

Dietary Selenium reduces the toxic effect of Mercury on different organs (Brain, Gills, Kidney and Liver) of Rohu fish (*Labeo rohita*)

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ABSTRACT

Because of improbably high partiality with antioxidant selenium to mercury, selenium isolates mercury and reduces its biological handiness to organisms. The present study investigates the effect of mercury (Hg) and selenium (Se) on the fingerling of *Labeo rohita*. The various sub-lethal concentrations of Hg i.e. 0.125µg/g, 0.250µg/g, and 0.500µg/g was used and incorporated in diet with a commercial diet (fish meal 40%, soya bean 33%, 3 % of vitamins premix, mineral premix and oil each and rice polish 18%). To understand how selenium reduces the toxic effect of mercury, fingerlings were exposed with 6µg/g Se singly and combined with doses of Hg. The effect of these heavy metals was observed after 24, 48, 72, and 96 hours in different organs of (Brain, Gills, Kidney and Liver) of *Labeo rohita*. The organs of exposed rohu showed significant changes in their microscopic anatomy in comparison to control. Prominent changes were observed in Hg treatments and found embody shrinkage of capillary, and dilation of the hollow lumen. In addition to the aforementioned changes vacuolation, peeling, hydropic swelling, and hyaline degeneration of hollow epithelium were also observed. Cysts and hamorrhage developed additionally seem in the organs of fish. Length of exposure seems to own a profound impact on organs because the increase in length of exposure enhanced the severity of histopathological damages. However, combined doses of metals cause reduction in the toxicity of mercury results in decrease damage in the shrinkage of capillary and dilation of the hollow lumen. During histological study vacuolation, hydropic swelling, and hyaline degeneration of hollow epithelium were also reduced. In Hg, the lethal concentration (LC₅₀) was 0.374ug/g while when Hg and Se were combined, LC₅₀ drops to 0.491ug/g. Results of this study suggest that exposure to Se helps to scale back the impact of mercury on mortality and different organs of *Labeo rohita* fingerling.

Keywords: Brain, Gills, Kidney, *Labeo rohita*, Liver, Mercury and Selenium,

Accepted

1. INTRODUCTION

Fish shows almost sixteen percent of the universal population's intake of animal protein and six percent of all protein used. Fish is a healthy and fair diet because it contains a high level of omega-3 fatty acids and macromolecules that the physical body needs to remain energetic and functioning. Fish culture provides an ample opportunity to produce wonderful quality food for utilization by masses which is free from contagion [1]. Due to violation of an existing set of rules and regulations there is a continuous rise in the concentration of contagions in the freshwaters because of household squanders, bug sprays and herbicides, nourishment handling waste, toxins domesticated animals tasks, unstable natural mixes, substantial metals, concoction waste and others [2]. Among natural contagions, substantial metals are a matter of grave concern due to their potential dangerous impact and capability to bio-aggregate in aquatic environments [3]. The pollution inside the water ambience with substantial metals has become an overall issue from the previous few years as a result of their indestructible and a deadly impact on living organisms [4].

Due to its possible hazards to higher biological process levels, mercury has attracted greater attention. Methyl mercury is predominant in fish organs and is biologically available in greater concentrations than inorganic Hg. The high degree of mercury (Hg) in aquatic living beings, primarily fish, correlates with nursing environmental and human concern [5]. Hg reflection in fish is inclined by fish age [6], Hg concentrations in water food contagion, chemical, organic and objective processes in water environment, and seasonal alterations [7, 8]. In fish, as higher vertebrates, the excretory organ plays out an important role in balancing the water and other solutions ultimately maintaining the internal environment stability. The excretory organ secretes nitrogen-containing waste products from digestion, for instance, carbamide and creatinine. Following exposure of fish to compounds like pesticides, histological changes are visible in animal tissue and blood [9]. The fish is very sensible to the external environment and sensible measures should be taken to give compound checking of the getting from its usage.. Histopathological studies can be used for watching the effects of maritime pollutions particularly considerable metals [10]. The toxic nature of total mercury (Hg) and methylmercury (MeHg) was observed via histopathological studies of living things which identify the degree of cell damage. [9].

Selenium is a basic element for living organisms and has been seemed to overcome the toxic effects of mercury and other heavy metals like arsenic, cadmium, and probably lead [11]. Selenium is incorporated into the twenty initial amino corrosive L-selenocysteine that could be a constituent of selenoproteins [12]. Selenium sensitizes the gathering and harmfulness of mercury in oceanic living beings in a very intricate manner. In some marine well evolved creatures, demethylation of the alkyl group of mercury within the liver prompts arrangement of insoluble mercury selenium giving ascend to a 1:1 molar proportion Mercury: Selenium [13]. It has been projected that selenium detoxifies MeHg by shaping edifices containing the two elements are present at equimolar proportion. It has been well documented that metallic element mercury selenide (HgSe) has been found within the liver of marine well-evolved creatures and seabirds. This element is believed to be a dormant consequence of the detoxification procedure in these marine creatures [14]. The objective of the present study was to determine the Hg toxicity and its effect on *Labeo rohita* species and the combined effect of Hg and Se as well. The study helps how the *L. rohita* species respond to different level of heavy metal (Hg).

2. MATERIALS AND METHODS

2.1. Sample Collection and Maintenance

The fingerlings of *Labeo rohita*, were obtained from a fish farm settled some 40 kilometres from the city (latitude 31°58 N, longitude 74°13'E), on the North certain GT road, Manawa.

The collected fish were raised in a polyculture of major carps under well-established procedures and practices. The fishes were transported in air-packed polythene bags from the farm. The fish were then given a shower in 0.1% methylene blue before moving them to the aquaria. This prophylactic action was done to keep the fish away from any infection from bacteria that can badly influence fish. Prophylaxis was followed by the transfer of fish in neat aquaria supplied with dechlorinated water. Artificial aeration was provided to the fish [15]. The fish were fed *ad libitum* with commercial fish feed.

