzimática e expressão neurocomportamental em ratos Charles Foster*

[1] Jai Prakash University (JPU), Chapra, Bihar, India
[2] Mahavir Cancer Sansthan and Research Centre, Patna, Bihar India
[3] Patliputra University (PPU), Patna, Bihar, India

***Correspondence:** drarunk31@gmail.com
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Abstract

Arsenic, a highly hazardous metalloid present ubiquitously in the Earth's crust, poses a significant threat to global groundwater contamination. This study aimed to evaluate the hepato-nephro alterations, enzymatic inhibition, and neurobehavioral flux in Charles Foster rats exposed to arsenic. The rats were divided into control and arsenic-exposed groups, and their hepatic and renal functions were assessed. Enzymatic activity was measured to determine the inhibitory effects of arsenic, and neurobehavioral assessments were conducted to evaluate any behavioural changes. The results revealed significant alterations in hepatic and renal parameters, including biochemical and histopathological changes. Enzymatic inhibition and neurobehavioral abnormalities were observed in the arsenic-exposed rats. These findings provide valuable insights into the toxic effects of arsenic on hepato-nephro function and neurobehavioral outcomes.

Keywords: Anxiety. Antioxidants. Hepato-nephro enzymes. Rats. Sodium arsenite.

Resumo

O arsênio, um metaloide altamente perigoso presente onipresentemente na crosta terrestre, representa uma ameaça significativa à contaminação global das águas subterrâneas. Este estudo teve como objetivo avaliar as alterações hepato-nefro, inibição enzimática e fluxo neurocomportamental em ratos Charles Foster expostos ao arsênio. Os ratos foram divididos em grupo controle e grupo de expostos ao arsênio, e suas funções hepática e renal foram avaliadas. A atividade enzimática foi medida para determinar os efeitos inibitórios do arsênio, e avaliações neurocomportamentais foram conduzidas para avaliar quaisquer alterações comportamentais. Os resultados revelaram alterações significativas nos parâmetros hepáticos e renais, incluindo alterações

bioquímicas e histopatológicas. Inibição enzimática e anormalidades neurocomportamentais foram observadas nos ratos expostos ao arsênio. Essas descobertas fornecem insights *valiosos sobre os efeitos tóxicos do arsênio na função hepato-nefro e nos resultados neurocomportamentais.*

Palavras-chave: *Ansiedade. Antioxidantes. Enzimas hepato-nefro. Ratos. Arsenito de sódio.*

Introduction

Arsenic (As), highly adaptable metallic element found worldwide, is classified as a class 1 human carcinogen. It has been linked to various forms of cancer (Chen and Costa, 2021). In urban and occupational settings, arsenic exposure primarily arises via inhalation. Conversely, in rural regions, individuals are frequently subjected to direct exposure through consumption of contaminated drinking water and the utilization of irrigation systems affected by arsenic pollution (Chakraborti et al., 2018; Upadhyay et al., 2021). Global records reveal that despite the limit of 10 parts per billion prescribed by the Environmental Protection Agency and the World Health Organization, millions of people continue to be consistently exposed to arsenic-contaminated drinking water (Richards et al., 2020; Kumar et al., 2021). These metalloid contamination comes in nature through natural and anthropogenic sources. Chemically, it has three common forms, i.e. inorganic, organic and the gaseous state (Rahman et al., 2019; Balali-Mood et al., 2021). The trivalent arsenic is regarded to be more toxic compared to pentavalent (Balali-Mood et al., 2021; Raju, 2022). Trivalent arsenic or arsenite (As^{3+}) toxicity can be triggered by direct interaction with vicinal thiols or organic ligands containing sulphur group compounds and as an outcome it produces free radicals that increase significantly during cellular redox reactions (Hug et al., 2020; Kim et al., 2020).

The phenomenon of uncontrolled arsenic concentration escalation has been observed in multiple countries like India, Bangladesh, Taiwan, Thailand, Nepal, China, Chile, Argentina, Mexico, Romania, and Hungary in a substantial progressive duration (Rahman et al., 2019; Kim et al., 2020; Chakraborty et al., 2022). Asia's worst-hit countries, India and Bangladesh are especially affected by the catchment area of Ganga-Meghana-Brahmaputra (Rahman et al., 2019; Kumar et al., 2021; Chakraborty et al., 2022). Indian states that are nearby the Ganga basin, such as Bihar, West Bengal, Assam, Uttarakhand and Uttar Pradesh, are severely affected by groundwater arsenic poisoning (Kumar et al., 2021; Penke et al., 2021). In Bihar, eighteen districts were reported to have high level of arsenic contamination in groundwater and approximately 10 million are facing the risk of serious health concern caused by arsenic poisoning (Palma-Lara et al., 2020; Kumar et al., 2022; Richards et al., 2022). The majority of those people who grew up near arsenic exposed hotspot area exhibited a range of symptoms including skin ma-nifestations (melanosis, keratosis, rain drop pigmentation, etc.), loss of appetite, gastrointestinal disorders, hepato and nephro disorders, generalized body weakness, hormonal imbalance and reproductive disorders (Dani and Walter, 2018).

The exposure to arsenic toxicity and its associated impacts elicits significant deleterious effects on the metabolic processes within the body, consequently leading to an increased susceptibility to micro-vascular disease and neurological disorders (Podgorski and Berg, 2020).The arsenic toxicants have the potential to disrupt the mitotic process of granule cells and impede the typical development of the cerebellum (Berntsen et al., 2021). The cerebellum is a central region of the brain that involves in coordinating and regulating voluntary movement, balance, posture, motor learning and skilled voluntary actions (Garza-Lombó et al., 2019; Thakur et al., 2021). Recent research suggests that it plays a role in cognitive, language, memory and specific executive functions. Whenever arsenic enters in blood-brain barrie, accumulates in cortex, cerebellum and hippocampus causes' abnormal neurobehavioral performance (Iqubal et al., 2020; Tahir et al., 2023). Additionally, it induces an elevation in the generation of reactive oxygen species within the brain, disrupting the delicate equilibrium between the antioxidant defense system and the production of free radicals, consequently leading to neurotoxicity (Smeester and Fry, 2018; Zhang et al., 2020).

