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Sub-Lethal Sodium Arsenite Elicits Pronounced Lipid Peroxidation in Vital Organs of Zebrafish (*Danio rerio*)

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Abstract: ***Arsenic is a pervasive environmental contaminant that exerts profound toxicological effects on aquatic organisms, primarily through oxidative stress and disruption of redox homeostasis. Sub-lethal exposures are of particular concern, as they can impair physiological integrity without inducing overt mortality, thereby threatening population health and ecosystem stability. In the present study, we investigated lipid peroxidation (LPO) as an indicator of oxidative damage in the liver, kidney, and brain of adult zebrafish following sodium arsenite exposure at Sub-lethal levels. Fish were exposed to 10, 300, and 800 ppb sodium arsenite for 7, 21, and 42 days, and LPO levels were quantified by measuring malondialdehyde (MDA) formation via the thiobarbituric acid reactive substances (TBARS) assay. A significant, dose- and duration-dependent increase in MDA levels was observed across all tested tissues, reflecting progressive oxidative stress with increasing arsenite burden. The brain showed the highest vulnerability to lipid oxidative injury, followed by the liver and kidneys. The results establish that even sub-lethal concentrations of sodium arsenite trigger measurable oxidative perturbations, with organ-specific variations in sensitivity. Collectively, this study demonstrates that LPO is a robust and sensitive biomarker of arsenic-induced oxidative stress in zebrafish, providing mechanistic insights into sub-lethal arsenic toxicity and reinforcing the utility of Danio rerio as a sentinel species for aquatic ecotoxicological assessment.***

Keywords: Sodium arsenite, Zebrafish (*Danio rerio*), Lipid peroxidation, Oxidative stress, Malondialdehyde (MDA), and TBARS assay

1. Introduction

Arsenic contamination represents a critical environmental issue, posing escalating risks to aquatic ecosystems and global public health. It is estimated that nearly 2.5 billion people, representing 32% of the world's population, are exposed to arsenic at levels associated with significant health risks, predominantly through arsenic-laden water sources (Aryan et al. 2024). This problem is especially severe in South and Southeast Asia, including significant parts of India and Bangladesh, where persistent contamination of groundwater and surface water has led to widespread arsenic exposure (Shaji et al. 2021; Aryan et al. 2024; Sultan et al. 2025) In India, regions including Punjab, West Bengal, Assam, Bihar, Chhattisgarh, and Uttar Pradesh have documented among the highest arsenic burdens in their drinking and irrigation sources (Kumar & Nayak 2025). Vulnerable populations within these regions are susceptible to toxicity due to reliance on contaminated water for daily consumption, agriculture, and fisheries.

Both organic and inorganic forms of arsenic are found naturally and as a result of anthropogenic activities, but it is the inorganic forms, particularly trivalent (As(III)) and pentavalent (As(V)) arsenic, that are associated with higher toxicity and pose a more serious threat to biological systems. Among the diverse mechanisms underlying arsenic toxicity, oxidative stress has been identified as a principal mechanism of cellular injury (Lantz & Hays 2006; Ganie et al. 2024; Thakur et al. 2025). Central to this mechanism is the generation of reactive oxygen species (ROS), which trigger the peroxidation of vital biomolecules, including lipids, proteins, and nucleic acids. LPO is a key event in oxidative stress, commencing when ROS or free radicals oxidize polyunsaturated fatty acids in cell membranes, leading to their degradation and the formation of MDA, a well-established biomarker of LPO. This oxidative injury compromises membrane integrity and disturbs vital signaling pathways necessary for cellular homeostasis (Ganie et al. 2024; Thakur et al. 2025).

Importantly, aquatic organisms in natural environments are commonly subjected to chronic exposure to sub-lethal concentrations of arsenic, which may result in the gradual onset of oxidative stress and subsequent tissue damage. Also, the extent of oxidative damage and susceptibility to arsenic varies significantly among different tissues, depending on their physiological and biochemical interactions with arsenic (Malik et al. 2023). This underscores the necessity for comparative evaluations across key organs under sub-lethal arsenic exposures to elucidate the mechanistic basis of arsenic toxicity. To address this gap, the present investigation evaluates the effects of sodium arsenite at 10 ppb (consistent with WHO guidelines), 300 ppb, and 800 ppb on LPO in the liver, kidney, and brain tissues of adult zebrafish, with exposure durations of 7, 21, and 42 days, thereby capturing the spectrum of contamination commonly observed in aquatic ecosystems.

