Bulletin of Pure and Applied Sciences
Vol.40A (Zoology), No.2,
July-December 2021: P.215-225

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI: 10.5958/2320-3188.2021.00026.7

Content Available online at: https://www.bpasjournals.com/zoology

Original Research Article

Assessment of 8-hydroxy-2′deoxyguanosine Activity and Histopathological Alteration in Liver and Intestine of Zebrafish (*Daniorerio*) Exposed to Arsenic Trioxide

Thakur Swati Singh[1], Jain Abhishek[2], Jain Roshni[3], Jain Swati[4], *Jain Subodh Kumar[5]

Author's Affiliation:
[1,2,3]Department of Biotechnology, Dr Harisingh Gour University, Sagar, Madhya Pradesh 470003, India
[4,5]Department of Zoology, Dr Harisingh Gour University, Sagar, Madhya Pradesh 470003, India

***Corresponding author:**
Prof. Subodh Kumar Jain
Department of Zoology, Dr Harisingh Gour University, Sagar, Madhya Pradesh 470003, India
E-mail: subjain@gmail.com

Article Info:
Received on 13.05.2021
Accepted on 18.08.2021
Published on 15.12.2021

ABSTRACT:
The present study is aimed at assessing the chronic toxic effects of arsenic trioxide on liver and intestine of the zebrafish. For this purpose, histopathological changes in the liver and intestine tissues of adult zebrafish exposed to waterborne arsenic trioxide concentrations (50ppb and 500ppb respectively) for 90 days evaluated. Activation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was also assessed by immunofluorescence assay. We observed severe histopathological alterations, including cellular degeneration, vacuolization of hepatocytes, and an increase in the hepatic plate in the liver. Histopathological changes in the intestine includes vacuolization of the enterocytes, hyperplasia of goblet cells, displacement of the lamina propria, and disruption of the apical cytoplasm of epithelial cells that cover the intestinal villi were observed. Strong signs of immunofluorescence reaction for 8-OHdG in the exposed liver and intestine tissues in a dose-dependent manner has also been detected. Results of the study indicate that toxicity of arsenic trioxide lead to crucial histopathological alteration and induce oxidative DNA damage in both liver and intestine of zebrafish.

Keywords: *Arsenic Trioxide, Histopathology, Immunofluroscence, 8-OHdG, Zebrafish*

How to cite this article: Thakur, S.S., Jain, A., Jain, R., Jain, S., & Jain, S.K. (2021). Assessment of 8-hydroxy-2′deoxyguanosine Activity and Histopathological Alteration in Liver and Intestine of Zebrafish (*Daniorerio*) Exposed to Arsenic Trioxide. *Bulletin of Pure and Applied Sciences-Zoology,* 40A (2), 215-225.

INTRODUCTION

Arsenic is an environmental pollutant listed as a top hazardous substance to human health according to the Agency for Toxic Substances and Disease Registry (ATSDR, 2013). Arsenic has been released in many anthropogenic activities such as mining and metallurgy, wood preservation, urban and industrial wastes as well as sewage sludge management and agricultural pesticide production (Klancko, 2003; Donget al., 2018). Groundwater which is used for drinking is the major source of arsenic exposure for several millions of people in the world (Klancko, 2003). Even 200 million people worldwide suffer from chronic arsenicosis due to drinking arsenic-contaminated water (Flora, 2011).In natural water; the concentration of arsenic normally ranges from 1 to 2µg/L (WHO,

2011). However, arsenic contamination in groundwater exceeds the recommendations of the World Health Organization and the US Environmental Protection Agency which is ≤10µg/L (WHO, 2011; EPA US, 2001), whereas 50µg/L arsenic is considered as an acceptable level for drinking water in many developing countries including India (Sarkar et al., 2017; BIS, 2010). Such levels reach 800µg/L to 1980µg/L in different contaminated area of the world (Dipp et al., 2018).

Arsenic trioxide is helpful for aquatic weed control but its excess use is detrimental to the aquatic ecosystems that may cause adverse effects on aquatic organisms health, due to their suitability to accumulate in aquatic organisms especially fish because in aquatic environment fish is considered as the top predators which is exposed to more arsenic than other species (Dong et al., 2018; Roy & Bhattacharya, 2006; Pei et al., 2019). Arsenic change normal physiological condition of the cell leading to generation of reactive oxygen species (ROS), which disorganize cellular redox balance leading to oxidative stress, further, modulates antioxidant system that is well documented in fish (Jomova et al., 2011; Ventura-Lima et al., 2011). In addition it also damages biomolecules such as DNA and protein resulting in to physiological and histopathological alteration in fish (Mondal et al., 2021). For instance earlier reports manifested that exposure to low arsenic trioxide concentrations generate oxidative stress and decrease zebrafish growth and also induce apoptosis in zebrafish (Dong et al., 2018).

Liver is the principal organ of several key metabolic pathways. The teleost liver considered as one of the most sensitive organs, which plays a major role in the uptake, biotransformation, and excretion of xenobiotic or aquatic pollutants (Roy & Bhattacharya, 2006). Zebrafish liver resembles the human liver in cellular structure, function, and genetics (Teame et al., 2019). Previous studies on different species showed that the ingested arsenic mainly accumulates in the intestine and liver (Pei et al., 2019; Sims et al., 2019; Shi et al., 2020). Dubey et al. (2014) demonstrated that chronic exposure of arsenic trioxide induced hepatotoxicity in relation to arsenic accumulation in liver of rats. Likewise, Intestine is the multifunctional organ crucial for water and electrolyte balance, digestion, metabolism, and immunity (Buddington et al., 1997). Morphogenesis and development of the intestine are indistinguishable in fish and mammals, even though most of the fishes lack crypts but produce the same main cell types such as enterocytes, goblet cells and enteroendocrine cells (Sims et al., 2019). Fish intestine plays a principal role in osmoregulation (Zhao et al., 2019) and absorb xenobiotics (Painefilú et al., 2019). Fish can intake arsenic directly dissolved in the aquatic medium, primarily mediated into cell by aquaglyceroporins (AQPs), which plays an important role in cellular arsenic uptake in teleost fish including zebrafish (Jung et al., 2015; Hamdi et al., 2009).

