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Research Article

Effect of Arsenic Induction on Hematology of Albino Mice (*Mus musculus*) and assessment of DNA damage by micronucleus assay

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ABSTRACT Arsenic is a persistent environmental pollutant with serious health implications due to its non-biodegradable nature and bioaccumulation through water, soil, and food chains. Chronic exposure to arsenic is associated with various carcinogenic and genotoxic effects. This study aimed to evaluate the hematological and genotoxic impacts of arsenic in albino mice (*Mus musculus*) to better understand its systemic toxicity. A total of 24 healthy albino mice were randomly assigned into four groups: one control and three treatment groups. Mice in treatment groups received daily oral doses of sodium arsenate at concentrations of 3.5 µg, 6.5 µg, and 9.5 µg for 30 days. Standard housing and feeding conditions were maintained throughout the trial. Blood samples were collected post-treatment for hematological analysis using an automated hematology analyzer. Genotoxic effects were assessed using the micronucleus assay to evaluate DNA damage and nuclear abnormalities. Results revealed significant dose-dependent hematological alterations in arsenic-treated groups compared to controls. Notable declines were observed in red blood cell (RBC) counts, hemoglobin (HGB), and platelet (PLT) levels, especially at higher arsenic doses. Conversely, white blood cell (WBC) counts increased significantly with increasing arsenic concentration. Micronucleus assay results indicated marked genotoxic effects, with higher frequencies of micronuclei, binucleated cells, blebbed, and notched nuclei in arsenic-exposed groups. The highest genotoxic response was seen in the group receiving 9.5 µg arsenic, showing a micronucleus frequency of 37.85% compared to 0.267% in the control group ($p < 0.001$). In conclusion, arsenic exposure induces significant hematological disruptions and genotoxic damage in albino mice in a dose-dependent manner. These findings highlight the potential of arsenic to impair physiological and genetic functions even at low concentrations. This study underscores the urgent need for effective arsenic monitoring and mitigation strategies to safeguard environmental and public health.

KEYWORDS Arsenic toxicity, Hematological parameters, Micronucleus assay, DNA damage, Genotoxicity

Introduction

Heavy metal pollution is a serious environmental concern due to the non-biodegradable nature of metals. Unlike organic pollutants, metals cannot be broken down by microorganisms, leading to their accumulation in ecosystems (Briffa *et al*, 2020). Arsenic, a toxic and carcinogenic metal, poses a significant health risk through contaminated water, soil, and food chains (Liu and Wang, 2024). It has been historically used in traditional medicine and industry, yet it remains hazardous to both human and environmental health. The persistence of metals like arsenic in the environment causes long-term exposure risks (Khosravi-Darani *et al*, 2022). Arsenic exposure is associated with various cancers and non-carcinogenic disorders. Due to its widespread use and presence, managing arsenic contamination has become a global environmental challenge.

Arsenic enters the environment through both natural sources and human activities such as mining and industrial waste disposal (Xing *et al*, 2024). Once in the environment, arsenic is absorbed by plants and accumulates in animal and human tissues through the food chain. Aquatic organisms are especially vulnerable and may transfer arsenic to offspring. Arsenic exists in various oxidation states, with all forms being toxic. It interferes with cellular respiration, enzyme function,

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and DNA synthesis (Ganie *et al*, 2024). Arsenic-induced toxicity leads to diseases such as Guillain-Barre syndrome and liver damage (Kim *et al*, 2012). Environmental exposure is further compounded by the metal's ability to form stable, non-degradable compounds with other elements, making remediation complex and costly.

Despite efforts to reduce environmental exposure, arsenic contamination remains a critical public health issue. Its genotoxic and carcinogenic effects are still not fully understood, especially in relation to hematological and DNA-level damage. The present study aims to evaluate the impact of various concentrations of arsenic on hematological parameters in albino mice. This research will contribute to understanding arsenic toxicity and support efforts to monitor and mitigate its health effects. Specific objectives include assessing changes in blood composition and identifying DNA damage through micronucleus assay. The findings may inform future strategies for environmental protection and public health safety.

Materials and Methods

Ethical Considerations

Present study received ethical clearance from the Ethical committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan. All the subjects were handled in a humane way as per guidelines mentioned in the previous studies (Guidobono *et al.* 2010). All the aspects of basic living necessities for animals were monitored and assured. Additionally, drug administration methods were further monitored.