2.2. Experimental Plan

After acclimatization, fish were placed in the glass aquariums. Glass aquarium was filled with 15 liters of water about $\frac{3}{4}$ of the capacity of each aquarium. The water was replaced with fresh clean water once a day. Fish was weighed in gm and measured in cm and then randomly distributed in each aquarium 25 fishes per aquarium. Fish was then exposed to three sub-lethal concentrations of mercuric chloride i.e. 0.125µg/g, 0.250µg/g, and 0.500µg/g respectively with a commercial diet for 24, 48, 72, and 96 hours. Then commercial diet was mixed with both HgCl₂ and Se i.e. (0.125+6 µg/g, 0.250+6 µg/g and 0.500+6 µg/g). The test concentrations were restored after every 24 hours by cleaning and replacement of dechlorinated water. The above protocol was repeated after every 24 hours. The fish of each aquarium was fed thrice a day. After each exposure, the fish specimens were measured, weighed, and dissected to obtain organs (Brain, Kidney, Gills and Liver) for histological processes. The fish was anesthetized immediately to reduce stress. The Clove oil (National Chemicals, Pakistan) was use as anaesthesia[16]. The fish was put in the solution for 3 to 5 minutes nonetheless, time period of anaesthesia could be changed according to the age and size of the fish.

2.3. Dissection and Preparation of Organs

The lower abdomen of the fish was cut from the posterior to the anterior end by a sharp surgical scalpel and scissors. Kidney tissue was removed with small scissors and freed from any extraneous tissue. The kidney of fish was removed and weighed on an electric balance. Examination of the microanatomy of tissue was started with surgery of animal Brain, Kidney, Gills and Liver. The tissues were preserved in one hundred millilitres of 100% unbiased buffered formol which is four percent aldehyde in phosphate-buffered brackish mixture until processed for histological examination. The embedding was done to pieces of approximately 1 cm³ before loading the cassettes for histological processing. The sample-loaded cassettes were passed for dehydration of the tissues in the following pattern of alcohol (30% → 50% → 70% → 90%). The sample-loaded cassettes were passed for dehydration of the tissues in following pattern of alcohol (30% → 50% → 70% → 90%). The samples were cleared in two applications of xylene followed by applications of molten wax (58°C) for impregnation. The resultant tissues were then placed in a very mold containing additional liquefied wax (embedded) and allowed to cool down until wax hardened. Tissues were then removed from the cassettes and molded with molten paraffin wax at 58°C melting point, for making blocks. The blocks were attached to the cassettes, trimmed, frozen and sectioned [17].

2.4. Microtome and Staining

The tissues were cut at 3-4 micrometer thickness by a rotating mechanized microtome (Shandon, Thermo, CD-2235) fixed with microtome blade. The strips of segments were shaped and extended in warm water. The slides were mounted with egg albumen, following the placement of tissues on the slide. Slides were then placed under warm water letting the wax to melt and fix tissue on the slide.

Hematoxylin and fluorescent dye (Hematoxylin & Eosin Stain) is the most typically used light weight mark in microscopic anatomy & histology. Hematoxylin, a dyestuff, mark nucleus indigo as a result of its resemblance to nucleic acids within the cell centre; eosin, a bitter stain, dyes the protoplasm crimson. The mandatory portion was photographed by using a digital camera mounted on a Leica DM-500 microscope.

2.5. Estimation of lethal concentration (LC₅₀)

Keeping in view the scope of the study it was necessary to estimate LC₅₀ for the selection of different dosages of mercury for further initiatives in these studies. LC₅₀ test (24, 48, 72, and 96 hours) based on fish mortalities when fishes were exposed to known toxicant concentration sequence and then its comparison with parallel control that did not receive any toxicant. During this cycle, the fish was not fed [18].

Seven groups of fish were exposed to increased concentrations of HgCl₂. An appropriate quantity of HgCl₂ was added to the required lethal concentration based on the understanding that there would be no mortality up to 0.125 µg/g. But almost all the fishes died within 96 hours at 0.500 µg/g exposure to Hg [19].

2.6. Statistical analyses

Statistical analysis was performed by using SPSS (Ver.19). LC₅₀ was detected by probit analysis using Statplus 5.

3. Results

The percentage survival rate and lethal concentration were assessed. No mortality was observed in the control group but due to increases in Hg survival rate decreases from 24 h to 96 h. When Se had added in all Hg concentrations the survival rate increases when Hg concentration increases from 24 h to 96 h. LC₅₀ and LC₉₀ were also high in Hg groups but when Se was added lethal concentration increases. This shows that the addition of Se reduces the effect of Hg in feed (Table 1).

In the brain, the cell was observed with no change in all-time duration in control while granular cell loss, purkinje cells started aggregation and neutrophil cells loss were observed in 24 h exposure time. In 48 h, the presence of neural cells, pyramidal cells, and nissl substances were observed. In 72 h, delicate chronic changes were discovered within the neural cells, Gangrene of neurons, living hydrops, and congestion of neural cells were noticed. In 92 h, initiation of the degeneration of neural cells and protoplasm vacuolation occurs. The severity was observed in brains cell when Hg concentration increases. When Se was added with Hg, improvement was observed in all brains cell (Fig 1a).

In the kidney, no change was observed in the tissue after 24, 48, 72, and 92 h with any treatment. In 24 h, slight inflation was observed in the area between the capillary vessel and capsule glomeruli while in 48 h, shrinkage was observed. After 72 h, numerous degenerative changes were observed in some areas i.e. tubular epithelium with desquamation in tubes, Hyaline degeneration, hydropic swelling, vacuolization, and necrosis. In 92 h, shrinkage of the glomerulus, shortening and narrowing of lumen space and reduction of renal cell number count. But when both metals were exposed to fish, some minor changes were observed but the improvement was observed in low Hg concentration but in high concentration Hg cause extreme shrinkage of capillary, capillary and membrane was conjointly exaggerated, Gangrene and pycnosis was ascertained in animal tissue lining, epithelium of kidney tubules were extremely denatured (Fig 1b).