Assessment of pollutant's impact and their environmental risk on the aquatic ecological system is necessary for the safety of human health. The data obtained as a response of model organism to toxic substances are essential for risk management (Wei et al., 2021). The zebrafish is one of the popular vertebrate models for rapid toxicology screening as well as to study developmental biology, cancer biology, drug discovery, and molecular genetics (Khan & Alhewairini, 2018). Zebrafish has come out as an important model for aquatic toxicology research because the degree of homology with the human genome is approximately 87% and specific organ systems are strikingly conserved with mammalian organ system, alsoit has been widely used as tool forfinding toxins in water samples. These points make zebrafish a tremendous complementary system for research in animal health (Wei et al., 2021; Khan & Alhewairini, 2018; Bambino & Chu, 2017).

The present study has been planned to investigate the morphological description of liver and intestine tissue through histopathological and 8-OHdG analysis. Histopathological investigationis a direct and efficient tool for physiological evaluation, selected in order to assess the detrimental effect of arsenic trioxide (Lam et al., 2006). 8-OHdG activity was also evaluated as a biomarker of oxidative DNA damage, which is broadly employed for assessment of arsenic induced genotoxicity (Faita et al., 2013).

MATERIAL AND METHODS

Zebrafish Maintenance

Adult wild-type zebrafish (*Daniorerio*) were obtained from the authorized fish supplier, housed, and acclimatized in a glass tank (15 lit.) under laboratory conditions for two weeks. All exposure conditions were maintained the same, with a density of 3fish/L water, with a water temperature of 27 ± 1.5°C, photoperiod of 14-h light/10-h dark, and pH 7.0± 0.5. The commercially available fish feed was provided twice a day and aquarium water was renewed every day consistently. For experimentation on zebrafish prior permission was obtained by the Institutional Animal Ethics Committee of the University (No. 379/S/01/CPCSEA). During experimentation standard protocol and procedure followed.

Chemical Exposure Protocol

Arsenic trioxide (CAS NO: A1010, purity 99% Sigma Aldrich) was dissolved in 1N NaOH to obtain a stock solution, which was further used to prepare the fresh diluted solution. After acclimatization, zebrafish were randomly divided into three groups, one control and the other two groups were exposed to arsenic trioxide (50ppb, and 500ppb). Followed the 90 days arsenic trioxide (As_2O_3) exposure, zebrafish were anesthetized using ice-cold water, targeted organs liver and intestine were dissected out for further experimentation. The choice of a treatment regimen was based upon the literature (Sarkar et al., 2014, 2017; Dipp et al., 2018; Babich& Van Beneden 2019).

Histopathological Examination

After the sacrifice of fish, liver and intestine were dissected out from control and arsenic trioxide exposed fish and fixed immediately with freshly prepared 4% paraformaldehyde for 24 hrs. Following fixation, tissue were dehydrated in an ascending series of ethanol, cleared in xylene, embedded in paraffin, and sectioned at 6 μm thickness using microtome and finally mounted on gelatine-coated slides. After deparaffinization, staining was carried out with Haematoxylin and Eosin (H&E) staining; the stained sections were mounted in DPX and covered with cover glass. Images of the stained sections were captured at 400x magnification using an inverted microscope (Magnus INVI trinocular microscope).

Immunohistochemical Examination

The immunofluorescence was performed with the primary antibodies against 8-OHdG (Sc-66036, Santa Cruz Biotechnology) in 1:500 dilution. A secondary antibody conjugated with Alexa Fluor 488 was used in a 1:500 dilution and counterstained with 4', 6-diamidino-2-phenylindole (DAPI) to label nuclei. Specimens were processed as described by Maurya & Mishra (2017). The immunofluorescence images were obtained with a confocal microscope (Confocal Laser Scanning Microscope (CLSM) MEA53100, Nikon Corporation). The images were further quantified using the software Image J.

Statistical Analysis

The data obtained was analyzed with the one-way analysis of variance (ANOVA) using SPSS software (version Statistics V26, Windows XP) followed by Tukey'spost hoc test at $P<0.05$ for multiple comparisons of mean.

RESULT

Histopathological Analysis

The histological examination of liver sections of control group presented normal histoarchitecture in which hepatocytes arranged compactly with centrally located nucleus (Fig. 1A). In the experimental groups exposed to different doses of arsenic trioxide, histopathological changes were observed increased with dose-dependent manner, exhibiting a large number of vacuolization of hepatocytes and pyknotic nuclei in 50ppb exposure group (Fig. 1B) whereas in 500ppb exposure group, shrinkage of hepatocytes with increased hepatic plate gap in the liver, blood spaces were observed (Fig. 3C). Microscopic examination of the intestine of control zebrafish exhibited normal intestine histoarchitecture with villi, surrounded by a mucous layer. Within the villi mucus-producing goblet cells and the enterocytes and lamina propria is present (Fig. 1D). In case of arsenic trioxide exposed groups, vacuolization of the enterocytes, hyperplasia of goblet cells, displacement of the lamina propria, and disruption of the apical cytoplasm of epithelial cells have been observed (Fig. 1E, 1F).

