Journal of Applied and Natural Science
15(2), 685 - 691 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Effect of mercury on histological alterations in gill, liver and stomach tissues of Indian catfish, *Clarias batrachus*

Akansha Mishra
Department of Zoology, Fakir Mohan University, Balasore-756089 (Odisha), India
Bhaskar Behera*
Department of Biosciences and Biotechnology, Fakir Mohan University, Balasore-756089 (Odisha), India

* Corresponding author. E-mail: drbhaskarbehera@gmail.com

Article Info
https://doi.org/10.31018/jans.v15i2.4467
Received: February 15, 2023
Revised: May 23, 2023
Accepted: May 27, 2023

How to Cite
Mishra, A. and Behera, B. (2023). Effect of mercury on histological alterations in gill, liver and stomach tissues of Indian catfish, *Clarias batrachus*. *Journal of Applied and Natural Science*, 15(2), 685 - 691. https://doi.org/10.31018/jans.v15i2.4467

Abstract
Heavy metal pollution poses a great threat to aquatic ecosystem, ultimately threatening the survival of safe and healthy life on earth. Contaminated water persuades pathological alterations and behavioural changes in fish. Mercury is highly toxic and seriously affects aquatic organisms, ultimately affecting humans.The present study aimed to assess the histological alterations in tissues like gill, liver and stomach of Indian catfish *Clarias batrachus*. The fish were divided in to four groups i.e. one control (Group I) and three groups of different concentration of mercury chloride treated groups, i.e.0.0002mg/l (Group II), 0.002mg/l (Group III), and 0.02mg/l (Group IV) for 14 days. Each group contained 25 number of fish.The results indicated degenerative changes in gill, liver and stomach tissues of exposed fish. Level of changes were higher with respect to higher in concentration. Rupture of epithelial layer, club shaped gill filament, broken gill lamellae, hyperplasia of interlamellar epithelium, deletion of secondary lamellae, formation of vacuoles, blood conjugation and necrosis in gill filaments were observed in gill tissues of exposed fish. Liver damage included hepatocellular dissociation, inflamed hepatocytes, cloudy swelling, vacuole formation and hydropic degeneration. Besides, stomach tissues showed alterations such as thinner serosa, vacuolization, muscle damage, and necrosis at several points. The study confirmed that mercury is life hazardous even at very low concentration.

Keywords: *Clarias batrachus,* Histological alteration, Mercury choride, Toxicity

INTRODUCTION

Aquatic ecosystem is delicate and its pollution by heavy metals has beena major global problem in recent times. The cluster of communities in the cities and elevated industrial practices are the two main sources of thousands of metals and chemicals that come into the environment. Although heavy metals, in minimal amounts, are essential for our normal body functions, still slightly higher amounts than necessary of any heavy metal can cause toxicity resulting in variations in the genetics of living beings (Vardhan *et al.,* 2019, Kumar *et al.,* 2019). Direct discharge of effluents from industries and sewerage contains heavy quantities of organic and inorganic chemicals, suspended solids, high BOD and COD, toxic oils etc. that cause ruinous effects on the aquatic environment (Dutta *et al.,* 2018, Jha and Jha, 1995, Chagas *et al.,* 2019). Problems in aquatic environment aggravate more rapidly than in other environments, affecting aquatic life and posing a threat to terrestrial lives directly or indirectly (Jiyavudeen and Puvaneswari, 2016, Abiona *et al.,* 2019). Heavy metals are persistent environmental toxins. They rapidly accumulate but are slowly metabolised and excreted from animal bodies (Jaiswal *et al.,* 2018).

Mercury is very much toxic in its every form and is ablack-listed element by eco-activists. It is released into our surroundings by different natural sources such as volcanic eruptions, weathering of rocks, forest fires etc, while anthropogenic sources are the combustion of mining and fossil fuels, thermal power plants, medical wastes, use of pharmaceuticals etc (Maheswaran *et al.,* 2008, Zulkipli *et al.,* 2021, Zheng, *et al.,* 2019). In water, heavy metals accumulate in fish tissues, resultingin the cumulative deleterious function of various body organs leading to their death (Vutukuru and Basani, 2013). Consumption of these organisms leads tofood chain contamination.The Indian walking catfish

Clarias batrachus has high demand throughout our country as it is economical as well as it has high nutritive value (Mudgal *et al.,* 2010, Jaishankar *et al.,* 2014). Since it can survive less amount of water for some days it can be a good model for scientific study. In aquatic toxicology, histopathology is a gifted field for research as it provides real image of changes in specific target organ, cells and organelles infected *in vivo*. It is one of the easiest methods of assessing short- and long-term toxic effects on body organs (K AL Taee and Al-Mallah, 2020, Sun *et al.,* 2018, Jayakumar and Subburaj, 2017). The study aimed to highlight various histological changes in the gill, liver and stomach tissues of *Clarias batrachus* exposed to different concentrations of mercury chloride.