Arsenic toxicity induces notable morphological and physiological alterations in cellular structures. Due to its heightened energy demands, the brain

is particularly susceptible to oxidative stress, which is provoked by arsenic exposure. This oxidative stress response is characterized by the reduction of antioxidant enzyme levels within the brain (Iqubal et al., 2020; Zhang et al., 2020). Therefore, the objective of the current investigation was to evaluate the hepatic and nephrological changes induced by arsenic exposure, along with examining the inhibitory effects on enzymatic activity, oxidative stress levels, and neurobehavioral instability in Charles Foster rats.

Material and methods

The proposed experiment had been reviewed and approved by the Mahavir Cancer Sansthan and Research Centre (Institutional Animal Ethics Committee), assigned with No. 2020/ID-27/08/20). Approval was granted in view of obtaining compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines, Ministry of Environment, Forest and Climate changes, Government of India, New Delhi.

Animals

Healthy 2 to 2.5 months old Charles Foster rats, male, weighing 160 ± 20 g, were obtained from the breeding house of Mahavir Cancer Sansthan and Research Centre, Phulwarisharif, Patna (Bihar), India. The obtained rats were comfortably accommodated in clean polypropylene cages which has the stainless-steel grill top facelifts. In animal house laboratory, temperature was regulated at 24 ± 2 °C for 12h light and dark cycles. Feed-nutrition was nurtured by laboratory itself (freshly prepared animal feed) and water was provided as per *ad libitum* procedure.

Chemicals

Sodium arsenite (Meta) ≥ 90%, listed with the CAS No. 7784-46-5. S7400-100G, Lot# SLBH5736V, P Code 1001683292, manufactured by Sigma-Aldrich, USA, were purchased from the certified and listed scientific counter of Patna, India.

Experimental design

A total of 24 Charles Foster rats were randomly assigned to four groups, with six rats in each group. The control group received distilled water without any arsenic, while the other three groups, namely T1, T2, and T3, were administered sodium arsenite ($NaAsO_2$) at doses of 5, 8 and 12 mg per kilogram of body weight, respectively, in combination with distilled water. This treatment regimen was continued for duration of 90 days.

Dose selection

The inorganic compound $NaAsO_2$ was selected for the treatment on experimental rats' group. To frame the arsenic model of rats, a medial lethal dose (LD_{50}) was utilized after experimental calculation (Verma et al., 2022).

Sample preparations

Upon completion of the entire experiment, the animals were subjected to an overnight fast and subsequently euthanized under anesthesia by using Ketamine drug (Rayner et al., 2020). Cardiac puncture was performed to collect blood, and to separate the serum it was centrifuged at 3,000 g and was stored at -20 °C for future biochemical analysis. A portion of the whole blood was utilized for haematological studies.

The liver, kidney, and brain of the euthanized rats were promptly removed and preserved individually using ice-cold saline solution. Tissue samples from each selected organ were then homogenized in a 10% (w/v) solution in 0.1M Tris-HCl buffer (pH 7.4) and centrifuged at 4,000 g for 15 minutes at 4°C. The resultant supernatant was collected for subsequent experimental assays.

Body weight analysis

Analytical balance was used to the weight analysis of each rat's group. The initial and final weight was recorded to the detection of body weight changes. Examined rat's body weight was determined at mean ± standard deviation (SD) in g/kg.

Organ's morphological analysis

After the completion of entire experiment, the hepatic and renal organs were surgically removed from each group. The collected organs were carefully cleaned to remove any fats and connective tissue.

In order to assess general morphology, the organs were observed. The primary factors taken into account in the gross morphological assessment were organ's weight, shape, size, color, and consistency. The organs were stored in different petri plates based on their morphological characteristics. The organs were then compared between the control group and all arsenic treated groups using a centimeter scale to measure organ size. The organs were weighed using an analytical balance, and the measurements were recorded to determine the mean ± SD (mg/g).

Haematological analysis

Whole blood samples were obtained from all experimental groups to assess haematological parameters, i.e. hemoglobin (HGB) levels, red blood cell (RBC) count, white blood cell (WBC) count, hematocrit (HCT) levels, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) count. These parameters were measured and analyzed to evaluate the effects of the experimental conditions on the blood composition (Gunčar et al., 2018).

Biochemical analysis

Determination of electrolyte and nitric oxide

Electrolytes are minerals that carry an electrical charge and play a crucial role in fluid retention and maintaining the proper acid-base balance in the body. They are involved in regulating heart rhythm, muscle and neuron activity, and other vital physiological processes. The levels of electrolytes in the serum, including potassium (K^+), sodium (Na^+), and chloride (Cl^-), were determined as part of the study (Bazzi et al., 1952; Heidari et al., 2018).

Nitric oxide (NO) is a signaling molecule that coordinates a variety of physiological activities, including those that alter neurotransmission and vascular smooth muscle tone. As a potent vasodilator, NO's continuous release into the circulatory system significantly reduces peripheral resistance, making it essential for maintaining basal blood flow in many tissues. The serum level of NO was also determined as part of the study (Zhang et al., 2019).

Determination of hepato-nephro markers

In order to determine hepatic marker, Reitman and Frankel's method (Reitman and Frankel, 1957) was used to determine the serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT). The alkaline phosphatase (ALP) was analysed by Kind and King (1954) technique. Total bilirubin was analyzed by Gambino et al. (1967) method.

Mandate to determine nephro markers, urea level was measured by the method of Fawcett and Scott (1960), the level of uric acid was measured by the method of Fossati et al. (1980), and creatinine by Moore and Sharer (2017) method, while Hill (1985) method was used to determine albumin levels.

Determination of reactive oxygen species

Under the detection of reactive oxygen species (ROS) generation serum/tissue supernatant sample were used. By using tissue supernatant, the level of protein carbonyl (PC) was detected from Reznick and Packer (1994) method. The serum level of malondialdehyde (MDA) determination was evaluated as per the procedure (double heating method) described by Draper and Hadley (1990). Glutathione (GSH) level (using tissue supernatant) was analyzed by Giustarini et al. (2013) method. The serum level of superoxide dismutase (SOD) was performed by Misra and Fridovich (1972) technique, and the serum level of catalase (CAT) activity was determined by Góth (1991) method.

