The Pharma Innovation Journal 2022; 11(12): 1005-1008

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 1005-1008

www.thepharmajournal.com
Received: 10-09-2022
Accepted: 12-10-2022

Harsh R Patel
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Harshad B Patel
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Bhulesh V Paida
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Pavan M Patel
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Divya M Ramchandani
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Urvesh D Patel
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Chirag M Modi
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Corresponding Author:
Harsh R Patel
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

Assessment of respiratory toxicity following 28 days exposure of acrylamide in adult male zebrafish: Histological impairment in gills

Harsh R Patel, Harshad B Patel, Bhulesh V Paida, Pavan M Patel, Divya M Ramchandani, Urvesh D Patel and Chirag M Modi

Abstract
Acrylamide is a widespread an environmental pollutant that can produce severe negative effects on fish even at very low and high concentrations. In the present study, Histopathological changes in the gills of zebrafish were evaluated following exposure of ACR (8.5 and 17 mg/L) for 28 days in adult male zebrafish. Total 18 adult male zebrafish were randomly divided into three groups. The zebrafish of control group were maintained in normal condition without any treatment (Reverse osmosis water with standard range of temperature and pH). The zebrafish of second group were maintained in R.O. water containing ACR at the strength of 8.5 mg/L. The zebrafish of third group were maintained in R.O. water containing ACR at the strength of 17 mg/L of water. In conclusion, Acrylamide exposure of second group (8.5 mg/L) shown normal architecture of gills of adult male zebrafish, However third group (17 mg/L) showed congestion (C) in primary lamellae along with fusion/clubbing (F) of secondary lamellae, epithelial hyperplasia (H) at the end of secondary lamellae and vacuolization (V) formation of secondary lamellae in gills of adult male zebrafish. In conclusion, Acrylamide exposure at 17 mg/L for 28 days produced remarkable histopathological changes in gills of adult male zebrafish.

Keywords: Acrylamide, gill, histopathology, zebrafish

Introduction
Acrylamide (ACR) is a water-soluble alkene substance primarily used in the production of polyacrylamides. Acrylamide (C_3H_5NO) polymers and copolymers are widely used in the paper and textile industries, as flocculants in wastewater treatment, as soil conditioners, in ore processing and cosmetics (Friedman, 2003) [7]. In fish, gills are one of the most important sites for the entry of pollutants, due to having very large surface and their morphological characteristics. Generally, the gill is the first organ contacting with chemicals dissolved into water as if is the respiratory organ of fish (Evans *et al*., 2005) [4]. In gills, large surface area and very thin water-blood diffusion distance favour xenobiotic uptake in freshwater fish, due to the great water volume flowing into the gill lamellae to obtain the necessary O_2 for aerobic metabolism. Furthermore, the gills also play important role in osmotic and ionic regulation, acid-base equilibrium and nitrogen excretion and being sensitive to chemicals into water (Wendelaar-Bonga, 1997) [12].
In the gills, chemicals may be metabolized, accumulated, and/or transferred to blood stream reaching to other organs (van der Oost *et al.*, 2003) [11]. Higher level of ACR in the environment is due to industrial development near by the residential areas. Acrylamide has been detected at the level of < 5µg/L in both river water and tap water in an area where polyacrylamides are used in the treatment of potable water. Samples from public drinking-water supply wells in West Virginia in the United States of America (USA) were found to contain 0.024-0.041 µg ACR/L (Brown and Rhead, 1979) [3]. Mean ACR intake in women of Sweden has been reported to be 25.9 µg/day and found positively correlated with cancer (Mucci and Wilson, 2008) [9]. The ACR intake for the general population was estimated to be in the range of 0.3 to 0.8 µg/kg/day (Anonymous, 2002) [1].
In recent past, zebrafish is recognized as good animal model for toxicological study. The zebrafish has many advantages as a model organism, such as small size, short reproductive cycle, high fecundity, ex utero development of the embryo and transparent embryos (Feitsma and Cuppen, 2008) [6].