In gills, the tissue did not display any change after 24, 48, 72 and 92 hours of zero dose. In 24 h, exposure the changes in the gill histology was not very conspicuous. In 48 h, *Eubacteria cholerae* showed deformities within the gills. The gill filaments degenerated and sphacelus. In 72 h, cellular damage was observed within the gills in conditions of animal tissue propagation. In 92 h, gills abnormalities increased which incorporated dysplasia of animal

tissue cells, a mixture of derivative lamellae, enlivening of the lamellar epithelial hankie, blood obstruction, proliferation of animal tissue cells of primary and secondary lamellae, and sphacelus. The increased effects on these cells were observed with respect to increase in Hg concentrations. When Hg was mixed with Se, cell growth was observed normal in low Hg concentrations. Disruption of cell damage was observed in high concentrations but not as much as Hg (Fig 1c).

In the liver, no change was observed in the tissue after 24, 48, 72, and 92 hours in the control group. In 24 h, few mirror changes were revealed like degradation of viscous parenchyma cells and viscous cells did not create distinct lobules. In 48 h, there was structural harm to the central vein, busted and irregular viscous plate, granulation in living substance, necrosis, vacuolization, and disruption of hepatocytes. In 72 h, deterioration, mortification of the hepatocytes, vacuoles degenerated within the hepatocytes, occlusion formation in central veins, and dilation occurred. In 92 h, tissue displayed several alterations such as mild necrosis, pyknosis, cytoplasmic degeneration, and infiltration of leukocytes. In high concentrations of Hg, extreme alterations were observed in nuclear structures like nuclear degradation. The structure of the liver was completely denatured; the cytoplasm appeared degraded with the appearance of adverse necrosis and pyknosis. When Se was mixed with Hg, improvements were observed in cells with respect to Hg alone (Fig 1d).

4. DISCUSSION

The present study was very useful in understanding how to reduce the mercury level in the organs of fish (*Labeo rohita*) by the use of selenium.. During the investigation, mercury was combined with selenium and showed minor alterations in organs as compared to those groups which were solely exposed to mercury.

It has been conjointly ascertained that if the concentration of mercury is incredibly high within the tissue, it should cause severe structural harm [20]. The current study revealed that Hg change the structure of the brain, gills, kidney, and liver even when they are in low concentrations but denaturation occurs when they are in high quantity. Similar findings with dilation, oedema, and enlarged nuclei of nephritic tubules were additionally reported within the excretory organ of *Mugil auratus* [21]. [22] also studied that toxicity of HgCl_2 in fish excretory organ varies from slight disruption of cannular cells to swelling in cells with delicate to high dose in major carps. They also reported a similar effect of HgCl_2 on the liver of *C. mrigala* [23] also, study the effect of Hg on *Danio rerio* and observed a negative impact on growth and survival. All the fishes died within 96 hours at high exposure of Hg [19]. The LC_{50} determines the population died in the presence of xenobiotics. Our study shows that LC_{50} of Hg is 0.374 ug/g and LC_{90} is 0.704 ug/g. [24] have reported that LC_{50} of Cd for zebrafish (*Danio rerio*) after 96 h exposure was 9.68 mg/L. So far, 96 h LC_{50} of Cd showed considerable differences among fish species. [28] studied that when total Hg and MeHg levels increases, 40 % accumulation increases in liver of *Brosme brosme* as compared to normal fish. [29] reported that when Hg exposed to fish for 96 h, it cause tissue anomalies like blood congestion in gills, swelling of liver, lesion in kidney, vacuolation and exfolitation. Many studies were conducted to estimate the effect of Hg in different organs of fish [30] i.e. gills [31-33], liver [34-37], kidney [38-40], and brain [41, 42].

5. The present study was to observe the effect of Se on Hg toxicity in *Rahu* fingerling. [25] have reported that the kidney of *Trichomycterus brasiliensis* treated with selenium was inappropriate shape, there was an increase in the number of tubule cells which ultimately reshaped normally. The Se reduces the negative impact on *Danio rerio* in the presence of Hg [23]. [26] Studied that Se improves the bioavailability and intercept the selenium-dependent enzymes that's why Se can use to minimize the toxicity of Hg. [27] also studied that high the concentration of Hg weekly food intake exceeds in *Hippoglossus hippoglossus* and cause severe damage

but Se reduce the toxic effect of MeHg. Our study indicated that Se when mixed with Hg reduces the LC₅₀ and LC₉₀ up to 0.491 and 0.798, respectively. This shows that in the presence of Se more Hg concentration was required to eliminate 50% population of Rohu fingerling. [43] studied the ratio of Hg and Se, in commercial diet of fish was very crucial in safety of ecosystem. Similar studies were conducted to overcome the Hg toxicity with help of Se [44-50]

6. CONCLUSION

In view of present findings, it is concluded that mercury at sub-lethal concentrations caused considerable histological damages in different organs in *L. rohita*. Mercury entered in fish caused severe injury to the cellular structural integrity in organs of *L. rohita*. However, once the fish were exposed to Se the tissue recovers and damage to organ cells decreased gradually. Our findings reveal that selenium plays an important role in reducing the toxic metal in fishes and can play an important role in mending this prevalent and dominant problem of the fisheries sector. In future, more investigation is required to understand the importance of selenium in fisheries sector. Current histopathological investigations suggest that selenium can help in reducing the toxic level of mercury and other heavy metals. Nonetheless, further studies need to be undertaken to clarify exactly the toxicity of mercury to fish and counteracting effects of selenium in neutralizing the toxicity of heavy metals.