Zebrafish were selected as the experimental model organism owing to their widespread adoption in toxicological research, driven by their high genetic homology to humans, and ease of laboratory manipulation (Ferrandino 2024; Zhao et al. 2024). Additionally, zebrafish are extensively utilized in studies evaluating the toxicity of environmental pollutants, including metals and metalloids such as arsenic (Bambino & Chu 2017).

2. Materials and Methods

2.1. Experimental Animals and Husbandry

Adult zebrafish, measuring 3–4 cm in standard length and weighing approximately 0.4–0.5 g, were procured from a certified fish supplier from Howrah, India. Upon arrival, the fish were subjected to a mild potassium permanganate (Sigma-Aldrich) bath to mitigate the risk of external infections and parasites. Subsequent to this treatment, the fish were acclimated to laboratory conditions for a period of 14 days. The fish were maintained in dechlorinated tap water within aerated glass tanks, maintained at 26 ± 2 °C. They were fed *ad libitum* with regular monitoring throughout the study. The lethal concentration 50 (LC50) values for sodium arsenite were determined according to the protocols described by Prasad (2024), providing essential toxicity baselines for the subsequent sub-lethal exposure experiments.

2.2. Study Design and Exposure Regimen

A total of 60 zebrafish were randomly allocated into four experimental groups (n = 15 per group): one unexposed control and three treatment groups receiving sodium arsenite (Loba Chemie) at concentrations of 10 ppb, 300 ppb, and 800 ppb, respectively. Each group was subdivided into three subgroups corresponding to exposure durations of 7, 21, and 42 days (n = 5 per time point). Exposures were conducted in 20-liter glass aquaria following a semi-static renewal regime, with complete water and arsenite replacement every 48 hours to maintain consistent toxicant levels and water quality parameters. No mortality was recorded during the exposure period across all experimental groups.

2.3. Dissection and Tissue Harvesting

At designated time points, zebrafish (n = 3 per subgroup) were euthanized by immersion in an overdose of MS-222 (tricaine methanesulfonate: Sigma-Aldrich). Liver, kidney, and brain tissues were rapidly dissected under sterile conditions, rinsed in phosphate-buffered saline (PBS, pH 7.4), gently blotted to remove excess moisture, weighed, and stored at −20 °C until further biochemical analyses.

2.4. Lipid Peroxidation Assay

Lipid peroxidation levels were assessed by quantifying MDA in tissue samples. Equal amounts of liver, kidney, and brain tissues were homogenized using a borosilicate glass tissue homogenizer to prepare tissue homogenates. Subsequently, aliquots of these homogenates from each experimental group were subjected to MDA determination using the TBARS assay. The protocol was adapted from Ohkawa et al (1979) with minor modifications optimized for zebrafish tissue matrices.

2.5. Statistical Analysis

Data are presented as mean ± standard deviation (SD) for three biological replicates per treatment and time point. Statistical comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). A significance threshold was set at $p < 0.05$. All statistical analyses and graphical representations were conducted using GraphPad Prism software (version 4.0).

3. Results

3.1. Sodium Arsenite Elevates Lipid Peroxidation in Liver, Kidney, and Brain Tissues

Exposure to sodium arsenite resulted in a marked, dose- and duration-dependent increase in LPO, as quantified by MDA levels, across liver, kidney, and brain tissues of adult zebrafish. Control groups maintained stable baseline MDA concentrations across all time points, confirming the absence of oxidative perturbation under normal physiological conditions.

3.2. Liver Tissue

The liver, as a primary organ involved in detoxification, is highly susceptible to heavy metal exposure. To evaluate the effects of sodium arsenite on LPO in hepatic tissue, LPO levels were measured across varying doses and exposure durations. Our results demonstrated a dose- and time-dependent progressive increase in hepatic LPO, with MDA concentrations significantly elevated in response to higher arsenite concentrations and longer exposure periods. Statistical analysis confirmed these increases to be significant ($p < 0.05$ and $p < 0.001$), as detailed in Table 1 and illustrated in Figure 1.