In addition, the zebrafish shares a high degree of homology with the human genome (Howe *et al*., 2013) [8]. Thus, the zebrafish is becoming a powerful model organism for studying genetics, development, environmental toxicology, pharmacology of drugs, DNA damage repair, cancer and other disease processes.

Therefore, the present experiment was conducted to evaluate the toxicity of acrylamide on adult male zebrafish following individual exposure with reference to histological impairment in the developed gills.

2 Materials and Methods

2.1 Chemicals

Acrylamide (Lot No: 0000304213) of analytical grade was purchased from Himedia, Mumbai. Other chemicals used in the study were of analytical grade.

2.2 Experimental Animals and Environment

The study was conducted on adult male zebrafish (*Danio rerio*) of 5–6 months of age. Fish were kept in a 20-L housing tank fitted with aeration (temperature of water 25–28 °C, and light/dark cycle 14:10 h) for 2 weeks (acclimatization) before starting the experiment. All fish has been fed (10 mg/fish twice a day) with fish pellets (Tetra bits complete®, Tetra GmbH-Germany) during the study period. Toxicant free filtered reverse osmosis (RO) water (pH of water 6.8–7.4, hardness 200–250 mg/L and electrical conductivity 500–600 µs/cm) was used during the experiment. Total water from each fish tank was drained off and then replaced with fresh water with particular concentration of ACR. The experimental procedure was approved by the Institutional Animal Ethics Committee of the college.

2.3 Experimental Design

The exposure level of ACR was selected based on previous studies (Faria *et al*., 2018) [5]. The exposure to ACR in the present experiment was around 1/10 and 1/5 of 72 h LC_{50} value (Faria *et al*., 2018) [5]. A total of 18 adult male zebrafish were randomly divided into three groups (06 zebrafish in each group). Groups of fish were treated with different treatments *viz*. Normal Control group (C1) was maintained under normal R.O. water only, Toxicity group 1 (T1) was maintained under 8.5 mg/L ACR and Toxicity group 2 (T2) was maintained under 17 mg/L ACR exposure in aquarium for 28 days. Accurate weighing of ACR was done using a precise analytical weighing balance (Model: MS 204S/A01, Mettler Toledo, USA). The level of ACR was maintained by changing the water daily with fresh water containing particular strength of ACR. Stock solution was prepared by dissolving 25.5 mg of ACR in 1 mL of milli-Q-water. After an exposure period of 28 days, a total of 18 zebrafish (06 in each group) were used for evaluation of histopathological alterations in the gills.