Declarations

Conflict of Interest: The authors declare no conflict of interest

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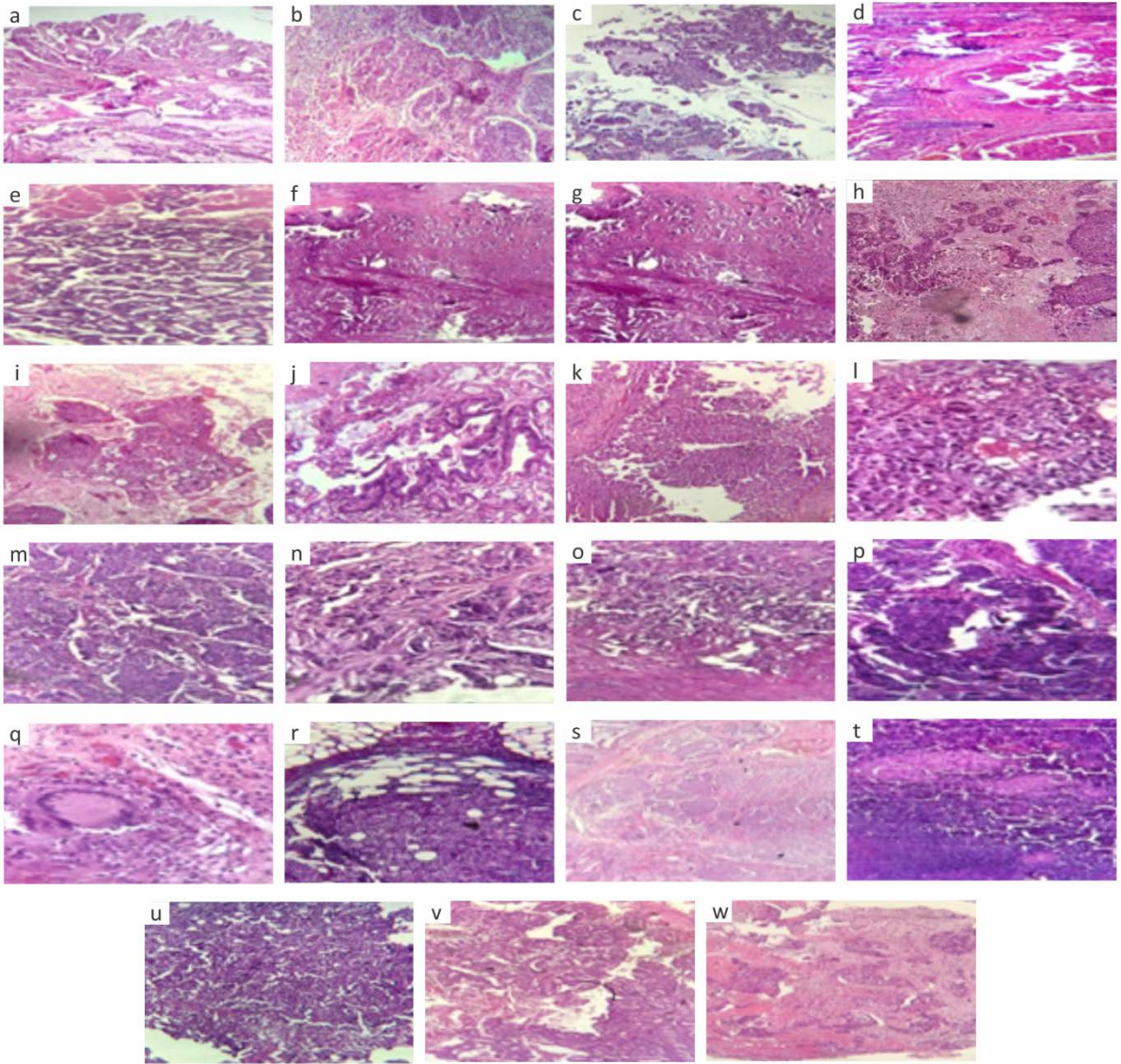
7. REFERENCES

- [1] Olojo E, Olurin K, Mbaka G, Oluwemimo A. Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to lead. *African Journal of Biotechnology*. 2005;4(1):117-122.
- [2] Rauf A, M. Javed, M. Ubaidullah and S. Abdullah,. Assessment of heavy Metals in sediments of the river Ravi, Pakistan. . *Int J Agric Biol*. 2009;11:197-200.
- [3] Censi P, Spoto S, Saiano F, et al. Heavy metals in coastal water systems. A case study from the northwestern Gulf of Thailand. *Chemosphere*. 2006;64(7):1167-1176.
- [4] MacFarlane G, Burchett M. Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Aquat Bot*. 2000;68(1):45-59.
- [5] Martin T, Chandre F, Ochou O, Vaissayre M, Fournier D. Pyrethroid resistance mechanisms in the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) from West Africa. *Pestic Biochem Physiol*. 2002;74(1):17-26.
- [6] Boening DW. Ecological effects, transport, and fate of mercury: a general review. *Chemosphere*. 2000;40(12):1335-1351.
- [7] Ipolyi I, Massanisso P, Sposato S, Fodor P, Morabito R. Concentration levels of total and methylmercury in mussel samples collected along the coasts of Sardinia Island (Italy). *Anal Chim Acta*. 2004;505(1):145-151.
- [8] Sarica J, Amyot M, Hare L, et al. Mercury transfer from fish carcasses to scavengers in boreal lakes: the use of stable isotopes of mercury. *Environ Pollut*. 2005;134(1):13-22.
- [9] Storelli M, Storelli A, Giacomini-Stuffler R, Marcotrigiano G. Mercury speciation in the muscle of two commercially important fish, hake (*Merluccius merluccius*) and striped mullet (*Mullus barbatus*) from the Mediterranean sea: estimated weekly intake. *Food Chem*. 2005;89(2):295-300.