Selection and management of animals

Current research was conducted on albino mice (*Mus musculus*) as model animal at Lab Animal Enclosure Farm, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki. A total of 24 albino mice, with average 7.5 weeks of age and 35-40 g weight, were included in this study. The animals were screened for any kind of disease or any physical harm. The diseased animals were rejected for research work and only healthy animals with no disease symptoms were selected. Mice were raised in sterile stainless-steel cages in a properly aerated animal room under standard conditions. Considering stock density issues, 4 animals were placed in each cage. The floor of each cage covered with sterile saw dust bedding which was regularly changed after every three days. Commercial laboratory pelleted feed was provided all subjects along with free access to drinking water.

Study design and randomization

A preclinical multi-arm randomized controlled trial with a parallel-group design was conducted. The subjects were randomly divided into four groups (arms) with 6 albino mice in each. To compare the exposure and outcome, these four arms included placebo and treatment groups based on dose increasing manner. Following the SPIRIT recommendations (https://spirit-statement.org/), randomization was performed using random generation in R-statistical language.

Intervention

In order to study the effects of Arsenic, Sodium arsenate was administered orally at a dose of 3.5µg, 6.5µg, and 9.5µg among three treatment groups. The doses for each group were prepared by dissolving Sodium arsenate in 10 ml water. The concentration of Sodium arsenate dose to each mouse was 0.2 ml per day. As placebo control, normal saline was administered in similar dose preparation. Interventions were administered to all the groups for 30 days. Mice were monitored every day during the study for any severe and chronic side effects.

Blood collection

Mice were sacrificed on day 31st, after completion of trial, blood samples were collected from heart of mice. Anaesthesia was given to mice to minimize discomfort before blood collection. The blood was taken by sinus puncture with care. Approximately 1 ml blood was collected from each mouse and was transferred to blood vacutainers with EDTA for storage of blood and to prevent coagulation of blood.

Haematological analysis

To compare the characteristics and counts of blood cells among groups, the haematological analysis was performed. The following haematological parameters were included for analyses: Red Blood Cells (RBCs), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Hematocrit (HCT), White Blood Cells (WBC) and platelet counts. The hematological analysis was performed using DxH 690T Hematology Analyzer (Beckman Coulter Inc., USA).

Micronucleus Assay Test

The micronucleus assay was performed to determine the mutagenic effects of arsenic that causes the formation of small membrane bounded fragments in interphase cells. The micronuleus assay was performed as per previous published literature with little modifications. A total of 500 µl histopaque was added in 500 µl blood sample. The centrifugation of mixture was done at 2100rpm for 20 minutes. The central layer was taken and added equal amount of 10x PBS. The centrifugation of mixture was done for 10 minutes at 1600rpm. The supernatant was discarded. The pellets were collected and mixed with 1% KCl solution. The incubation of this mixture was done for 5-10 minutes. The centrifugation of this mixture was done for 5-6 minutes at 1500rpm. The pellets were taken and add 1ml fixative 1 followed by centrifugation for 5-6 minutes at 1500rpm. The 1ml fixative 2 was added in pellets followed by centrifugation for 5-6 minutes at 1500rpm. The pellets were collected with some supernatant followed by vigorous mixing. The mixture was taken on wet slide and allowed to dry for 24 hours. The slides were dipped in beaker containing Giemsa stain (10ml Giemsa + 90ml distilled water) for 10 minutes. The slides were washed by distilled water and allow drying. The slides were observed under light microscope (Kumar *et al.* 2015).

The nuclear abnormalities and frequency of micronuclei were evaluated by light microscope under oil emersion lens carefully. Approximately 500±50 cells were examined for each sample. These cells were observed for the frequency of micronuclei and other nuclear abnormalities, including binucleated, blebbed and notched shaped nuclei. The

frequency of micronuclei were evaluated by following formula devised by Fenech *et al* (2003).

$$\text{Micronucleus frequency (\%)} = \frac{\text{No.of cells with micronuclei}}{\text{Total No.of cells counted}} \times 100$$

Data analysis

Data collected were analysed using R-statistical language (version 4.3) Descriptive results for quantitative data were presented using mean and standard deviation. The data collected were analysed statistically through one-way ANOVA tests followed by Tukey post-Hoc tests on significa--nt groups (p-value <0.05).

Results

Hematological parameters

The results of this study revealed that there are negative effects of arsenic on the hematological parameters. There are significant differences in hematological parameters between control group and three different treatment groups of having different concentrations of arsenic respectively (3.5µg, 6.5µg and 9.5µg). The mean concentrations of hematological parameters with standard deviation for all treatments and control group are given in table 1. The highest and significant variation was observed in the levels of RBCs, WBCs, and HGB in third treatment, compared to the control group. The lowest variation observed in number of MCV, MCH and MCHC of all kind of groups. As concentration of arsenic increase the variation of blood counts significantly varies as compare to the control group.