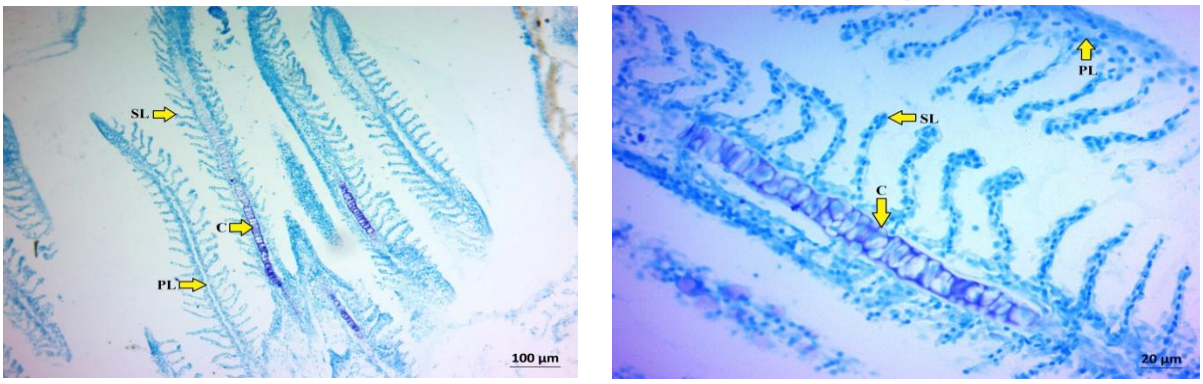
2.4 Histopathological Examination

At the end of experiment, all fish were humanely sacrificed by ice cold method (Wilson *et al*., 2009). 18 zebra fish (6 in each group) were used for the evaluation of histopathological changes in gills. After sacrifice, the whole fish was fixed in neutral buffered formalin (10 %) for 2 days. The formalin fixed fish was placed in sodium EDTA (0.35 M, pH 7.8) (Merck Ltd., Mumbai) solution for decalcification for 10 days. The decalcified fish were embedded in high quality paraffin (Thermofisher scientific, Mumbai, India) and sectioned at 5 µ thickness with semi-automated rotary microtome (RM 2245, Leica Biosystems, Germany). Slides were stained with toluidine blue stain. The toluidine blue stained slides were observed under microscope and microscopic pathological lesions were recorded.

3. Results and Discussion

Upon microscopic examination, Toluidine blue stained of gills of adult male zebrafish of control group showed normal architecture of gills with well-organized gill arch (GA), gill racker (GR), primary lamellae (PL), secondary lamellae (SL), which generally originated from the primary lamellae forming the main axis and chondrocytes (C) (Fig. 1A and 1B). The gills of zebrafish in T_1 group showed almost normal architecture of gills of zebrafish with well-organized primary lamellae (PL), secondary lamellae (SL) and chondrocytes (C) (Fig. 1C and 1D). The histopathological changes of gills of zebrafish in T_2 group showed congestion (C) in primary lamellae along with fusion/clubbing (F) of secondary lamellae, epithelial hyperplasia (H) at the end of secondary lamellae (Fig. 1E). The histopathological changes of gills of zebrafish in T_2 group showed vacuolization (V) formation of secondary lamellae (Fig. 1F).