MATERIALS AND METHODS

Test fish

The healthy fingerlings of freshwater Indian catfish (*Clarias batrachus*) with mean body weight of 7.2 ±2 g and length of 6.1±2 cm were procured from the fishermen in June 2022, raised at a nearby commercial hatchery in Balaramgadi, Balasore. The walking catfish, *C. batrachus* does not have a special status on the US Federal List or by CITES, and it is classified as a species of "Least Concern" on the IUCN Red List. Thus no animal ethical approval was required for present study.

The fish were cleansed with a 0.1% $KMnO_4$ solution to prevent cutaneous infections. Now they are acclimated to the laboratory environment for 15 to 20 days in large plastic tubs.

Test medium

After the acclimatization period, these healthy and equal-sized fish were sorted into 4 groups (Groups I to IV) for 14 days of the experimental period with a control (Group I) and mercury chloride exposed i.e- 0.0002 mg/l (Group II), 0.002mg/l (group III), and 0.02mg/l (Group IV) groups. 25 fish were there for each group and each group of fish were transferred into the same size tubs, each with 30 litre capacity and non-chlorinated tap water (APHA, 2005). The experiment was conducted in the P.G Department of Zoology laboratory in July and August, 2022. Once each day, the fish were fed with commercial fish food. The chemical used for the exposure study was Sisco research laboratories Pvt Ltd mercury chloride. The water of each experimental tub was changed at regular intervals along with waste feed and faecel material and aerated with the mechanical air pump.

Every day throughout the exposure period, the condition and behaviour of the treated fish were documented. The water quality was measured and the photoperiod was kept at 12 hours of light and 12 hours of darkness during the acclimation and exposure period. The average values for the test water's characteristics, which included pH of 7.1±0.02; dissolved oxygen of 6.2±0.5mg/l; temperature of 27±2 ºC; alkalinity of 224±2.6 mg/l; Total suspended solid of 43±2 (mg /L) and total hardness as $CaCO_3$ of 42±5 mg/l, were established. The test medium and dead fish were taken out right away after the LC50 values (0.04 mg/l) were determined with the formula-

LC50 = LC100 - ∑ conc. diff. × mean % mortality / % control. Eq.1

Fish from each group are collected and offered as sacrifices after 14 days. Carefully harvested tissues for sectioning from the treatment and control groups included gills, liver and stomach tissues.

Histopathology

The collected gill, liver and stomach tissues were rinsed with physiological saline (0.75% NaCl) to get rid of the blood and clean the mucus that was adhered to the tissues. After that, the tissues were preserved for 24 hours in fixative (Bouin's fluid). Following that, both the controls and treated tissues were immediately transferred to 70% alcohol, followed by repeating this alcohol transfer process twice more with an 8-hour gap between each transfer to remove the fixative completely. The tissues were subsequently prepared for paraffin blocks at a temperature of 56 to 60 °C and sectioning was made at 3-5 microns with a semi-automatic microtome of Radical scientific equipment, model RNT-35. The various tissues were stored using haematoxylin following the standard laboratory method (Younis *et al.,* 2013).

RESULTS

The gill tissue of the control fish (Group I) showedlong filaments with well-distinguished primary and secondary lamellae (Fig.1A). Fish (Group II) exposed to mercury chloride (0.0002 mg/l) showed mild degenerative changes, including rupture of the epithelial layer, club shaped gill filament, broken gill lamellae, hyperplasia of interlamellar epithelium and deletion of some secondary lamellae (Fig.1B). Similarly, the effect of mercury(0.002 mg/l) on gill (Group III) where damages were more pronounced. The formation of vacuoles, blood conjugation, necrosis in gill filaments were the additional features (Fig.1C). Further, exposed to mercury (0.02 mg/l) gill (Group IV) showed loss of normal structure which included complete necrosis of gill filaments and fusion of lamellae at several points (Fig.1D).