Table 1. Mean Blood counts with standard deviation of all treatments and control group

Hematological Parameters	Control Group	Treatment 1	Treatment 2	Treatment 3	p-value
RBCs (10^6/µL)	8.58 ± 0.216^{a}	8.5 ± 0.062^{a}	7.4 ± 0.127^{b}	6.97 ± 0.043^{c}	<0.001
WBCs (10^3/µL)	25.83 ± 1.139^{a}	26.12 ± 0.904^{a}	36.38 ± 0.050^{b}	40.36 ± 0.103^{b}	<0.001
HGB (g/dL)	14.21 ± 0.516^{a}	12.14 ± 0.166^{b}	12.39 ± 0.231^{b}	10.24 ± 0.205^{c}	<0.001
HCT (%)	43.54 ± 2.764^{a}	36.95 ± 0.947^{b}	41.23 ± 0.309^{ab}	36.03 ± 0.901^{b}	<0.01
MCV (fL)	51.67 ± 1.291^{a}	43.81 ± 0.774^{b}	51.34 ± 2.820^{a}	51.07 ± 0.469^{a}	<0.001
MCH (pg)	15.66 ± 0.953^{a}	13.82 ± 0.160^{a}	14.41 ± 0.215^{a}	15.16 ± 0.193^{a}	<0.075
MCHC (g/dL)	28.51 ± 0.273^{a}	30.71 ± 0.344^{b}	30.57 ± 0.068^{b}	29.34 ± 0.248^{ab}	<0.001
PLT (10^3/µL)	742.33 ± 1.764^{b}	686.75 ± 12.73^{a}	700.33 ± 1.453^{a}	728.67 ± 5.696^{b}	<0.001

Body parts affected and impact of daily activities

According to the findings of our study the increased arsenic level induced DNA damages in peripheral lymphocytes. There were many different types of abnormalities observed in prepared slides but only total micronucleus frequency and three types of nuclear aberrations (binucleated, blebbed and notched nuclei cells) were mainly focused (Fig 1). The frequencies of nuclear abnormalities were significantly varied in different treatments. The results revealed that the frequency of micronuclei was higher in third treatment group than other treatment groups (table 2). There was minor difference for micronucleus frequency between treatment two and three and control group. Similarly, the binucleated frequency less as compare to micronuclei but within treatments there were mean difference found then control group. Blebbed and notched nuclei cells frequency were significantly lower as compare to other nuclear abnormalities. However total analyzed cells mean differences was significant in all types of treatments and control group.

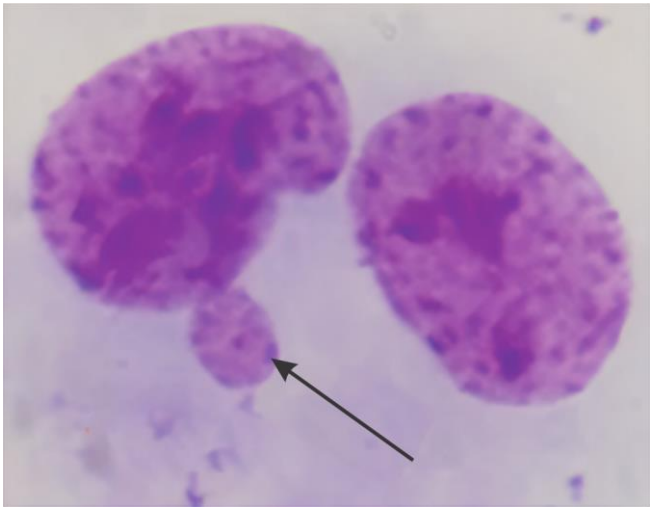

Fig. 1: Micronucleus formation in the treated cells.