Table 1: Mean MDA concentration (µmol/mg protein) in liver tissue, expressed as mean ± SD.

Exposure Duration	Concentration (ppb)	Mean MDA Level (µmol/mg protein)	Standard Deviation
7 days	Control	0.71	0.09
	10ppb	1.13	0.07
	300ppb	2.57	0.08
	800ppb	4.41	0.25
21 days	Control	0.73	0.10
	10ppb	1.33	0.08
	300ppb	3.16	0.14
	800ppb	5.34	0.36
42 days	Control	0.86	0.11
	10ppb	1.49	0.09
	300ppb	3.69	0.22
	800ppb	6.69	0.28

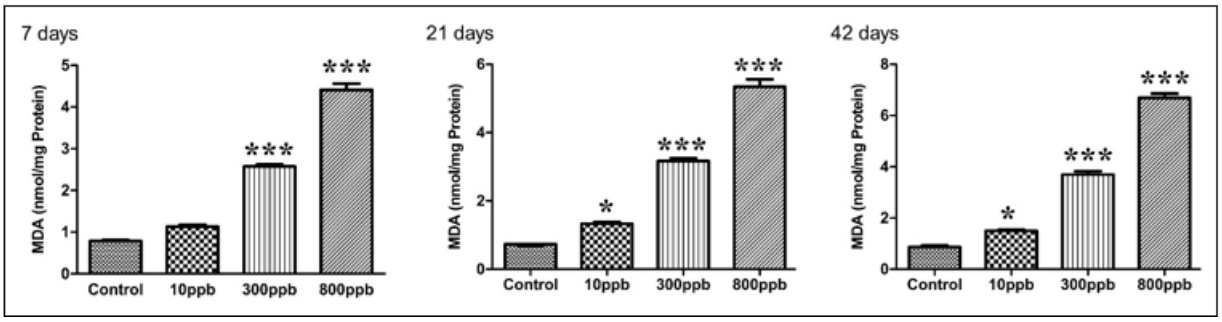

Figure 1: Effects of sodium arsenite exposure on LPO in zebrafish liver tissue over varying doses and periods

MDA concentrations, a marker of oxidative stress, were recorded at 10, 300, and 800 ppb arsenic concentrations for 7, 21, and 42 days. Data is presented as mean ± SD with statistical significance of *p≤0.05 and ***p≤0.001.

3.3. Kidney Tissue

Renal tissue demonstrated an early onset of oxidative damage subsequent to sodium arsenite exposure. Our study revealed a dose- and time-dependent progressive elevation of LPO in the kidney, with MDA levels significantly increasing in correlation with higher arsenite concentrations and prolonged exposure durations. Statistical analyses confirmed these elevations to be significant ($p < 0.05$, $p < 0.01$, and $p < 0.001$), as presented in Table 2 and depicted in Figure 2.

Table 2: Mean MDA concentration (µmol/mg protein) in kidney tissue, expressed as mean ± SD.

Exposure Duration	Concentration (ppb)	Mean MDA Level (µmol/mg protein)	Standard Deviation
7 days	Control	0.59	0.07
	10ppb	0.83	0.07
	300ppb	1.72	0.22
	800ppb	2.91	0.33
21 days	Control	0.42	0.03
	10ppb	0.91	0.10
	300ppb	2.27	0.24
	800ppb	3.64	0.11
42 days	Control	0.46	0.04
	10ppb	1.27	0.12
	300ppb	2.55	0.27
	800ppb	4.29	0.21