Determination of arsenic concentration in blood and cerebellum tissue

To the estimation of arsenic concentration in blood, 500 µl blood sample were collected in a conical flask and added with 5 ml concentrated nitric acid (HNO_3). Similarly, 500 mg cerebellum tissue were collected to the detection of arsenic concentration in a conical flask and added 5ml of concentrated HNO_3 for further procedure. Both samples were allowed to react overnight at ambient temperature. The following morning, a conical flask was positioned on a hot plate (Thermotech PID 420, SSI LASTh) for digestion, following the guidelines outlined in the NIOSH protocol (1994). The observations

were recorded using a graphite furnace atomic absorption spectrophotometer (GF-AAS) with the model number Pinnacle (Perkin Elmer, Singapore) at the Mahavir Cancer Sansthan and Research Centre (Kumar et al., 2020).

Neuro-behavioural observation

Spontaneous motor activity test: the analysis of rat's spontaneous motor activity was performed by Karthikeyan et al. (2019) method. Rats were kept in the cage connected with computerized Multi Mouse Monitor and provides 24 ± 2 °C temperature. The effect on variety of measures including total distance travelled, resting time, stereotypic time moving and rearing was observed in control and treated groups of rats.

Rotarod activity test: by the Rafiee et al. (2018) technique, rotarod activity test were performed. In this test rats were placed on a horizontal rod, which rotates about its long axis. Task was performed by both control and treated group. The descent off time from the rotating rod was measured accordingly with normal practice.

Analysis of grip strength: the standard approach as stated by Adedara et al. (2018) was followed to test the forelimb grip strength in the control and treated group of rats using a computerized grip strength meter.

Histopathological determination

For the purpose of histopathological examination, selected organs (hepatic and renal sections) were collected from each group and washed with ice-cold normal saline solution. The tissues were then cut into small pieces, fixed in 10% formalin for 24 hours, and further processed with appropriate coding. These coded tissues were embedded in paraffin wax using standard block size measuring 10 × 5 × 3 mm and preserved at -20 °C until further use. Following the laboratory manual, all blocks were kept at room temperature for 15 minutes and then sectioned to a thickness of 4 to 5 µm using a water bath. The sectioned tissues were placed on slides with the assistance of a water bath. Any residual wax surrounding the tissues was removed using a microwave oven, and subsequently, the tissues were stained with haematoxylin-eosin using a polypropylene staining Coplin Jar.

Statistical analysis

All the experimental results were examined through one-way analysis of variance (ANOVA) and Turkey's multiple range test. The computations were executed by Graph Pad Prism (Graph Pad software, Inc., San Diego, USA). The value of $p < 0.05$ were deemed to be significant where the findings were presented through mean ± SD.

Results

Effects on body weight

A significant change in body weight was observed among the rats in all experimental groups. Prior to euthanasia, the rats in each group were weighed one final time. The analyzed results indicated that body weight of rats exposed to arsenic (T1, T2, and T3 groups) was significantly reduced ($p < 0.0001$) as compared to the control group. Furthermore, the body weight of T2 and T3 rats was significantly ($p < 0.0001$) lower than in T1 (Figure 1).

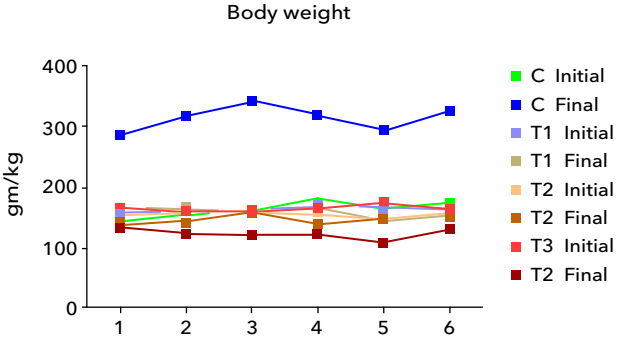

Figure 1 - Comparative study of sodium arsenite-induced (T1, T2 and T3) vs. control (C) rat's body weight.

Note: Value expressed in mean ± standard deviation.

Effect on organ's morphology

The liver and kidney morphology weight of As-exposed (T1, T2 and T3 groups) were significantly ($p < 0.0001$) increased than control. Compared to control, As-induced liver of T1 had diminutive variation in shape and size. The liver of As-exposed T2, and T3 group was dark in color, severely enlarged

in size. These both groups were significantly overweight ($p < 0.0001$) than the As-exposed T1 and control groups (Table 1). In view of renal position in arsenic exposed group mild changes were observed in shape and size. On the basis of color comparison, no much changes were observed in T1, T2 and T3 group vs. control group.

Haematological consequences

The outcomes of haematological consequences in As-exposed (T1, T2 and T3 groups) showed significant ($p < 0.0001$) changes in the level of HGB, RBC, HCT, MCH, MCHC, and non-significant with PLT in compare to control group. The level of WBC was significantly ($p < 0.0001$) increased than the control (Table 2).

Biochemical observation

Effect on electrolyte and nitric oxide

As-exposed (T1, T2 and T3) rat's Na^+, Cl^- and NO was significantly ($p < 0.0001$) reduced than control, but the level of K^+ was significantly ($p < 0.0001$) increased. Some of the mild alterations was observed in between As-exposed T1 vs. T2 and T2 vs. T3 rat's groups (Table 3).

Table 1 - Variations in organ weight: control (C) vs. As-treated groups of rats (T1, T2 and T3)

Parameter	C	T1	T2	T3
Liver (mg/g)	4.925 ± 0.113	6.365 ± 0.086	8.783 ± 0.340	16.290 ± 0.609
Kidney (mg/g)	0.800 ± 0.037	1.234 ± 0.047	1.723 ± 0.095	1.962 ± 0.072

Note: As = sodium arsenite. Data presented as mean ± standard deviation. Differences were analyzed by one-way ANOVA.

Table 2 - Haematological changes in control (C) vs. As-treated groups of rats (T1, T2 and T3)

Parameter	C	T1	T2	T3
HGB (g/dL)	13.780 ± 0.333	11.210 ± 0.191	8.333 ± 0.110	6.170 ± 0.098
RBC ($\times 10^6/mm^3$)	7.445 ± 0.064	6.132 ± 0.039	4.467 ± 0.048	4.260 ± 0.430
HCT (%)	272.000 ± 4.180	148.000 ± 0.340	113.000 ± 1.530	98.000 ± 1.320
MCH (pg)	124.000 ± 1.140	108.000 ± 1.030	98.000 ± 1.720	73.000 ± 1.390
MCHC (g/L)	33.400 ± 0.032	31.600 ± 0.240	28.400 ± 0.034	24.500 ± 0.017
PLT ($\times 10^3/mm^3$)	732.400 ± 103.600	612.000 ± 102.600	452.000 ± 122.500	353.100 ± 123.400
WBC (x $10^3/mm^3$)	7376.000 ± 31.140	12880.000 ± 242.700	15319.000 ± 65.690	18324.000 ± 299.700

Note: As = sodium arsenite; HGB = hemoglobin; RBC = red blood cell; HCT = hematocrit; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet; WBC = white blood cell. Data presented as mean ± standard deviation. Differences were analyzed by one-way ANOVA.