Table 2. Mean nuclear abnormalities with standard deviation of all treatments and control group

Parameters	Control Group	Treatment 1	Treatment 2	Treatment 3	p-value
Micronuclei frequency (%)	0.267 ± 0.092^{a}	14.72 ± 1.375^{b}	18.59 ± 2.149^{c}	37.85 ± 2.724^{d}	<0.001
Binucleated cells	0.184 ± 0.088^{a}	2.689 ± 0.447^{b}	4.018 ± 0.466^{c}	6.612 ± 0.276^{d}	<0.001
Cells with blebbed nucleus	0.032 ± 0.008^{a}	1.453 ± 0.214^{b}	2.703 ± 0.29^{c}	4.410 ± 0.346^{d}	<0.001
Cells with notched nucleus	0.023 ± 0.005^{a}	0.066 ± 0.012^{a}	0.119 ± 0.021^{a}	1.761 ± 0.256^{b}	<0.001
Total number of analyzed cells	553 ± 25.48^{ac}	452 ± 25.29^{ab}	535 ± 28.32^{c}	436 ± 20.28^{b}	<0.014

Discussion

The toxicity due to Arsenic consumption affects millions of people around the world. The major source being leaching out of Arsenic into aquifers, contamination into drinking water, mining activities, burning of fossil fuels, metal smelting and from various other industrial processes (Ratnaike, 2003). Other sources of Arsenic pollution include the commercial usage of Arsenic as preservative for timber, this exposes out the Arsenic to our environment (Chung *et al*, 2014; Khosravi-Darani *et al*, 2022). The key purpose of the following study was to determine the hematology and DNA damage due to the intoxication of Arsenic (As) in Albino mice (Mus musculus). In this study, different levels of Arsenic were made and the toxicity consequences were analyzed on hematological parameters and DNA damage *of Mus musculus*. *Mus musculus* being a mammalian specie, the results carried out were supposed to be identical as other mammalian specie such as *Homo sapiens*. The results of these trials will be in correspondence to all the mammalian biota on this planet.

With the increase of global population, there is rapid increase in urbanization which also resulted in abrupt increase of industrialization. Higher industrialization rate put half of the earth's population to Arsenic exposure. The estimate human population in 2019 was 7.7 billion, which was once 8.5 billion in 2012-13. According to estimation, the world population will increase to 9.7 billion in 2050 and 10.9 billion in 2100 (Gu *et al*, 2021). According to World Bank, almost half of the world population (4 billion) lives in urban areas in 2019, which is likely to increase 6 billion in 2019 which is almost 1.5 times higher than the 2019's world population (Gu *et al*, 2021). With the increase of urbanization, there is increase of industrialization rate hence more pollution of underground water. The arsenic level in 50 countries concluding 140 million people is 10 µg/L in commonly drinking water.

To check out the oral toxicity of Arsenic in *Mus musculus*, various concentration of Arsenic metal were dissolved in water. Keeping in mind the guideline of WHO, the concentration of Arsenic was kept below 10 µg/L.

The hematological parameters were determined by hematological analyzer. During analyzing hematological results, it was found that number of RBCs decreasing as with increasing time interval. It was also found that WBCs increasing with increasing time interval most probably for natural defense of the body. Similar results were reported by Zhu *et al* (2024) when they treated Tatera indica and Bandicota begalensis with zinc phosphide and vacor (Sridhara and Srihari, 1980). Similar results were also obtained by Ho (2004) while experiment on *Rattus rattus* when giving rodenticide named rocumin which shows an increase in WBCs and decrease in RBCs, hemoglobin, and platelets count (Rahmawati *et al*, 2022). These results also matched with Jesri *et al* (2005) when they found that there was increase in WBCs and decrease in platelet count.

Increasing level of Arsenic causes hemorrhage (destruction of blood vessels and leakage of blood from body organs). Fibrinogen is the blood clotting protein only formed in organism's liver, but due to the attachment of dicoumarol as an inhibitor blocks the vitamin K cycle, so in these conditions the blood from the different organs lost and blood pressure in the body of the mice eventually falls out and results in the death of that animal Fibrinogen is the blood clotting protein only formed in organism's liver, but due to the attachment of dicoumarol as an inhibitor blocks the vitamin K cycle, so in these conditions the blood from the different organs lost and blood pressure in the body of the mice eventually falls out and results in the death of that animal (Hanington and Zhang, 2011).

The micronucleus results of current study revealed that with increasing concentration of sodium arsenate the numbers of abnormal cells increased. The arsenic induces DNA damages in cells. There were different types of abnormal cells, but we focus on some types such as binucleated, blebbed and notched nuclei cells and total micronucleus frequency. There were significant variations between different groups for the frequency of micronucleus cells.