Histopathological images of Liver and Intestine

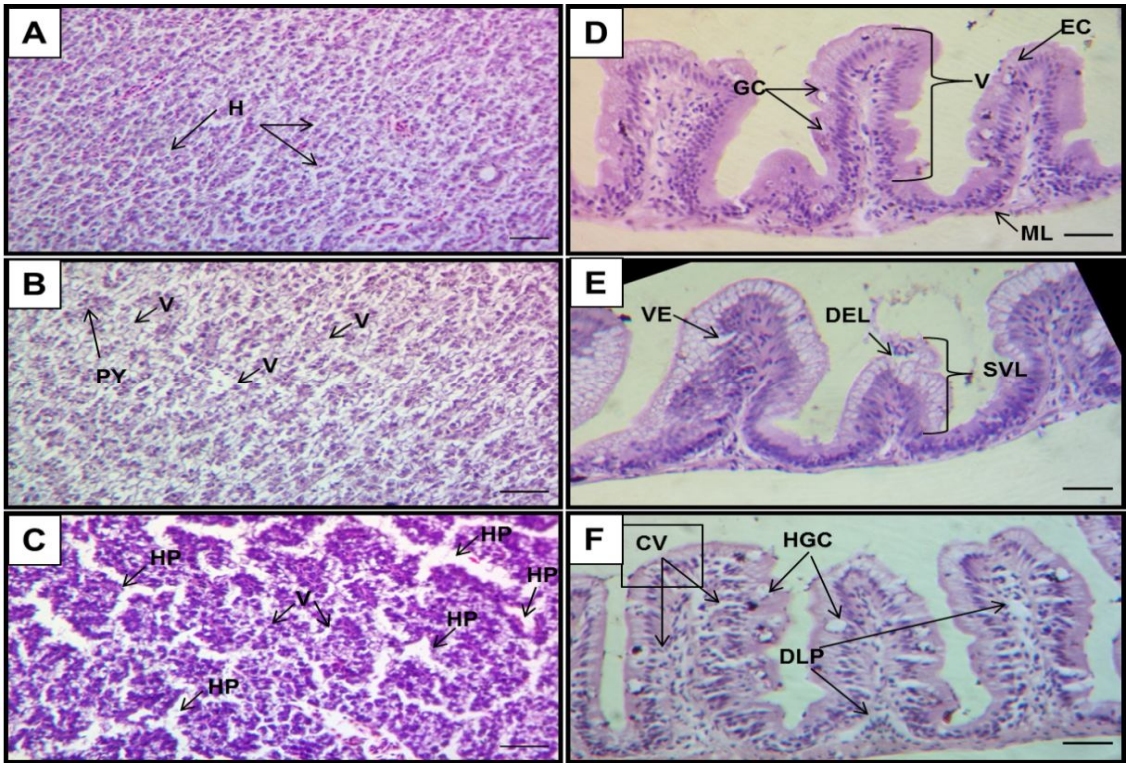

Figure 1: Histopathological study of zebrafish Liver (A, B, C) and Intestine (D, E, F) stained with Haematoxylin and Eosin (400X). Liver: A. Control; B& C. 50ppb and 500ppb As_2O_3exposed respectively. Arrow indicates (H) hepatocytes; (V) vacuolization in hepatocytes; (Py) Pyknotic nuclei; (HP) hepatic plate. Intestine: D. Control; E& F. 50ppb and 500ppb As_2O_3exposed. Arrow indicates (V) is the villi; (EC) enterocytes; (GC) goblet cells; (ML) muscle layer; (VE) vacuolization of the enterocytes; (DEL) detachment of the epithelium; (SVL) shorten villi length; (DLP) displacement of the lamina propria; (HGC) hyperplasia of goblet cells; (CV) cracking of villi.

Immunohistochemical Analysis

An immunofluorescence study revealed a statistically significant difference in terms of fluorescence intensity in the liver and intestine. In case of control tissues a very slight immunoreactivity observed. The immunofluorescence reaction of 8-OHdG enhancement was directly proportioned to arsenic trioxide concentrations when compared to the control group. Moderate to high level immunofluorescence reaction of 8-OHdG was detected in the liver exposed to 50 ppb and 500ppb As_2O_3 groups respectively (Fig. 2A, C $p<0.05$). An immunofluorescence reaction was detected at a moderate level in the intestine tissues exposed to 50ppb of As_2O_3, whereas immunofluorescence reaction increase severely in the intestine tissues exposed to 500ppb of As_2O_3in comparison to control (Fig. 2B,D $p<0.05$).