Table 3 - Variations in electrolytes and nitric oxide in control (C) vs. As-treated group of rats (T1, T2 and T3)

Parameter (mmol/L)	C	T1	T2	T3
Sodium	143.24 ± 3.25	114.20 ± 1.38	103.22 ± 0.47	98.52 ± 0.18
Potassium	4.53 ± 0.38	7.64 ± 0.40	9.38 ± 0.62	10.94 ± 0.75
Chloride (-)	109.16 ± 3.29	102.58 ± 1.45	102.24 ± 0.39	101.32 ± 0.56
Nitric oxide	13.32 ± 1.03	11.44 ± 0.63	7. 25 ± 0.73	5.19 ± 0.46

Note: As = sodium arsenite. Data presented as mean ± standard deviation. Differences were analyzed by one-way ANOVA.

Effect on hepato-nephro enzymes

As-exposed rats' group had chronic variations in hepato-nephro enzyme. The serum level of SGPT, SGOT, total bilirubin, urea, uric acid and creatinine was significantly ($p < 0.0001$) increased than the control. T3 group had non-significant ($p < 0.0001$) changes in ALP level compared to control, T1 and T2. Level of serum albumin was significantly decreased ($p < 0.0001$) in between control group vs. T1, T2 and T3 groups (Table 4).

Effect on reactive oxygen species generation

Changes in ROS levels are closely linked to oxidative stress that progressively damage lipids, proteins, DNA and ultimately cell death. The outcomes of this study reveal that compared to control, the As-exposed group T1, T2 and T3 showed significantly ($p < 0.0001$) increased lipid (MDA) and PC level. A significant ($p < 0.0001$) reduction in GSH, SOD and CAT level was observed in comparison to control vs. T1, T2 and T3 groups (Figures 2A and B).

Table 4 - Variations in hepatic enzymes and renal enzymatic expressions in control (C) vs. As-treated groups of rats (T1, T2 and T3)

Parameter	C	T1	T2	T3
Liver (U/L)				
SGPT	35.620 ± 1.250	38.010 ± 2.070	48.220 ± 1.320	67.310 ± 2.430
SGOT	51.340 ± 1.570	63.220 ± 1.230	68.220 ± 2.470	83.060 ± 1.540
ALP	142.090 ± 3.280	147.320 ± 2.480	164.110 ± 2.060	213.260 ± 4.130
Total bilirubin	0.722 ± 0.025	1.005 ± 0.050	1.833 ± 0.1800	2.2570 ± 0.030
Kidney (mg/dl)				
Urea	20.980 ± 0.774	28.140 ± 0.677	37.900 ± 1.832	45.130 ± 0.478
Uric-acid	3.528 ± 0.304	4.548 ± 0.272	6.630 ± 0.238	7.390 ± 0.197
Creatinine	0.593 ± 0.074	1.062 ± 0.049	1.514 ± 0.169	2.145 ± 0.107
Serum albumin	3.528 ± 0.304	3.448 ± 0.272	2.832 ± 0.135	2.023 ± 0.222

Note: As = sodium arsenite; SGPT = serum glutamate pyruvate transaminase; SGOT = serum glutamate oxaloacetate transaminase; ALP = alkaline phosphatase. Data presented as mean ± standard deviation. Differences were analyzed by one-way ANOVA.

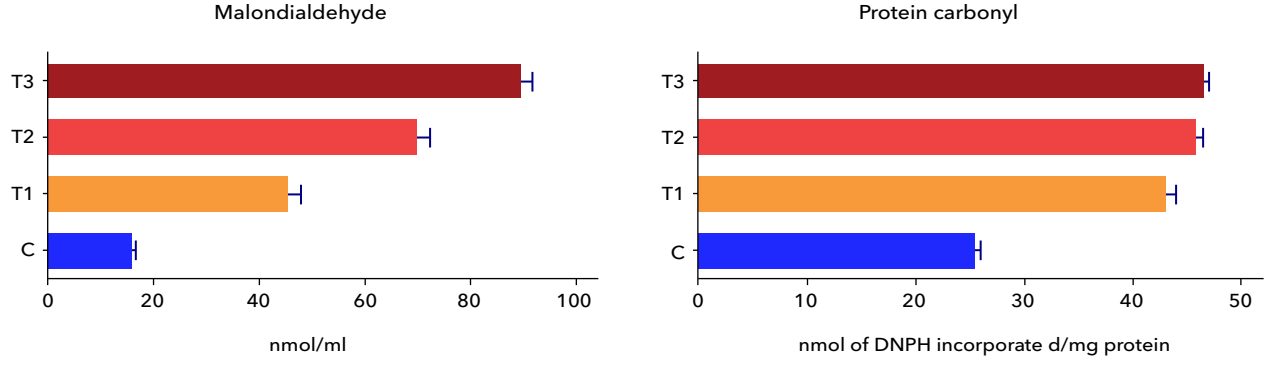

Figure 2A - Comparative study of malondialdehyde and protein carbonyl on As-induced rats (T1, T2 and T3) vs. control (C).

Note: DNPH = 2,4-Dinitrophenylhydrazine; AS = sodium arsenite. Data presented as mean ± standard deviation. Differences were analyzed by one-way ANOVA.