Hepatic tissue ofthe Control fish (Group I) showed normal hepatocytes with granular cytoplasm and darkly stained nuclei. Typical hepatic central veins were seen with thin endothelium, flattened nucleiand endothelial opening (Fig.2A). Fish liver (Group II) exposed to mercury (0.0002 mg/l) showed hepatocellular dissocia-

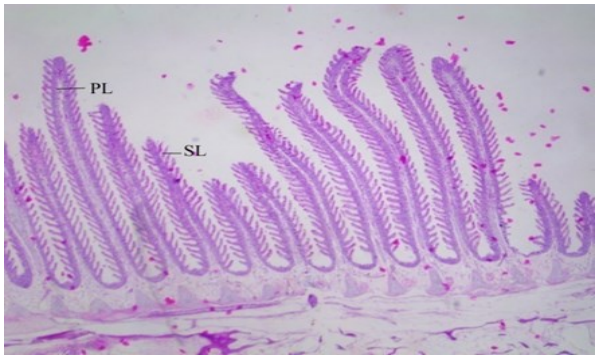

Fig.1A. *Section of gill showing normal structure (Control)*

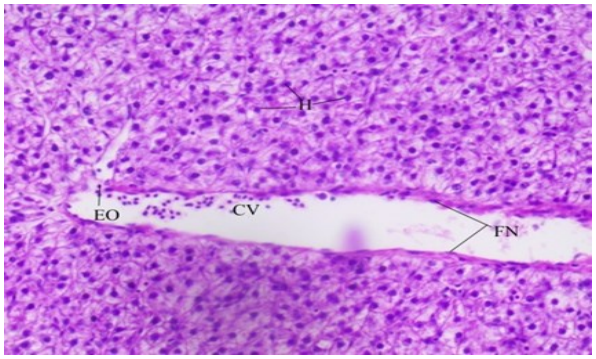

Fig.2. A. *Section of liver showing normal structure (Control)*

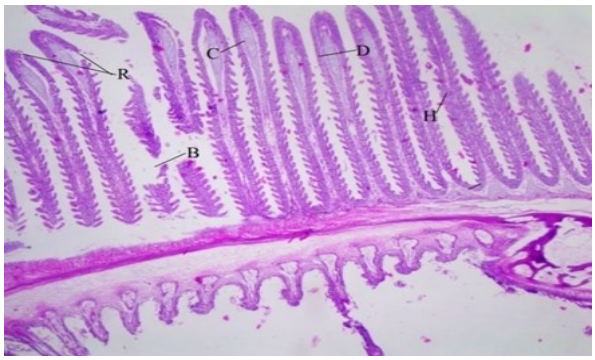

Fig. 1B. *Section of gill showing small degeneration of tissue exposed to mercury at 0.0002 mg/l conc.*

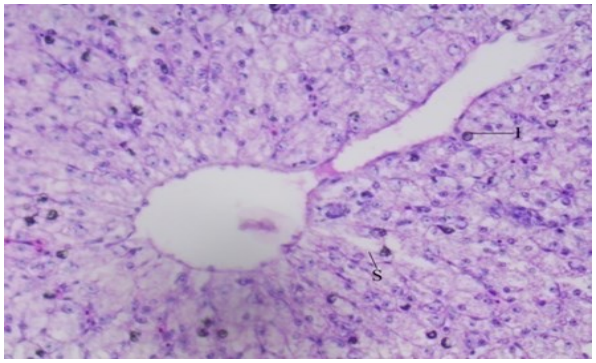

Fig. 2B. *Section of liver showing a bit of degeneration of tissue exposed to mercury at 0.0002 mg/l Conc.*

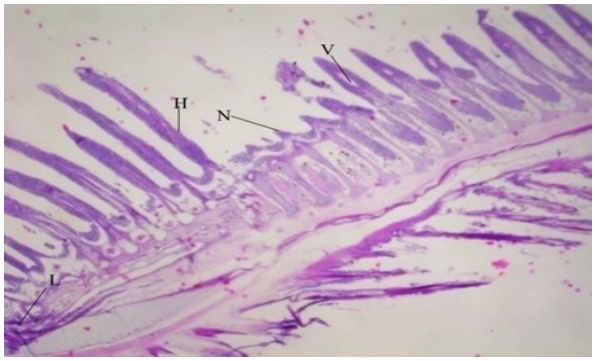

Fig. 1C. *Section. of gill showing moderate degeneration of tissue exposed to mercury at 0.002 mg/l conc.*