Immunohistochemistry Images of Liver and Intestine

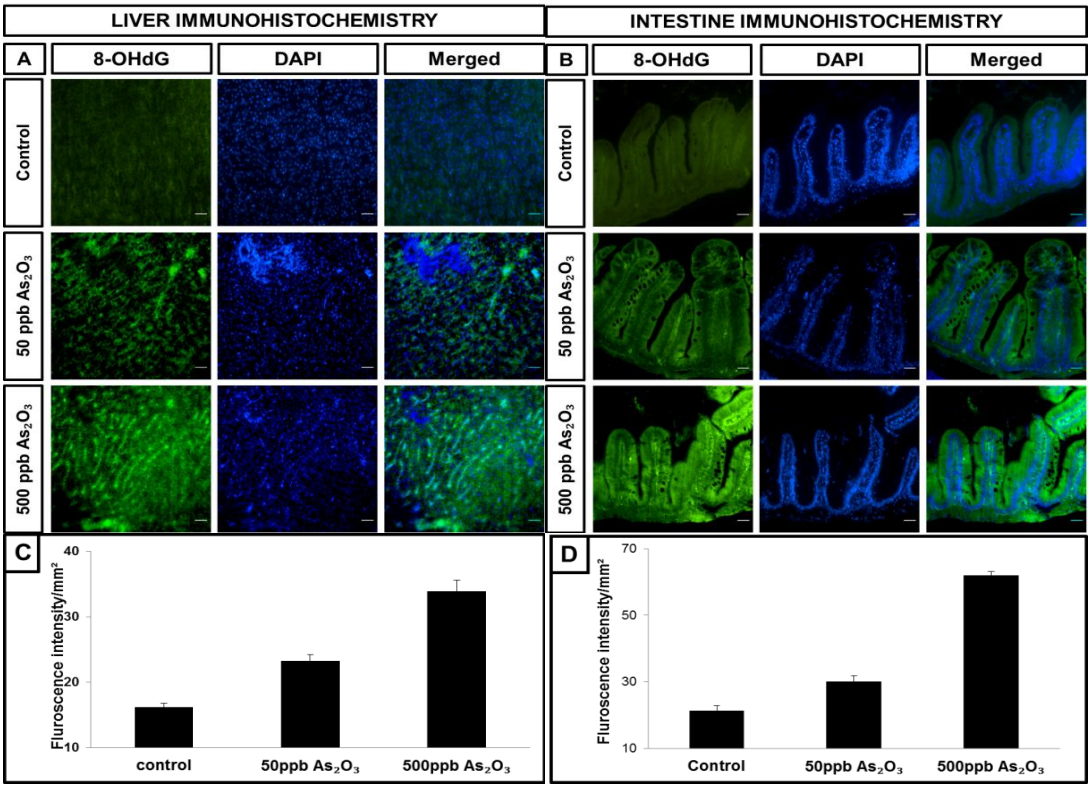

Figure 2: Representative photographs showing Immunofluorescence stains with 8-OHdG (green), DAPI (blue) and Merged (8-OHdG + DAPI). Image A and B represent 8-OHdG immunofluroscence intensity in Liver and Intestine, respectively. C and D Bar charts showing the statistical analysis of the immunofluroscence staining in Liver and Intestine respectively calculated through image J software. The relative fluorescence intensity was expressed as the mean ± SD with $p < 0.05$ (Scale bar = 20 μm).

DISCUSSION

Arsenic trioxide is a worldwide metalloid pollutant in the environment, which is a major threat to animal health (Sun et al., 2020). Fish considered as the best model organism for evaluation of aquatic environmental toxicityand very sensitive to environmental change also have an evolutionary relationship with mammals and play an important role in toxicity assessment associated with pollution in the aquatic environment (Carvan et al., 2007). Zebrafish is one of the popular vertebrate models to study toxicogenomics (Khan &Alhewairini, 2018).

There are many studies on the arsenic toxicity in the teleost liver, whereas studies on the intestine toxicity due to arsenic are rather insufficient. In the present study, the chronic effects of arsenic trioxide from low to high concentration observed in zebrafish, which showed organ-specific and dose-dependent histopathological alterations and DNA damage. These alterations were observed even at low concentration exposed group but more pronounced in the group exposed to the high concentration of arsenic trioxide. The present histopathological study of liver showed severe changes in the fish exposed to arsenic trioxide. The intensity of histopathological changes gradually increases with increased concentration of arsenic trioxide, revealed a large number of vacuolization, shrinkage of hepatocytes with increased hepatic plate gap. Vacuolization of hepatocytes is linked with the inhibition of protein synthesis, disaggregation of microtubules which is common in metal toxicities (Ahmed et al., 2013). Lipid / glycogen deposition is the origin of hepatic vacuolization, because lipid metabolism / transport pathway in zebrafish liver is sensitive to perturbation via arsenic exposure (Li et al., 2016). Previous study suggested that arsenic exposure caused hepatotoxicity, with disturbance in

cytochrome P-1A (CYP1A) activity, which is functional in detoxification and biotransformation (Zhao et al., 2019a). Similar histopathological observations were reported in a previous study on zebrafish (Sarkar et al., 2017).Histopathological changes have also been recorded in several other fish species such as tilapia (*Oreochromismossambicus*) in which macrophage infiltration, congestion, vacuolization and shrinkage of hepatocytes, dilation of sinusoids, and vacuolar degeneration after arsenic exposure were observed (Ahmed et al., 2013). Another study on the liver of shingi fish (*Heteropneustesfossilis)* showed histopathogical alteration after chronic arsenic exposure (Begum et al., 2014). Roy & Bhattacharya (2006) observed a significant histopathological alteration in the liver of freshwater channafish *(Channapunctatus*) after exposure to nonlethal concentrations of arsenic trioxide.

Based on the present histopathological analysis of intestine, vacuolization and splitting of the enterocytes, cracking of villi and epithelial damage were observed in the arsenic trioxide exposed zebrafish intestine tissue after 90 days. The previous results showed that the most pronounced accumulation of arsenic occurred at day 90 in zebrafish (Liu et al., 2006). It has also been demonstrated that arsenic could induce microbiotadysbiosis in the gut of zebrafish (Dahan et al., 2018). Gut microbiome aberrations or dysbiosis can affect the health status of the host because gut microbes can metabolize xenobiotics and alter their bioavailability thus, gut microbes can perform xenobiotic reductions, but disruption of intestine microbes may alter the permeability of the gut leading to abnormal absorption of xenobiotics (Shi et al., 2020; Qiao et al., 2019; Bertotto et al., 2020) and this might be the basis of arsenic induce intestine toxicity. The present observations are in accordance with the Begum et al. (2014) study where the effects of arsenic on fish intestine show damaged serosa and mucosa, increase in the number of goblet (mucosal) cells and degeneration of villi after 60 days of arsenic exposure in shingi fish (*Heteropneustesfossilis)*. Another study exhibited a significant reduction in intestinal villus height and decrease in proliferating cell nuclear antigen (PCNA+) in the intestinal cells ofkilli fish (*Fundulusheteroclitus*) exposed to arsenic (Sims et al., 2019). In one of the study, histopathological damage observed in intestine of common carp (*Cyprinuscarpio*) insulted by arsenic showed infiltrated inflammatory cells, shedding and swelling intestinal villi, and massive goblet cells (Zhao et al., 2019b).