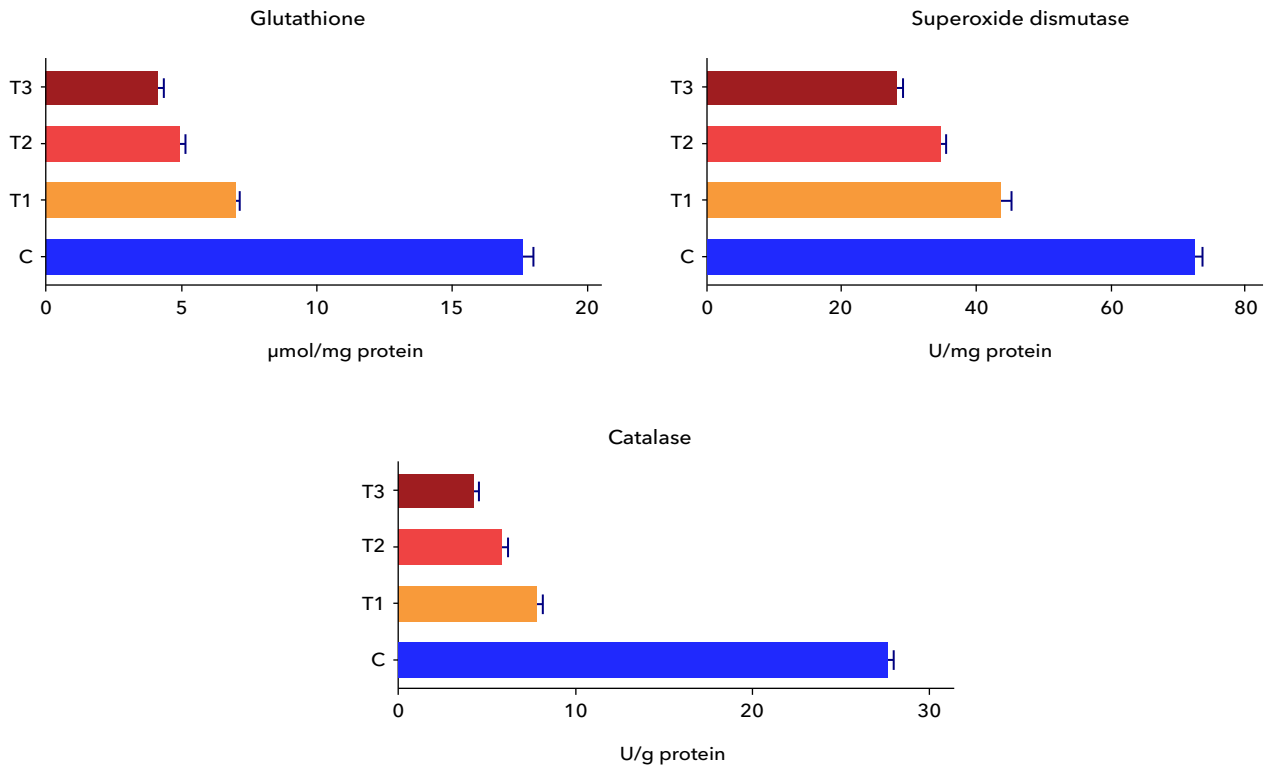

Figure 2B - Comparative study of glutathione, superoxide dismutase and catalase on As-induced rats (T1, T2 and T3) vs. control (C).

Note: AS = sodium arsenite. Data presented as mean ± standard deviation. Differences were analyzed by one-way ANOVA.

Arsenic concentration in blood and cerebellum tissue

Dose dependent As-exposed (T1, T2 and T3) rats' group had significant ($p < 0.0001$) high arsenic concentration in blood, but the non-significant changes were observed in As-exposed T1 vs. T3 group. Significant elevated ($p < 0.0001$) arsenic accumulation in cerebellum tissue were observed in between control vs. T1, T2 and T3 groups (Figure 3).

Neurobehavioral observation

Effect on spontaneous motor activity: The finding of spontaneous motor activity indicates that the As-exposed (T1, T2 and T3) rats had chronic similarity to each other. On the basis of total covering distance (TCD), resting time, stereotypic time, moving time,as well as; rearing, the significant ($p < 0.0001$) reduction had been seen in arsenic exposed group than control. (Table 5).

Table 5 - Comparative differentiation on spontaneous motor activity in between As-induced rats (T1, T2 and T3) vs. C

Parameter	C	T1	T2	T3
TCD (cm)	1871.000 ± 8.820	997.000 ± 9.266	902.800 ± 15.38	798.800 ± 9.393
Resting time (s)	213.500 ± 3.836	256.800 ± 2.257	259.500 ± 1.839	298.300 ± 1.430
Stereotype time (s)	190.000 ± 1.414	172.700 ± 1.430	168.200 ± 1.740	154.700 ± 2.512
Time moving (s)	76.670 ± 1.892	57.330 ± 0.954	53.330 ± 1.116	42.330 ± 1.202
Rearing (s)	20.500 ± 0.763	12.670 ± 0.333	11.330 ± 0.333	10.670 ± 0.421

Note: As = sodium arsenite; C = control; TCD = total covering distance. n = 6, value expressed in mean ± standard deviation. Data analyzed by one way ANOVA.

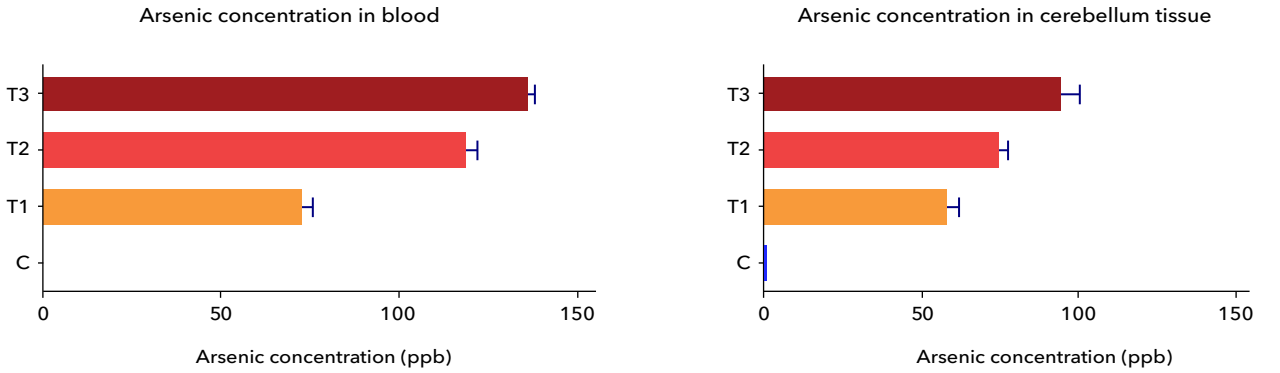

Figure 3 - Arsenic concentration in blood and in cerebellum tissue on As-induced rats (T1, T2 and T3) vs. control (C).

Note: AS = sodium arsenite. Data presented as mean ± standard deviation. Data analyzed by one way ANOVA.