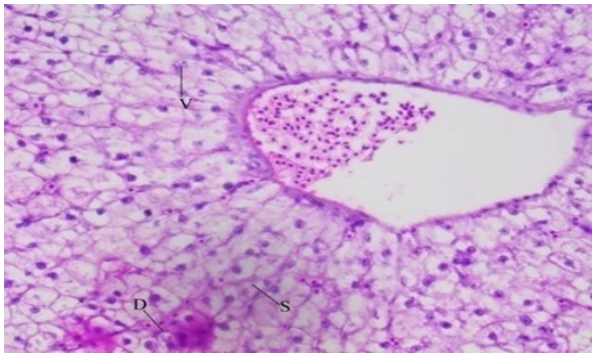

Fig. 2C. *Section of liver showing more degeneration of tissue exposed to mercury at 0.002 mg/l Conc.*

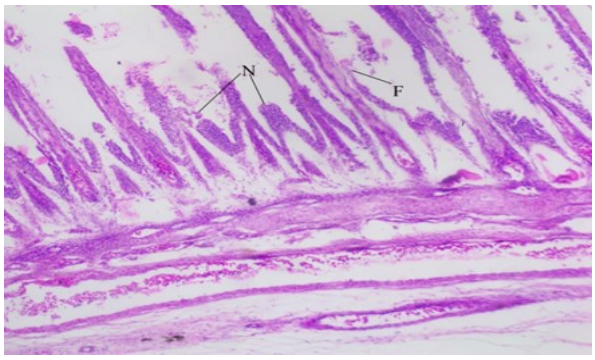

Fig. 1D. *Section of gill showing significant degeneration of tissue exposed to mercury at 0.02 mg/l conc.* ***[****Primary lamellae(PL) , secondary lamellae(SL), rupture of epithelial layer(R), club shaped gill filament(C), broken gill lamellae(B), hyperplasia of interlamellar epithelium(H), deletion of secondary lamellae(D), formation of vacuoles(V), blood conjugation(L), necrosis in gill filaments(N), fusion of lamellae(F)]*

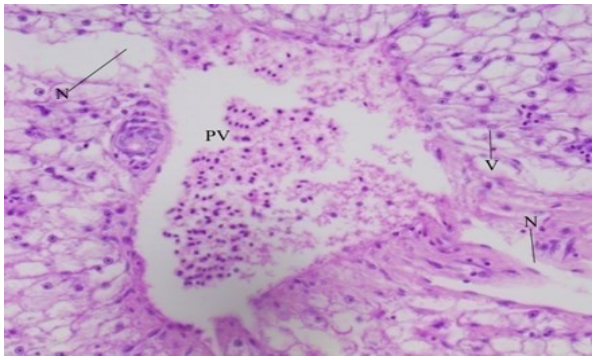

Fig. 2D. *Section of liver showing prominent degeneration of tissue exposed to mercury at 0.02 mg/l Conc. [hepatocytes(H), central vein (CV), portal vein(PV), flattened nuclei(FN), endothelial opening(EO), , inflamed hepatocytes(I), cloudy swelling(S), vacuole formation(V), hydropic degeneration(D), necrosis(N)]*

tionand some inflamed hepatocytes (Fig.2B). Liver tissue (Group III) treated with mercury (0.002 mg/l) showed higher damage level which included cloudy swelling, vacuole formation and hydropic degeneration (Fig.2C). Further, mercury treated (0.02 mg/l) liver tissue (Group IV) showed abnormal structure with higher level of necrosis, vacuolization and demolition of portal vein (Fig.2D).

In Control fish (Group I), stomach showed usual mucosa, submucosa, circular muscle, longitudinal muscleand serosa (Fig.3A). Fish (Group II) exposed to mercury (0.0002 mg/l) explained small modifications in stomach tissue like thinner serosa and vacuolization in circular muscle layer (Fig. 3B). Mercury (0.002 mg/l) treated stomach (Group III) showed cellular dissociation in mucosa and submucosa, vacuolization in submucosa, muscle damage in the circular muscle layer and necrosis (Fig.3C). Again stomach tissue (Group IV) exposed to mercury (0.02 mg/l) showed unusual structure with necrosis at several points on tissue (Fig.3D).