A **B**



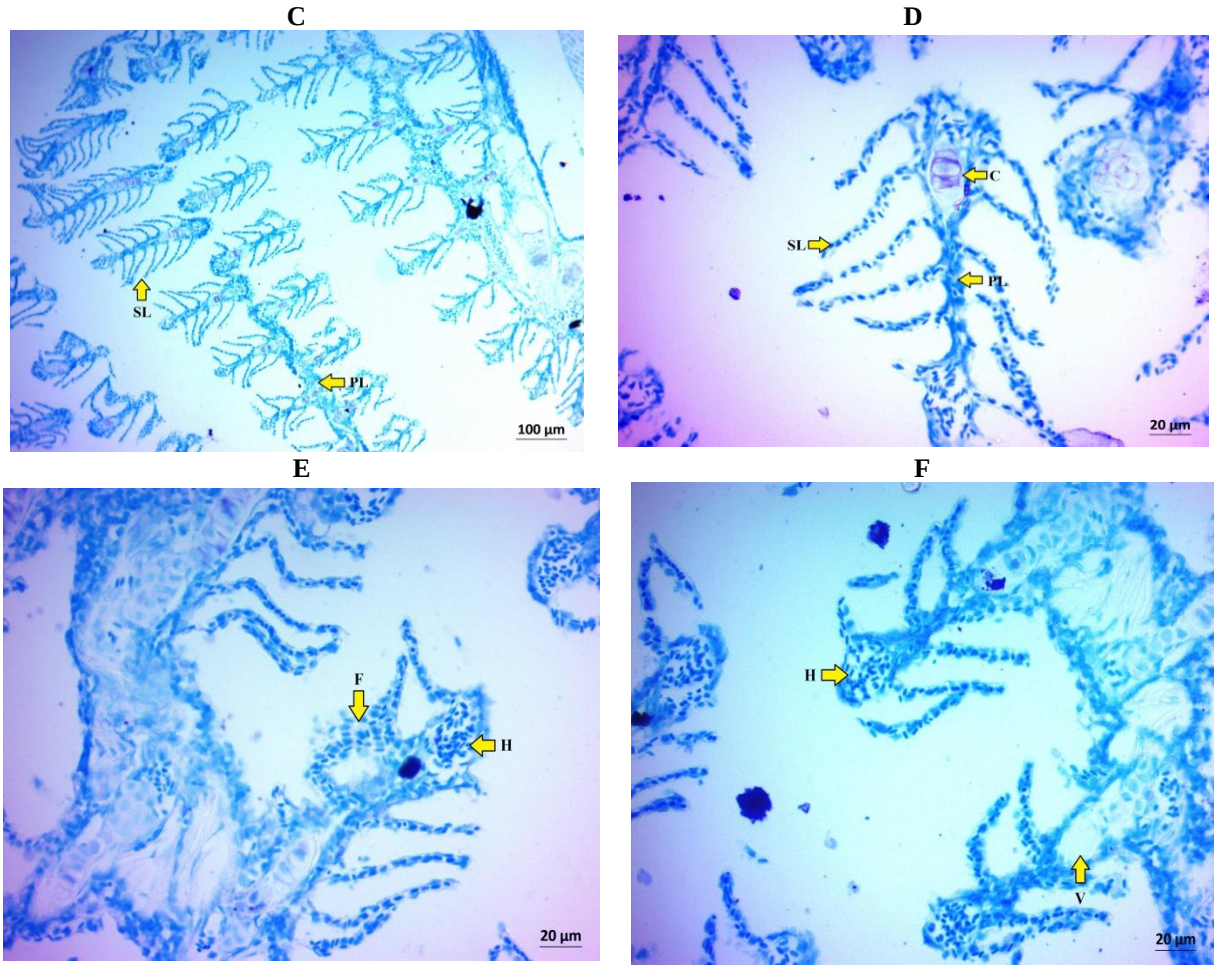


Fig 1: Microscopic view of zebrafish gills **(A and B)** control group showed normal structure of gill arch (GA), gill racker (GR), primary lamellae (PL) and secondary lamellae (SL); **(C and D)** ACR (8.5 mg/L, for 28 days) exposure group showed almost normal architecture of chondrocytes (C), primary lamellae (PL) and secondary lamellae (SL); **(E)** ACR (17 mg/L, for 28 days) exposure group showed congestion (C) in primary lamellae along with epithelial hyperplasia (H) at the end of secondary lamellae, as well as fusion **(F)** of secondary lamellae; (F) ACR (17 mg/L, for 28 days) exposure group also showed vacuolization (V) formation of secondary lamellae (100 x and 400 x, Toluidine blue staining).

Xenobiotic metabolism pathways in fish are similar to higher vertebrates and equally play and important role in eliminating xenobiotic induced stress (Bhattacharya *et al*., 2007). In support of the present observation, Petersen *et al*. (1987) [10] evaluated the effects of ACR (12.5, 25, and 50 mg/L) exposure for 15 days on histological changes in gills of rainbow trout. The ACR had produced pathological changes in gills. Dose-dependent lesions in gills like metaplasia and hyperplasia in the distal ends of the secondary lamellae were observed. In the most severely affected animals, the lamellae were hyperplastic and fused multiple gill lamellae. Xiuming *et al*. (2016) evaluated the toxic effects of ACR (2.04, 6.12 and 18.36 mg/L) exposure in gill of zebrafish for 40 days. The results showed that the gill filaments and gill cells were damaged. Kilicle *et al*. (2020) investigated the histopathological effects of acrylamide (10 mg/L, 20 mg/L, and 30 mg/L) exposure for 4 days on *Capoeta capoeta* fish. Histopathological examinations revealed mild edema (10 mg/L); vacuolization and edema (20 mg/L) and 30 mg/L exposure revealed irregular structure of secondary lamellae and epithelial separation was also observed. The significant separation was observed between the main axis of the cartilage of the primary lamellae and the epithelial layer. Necrosis and degeneration of epithelial layer of primary lamellae in gills of *Capoeta capoeta* fish were recorded.

5. Conclusion

Overall, acrylamide exposure at 17 mg/L for 28 days produced remarkable histopathological changes in gills of adult male zebrafish.