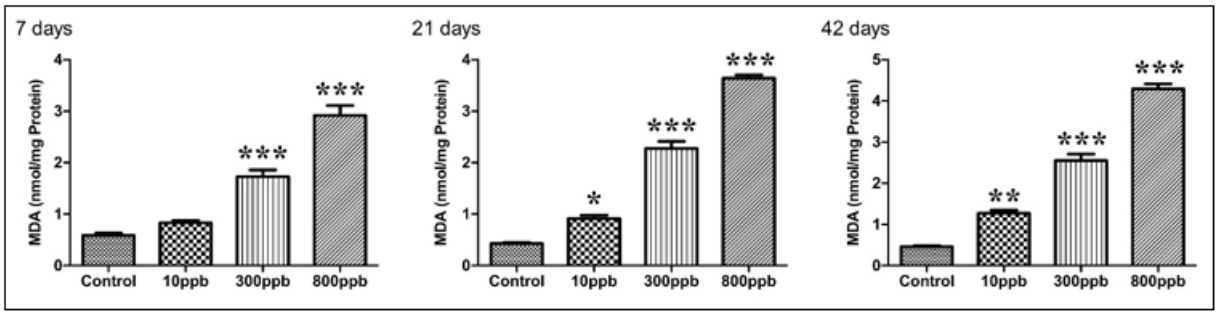

Figure 2: Effects of sodium arsenite exposure on LPO in zebrafish kidney tissue over varying doses and periods

MDA concentrations, a marker of oxidative stress, were recorded at 10, 300, and 800 ppb arsenic concentrations for 7, 21, and 42 days. Data is presented as mean ± SD with statistical significance of *p≤0.05, **p≤0.01, and ***p≤0.001.

3.4. Brain Tissue

In this study, the brain was identified as the most vulnerable organ to sodium arsenite-induced toxicity, demonstrated by the highest dose- and time-dependent increases in MDA levels. Statistical analysis confirmed these elevations to be significant ($p < 0.05$, $p < 0.01$, and $p < 0.001$), as detailed in Table 3 and illustrated in Figure 3.

Table 3: Mean MDA concentration (µmol/mg protein) in brain tissue, expressed as mean ± SD

Exposure Duration	Concentration (ppb)	Mean MDA Level (µmol/mg protein)	Standard Deviation
7 days	Control	1.20	0.195
	10ppb	1.57	0.077
	300ppb	3.16	0.045
	800ppb	5.46	0.224
21 days	Control	1.21	0.065
	10ppb	1.85	0.085
	300ppb	4.38	0.345
	800ppb	6.85	0.165
42 days	Control	1.16	0.109
	10ppb	2.54	0.16
	300ppb	4.85	0.545
	800ppb	8.40	0.358

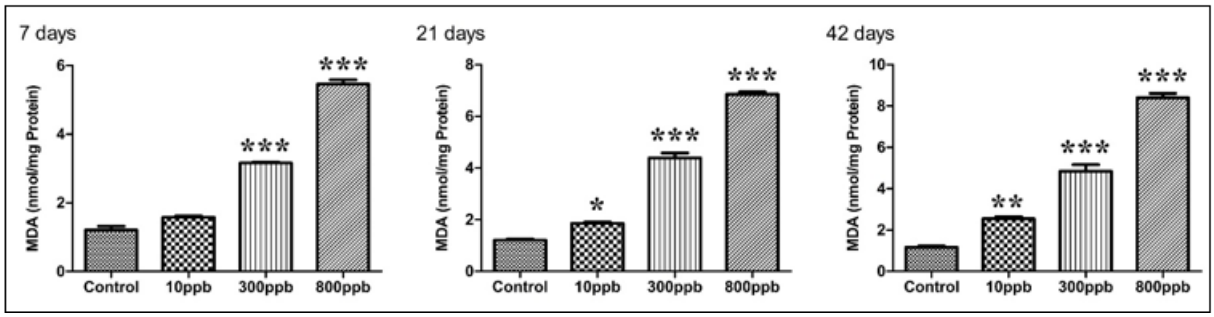

Figure 3: Effects of sodium arsenite exposure on LPO in zebrafish brain tissue over varying doses and periods

MDA concentrations, a marker of oxidative stress, were recorded at 10, 300, and 800 ppb arsenic concentrations for 7, 21, and 42 days. Data is presented as mean ± SD with statistical significance of *p≤0.05, **p≤0.01, and ***p≤0.001.