DISCUSSION

Panigrahi *et al. (*2021) stated that fish could accumulate trace metals and act as indicators of pollution. Depending on the amount and length of exposure, as well as other factors, including temperature, age, the interaction of one metal with another, water biochemistry, and the metabolic activity of the fish, metals accumulate in fish tissues (Sonone *et al.,* 2020). In target fish organs, heavy metal-induced cell harm at the molecular and subcellular levels results in degenerative and cancerous disorders (Malik *et al.,* 2020). In order to assess the harmful consequences, numerous studies have been made on the histopathological changes in different fishes like *Clarias gariepinus*, *Salvelinus alpines, Cyprinus carpio* etc., which involved microscopic analysis of tissues from various affected organs such as gill, kidney, liver, olfactory epithelium etc. (Alimba *et al.,* 2019, de Oliveira Ribeiro *et al.,* 2002, Al-Tamimi *et al.,* 2015).

Since the gill comes into contact with contaminated water regularly, it is a crucial tissue for histology research. Therefore, the response in gill cells is uncontrolled. Some chemicals can quickly destroy Gill lamellae after only a few hours of exposure (Taee and Al-Mallah, 2020, Pariza *et al.,* 2019). *C. batrachus* gill tissue exposed to mercury and cadmium at levels below lethal levels displayed lamellar disintegration, vacuolization, fusion of secondary lamellae, and hyperplasia of the epithelial surface (Selvanathan *et al.,* 2013). Fish, *C. gariepinus* exposed to effluent water containing aluminium, lead, cadmium, zinc, and heavy metal showed signs of lamellar atrophy, telangiectasia, and

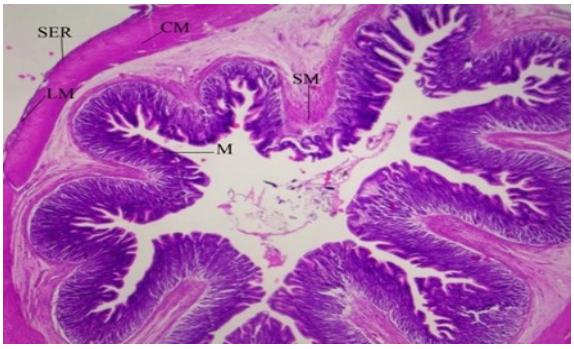

Fig. 3A. *Section of stomach showing normal structure (Control)*

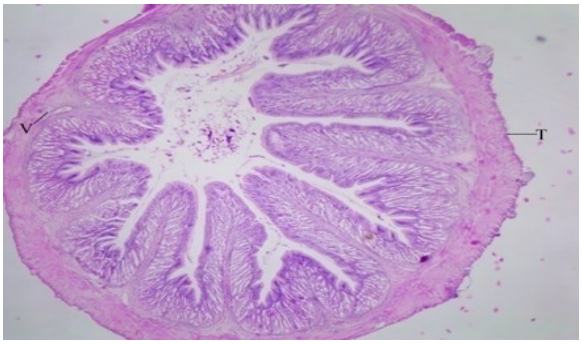

Fig. 3B. *Section of stomach showing slight degeneration of tissue exposed to mercury at 0.0002 mg/l Conc.*

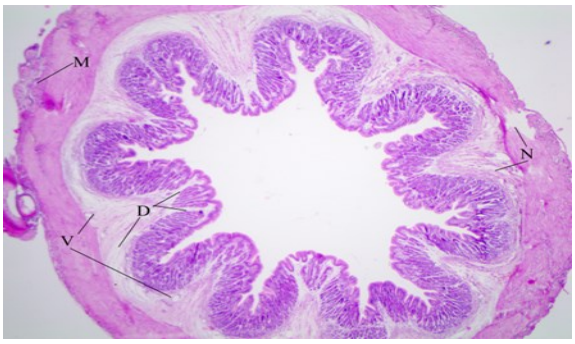

Fig. 3C. *Section of stomach showing degeneration of tissue exposed to mercury at 0.002 mg/l Conc.*

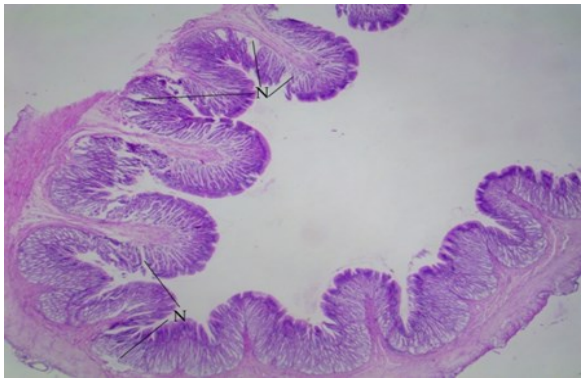

Fig. 3D. *Section of stomach showing highest degeneration of tissue exposed to mercury at 0.02mg/l Conc.[mucosa (M), submucosa(SM), circular muscle(CM), longitudinal muscle(LM), serosa(SER), thinner serosa(T), vacuolization (V), cellular dissociation(D), muscle damage(M), necrosis (N)]*

necrosis of lamellar epithelial cells upon microscopic examination of the gills (Van Dyk *et al.,* 2009). According to Hussan *et al.* (2018), histological changes in the gills of freshwater fish, *Catla catla*, included oedema, haemorrhage, hyperplasia, hypertrophy, necrosis, curling, and fusion.