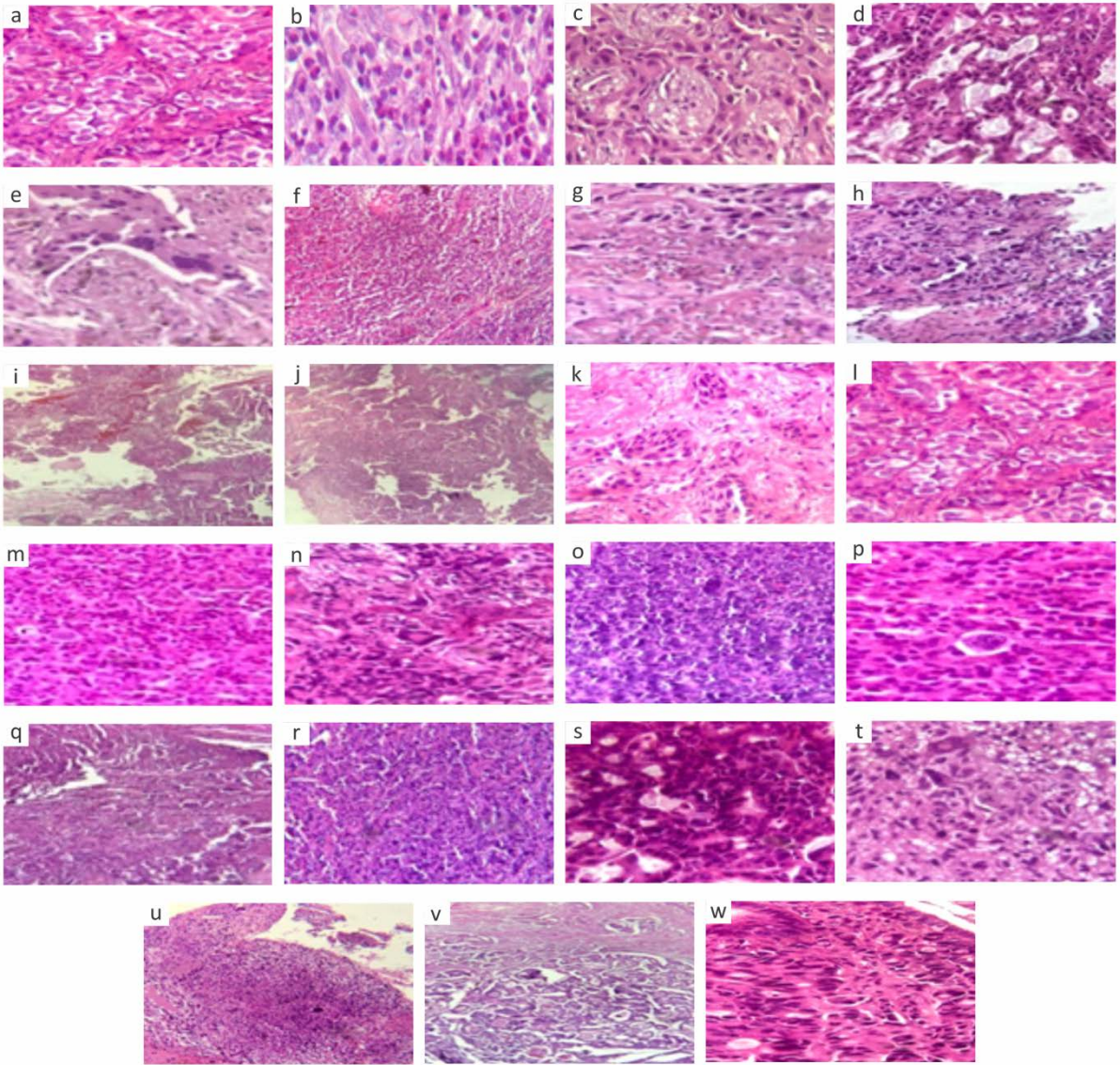
- [10] Manosathiyadevan M, Divya V. Histopathological alterations of the gills of the freshwater prawn, *Macrobrachium malcolmsonii* (Milne Edwards) exposed to Paravanar River pollutants. *Journal of Experimental Zoology, India*. 2012;15(1):233-239.
- [11] Hatfield DL, Tsuji PA, Carlson BA, Gladyshev VN. Selenium and selenocysteine: roles in cancer, health, and development. *Trends Biochem Sci*. 2014;39(3):112-120.
- [12] Gailer J. Arsenic–selenium and mercury–selenium bonds in biology. *Coord Chem Rev*. 2007;251(1-2):234-254.
- [13] Southworth GR, Peterson MJ, Ryon MG. Long-term increased bioaccumulation of mercury in largemouth bass follows reduction of waterborne selenium. *Chemosphere*. 2000;41(7):1101-1105.
- [14] Ikemoto T, Kunito T, Tanaka H, Baba N, Miyazaki N, Tanabe S. Detoxification mechanism of heavy metals in marine mammals and seabirds: interaction of selenium with mercury, silver, copper, zinc, and cadmium in liver. *Arch Environ Contam Toxicol*. 2004;47(3):402-413.
- [15] Bjerregaard P, Fjordside S, Hansen MG, Petrova MB. Dietary selenium reduces retention of methyl mercury in freshwater fish. *Environ Sci Technol*. 2011;45(22):9793-9798.
- [16] Kaiser H, Brill G, Cahill J, et al. Testing clove oil as an anaesthetic for long-distance transport of live fish: the case of the Lake Victoria cichlid *Haplochromis obliquidens*. *J Appl Ichthyol*. 2006;22(6):510-514.
- [17] Ganjali H. aG, M. Fixation in tissue processing. *International Journal of Farming and Allied Science*. 2013;2:686-689.
- [18] Gooley G, Gavine F, Dalton W, De Silva S, Samblebe M. Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. Final Report DRDC Project No. MAF001. *Marine and Freshwater Research Institute Snobs Creek*. 2000;
- [19] Hedayati A, Safahieh A, Savari A, Marammazi JG. Detection of range finding test of mercury chloride in yellowfin sea bream (*Acanthopagrus latus*). *Iranica Journal of Energy and Environment*. 2010;1(3):228-233.
- [20] Kehrig HA, Seixas TG, Malm O, Di Benedetto APM, Rezende CE. Mercury and selenium biomagnification in a Brazilian coastal food web using nitrogen stable isotope analysis: a case study in an area under the influence of the Paraiba do Sul River plume. *Mar Pollut Bull*. 2013;75(1-2):283-290.
- [21] Nath R, Banerjee V. Effects of various concentrations of lead nitrate on haematological parameters of an air breathing fish, *Clarias batrachus*. *Journal Fresh Water Biology*. 1995;7:267-268.
- [22] Gupta P, Srivastava N. Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish *Channa punctatus*(Bloch). *J Environ Biol*. 2006;27(2):211-215.
- [23] Penglase S, Hamre K, Ellingsen S. Selenium and mercury have a synergistic negative effect on fish reproduction. *Aquat Toxicol*. 2014;149:16-24.
- [24] Liu X-J, Luo Z, Li C-H, Xiong B-X, Zhao Y-H, Li X-D. Antioxidant responses, hepatic intermediary metabolism, histology and ultrastructure in *Synechogobius hasta* exposed to waterborne cadmium. *Ecotoxicol Environ Saf*. 2011;74(5):1156-1163.
- [25] Ribeiro CO, Fanta E, Turcatti N, Cardoso R, Carvalho C. Lethal effects of inorganic mercury on cells and tissues of *Trichomycterus brasiliensis* (Pisces; Siluroidei). *Biocell: official journal of the Sociedades Latinoamericanas de Microscopia Electronica et al*. 1996;20(3):171-178.
- [26] Ralston N. Ralston., LJ; Lloyd Blackwell, J., III; Raymond, LJ Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology*. 2008;29:802-804.