The assessment of the oxidative stress has been regarded as a sensitive tool for the evaluation of toxicity produced by various aquatic environmental pollutants including metals in fish (Ratn et al., 2018). When the Reactive oxygen species (ROS) surpassed the antioxidant defense system, subsequently oxidative stress generated. Oxidative stress mediated by reactive oxygen species (ROS) is a common impression of arsenic toxicity (Khafaga et al., 2020; Hu et al., 2020). For instance, one study suggested that arsenic trioxide induce extensive oxidative stress in the brain, liver and kidney of zebrafish (sarkar et al., 2014, 2017). It can also cause gills toxicity and alter the antioxidant system in zebrafish exposed to arsenic for two days (Ventura-Lima., 2009a).Whereas exposure of arsenite and arsenate to common carp (*Cyprinuscarpio*) leads to imbalance in antioxidant system of gills and liver (Ventura-Lima et al., 2009b). The antioxidant system in the liver of goldfish (*Carassiusauratus*) also modulates due to arsenite induced oxidative stress (Bagnyukova et al., 2007). Antioxidant enzymes considered as the first line of defensive mechanisms against oxidative stress for detoxification and scavenging the ROS. Superoxide dismutase (SOD) catalyzes the transformation of superoxide (O2-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), among which the former itself is a highly toxic free radical responsible for the formation of another deleterious ROS, hydroxyl radical (OH-) through Fenton reaction (Mondal et al., 2019). Hydroxyl radicals are known to cause 8-OHdG or 8-OHG through hydroxylation at the C-8 position of deoxyguanosine or guanosine residues in DNA (Baran et al., 2021). 8-OHdG is known as a biomarker of oxidative stress and DNA damage (Arslan et al., 2017). It has been described earlier that there is a linear correlation between ROS generation and 8-OHdG formation and this occurrence indicate that ROS can result in to the formation of 8-OHdG (Topal et al., 2017).

According to observations of the present study arsenic trioxides cause 8-OHdG

immunofluorescence positivity in the liver and intestine tissue in a dose-dependent manner. Similar observations have been found in other species e.g. arsenic trioxide induced DNA damage in the brain of mice when 4ppm of arsenic was given through drinking water for 60 days (Hong et al., 2009). Study showed that arsenic-induced hepatoxicity is related to its potential to induce aberrant DNA methylation during hepatocarcinogenesis (Bagnyukova et al., 2007). Arsenite also may interfere with the DNA repair system, cause DNA damage via production of ROS (Seok et al., 2007). Moreover, it can be hypothesized that DNA damage in different tissue of zebrafish might also be accompanying to genic abnormalities induced by arsenic trioxide.

CONCLUSION

The present study demonstrates that exposure of arsenic trioxide to zebrafish induce oxidative stress leads to the 8-OHdG adduct formation, a marker of DNA damage and also induce severe histopathological changes in intestine and liver of zebrafish. Therefore zebrafish species can be used as bio indicator of heavy metals in environment by studying the genotoxicity and histopathological changes to elucidate the mechanism of hepatoxicity and intestinal toxicity.

Acknowledgement

Financial assistance from UGC, New Delhi is gratefully acknowledged.

Conflict of Interest

The authors have no conflicts of interest

REFERENCES

1. Agency for toxic substances and disease registry (ATSDR), 2013. Toxicological Profilefor Arsenic. U.S. Department of Health and Human Services, Public Health Services, Atlanta, GA. Available at:http://www.atsdr.cdc.gov/spl/index.html(Accessed 8 June 2019).
2. Ahmed MK, Habibullah-Al-Mamun M, Parvin E, Akter MS, & Khan MS. (2013). Arsenic induced toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (Oreochromismossambicus). *Experimental and toxicologic pathology: official journal of the GesellschaftfurToxikologische Pathologie,* 65(6), 903–909. Available at:https://doi.org/10.1016/j.etp.2013.01.003(Accessed 01 December 2020).
3. Arslan H, Özdemir S, &Altun S. (2017). Cypermethrintoxication leads to histopathological lesions and induces inflammation and apoptosis in common carp (Cyprinuscarpio L.). *Chemosphere,* 180, 491–499.Available at:https://doi.org/10.1016/j.chemosphere.2017.04.057(Accessed 25 November 2020).
4. BabichR. & Van Beneden RJ. (2019). Effect of arsenic exposure on early eye development in zebrafish (Danio rerio). Journal of applied toxicology: JAT, 39(6), 824–831.Available at:https://doi.org/10.1002/jat.3770(Accessed 11 March 2020).
5. Bagnyukova TV, Luzhna LI, Pogribny IP &Lushchak VI. (2007). Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. Environmental and molecular mutagenesis, 48(8), 658–665. Available at:https://doi.org/10.1002/em.20328(Accessed 20 March 2021) .
6. Bambino K, & Chu J. (2017). Zebrafish in Toxicology and Environmental Health. *Current topics in developmental biology,* 124, 331–367. Available at: https://doi.org/10.1016/bs.ctdb.2016.10.007(Accessed 02 January 2021).
7. Baran A, Yildirim S, Ghosigharehaghaji A, Bolat İ, Sulukan E, &Ceyhun SB. (2021). An approach to evaluating the potential teratogenic and neurotoxic mechanism of BHA based on apoptosis induced by oxidative stress in zebrafish embryo (Danio rerio). *Human & experimental toxicology*, 40(3), 425–438. Available at:https://doi.org/10.1177/0960327120952140(Accessed 20 April 2021).
8. Begum A, Mustafa A, Amin MN, Banu N, &Chowdhury T. (2014). Accumulation and histopathological effects of arsenic in tissues of shingi fish (Stinging Catfish) Heteropneustesfossilis (Bloch, 1794). *Journal of the Asiatic Society of Bangladesh, Science,* 39(2), 221-230Available

at:https://doi.org/10.3329/jasbs.v39i2.17861.(Accessed 11 April 2021).