The principal organ for the detoxification of organic xenobiotics is the liver. It tends to accumulate a variety of metals and other hazardous byproducts in large concentrations, which has detrimental consequences on the organ (Morovvati *et al.,* 2012, Jabeen *et al.,* 2018). According to research by Zulkipli *et al.* (2021), exposure to heavy metals caused a number of pathological changes in different fish livers, including hyalinization, hepatocyte vacuolation, cellular expansion, and blood channel congestion. Hepatic cell degeneration, congestion, and haemorrhages were all seen during the histopathological analysis of the liver tissues. Similar results were also discovered by Naz *et al*. (2021), for big carp treated to two separate heavy metals. Histological study of liver tissue of freshwater fish *Clarias batrachus* exposure to mercury and cadmium showed degeneration of hepatocytes showing distinct vacuoles, necrosis and sinusoidal lesion (Selvanathan *et al.,* 2013).

The digestive tract is the first organ to be exposed when contaminated food is consumed. Therefore, the fish stomach is one of the tissues required for the histology analysis to determine the impact of pollution in the water (Dane and Şişman, 2020, Shahin *et al.,* 2013). The effects of the histopathological alteration in the stomach tissues of fish, *Channa punctatus,* treated with various concentrations of the insecticide endosulfan were detailed by Haloi *et al.* (2013). In an increasing order towards the higher tested doses, the degenerative alterations comprised fused microvilli, fractured microvilli's outer membrane, haemorrhage in the submucosa region, swollen cells, and vacuoles. Microscopic examination of the gut tissues of *Catla catla* subjected to various heavy metals revealed significant alterations, including villi atrophy, epithelial villi sloughing, and congestion (Naz *et al.,* 2021).

In the present study, the gill tissue of *Clarias batrachus* exposed to mercury (0.0002 mg/l) showed mild alterations involving a break in the epithelial layer, damage in gill lamellae, and club-shaped gill filament, hyperplasia of interlamellar epithelium, deletion of some secondary lamellae. But gill tissues exposed to higher concentration of mercury (i.e. 0.002 mg/l and 0.02 mg/l) described elevated levels of damage. Here vacuole formation, blood conjugation and necrosis of gill lamella were the indication of unusual structure of gill tissues (Fig.1A-D). Again, the present study showed some remarkable degenerative pathological changes in the liver tissues of freshwater walking catfish exposed to different mercury concentrations (0.02 mg/l, 0.002 mg/l, 0.0002 mg/l). Hepatocellular dissociation, vacuole formation and necrosis intensity were higher in treated liver tissues with respect to the rise in metal concentration (Fig. 2A-D). The histological analysis of stomach tissues of *Clarias batrachus* exposed to different mercury concentrations (0.02 mg/l, 0.002 mg/l, 0.0002 mg/l) involved

cellular dissociation, vacuolization and tissue damage at several points. Here degree of damage and structural changes were higher at higher concentrations of the test medium (Fig.3A-D).