Effect on Rotarod activity: the Rotarod activity test is used to detect motor deficits in rodent brain injury and the power of consistency. Outcomes of this study shows that As-exposed (T1, T2 and T3) rats group had significant ($p < 0.0001$) reduction than control (Figure 4).

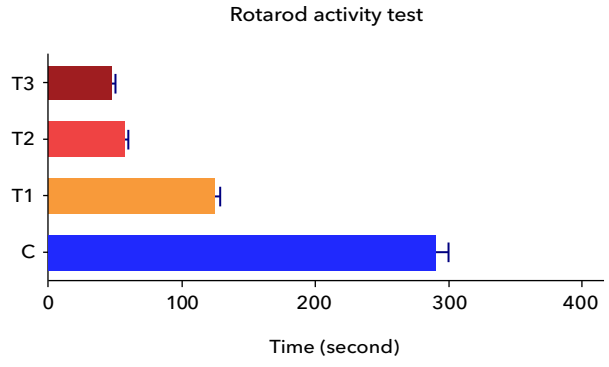

Figure 4 - Comparative level of rotarod activity in between As-induced (T1, T2 and T3) rats vs. control (C).

Note: AS = sodium arsenite. n = 6, value expressed in mean ± standard deviation. Data analyzed by one-way ANOVA.

Effect on grip strength activity: the grip strength activity is used to detect the muscle strength and the performance of rodents (rats). The finding of this study indicates that compare to control all of the dose dependent (at 5, 8, and 12 mg per kg body weight) arsenic exposed experimental rats had significant reduction ($p < 0.0001$) (Figure 5).

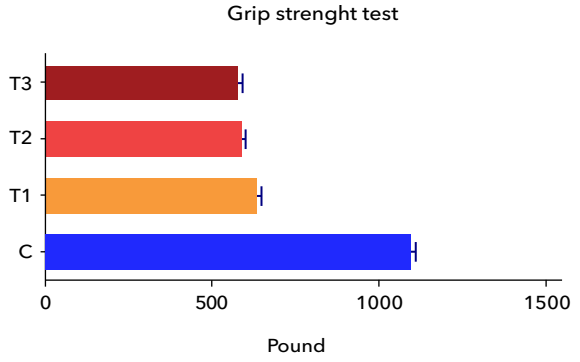

Figure 5 - Comparative level of Grip strength activity in between As-induced (T1, T2 and T3) rats vs. control (C).

Note: AS = sodium arsenite. n = 6, value expressed in mean ± standard deviation. Data analyzed by one-way ANOVA.

Effect on histopathological observation

Liver: the examined current study indicates the normal histopathological tissue along with its cellular morphology in hepatic cells and Kupffer's cells hyperplasia in control rat's hepatic section. The hepatocytes are well arranged in the sinusoids along the central lobular vein (Figure 6). Arsenic-induced (5 mg kg^{-1} body weight) rat's hepatic section exposed significant degradation, that were significant increase in the numbers of Kupffer's cells hyperplasia along with the degeneration in the central lobular vein and vacuolations in the sinusoids in comparison to control (Figure 6).

The arsenic-induced high dose (8mg Kg-1 and 12mg Kg-1 body weight) treated group showed heavy alteration in hepatocytes showing necrosis (Figure 6).

Kidney: The renal section of control group reveals normal histopathological tissues along with its cellular morphology of nephrons with precise proximal and distal convoluted tubules (Figure 7A). Compared to control arsenic (at 5mg Kg-1 body weight) induced rat's group significantly reveals mild degeneration in nephrocytes and elevated magnitude of degeneration in Bowman's capsule along with the glomerulus is observed (Figure 7B). However, arsenic-induced (at 8mg and 12mg Kg-1 body weight) rat's group revels the massive degeneration in nephrocytes and the magnitude of degeneration in Bowman's capsule as well as glomerulus (Figure 7C and 7D).