The results of our research found that the arsenic exert negative impact on hematological parameters of albino mice. The introduction to arsenic induced mutations in genetic material and resulting formation of micronuclei in albino mice. The hematological parameters can be used to assess the health status of an organism. The findings of this study

concluded that the micronucleus assay test can be performed to assess the genotoxic effects. The results concluded that arsenic is a genotoxic element. The findings of this study will be helpful to understand the effects of arsenic on hematological parameters and genetic material

Declaration of Competing Interest

The authors declare that they have no competing or conflict of interests.

Author Contributions

AA: Conceptualization, Methodology, Formal analysis, Writing—original draft preparation. **AI:** Conceptualization, Methodology, Writing—review and editing. **SS:** Methodology, Formal analysis. **MI:** Methodology, Formal analysis. All authors have read and agreed to the published version of the manuscript.

References

1. Briffa, J., E. Sinagra, and R. Blundell. (2020). *Heavy metal pollution in the environment and their toxicological effects on humans*. Heliyon 6(9):e04691. doi: https://doi.org/10.1016/j.heliyon.2020.e04691
2. Chung, J. Y., S. D. Yu, and Y. S. Hong. (2014). *Environmental source of arsenic exposure*. J Prev Med Public Health 47(5):253-257. doi: 10.3961/jpmph.14.036
3. Fenech, M., W. P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi, and E. Zeiger. (2003). *HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures*. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 534(1-2):65-75.
4. Ganie, S. Y., D. Javaid, Y. A. Hajam, and M. S. Reshi. (2024). *Arsenic toxicity: sources, pathophysiology and mechanism*. Toxicol Res (Camb) 13(1):tfad111. doi: 10.1093/toxres/tfad111
5. Gu, D., K. Andreev, and M. E. Dupre. (2021). *Major Trends in Population Growth Around the World*. China CDC Wkly 3(28):604-613. doi: 10.46234/ccdcw2021.160
6. Hanington, P. C., and S. M. Zhang. (2011). *The primary role of fibrinogen-related proteins in invertebrates is defense, not coagulation*. J Innate Immun 3(1):17-27. doi: 10.1159/000321882
7. Ho, C. H. (2004). *White blood cell and platelet counts could affect whole blood viscosity*. J Chin Med Assoc 67(8):394-397.
8. Jesri, A., E. C. Okonofua, and B. M. Egan. (2005). *Platelet and white blood cell counts are elevated in patients with the metabolic syndrome*. J Clin Hypertens (Greenwich) 7(12):705-711; quiz 712-703. doi: 10.1111/j.1524-6175.2005.04809.x
9. Khosravi-Darani, K., Y. Rehman, I. A. Katsoyiannis, E. Kokkinos, and A. I. Zouboulis. (2022). *Arsenic Exposure via Contaminated Water and Food Sources*. Water 14(12):1884.
10. Kim, S., A. Takeuchi, Y. Kawasumi, Y. Endo, H. Lee, and Y. Kim. (2012). *A Guillain-Barré Syndrome-like Neuropathy Associated with Arsenic Exposure*. Journal of Occupational Health 54(4):344-347. doi: https://doi.org/10.1539/joh.12-0023-CS
11. Liu, T., and Z. Wang. (2024). *Contamination and health risk assessment of heavy metals in soil surrounding an electroplating factory in JiaXing, China*. Scientific Reports 14(1):4097. doi: 10.1038/s41598-024-54620-w
12. Rahmawati, N., M. Syukri, D. Darmawi, I. Zachreini, U. Zulfiani, M. Yusuf, and R. Idroes. (2022). *Haematological Features of White Rats (Rattus norvegicus) Infected with S. pyogenes and Administered with Probiotics (Yogurt)*. ScientificWorldJournal 2022:2899462. doi: 10.1155/2022/2899462
13. Ratnaike, R. N. (2003). *Acute and chronic arsenic toxicity*. Postgrad Med J 79(933):391-396. doi: 10.1136/pmj.79.933.391
14. Sridhara, S., and K. Srihari. (1980). *Bait shyness towards zinc phosphide & vacor in the larger bandicoot rat Bandicota indica (Bechstein)*. Indian J Exp Biol 18(9):1029-1031.
15. Xing, M., D. Yan, M. Hai, Y. Zhang, Z. Zhang, and F. Li. (2024). *Arsenic Contamination in Sludge and Sediment and Relationship with Microbial Resistance Genes: Interactions and Remediation*. Water 16(24):3633.
16. Zhu, J., Z. Li, Y. Deng, L. Lan, and J. Yang. (2024). *Comprehensive reference intervals for white blood cell counts during pregnancy*. BMC Pregnancy and Childbirth 24(1):35. doi: 10.1186/s12884-023-06227-8