- [27] Azad AM, Frantzen S, Bank MS, et al. Effects of geography and species variation on selenium and mercury molar ratios in Northeast Atlantic marine fish communities. *Sci Total Environ*. 2019;652:1482-1496.
- [28] Olsvik PA, Azad AM, Yadetie F. Bioaccumulation of mercury and transcriptional responses in tusk (*Brosme brosme*), a deep-water fish from a Norwegian fjord. *Chemosphere*. 2021 Sep 1;279:130588.
- [29] de Oliveira Ribeiro CA, Belger L, Pelletier E, Rouleau C. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). *Environmental research*. 2002 Nov 1;90(3):217-25.
- [30] Zulkipli SZ, Liew HJ, Ando M, Lim LS, Wang M, Sung YY, Mok WJ. A review of mercury pathological effects on organs specific of fishes. *Environmental Pollutants and Bioavailability*. 2021 Jan 1;33(1):76-87.
- [31] Liew HJ, Sinha AK, Nawata CM, Blust R, Wood CM, De Boeck G. Differential responses in ammonia excretion, sodium fluxes and gill permeability explain different sensitivities to acute high environmental ammonia in three freshwater teleosts. *Aquatic toxicology*. 2013 Jan 15;126:63-76.
- [32] Monteiro SM, Oliveira E, Fontaínhas-Fernandes A, Sousa M. Effects of sublethal and lethal copper concentrations on the gill epithelium ultrastructure of Nile tilapia, *Oreochromis niloticus*. *Zool. Stud*. 2012 Dec 1;51(7):977-87.
- [33] 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 Jan;85(1):97-177.
- [34] Salamat N, Zarie M. Using of fish pathological alterations to assess aquatic pollution: a review. *World Journal of Fish and Marine Sciences*. 2012;4(3):223-31.
- [35] Moyson S, Liew HJ, Diricx M, Sinha AK, Blust R, De Boeck G. The combined effect of hypoxia and nutritional status on metabolic and ionoregulatory responses of common carp (*Cyprinus carpio*) Part A Molecular & integrative physiology.
- [36] Yancheva VS, Georgieva ES, Velcheva IG, Iliev IN, Vasileva TA, Petrova ST, Stoyanova SG. Biomarkers in European perch (*Perca fluviatilis*) liver from a metal-contaminated dam lake. *Biologia*. 2014 Nov;69(11):1615-24.
- [37] Liew HJ, Sinha AK, Nawata CM, Blust R, Wood CM, De Boeck G. Differential responses in ammonia excretion, sodium fluxes and gill permeability explain different sensitivities to acute high environmental ammonia in three freshwater teleosts. *Aquatic Toxicology*. 2013 Jan 15;126:63-76.
- [38] Moyson S, Liew HJ, Diricx M, Sinha AK, Blust R, De Boeck G. The combined effect of hypoxia and nutritional status on metabolic and ionoregulatory responses of common carp (*Cyprinus carpio*) Part A Molecular & integrative physiology.
- [39] Moyson S, Liew HJ, Fazio A, Van Dooren N, Delcroix A, Faggio C, Blust R, De Boeck G. Kidney activity increases in copper exposed goldfish (*Carassius auratus auratus*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2016 Dec 1;190:32-7.
- [40] Gehringer DB, Finkelstein ME, Coale KH, Stephenson M, Geller JB. Assessing mercury exposure and biomarkers in largemouth bass (*Micropterus salmoides*) from a contaminated river system in California. *Archives of environmental contamination and toxicology*. 2013 Apr;64(3):484-93.
- [41] Pereira P, Korbas M, Pereira V, Cappello T, Maisano M, Canário J, Almeida A, Pacheco M.
- A multidimensional concept for mercury neuronal and sensory toxicity in fish-From toxicokinetics and biochemistry to morphometry and behavior. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 2019 Dec 1;1863(12):129298.