6. Acknowledgements

All the authors are highly thankful to Dr. A. R. Bhadaniya, Associate Professor, Department of Veterinary Pathology.

7. Author Contribution

Harsh R. Patel: Investigation, validation, formal analysis, writing – original draft.
Harshad B. Patel: Conceptualization, writing – review and editing, supervision.
Bhulesh V. Paida, Pavan M. Patel, Divya M. Ramchandani: Dosing, sample collection.
Urvesh D. Patel: Planned, review and editing.
Chirag M. Modi: Review and editing.

8. Funding

The study was carried out using the fund provided to the Department by the Institute/University.

9. Data Availability

Data will be made available upon reasonable request.

10. Declarations

Conflict of Interest The authors declare no competing interests.

11. Consent for Publication All the authors approved the manuscript for publication.

12. Reference

1. Anonymous. Health implications of acrylamide in food. Report of a Joint FAO/WHO Consultation, WHO Headquarters, Geneva, Switzerland, 2002, 25-27 June. https://dx.doi.org/10.22101/JRIFST.2014.08.23.326
2. Bhattacharya, S., Bhattacharya, A. & Roy, S. Arsenic induced responses in fresh water *teleosts*. Fish Physiology and Biochemistry. 2007;33(4):463-473. https://doi.org/10.1007/s10695-007-9173-2
3. Brown L, Rhead M. Liquid chromatographic determination of acrylamide monomer in natural and polluted aqueous environments. The Analyst, 1979;104(1238):391-399. https://doi.org/10.1039/AN9790400391
4. Evans DH, Piermarini PM, Choe KP. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiological Reviews. 2005;85:97-177. https://doi.org/10.1152/physrev.00050.2003
5. Faria M, Ziv T, Gómez-Canela C, Ben-Lulu S, Prats E, Novoa-Luna KA, *et al*. Acrylamide acute neurotoxicity in adult zebrafish. Scientific Reports. 2018;8(1):1-14.
6. Feitsma H, Cuppen E. Zebrafish as a cancer model. Mol. Cancer Res. 2008;6(5):685-694. https://doi.org/10.1158/1541-7786.MCR-07-2167
7. Friedman M. Chemistry, biochemistry, and safety of acrylamide: A review. Journal of Agricultural and Food Chemistry. 2003;51(16):4504-4526. https://doi.org/10.1021/jf030204
8. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, *et al*. The zebrafish references genome sequence and its relationship to the human genome. Nature. 2013;496(7446):498-503. https://doi.org/10.1038/nature12111
9. Kiliçle PA, Evren KOÇ, Doğan A, Süleyman GÜL, Doğan ANC, Ersan Y. Investigation of the Effect of Acrylamide on *Capoeta Capoeta* (Guldensttead 1773) by Histopathological, Electrophoretic and Biochemical Methods. Caucasian Journal of Science. 2020;7(2):153-166.
10. Mucci LA, Wilson KM. Acrylamide intake through diet and human cancer risk. Journal of Agricultural and Food Chemistry. 2008;56(15):6013-6019.
11. Petersen DW, Cooper KR, Friedman MA, Lech JJ. Behavioral and histological effects of acrylamide in rainbow trout. Toxicology and Applied Pharmacology. 1987;87(1):177-184. https://doi.org/10.1016/0041-008X(87)90095-0
12. Van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology. 2003;13:57-149. https://doi.org/10.1016/S1382-6689(02)00126-6
13. Wendelaar-Bonga SE. The stress response in fish. Physiological Reviews. 1997;77:591-625. https://doi.org/10.1152/physrev.1997.77.3.591
14. Wilson JM, Bunte RM, Carty AJ. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science: JAALAS. 2009;48(6):785-789.
15. Xiuming C, Fei L, Yu F. Toxic effects of acrylamide on zebrafish organs and DNA damage of germ cell. Asian Journal of Ecotoxicology. 2016;10(1):382-388.