9. Bertotto LB, Catron TR, & Tal T. (2020). Exploring interactions between xenobiotics, microbiota, and neurotoxicity in zebrafish. *Neurotoxicology,* 76, 235–244. Available at: https://doi.org/10.1016/j.neuro.2019.11.008(Accessed 4 March 2021).

10. Buddington RK, Krogdahl A, Bakke-Mckellep AM. (1997). The intestines of carnivorous fish: Structure and functions and the relations with diet. *Actaphysiologica Scandinavica. Supplementum.* 638. 67-80. Available at: DOI:10.3329/jasbs.v39i2.17861(Accessed 4 March 2021).

11. Bureau of Indian Standard (BIS), 2010. Ground Water Quality in Shallow Aquifers of India. Central Ground Water Board, Ministry of Water Resources. Government of India, Faridabad. Available at:http://cgwb.gov.in/documents/waterquality/gw_quality_in_shallow_aquifers.pdf(Accessed 14 January 2018).

12. Carvan MJ, Gallagher EP, Goksøyr A, Hahn ME, & Larsson DG. (2007). Fish models in toxicology. *Zebrafish,* 4(1), 9–20. Available at: https://doi.org/10.1089/zeb.2006.9998(Accessed 11 March 2019)

13. Dahan D, Jude BA, Lamendella R, Keesing F, & Perron GG. (2018). Exposure to Arsenic Alters the Microbiome of Larval Zebrafish. *Frontiers in microbiology,* 9, 1323. Available at: https://doi.org/10.3389/fmicb.2018.01323(Accessed 4 March 2021).

14. Dipp VR, Valles S, Ortiz-Kerbertt H, Suarez JV, & Bardullas U. (2018). Neurobehavioral Alterations in Zebrafish Due to Long-Term Exposure to Low Doses of Inorganic Arsenic. *Zebrafish*, 15(6), 575–585. Available at: https://doi.org/10.1089/zeb.2018.1627. (Accessed 10 January 2019).

15. Dong WQ, Sun HJ, Zhang Y, Lin HJ, Chen JR, & Hong H C (2018). Impact on growth, oxidative stress, and apoptosis-related gene transcription of zebrafish after exposure to low concentration of arsenite. *Chemosphere,* 211, 648–652. Available at: https://doi.org/10.1016/j.chemosphere.2018.08.010(Accessed 16 July 2019).

16. Dubey NP, Jain SK, Maheshwari HS. (2014) Chronic study of arsenic trioxide-induced hepatotoxicity in relation to arsenic liver accumulation in rats, Toxicological & Environmental Chemistry, 96(3), 491-499.A vailable at: DOI: 10.1080/02772248.2014.949129(Accessed 20 July 2020).

17. Environmental Protection Agency, US (2001). Drinking Water Requirements for States and Public Water Systems, Drinking Water Arsenic Rule History, USA. Available at: https://www.epa.gov/dwreginfo/drinking-water-arsenic-rule-history(Accessed 14 January 2018).

18. Faita F, Cori L, Bianchi F, &Andreassi MG. (2013). Arsenic-induced genotoxicity and genetic susceptibility to arsenic-related pathologies. *International journal of environmental research and public health,* 10(4), 1527–1546. Available at: https://doi.org/10.3390/ijerph10041527(Accessed 2 June 2020).

19. Flora SJ, (2011). Arsenic-induced oxidative stress and its reversibility. *Free radical biology & medicine*, *51*(2), 257–281. Available at: https://doi.org/10.1016/j.freeradbiomed.2011.04.008(Accessed 12 January 2019).

20. Hamdi M, Sanchez MA, Beene LC, Liu Q, Landfear SM, Rosen BP, & Liu Z. (2009). Arsenic transport by zebrafish aquaglyceroporins. *BMC molecular biology,* 10, 104. (Accessed 3 January 2021)Available at: https://doi.org/10.1186/1471-2199-10-104

21. Hong Y, Piao F, Zhao Y, Li S, Wang Y, & Liu P. (2009). Subchronic exposure to arsenic decreased Sdha expression in the brain of mice. *Neurotoxicology,* 30(4), 538–543. Available at: https://doi.org/10.1016/j.neuro.2009.04.011. (Accessed 1 April 2018).

22. Hu, Y., Li, J., Lou, B., Wu, R., Wang, G., Lu, C., Wang, H., Pi, J., &Xu, Y. (2020). The Role of Reactive Oxygen Species in Arsenic Toxicity. *Biomolecules*, 10(2), 240. Available at: https://doi.org/10.3390/biom10020240(Accessed 20 January 2021).

23. Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ,

&Valko M. (2011). Arsenic: toxicity, oxidative stress and human disease. *Journal of applied toxicology: JAT,* 31(2), 95–107. Available at: https://doi.org/10.1002/jat.1649(Accessed 20 February 2018)

24. Jung D, Adamo MA, Lehman RM, Barnaby R, Jackson CE, Jackson BP, Shaw JR, & Stanton BA. (2015). A novel variant of aquaporin 3 is expressed in killifish (Fundulusheteroclitus) intestine. Comparative biochemistry and physiology. *Toxicology & pharmacology: CBP,* 171, 1–7. Available at: https://doi.org/10.1016/j.cbpc.2015.03.001(Accessed 11 December 2020).

25. Khafaga AF, Naiel M, Dawood M, & Abdel-Latif H. (2020). Dietary Origanumvulgare essential oil attenuates cypermethrin-induced biochemical changes, oxidative stress, histopathological alterations, apoptosis, and reduces DNA damage in Common carp (Cyprinuscarpio). *Aquatic toxicology (Amsterdam, Netherlands),* 228, 105624. Available at: https://doi.org/10.1016/j.aquatox.2020.105624(Accessed 19 February 2021).