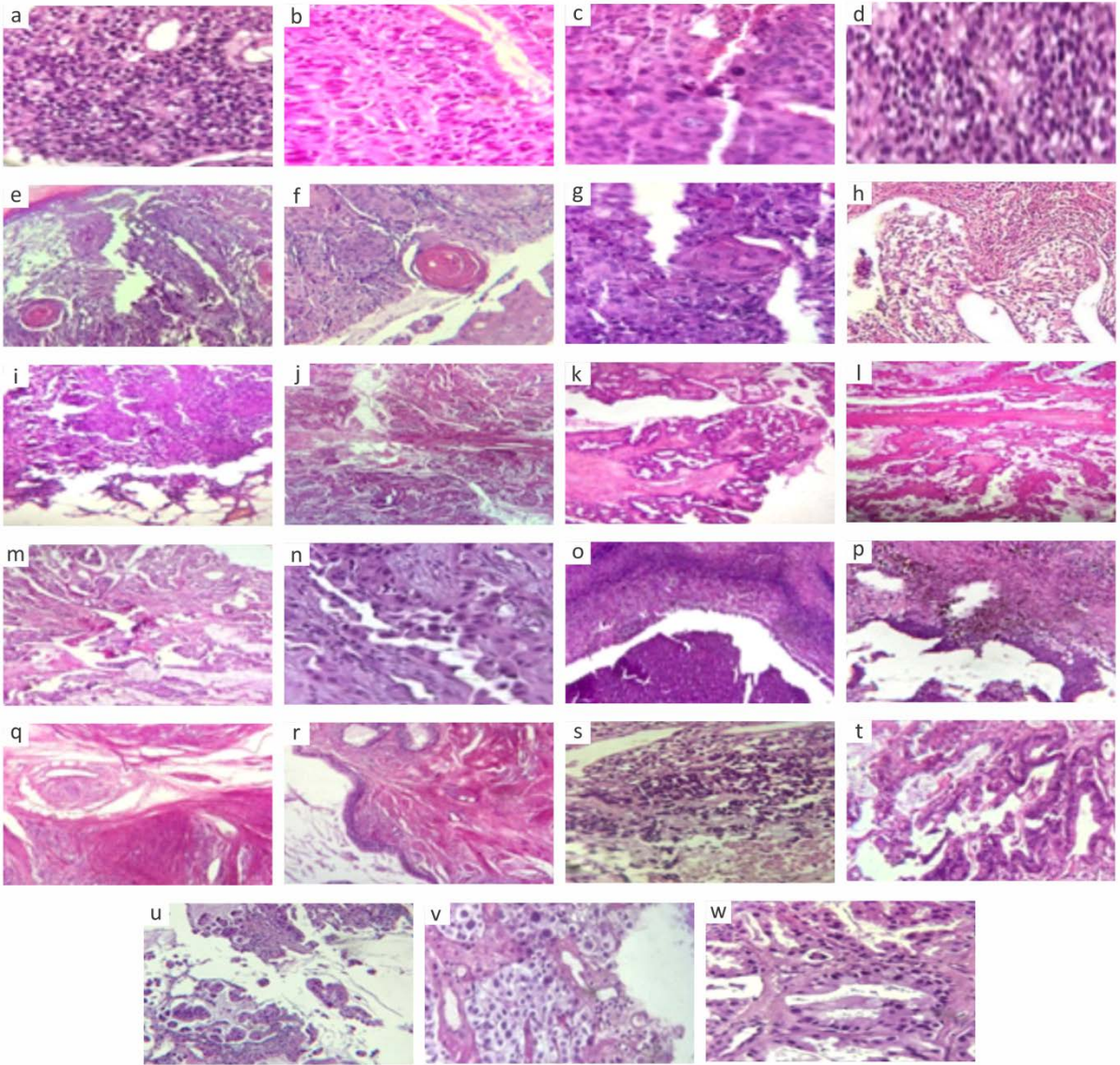
- [42] Berntssen MH, Aatland A, Handy RD. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquatic toxicology. 2003 Oct 8;65(1):55-72.
- [43] Barone G, Storelli A, Meleleo D, Dambrosio A, Garofalo R, Busco A, Storelli MM. Levels of Mercury, Methylmercury and Selenium in Fish: Insights into Children Food Safety. Toxics. 2021 Feb;9(2):39.
- [44] Grgec AS, Kljaković-Gašpić Z, Orct T, Tičina V, Sekovanić A, Jurasović J, Piasek M. Mercury and selenium in fish from the eastern part of the Adriatic Sea: A risk-benefit assessment in vulnerable population groups. Chemosphere. 2020 Dec 1;261:127742.
- [45] Mirlean N, Ferraz AH, Seus-Arrache ER, Andrade CF, Costa LP, Johannesson KH. Mercury and selenium in the Brazilian subtropical marine products: Food composition and safety. Journal of Food Composition and Analysis. 2019 Dec 1;84:103310.
- [46] Plessl C, Gilbert BM, Sigmund MF, Theiner S, Avenant-Oldewage A, Keppler BK, Jirsa F. Mercury, silver, selenium and other trace elements in three cyprinid fish species from the Vaal Dam, South Africa, including implications for fish consumers. Science of the Total Environment. 2019 Apr 1;659:1158-67.
- [47] Sofoulaki K, Kalantzi I, Machias A, Pergantis SA, Tsapakis M. Metals in sardine and anchovy from Greek coastal areas: Public health risk and nutritional benefits assessment. Food and chemical toxicology. 2019 Jan 1;123:113-24.
- [48] Cardoso C, Bernardo I, Bandarra NM, Martins LL, Afonso C. Portuguese preschool children: Benefit (EPA+ DHA and Se) and risk (MeHg) assessment through the consumption of selected fish species. Food and Chemical Toxicology. 2018 May 1;115:306-14.
- [49] Karimi R, Frisk M, Fisher NS. Contrasting food web factor and body size relationships with Hg and Se concentrations in marine biota. PloS one. 2013 Sep 3;8(9):e74695.
- [50] Olmedo P, Hernández AF, Pla A, Femia P, Navas-Acien A, Gil F. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury-selenium balance. Food and Chemical Toxicology. 2013 Dec 1;62:299-307.