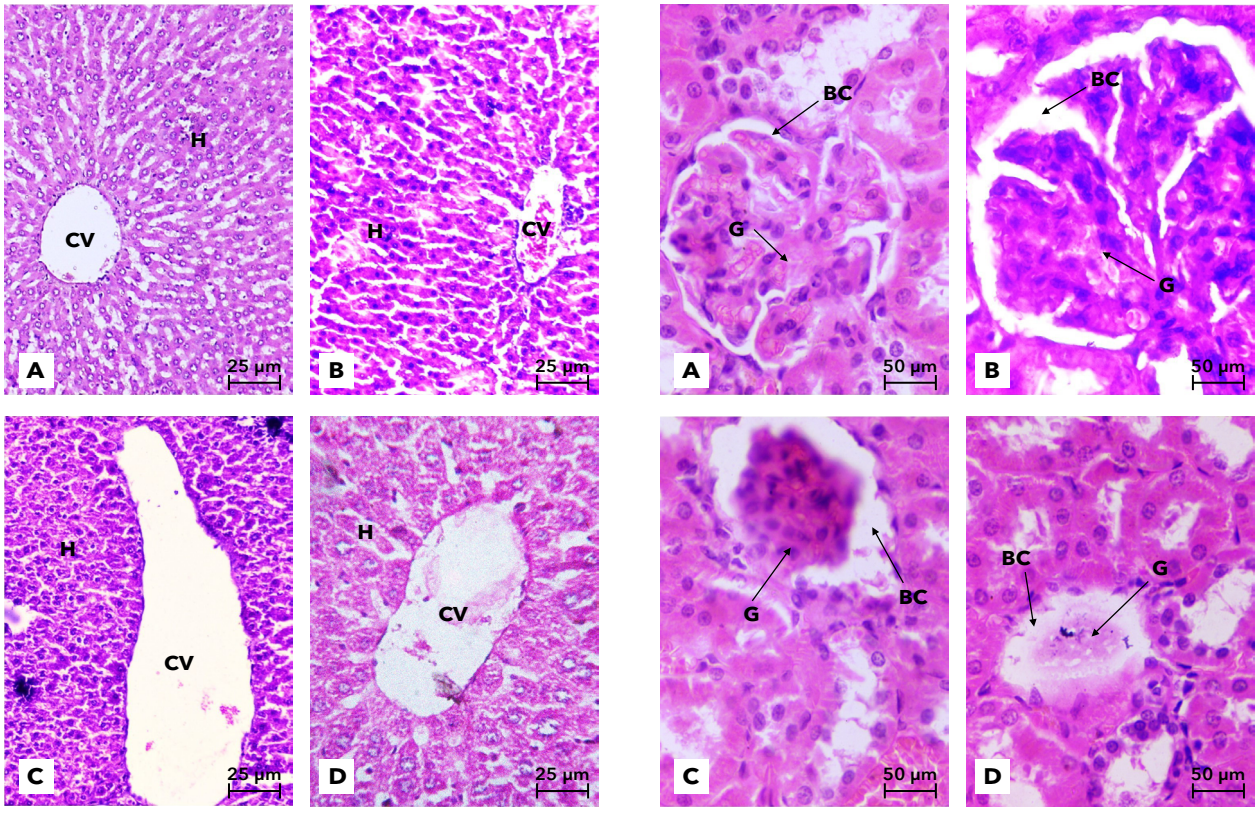

Figure 6 - The microphotograph of rat's hepatic section was stained using haematoxylin and eosin (H&E × 500). Section A indicates the control rat's hepatic section showing normal architecture of hepatocytes (H), central lobular vein (CV), portal vein, sinusoids etc. Section B indicates arsenic (5 mg kg^{-1} body weight) treated hepatic alteration showed significant degeneration in H and CV with increased Kupffer's cells. Sections C and D indicate arsenic-induced (8 mg kg^{-1} and 12 mg kg^{-1} body weight, respectively) rats have heavy hepatic cellular alteration and degeneration in portal vein.

Figure 7 - Renal microphotography of rat stained using haematoxylin and eosin (H&E). Section A: control group of rat's renal film showing normal architecture of nephrocytes with glomerulus (G) in Bowman's capsule (BC) (H&E × 800). Section B indicates arsenic (5 mg kg^{-1} body weight) treated rat's renal film reveals mild magnitude of degeneration in BC along with G (H&E × 1000). Sections C and D indicate arsenic-induced (8 mg kg^{-1} and 12 mg kg^{-1} body weight, respectively) rats have higher magnitude of degeneration in G and BC as there is severe hemorrhage in nephrocytes (H&E × 800).

Discussion

Consumption of arsenic contaminated drinking water alters metabolism leading to reduction in body weight, haematological alterations, highly production of free radicals to damage lipid, DNA, and protein (Adedara et al., 2018; Zhang et al., 2020; Tahir et al., 2023). Prolonged As-exposure creates toxicity that has potential to affects multi-organ failure, enzymatic inhibition, oxidative stress, mental impairment (neurobehavioral abnormalities) mainly in growing children, and cancer (Tchounwou et al., 2018; Thakur et al., 2021; Tahir et al., 2023). In the present study, LD_{50} based these all three doses (i.e., 5, 8, and 12 mg/kg body weight) administered for 90 days on the groups mentioned above was extremely effective (Verma et al., 2022). Across the dose duration, behavioural infirmities, weight and hair loss, keratosis and limb paralysis were seen in As-exposed rats' groups (Minatel et al., 2018; Karthikeyan et al., 2019). At the end of the experimentation, outcomes indicate that arsenic toxicant effects haematological parameters like HGB, RBC, HCT, MCH, MCHC, PLT and WBC (Bhardwaj et al., 2018). Study revealed significant ($p < 0.0001$) changes in HGB, RBC, HCT, WBC and PLT.

Scientifically, it has been suggested that prolonged exposure to arsenic leads to their accumulation in erythrocytes leading to inhibition of porphyrin or heam production, resulting reduction of RBC and HGB (Bhardwaj et al., 2018; Briffa et al., 2020). The As-exposed group showed elevated levels of WBC counts than control, which may be due to tissue damages, internal infection, severe physical stress, leukaemia or stimulation of immune system (Bhardwaj et al., 2018). The alteration in PLT counts indicates that arsenic inhibited the production of thromboprotein. The finding of the current study indicates that chronic As-exposure causes severe liver damage that may inhibit the production of thromboprotein, resulting in decrease in platelets (PLT) counts (Al Aboud, et al., 2021). Arsenic exposure interferes with blood electrolytes and nitric oxide (Sharma et al., 2022). The current study reveals that As-exposed rats' groups had significantly increased the serum level of potassium, but modestly decreased in the level of sodium, chloride and nitric oxide.

The elevated level of potassium in blood causes hyperkalemia, which is indicated by fatigue, muscle weakness, kidney disorders, heart rhythm etc. (Raffee et al., 2022). Nitric oxide (NO) reduction is a key regulator of cardiovascular function, neurotransmission, immunity and signaling (Lundberg and Weitzberg, 2022). Significant changes ($p < 0.0001$) in the level of electrolyte and nitric acid disrupts ROS generation, protein level, as well as lipid profile (Balarastaghi et al., 2023). The finding is in line with the reported study. Arsenic has been reported to metabolize in liver while the metabolized species are excreted through kidney. The high concentration of arsenic markedly increases the activity of SGPT, SGOT, ALP and bilirubin, which confirmed significant impairment of hepatic function (Mondal et al., 2018; Rafiee et al., 2018; Thakur et al., 2021). The outcomes of the present study reveals that As-exposed (T1, T2 and T3) rats on the different mentioned doses had significantly ($p < 0.0001$) increased levels than control. Consequently, these hepatic serum markers perturbations seems to be correlated with hepatic histopathology (Meyrier and Niaudet, 2018).

Investigation upon histopathology specimen revealed significant altered hepato-cellular architecture with the huge degenerated hepatocytes, vacuolizations in sinusoids and hemorrhages in the portal vein, which is consistent with previous reports (Mondal et al., 2018; Meyrier and Niaudet, 2018). Renal dysfunction was also detected in this study, where there were significant increase ($p < 0.0001$) observed in the serum levels of urea, uric acid and creatinine; in addition, significantly declined levels of albumin ($p < 0.0001$) were observed in comparison to the control (Heidari et al., 2018; Meyrier and Niaudet, 2018; Sharma et al., 2022; Verma et al., 2022). These outcomes were consistent with the previous reports. The elevated levels of urea and uric acid primarily damaged the renal function, but the increased level of serum creatinine significantly changed the cellular proliferation especially the decrease in glomerular filtration rate (Adedara et al., 2018; Mondal et al., 2018; Haidar et al., 2023). The severe renal toxicity of arsenic may be the reason for the lower levels of albumin in As-treated groups.