26. Khan FR & Alhewairini SS. (2018). Zebrafish (Daniorerio) as a Model Organism, Current Trends in Cancer Management. Intech Open, DOI: 10.5772/intechopen.81517. Available at: https://www.intechopen.com/books/current-trends-in-cancer-management/zebrafish-em-danio-rerio-em-as-a-model-organism(Accessed 18 May 2021).

27. Klancko RJ, (2003) Heavy Metals in the Environment. Sarkar B, ed. 2002. Marcel Dekker, New York. *Environmental Practice*, 5(2), 180-181, ISBN: 0-8247-06307Available at: DOI: 10.1017/S1466046603231124 (Accessed 12 June 2019).

28. Lam SH, Winata CL, Tong Y, Korzh S, Lim WS, Korzh V, Spitsbergen J, Mathavan S, Miller LD, Liu ET, & Gong Z. (2006). Transcriptome kinetics of arsenic-induced adaptive response in zebrafish liver. *Physiological genomics,* 27(3), 351–361. Available at: https://doi.org/10.1152/physiolgenomics.00201.2005 (Accessed 16 November 2020).

29. Li C, Li P, Tan YM, Lam SH, Chan EC, & Gong Z. (2016). Metabolomic Characterizations of Liver Injury Caused by Acute Arsenic Toxicity in Zebrafish. *PloS one*, 11(3), e0151225. Available at: https://doi.org/10.1371/journal.pone.0151225(Accessed 11 April 2021).

30. Liu FJ, Cobb GP, Anderson TA, Cheng QQ, & Theodorakis CW. (2006). Uptake, accumulation and depuration of sodium perchlorate and sodium arsenate in zebrafish (Daniorerio). *Chemosphere*, 65(10), 1679–1689. Available at: https://doi.org/10.1016/j.chemosphere.2006.05.030(Accessed 3 January 2021).

31. Maurya SK & Mishra R. (2017). Pax6 interacts with Iba1 and shows age-associated alterations in brain of aging mice. *Journal of chemical neuroanatomy,* 82, 60–64. Available at: https://doi.org/10.1016/j.jchemneu.2017.05.002(Accessed 11 April 2021).

32. Mondal P, Shaw P, Bandyopadhyay A, Dey Bhowmik A, Chakraborty A, Sudarshan M, & Chattopadhyay A. (2019). Mixture effect of arsenic and fluoride at environmentally relevant concentrations in zebrafish (Daniorerio) liver: Expression pattern of Nrf2 and related xenobiotic metabolizing enzymes. *Aquatic toxicology (Amsterdam, Netherlands),* 213, 105219 Available at: https://doi.org/10.1016/j.aquatox.2019.06.002. (Accessed 20 February 2020).

33. Mondal P, Shaw P, Dey Bhowmik A, Bandyopadhyay A, Sudarshan M, Chakraborty A, & Chattopadhyay A. (2021). Combined effect of arsenic and fluoride at environmentally relevant concentrations in zebrafish (Daniorerio) brain: Alterations in stress marker and apoptotic gene expression. *Chemosphere*, 269, 128678. Available at: https://doi.org/10.1016/j.chemosphere.2020.128678(Accessed 28 May 2021).

34. Painefilú JC, Pascual MM, Bieczynski F, Laspoumaderes C, González C, Villanueva S, & Luquet CM. (2019). Ex vivo and in vivo effects of arsenite on GST and ABCC2 activity and expression in the middle intestine of the rainbow trout Oncorhynchusmykiss. Comparative

biochemistry and physiology. *Toxicology & pharmacology: CBP*, 225, 108566.Available at: https://doi.org/10.1016/j.cbpc.2019.108566(Accessed 20 February 2021).

35. Pei J, Zuo J, Wang X, Yin J, Liu L, & Fan W. (2019). The Bioaccumulation and Tissue Distribution of Arsenic Species in Tilapia. *International journal of environmental research and public health,* 16(5), 757. Available at: https://doi.org/10.3390/ijerph16050757(Accessed 20 February 2020).

36. Qiao R, Sheng C, Lu Y, Zhang Y, Ren H, &Lemos B. (2019). Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. *The Science of the total environment*, 662, 246–253. Qiao Available at: https://doi.org/10.1016/j.scitotenv.2019.01.245(Accessed 18 January 2021).

37. Ratn A, Prasad R, Awasthi Y, Kumar M, Misra A, & Trivedi SP. (2018). Zn^{2+} induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver and kidney of the fish, Channapunctatus (Bloch, 1793). *Ecotoxicology and environmental safety,* 151, 10–20.Available at: https://doi.org/10.1016/j.ecoenv.2017.12.058 (Accessed 19 February 2021).

38. Roy S & Bhattacharya S. (2006). Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of Channapunctatus. *Ecotoxicology and environmental safety,* 65(2), 218–229. Available at: https://doi.org/10.1016/j.ecoenv.2005.07.005(Accessed 20 January 2018).

39. Sarkar S, Mukherjee S, Chattopadhyay A, & Bhattacharya S. (2014). Low dose of arsenic trioxide triggers oxidative stress in zebrafish brain: expression of antioxidant genes. *Ecotoxicology and environmental safety,* 107, 1–8. Available at:https://doi.org/10.1016/j.ecoenv.2014.05.012(Accessed 22 August 2017).

40. Sarkar S, Mukherjee S, Chattopadhyay A, & Bhattacharya S. (2017). Differential modulation of cellular antioxidant status in zebrafish liver and kidney exposed to low dose arsenic trioxide. *Ecotoxicology and environmental safety*, 135, 173–182. Available at: https://doi.org/10.1016/j.ecoenv.2016.09.025(Accessed 28 December 2019).