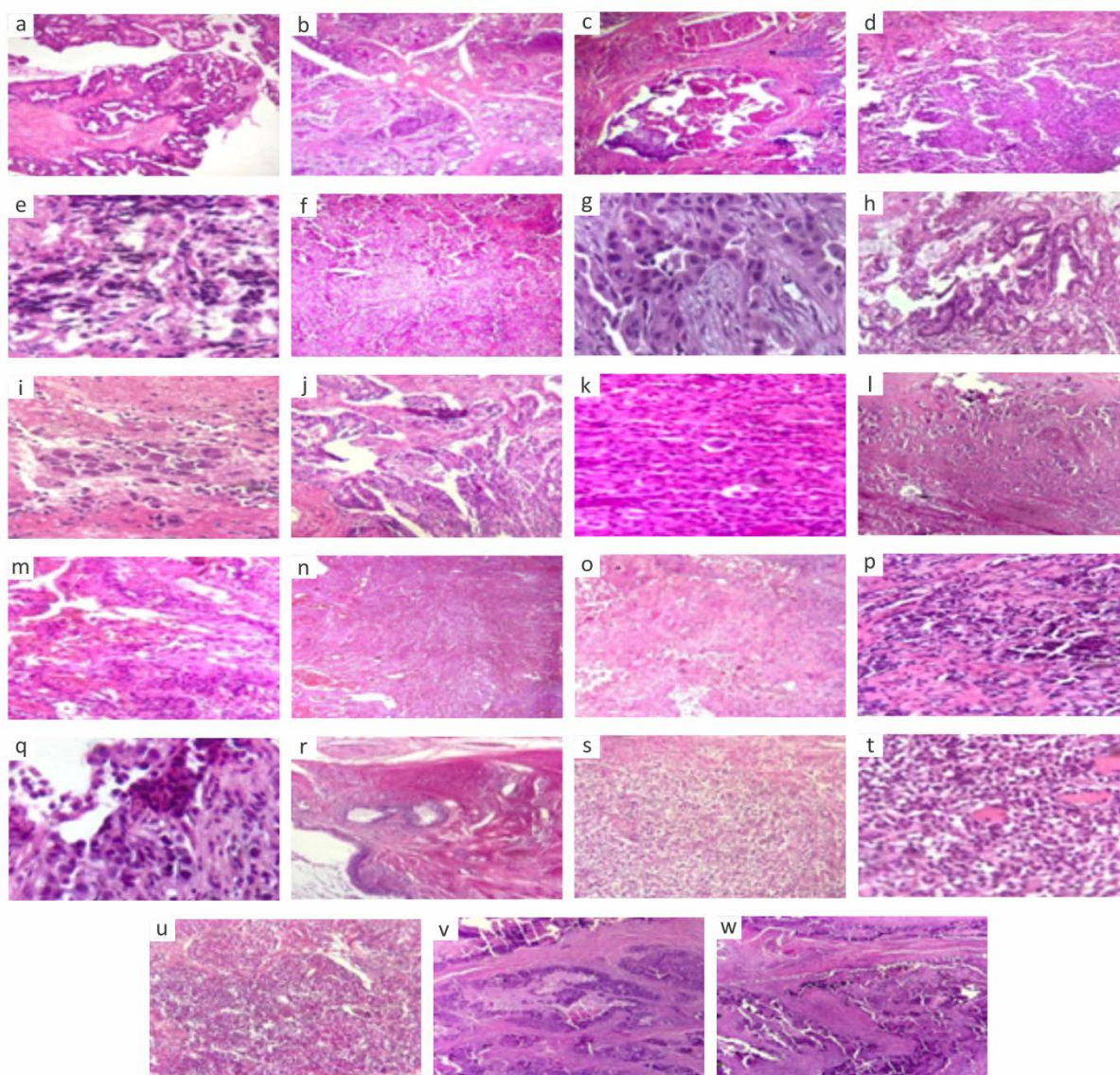
(1a)



(1b)



(1c)



(1d)

Fig 1. Effect of Mercury, Selenium and mixture of both metals (ug/g) on (1a) brain tissue, (1b) kidney tissue, (1c) gills tissue and (1d) kidney tissue *L. rohita* fingerling at different time intervals. **a.** control at 24 h. **b.** 0.125 at 24 h. **c.** 0.125 at 48 h. **d.** 0.125 at 72 h. **e.** 0.125 at 92 h. **f.** 0.250 at 24 h. **g.** 0.250 at 48 h. **h.** 0.250 at 72 h. **i.** 0.250 at 92 h. **j.** 0.500 at 24 h. **k.** 6 at 24 h. **l.** 6 at 48 h. **m.** 6 at 72 h. **n.** 6 at 92 h. **o.** 0.125+6 at 24 h. **p.** 0.125+6 at 48 h. **q.** 0.125+6 at 72 h. **r.** 0.125+6 at 92 h. **s.** 0.250+6 at 24 h. **t.** 0.250+6 at 48 h. **u.** 0.250+6 at 72 h. **v.** 0.250+6 at 92 h. **w.** 0.500+6 at 24 h.

Table 1. Survival rate and lethal concentration of *Labeo rohita* fingerling in presence of Hg and Se.

Treatments (ug/g)	24 h	48 h	72 h	92 h	LC 50	LC 90
	Survival rate (%)				(ug/g)	
0	100	100	100	100	0.374	0.704
0.125	100	75	66.6	50	(0.074)	(0.087)
0.250	80	75	66.6	50		
0.500	80	0	0	0		
6	100	100	100	100	0.491	0.798
0.125 + 6	100	100	80	75	(0.178)	(0.187)
0.250 + 6	100	80	75	66.6		
0.500 + 6	60	33.3	0	0		