4. Discussion

Sodium arsenite poses significant environmental risks as it can induce oxidative stress in both humans and aquatic organisms, even at concentrations below lethal thresholds (Kumari et al. 2017; Wang et al. 2021). Arsenic is well documented to interfere with redox homeostasis by generating excessive ROS and impairing antioxidant defense mechanisms, thereby promoting oxidative damage to cellular components and affecting overall health and functionality. Among these, LPO is a critical endpoint, as it reflects the vulnerability of polyunsaturated fatty acids in membrane lipids to free radical attack, ultimately compromising membrane integrity and cellular function. MDA, one of the most abundant and stable end products of polyunsaturated fatty acid oxidation, is widely employed as a biochemical index to assess the extent of oxidative damage in biological systems (Ganie et al. 2024; Thakur et al. 2025). In aquatic toxicology, elevation of MDA levels serves as a reliable indicator of compromised membrane integrity, altered redox homeostasis, and subsequent organ dysfunction (Rizzo 2024). Zebrafish, a well-recognized model for environmental toxicology, provides valuable insights into organ-specific oxidative responses to arsenic stress. Hence, evaluating MDA accumulation in vital organs following sub-lethal sodium arsenite exposure is essential to understand the mechanistic basis of arsenic-induced oxidative damage in fish physiology (Malik et al. 2023).

Numerous experimental studies have established a robust positive correlation between arsenic exposure and increased MDA levels, demonstrating this effect across a spectrum of vital organs including liver, kidney, brain and gastrointestinal tract of fish species (Malik et al. 2023; Rizzo 2024). In the current study, MDA levels were quantitatively assessed in the liver, kidney, and brain tissues of zebrafish that were subjected to different concentrations of sodium arsenite over varying durations, as detailed in section 2.2. We observed that, among three organs, the brain exhibited the highest lipid peroxidation, particularly at arsenic concentrations of 300 ppb and 800 ppb by the 21st and 42nd days of exposure. These results can be attributed to the brain's high rate of oxygen usage, abundance of lipid-rich membranes, and comparatively limited antioxidant defenses. Supporting literature describes similar neurotoxic outcomes in zebrafish and other teleost species upon chronic arsenic insult, underscoring the mechanistic role of oxidative stress in mediating arsenic-induced neurotoxicity (Sarkar et al. 2014; Malik et al. 2023; Saç & Yeltekin 2023).

In hepatic tissue, there was a notable and proportional increase in MDA levels, correlated with both dosage and duration of exposure. This observation aligns with the liver's essential role in detoxification and xenobiotic metabolism. The escalating MDA levels highlight the liver's susceptibility to oxidative stress induced by arsenic, particularly under extended exposure conditions where the protective antioxidant mechanisms are compromised. Moreover, similar hepatotoxic effects have been documented in various fish species subjected to arsenic and other heavy metals, reinforcing the consistency of these findings across different biological contexts (Khalid et al. 2024; Tang et al. 2024; Khalid & Azmat 2025).

Renal tissues in zebrafish displayed a moderate, yet statistically significant, upsurge in LPO, particularly evident at the highest dosages and extended arsenic exposure durations. Given the kidney's pivotal role in arsenic clearance and osmoregulation, the observed pattern of oxidative damage may reflect cumulative toxicant burden leading to impaired filtration and altered excretory function. These observations are consistent with previous studies that have reported similar manifestations of renal oxidative stress and functional deterioration following chronic exposure to metals in aquatic organisms, corroborating the current findings (Khalid et al. 2024; Concessao & Prakash 2025; Khalid & Azmat 2025)

A comparative analysis of MDA values revealed a distinct hierarchy of tissue susceptibility: brain > liver > kidney. Although all tissues experienced significant dose- and time-responsive increases in lipid peroxidation, the brain's consistently elevated MDA levels underscore its particular vulnerability to arsenic. These results suggest organ-specific dynamics in arsenic uptake, metabolism, and antioxidant capacity. Overall, the findings highlight the need for further research exploring the molecular underpinnings of arsenic-induced neural injury and reinforce the relevance of the central nervous system as a sentinel for oxidative damage triggered by environmental metal toxicity.

5. Conclusion

Sub-lethal exposure to sodium arsenite induces notable increases in lipid peroxidation, which are both dose- and time-dependent, in adult zebrafish. This effect appears to be organ-

specific, with the brain exhibiting the most significant changes. These results establish oxidative stress as a key pathway in arsenic toxicity and reinforce the utility of zebrafish models for mechanistic studies. Comprehensive lipid peroxidation biomarker profiling is crucial for understanding arsenic-induced effects in aquatic environments.

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