Conclusion

The present study showed that mercury in different sublethal concentrations (0.0002 mg/l, 0.002 mg/l, 0.02 mg/l) had severe consequences on tissues of *Clarias batrachus*, such as the gill, liver and stomach. The exposed tissues showed an elevated level of degradation where necrosis was clearly detected for the higher concentration of mercury chloride exposure. Thus, it was concluded that fishes were highly sensitive to mercury chloride ($HgCl_{2)}$. Damage to the fish tissues may obviously affect the regular physiological processes and activities that could ultimately result in death not only to fish but to other aquatic organisms. Thus, heavy metals at least in effluents and municipal waste must be thoroughly detoxified before being released into the water body.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Jiyavudeen, M. & Puvaneswari, S. (2016). A study on the effects of industrial effluents on the biochemical components of the fish *Mugil cephalus* in uppanar estuary south east coast of india. *Int. J. Modn. Res. Revs*, *4*(8), 1224-1227.
2. Chagas, T. Q., da Silva Alvarez, T. G., Montalvão, M. F., Mesak, C., Rocha, T. L., da Costa Araújo, A. P. & Malafaia, G. (2019). Behavioral toxicity of tannery effluent in zebrafish (*Danio rerio*) used as model system. *Science of the Total Environment*, *685*, 923-933.
3. Van Dyk, J. C., Marchand, M. J., Pieterse, G. M., Barnhoorn, I. E. & Bornman, M. S. (2009). Histological changes in the gills of *Clarias gariepinus* (Teleostei: Clariidae) from a polluted South African urban aquatic system. *African Journal of Aquatic Science*, *34*(3), 283-291.
4. Naz, S., Hussain, R., Ullah, Q., Chatha, A. M. M., Shaheen, A. & Khan, R. U. (2021). Toxic effect of some heavy metals on hematology and histopathology of major carp (*Catla catla*). *Environmental science and pollution research*, *28*(6), 6533-6539.
5. Haloi, K., Kalita, M. & Nath, R. (2013). The study on the histopathological changes of stomach of *Channa puncta-*

tus (Bloch) by used pesticide endosulfan. *Global J Sci Front Res Biol Sci*, *13*, 1-6.
6. Morovvati, H., Nikpour, Y., Zolgharneine, H., Ronagh, M., Abdi, R. & Roshan, A. (2012). Histological changes in the liver of reared spotted scat (*Scatophagus argus L.*) after exposure to mercury. *Comparative Clinical Pathology*, *21* (5), 745-753.
7. Hussan, A., Choudhury, T. G., Ahmed, I., Gita, S., Das, A., Udit, U. K.& Mandal, R. N. (2018). Effect of mercury and cadmium on the oxygen consumption and gill Histology of *Catla catla* (Ham. 1822). *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *88*(2), 729-738.
8. Al-Tamimi, A. H., Al-Azzawi, A. J. & Al-A'dhmi, M. A. (2015). Chronic toxicity assessment of histological changes and micronuclei in fish *Cyprinus carpio L*. after exposed to copper. *American Academic Scientific Research Journal for Engineering, Technology, and Sciences*, *13*(1), 194-210.
9. Dutta, J., Ahmad, A. & Singh, J. (2018). Study of industrial effluents induced genotoxicity on *Allium cepa L*. *Caryologia*, *71*(2), 139-145.
10. Jha, B. S. & Jha, M. M. (1995). Biochemical effects of nickel chloride on the liver and gonads of the freshwater climbing perch, *Anabas testudineus* (Bloch). *Proceedings-national academy of sciences india section B*, *65*, 39-46.
11. Jabeen, G., Manzoor, F., Javid, A., Azmat, H., Arshad, M. &Fatima, S. (2018). Evaluation of fish health status and histopathology in gills and liver due to metal contaminated sediments exposure. *Bulletin of Environmental Contamination and Toxicology*, *100*(4), 492-501.
12. de Oliveira Ribeiro, C. A., Belger, L., Pelletier, E. & Rouleau, C. (2002). Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). *Environmental research*, *90*(3), 217-225.
13. Mudgal, V., Madaan, N., Mudgal, A., Singh, R. B. & Mishra, S. (2010). Effect of toxic metals on human health. *The open Nutraceuticals journal*, *3*(1).
14. Jaiswal, A., Verma, A. & Jaiswal, P. (2018). Detrimental effects of heavy metals in soil, plants, and aquatic ecosystems and in humans. *Journal of Environmental Pathology, Toxicology and Oncology*, *37*(3),183-197.
15. Maheswaran, R., Devapaul, A., Muralidharan, S., Velmurugan, B. & Ignacimuthu, S. (2008). Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *International Journal of Integrative Biology*,2(1),50-54.
16. Vutukuru, S. S. & Basani, K. (2013). Acute effects of mercuric chloride on glycogen and protein content of Zebra fish, *Danio rerio*. *Journal of Environmental Biology*, *34*(2), 277-281.
17. Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2), 60.
18. APHA (2005), Standard Methods for the Examination of Water and Wastewater, 21th ed. Washington DC: American Public Health Association.
19. Selvanathan, J., Vincent, S. & Nirmala, A. (2013). Histopathology changes in freshwater fish *Clarias batrachus* (Linn.) exposed to mercury and cadmium. *International Journal of Life Sciences and Pharma Research*, *3*(2), 11-21.
20. Pariza, A. A. F. M., Ahmad, S. A., Fadzil, N. I., Basirun, A. A., Sha'arani, S. A. W., Asri, N. A. M. & Shukor, M. Y. (2019). Histopathological and cholinesterase changes in the gills of *Clarias gariepinus*as a result of cadmium exposure. *Journal of Environmental Biology*, *40*(4), 683-690.
21. Shahin, M. I. H., Chandra, K. J., Das, D. R. & Khalil, S. M. I. (2013). Morphology and histopathology of alimentary canal of *Clarias batrachus* (Linnaeus) and Heteropneustesfossilis (Bloch). *International Research Journal of Applied Life Sciences*, *2*(2), 11-20.
22. Abiona, O. O., Anifowose, A. J., Awojide, S. H., Adebisi, O. C., Adesina, B. T. & Ipinmoroti, M. O. (2019). Histopathological biomarking changes in the internal organs of Tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) exposed to heavy metals contamination from Dandaru pond, Ibadan, Nigeria. *Journal of Taibah University for Science*, *13*(1), 903-911.
23. Zulkipli, S. Z., Liew, H. J., Ando, M., Lim, L. S., Wang, M., Sung, Y. Y. & Mok, W. J. (2021). A review of mercury pathological effects on organs specific of fishes. *Environmental Pollutants and Bioavailability*, *33*(1), 76-87.
24. Zheng, N. A., Wang, S., Dong, W. U., Hua, X., Li, Y., Song, X. & Li, Y. (2019). The toxicological effects of mercury exposure in marine fish. *Bulletin of environmental contamination and toxicology*, *102*(5), 714-720.
25. Malik, D. S., Sharma, A. K., Sharma, A. K., Thakur, R. & Sharma, M. (2020). A review on impact of water pollution on freshwater fish species and their aquatic environment. *Advances in Environmental Pollution Management: Wastewater Impacts and Treatment Technologies*, *1*, 10-28.
26. Dane, H., & Şişman, T. (2020). A morpho-histopathological study in the digestive tract of three fish species influenced with heavy metal pollution. *Chemosphere*, *242*, 125212.
27. Sun, Y., Li, Y., Rao, J., Liu, Z. & Chen, Q. (2018). Effects of inorganic mercury exposure on histological structure, antioxidant status and immune response of immune organs in yellow catfish (*Pelteobagrusfulvidraco*). *Journal of Applied Toxicology*, *38*(6), 843-854.
28. Jayakumar, N. & Subburaj, A. (2017). Sub-lethal cadmium toxicity induced histopathological alterations in the gill, liver and kidney of freshwater catfish (*Heteropneustesfossilis*). *Journal of Entomolgy and Zoology Studies*, *5*(5), 1339-1345.
29. Alimba, C. G., Adekoya, K. O. & Soyinka, O. O. (2019). Exposure to effluent from pharmaceutical industry induced cytogenotoxicity, hematological and histopathological alterations in *Clarias gariepinus* (Burchell, 1822). *Excli journal*, *18*, 63-78.
30. Vardhan, K. H., Kumar, P. S. & Panda, R. C. (2019). A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. *Journal of Molecular Liquids*, *290*, 111197.
31. Kumar, V., Parihar, R. D., Sharma, A., Bakshi, P., Sidhu, G. P. S., Bali, A. S. & Rodrigo-Comino, J. (2019). Global evaluation of heavy metal content in surface water bodies: A meta-analysis using heavy metal pollution indices and multivariate statistical analyses. *Chemosphere*, *236*, 124364.

32. K AL Taee, S. & Al-Mallah, K. H. (2020). Review on some heavy metals toxicity on freshwater fishes. *Journal of Applied Veterinary Sciences*, *5*(3), 78-86.
33. Younis, E. M., Abdel-Warith, A. A., Al-Asgah, N. A., Ebaid, H. & Mubarak, M. (2013). Histological changes in the liver and intestine of Nile tilapia, *Oreochromis niloticus*, exposed to sublethal concentrations of cadmium. *Pakistan Journal of Zoology*, *45*(3), 833-841.
34. Panigrahi, A. K., Bakshi, A. & Pattanayak, S. (2021). A comprehensive review on the uptake by and accumulation of some heavy metals in fresh water fishes. *Biosc. Biotech. Res. Comm*, *14*(1), 10-21786.
35. Sonone, S. S., Jadhav, S., Sankhla, M. S. & Kumar, R. (2020). Water contamination by heavy metals and their toxic effect on aquaculture and human health through food Chain. *Lett. Appl. NanoBioScience*, *10*(2), 2148-2166.