The arsenic toxins have been proven to cause oxidative stress by producing ROS during the conversion of their valence, which bind to the Sulfhydal groups of enzymes, and thereby generate ROS or indirectly inhibits enzyme action. Exposure to arsenic produce free radicals that damage body's lipids,

proteins and DNA (Kumar et al., 2020; Liu et al., 2020; Juan et al., 2021). The outcomes of this pre-sent study confirms that inorganic arsenic causes
lipid peroxidation in targeted organs namely liver, kidney and brain. Elevated levels of MDA are important signs of oxidative damages in cells, which is thought to be consequence of oxidative stress that occurs when the dynamic balance between the per-oxidative and antioxidant mechanism is disturbed (Meyrier and Niaudet, 2018; Panneerselvam et al., 2020; Haidar et al., 2023). The enhanced level of lipid peroxidation has been reported even at low dose of arsenic treatment in rats (Prasanna et al., 2013). Presence of arsenic in the body system dramatically increases the synthesis of PC, a by-product of protein oxidation (Prasanna et al., 2013; Panneerselvam et al., 2020; Liu et al., 2020). The oxidation of protein is believed to lead to an excess of reactive species. Moreover, proteins simultaneously regulates the majority of biological functions in cells and exhibit a variety of functional effects of oxidation. In this study the As-exposed groups (T1, T2 and T3) had significant elevation ($p < 0.0001$) in the level of MDA and PC than control (Prasanna et al., 2013; Panneerselvam et al., 2020; Haidar et al., 2023).

The GSH depletion level demonstrates an image in which energy metabolism and antioxidant system endure negative transformations. This depletion concomitantly leads to mitochondrial dysfunction because mitochondria cannot synthesize GSH itself (Panneerselvam et al., 2020; Olfati and Tvrda, 2021). Antioxidant enzymes are considered the first line of cellular defense against oxidative damage. SOD is an antioxidant metalloenzyme that reduces superoxide radicals to water and molecular oxygen (Xu et al., 2017; Panneerselvam et al., 2020; Olfati and Tvrda, 2021). CAT is a hem protein, which reduces hydrogen peroxide to molecular oxygen and water guttridge. It plays a major role in regulating cellular level of hydrogen peroxide catabolism that protects cells from oxidative attack. The outcomes of the present study reveals that the As-exposed rat's groups (T1, T2 and T3) had significantly decreased ($p < 0.0001$) in the level of GSH, SOD and CAT than control. The finding is in line with Loureiro et al., 2002; Al Aboud et al., 2021; Olfati and Tvrda, 2021; and Haidar et al., 2023.

Compared to the control group, the GF-AAS tested arsenic-induced rat's (T1, T2 and T3) groups had significantly high concentration ($p < 0.0001$) in blood and cerebellum tissues. These findings support previous researchers as they found chronic arsenic exposure causing significant arsenic accumulation in cerebellum tissue as compared to the blood (Kumar et al., 2020). The absorbed arsenic circulates in the whole body through blood flow, and its accumulation in cerebellum tissue caused neurological disabilities (Canalejo et al., 2016; Cruz-Esquivel et al., 2019; Kumar et al., 2020). Excessive arsenic concentrations in mammals have raised the amount of free radicals, which can lead to cancer and liver damage (Shankar et al., 2024).

Exposure to arsenic concentrations affects spontaneous motor activity through which significant ($p < .0001$) reduction in parameters was observed in the parameters as total distance travel, resting time, stereotype time, time of moving and rearing (Karthikeyan et al., 2019; Medda et al., 2020). The outcomes of this study indicate that the rotarod activity test had a significant reduction ($p < 0.0001$) in the As-exposed rat's (T1, T2 and T3) groups than control (Medda et al., 2020; Shankar et al., 2024). Due to the high accumulation of arsenic in the body system, power of muscle strength and grip strength become poor through which the power of fore arm and hind arm deteriorates (Canalejo et al., 2016; Adedara et al., 2018; Kumar et al., 2020; Medda et al., 2020). In the present study, As-exposed groups had significant ($p < 0.0001$) reduction in the power of grip strength activity than control, findings supported by earlier research (Chandravanshi et al., 2018; Rehman et al., 2018; Medda et al., 2020).

The histopathological examination about As-exposed group indicates substantial deterioration in hepatic and renal region (Mondal et al., 2018; Haidar et al., 2023). Compared to control, the hepatic anatomy of As-exposed rat's groups T1, T2 and T3 was substantially deformed, with dilated and congested central lobular vein and hepatic sinusoids. In almost all sections that were examined under light microscope, Kupffer's cells hyperplasia activation and noticeable hepatocyte cytoplasmic vacuolization were seen (Mondal et al., 2018; Kumar et al., 2020; Al Aboud et al., 2021; Shankar et al., 2024).

In many of the examined sections, it was also quite evident that there were focal hepatocytes necrosis accompanied with mononuclear cell infiltration as well as hepatocytes death (Mondal et al., 2018; Al Aboud et al., 2021). The nuclei of some examined sections had karyomegaly. Portal regions exhibited portal fibrosis along with the development of newly formed bile ducts and significant infiltration of monocyte-derived inflammatory cells (Mondal et al., 2018; Al Aboud et al., 2021; Haidar et al., 2023).

On the other hand, the histopathology of renal sections revealed that compared to control, As-exposed rat's groups T1, T2 and T3 had alteration with renal tubules (PCT and DCT) and Bowman's capsule along with the high degree of degeneration and swelling in the glomerulus (Mondal et al., 2018; Sharma et al., 2022). These symptoms were also seen in As-exposed groups, which included vascular degeneration of epithelial lining renal tubules, congestion of renal blood vessels, and focal necrosis of epithelial lining renal tubules associated with mononuclear cells infiltration as well as cystic dilatation of some renal tubules (Kocadal et al., 2020; Kumar et al., 2020; Xing et al., 2022; Meyrier et al., 2023).

Conclusion

The study concludes that regular exposure to arsenic at low or high concentration significantly alters the level of haematological parameters, enzymes, antioxidants (ROS), and causes several cellular changes in the hepato-nephro region. High concentration has an impact on neuro-behavioural activities. Regular exposure to arsenic tends to get accumulated by tissues, and long-term accumulation causes tumors on various organs and cancer. Moreover, arsenic causes significant damage to the vital organs, causing impaired organ functions. Therefore, significant antidotes as therapeutic agent needs to be discovered, which can combat the toxic effect of arsenic.

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