41. Seok SH, Baek MW, Lee HY, Kim DJ, Na YR, Noh KJ, Park SH, Lee HK, Lee BH, Ryu DY, & Park JH. (2007). Arsenite-induced apoptosis is prevented by antioxidants in zebrafish liver cell line. *Toxicology in vitro: an international journal published in association with BIBRA,* 21(5), 870–877.Available at: https://doi.org/10.1016/j.tiv.2007.02.011 (Accessed 7 March 2019).

42. Shi L, Hu X, Wang N, Liang H, Wu C, & Cao H. (2020). Histopathological examination and transcriptome analyses to assess the acute toxic effects of arsenite exposure on rare minnows (Gobiocyprisrarus). *Ecotoxicology (London, England),* 29(5), 613–624. Available at: https://doi.org/10.1007/s10646-020-02222-3(Accessed 16 January 2021).

43. Shi L, Hu X, Wang N, Liang H, Wu C, & Cao H. (2020). Histopathological examination and transcriptome analyses to assess the acute toxic effects of arsenite exposure on rare minnows (Gobiocyprisrarus). *Ecotoxicology (London, England),* 29(5), 613–624. Available at: https://doi.org/10.1007/s10646-020-02222-3(Accessed 16 January 2021).

44. Sims KC, Schwendinger KL, Szymkowicz DB, Swetenberg JR, & Bain LJ. (2019). Embryonic arsenic exposure reduces intestinal cell proliferation and alters hepatic IGF mRNA expression in killifish (Fundulusheteroclitus). *Journal of toxicology and environmental health. Part A,* 82(2), 142–156. Available at: https://doi.org/10.1080/15287394.2019.1571465(Accessed 6 February 2021).

45. Sun HJ, Zhao WJ, Teng XQ, Shu SP, Li SW, Hong HC, & Guan DX. (2020). Antioxidant responses and pathological changes in the gill of zebrafish (Daniorerio) after chronic exposure to arsenite at its reference dose. *Ecotoxicology and environmental safety*, 200, 110743.A vailable at: https://doi.org/10.1016/j.ecoenv.2020.110743(Accessed 5 April 2021).

46. Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, Xie M, Gao C, Ye Y, Duan M, Zhou Z. (2019). The use of zebrafish (*Daniorerio*) as biomedical models, *Animal Frontiers*, 9(3), 68–77. Available at:

https://doi.org/10.1093/af/vfz020(Accessed 11 April 2021).

47. Topal A, Alak G, Ozkaraca M, Yeltekin AC, Comaklı S, Acıl G, Kokturk M, &Atamanalp M. (2017). Neurotoxic responses in brain tissues of rainbow trout exposed to imidacloprid pesticide: Assessment of 8-hydroxy-2-deoxyguanosine activity, oxidative stress and acetylcholinesterase activity. *Chemosphere,* 175, 186–191. Available at: https://doi.org/10.1016/j.chemosphere.2017.02.047(Accessed 20 April 2020).

48. Ventura-Lima J, Bogo MR, &Monserrat JM. (2011). Arsenic toxicity in mammals and aquatic animals: a comparative biochemical approach. *Ecotoxicology and environmental safety,* 74(3), 211–218. Available at: https://doi.org/10.1016/j.ecoenv.2010.11.002(Accessed 21 March 2018).

49. Ventura-Lima J, de Castro MR, Acosta D, Fattorini D, Regoli F, de Carvalho LM, Bohrer D, Geracitano LA, Barros DM, Marins LF, da Silva RS, Bonan CD, Bogo MR, & Monserrat JM. (2009a). Effects of arsenic (As) exposure on the antioxidant status of gills of the zebrafishDaniorerio (Cyprinidae). *Comparative biochemistry and physiology. Toxicology & pharmacology: CBP,* 149(4), 538–543. Available at: https://doi.org/10.1016/j.cbpc.2008.12.003(Accessed 20 October 2019).

50. Ventura-Lima J, Fattorini D, Regoli F, & Monserrat JM. (2009b). Effects of different inorganic arsenic species in Cyprinuscarpio (Cyprinidae) tissues after short-time exposure: bioaccumulation, biotransformation and biological responses. *Environmental pollution (Barking, Essex: 1987),* 157(12), 3479–3484.Available at: https://doi.org/10.1016/j.envpol.2009.06.023(Accessed 20 October 2019).

51. Wei Y, Meng Y, Huang Y, Liu Z, Zhong K, Ma J, Zhang W, Li Y, & Lu H. (2021). Development toxicity and cardiotoxicity in zebrafish from exposure to iprodione. *Chemosphere,* 263, 127860. Available at: https://doi.org/10.1016/j.chemosphere.2020.127860(Accessed 11 April 2021).

52. World Health Organization. 2011Arsenic . -in drinkingwater: background document for development of WHO guidelines for drinking-water quality. World Health Organization. Available at: https://www.who.int/water_sanitation_health/dwq/chemicals/arsenic.pdf5(Accessed 14 January 2018).

53. Zhao H, Wang Y, Guo M, Fei D, Mu M, Yu H, & Xing M. (2019a). Hepatoprotective effects of zinc (II) via cytochrome P-450/reactive oxygen species and canonical apoptosis pathways after arsenite waterborne exposure in common carp. *Chemosphere,* 236, 124869. Available at:https://doi.org/10.1016/j.chemosphere.2019.12486(Accessed 21 February 2021).

54. Zhao H, Wang Y, Yang X, Fei D, Mu M, Guo M, Yu H, & Xing M. (2019b). Zinc alleviates arsenism in common carp: Varied change profiles of cytokines and tight junction proteins among two intestinal segments. *Fish & shellfish immunology,* 94, 761–768. Available at: https://doi.org/10.1016/j.fsi.2019.09.069 (Accessed 3 January 2021).

****