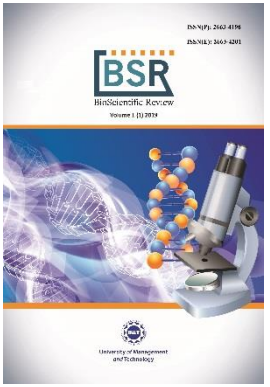

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Dietary Selenium Reduces the Toxic Effect of Mercury on Different Organs (Brain, Gills, Kidney and Liver) of Rohu Fish (*Labeo rohita*)

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Abstract

Due to improbably high partiality of antioxidant selenium to mercury, selenium isolates mercury and reduces its biological handiness to organisms. The current study investigates the effect of mercury (Hg) and selenium (Se) on the fingerling of *Labeo rohita*. Various sub-lethal concentrations of Hg including 0.125µg/g, 0.250µg/g, and 0.500µg/g were used and incorporated in fish diet with a commercial diet plan (fish meal 40%, soya bean 33%, 3% of vitamins premix, mineral premix and oil each and rice polish 18%). To understand how Se reduces the toxic effect of Hg, fingerlings were exposed to 6ug/g Se singly and combined with doses of Hg. The effect of these heavy metals was observed after 24, 48, 72, and 96 hours on different organs (brain, gills, kidney and liver) of *L. rohita*. The organs of the exposed rohu fish showed significant changes in their microscopic anatomy in comparison to control. Prominent changes were observed in Hg treatments and these included 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. Moreover, cysts and hamorrhage developed in the organs of the fish. The length of exposure seemingly has a profound impact on organs because the increase in length of exposure enhanced the severity of histopathological damages. However, combined doses of metals caused reduction in the toxicity of mercury, resulting in decreased damage in the shrinkage of capillary and dilation of the hollow lumen. During the histological study, vacuolation, hydropic swelling, and hyaline degeneration of the hollow epithelium were also reduced. For Hg, lethal concentration (LC_{50}) was 0.374ug/g. However, when Hg and Se were combined, LC_{50} dropped to 0.491ug/g. The results of this study suggest that exposure to Se helps to scale back the impact of Hg on the mortality and different organs of *L. rohita* fingerling.

1. Introduction

Fish comprises almost sixteen percent of the universal population's intake of animal protein and six percent of all protein used. Fish comprise a healthy diet because it contains a high level of omega-3 fatty acids and macromolecules that the body needs to remain energetic and functioning. Fish culture provides an ample opportunity to produce quality food for utilization by masses which is free from contagion [1]. Due to the violation of the existing set of rules and regulations, there is a continuous rise in the concentration of contagions in freshwaters because of household waste, bug sprays and herbicides, food waste, industrial toxins, animal waste, unstable natural mixes, heavy metals, concoction waste and others [2]. Among natural contagions, heavy metals are a matter of grave concern due to their potentially dangerous impact and their ability to bio-aggregate in aquatic environments [3]. Pollution inside water ambiance with heavy metals has become a universal issue for the last few years due to its deadly impact on living organisms [4].

Due to the possible hazards mercury (Hg) poses to higher biological processes, it has attracted a great deal of attention. Methyl Hg is predominant in fish organs and is biologically available in greater concentrations than inorganic Hg. The high degree of Hg in aquatic animals, primarily fish, directly impact human health and environment [5]. Hg reflection in fish is determined by fish age [6]. Water contaminated with Hg accumulates in the tissues of aquatic animals and is then transferred via the food web to other living organisms on the land as well [7, 8]. In fish, as higher vertebrates, the excretory organ plays an important role in balancing water and other solutions, ultimately maintaining the stability of the internal environment. The excretory organ secretes nitrogen containing waste products from the digestive system, for instance, carbamide and creatinine. Following the exposure of fish to compounds such as pesticides, histological changes are visible in its tissues and blood [9]. Fish are very sensitive to their external environment. Hence, sensible measures should be taken for the effective management of pollutants in their external environment. Moreover, histopathological studies can be used to discover the effects of maritime pollution, particularly considerable metals [10]. The toxic nature of total mercury (Hg) and methyl mercury (MeHg) was observed via histopathological studies of living beings to identify the degree of cell damage [9].

Se is a basic element for living organisms and is known to overcome the toxic effects of mercury and other heavy metals such as arsenic, cadmium, and lead [11]. It is incorporated into the twenty initial amino corrosive L-selenocysteine, that are a constituent of selenoproteins [12]. It mitigates the gathering and harmful effects of Hg in oceanic living beings in a very intricate manner. In some well-evolved marine creatures, demethylation of the alkyl group of Hg within the liver prompts the arrangement of insoluble mercury selenium, giving ascend to a 1:1 molar proportion Mercury: Selenium [13]. It has been established that Se detoxifies MeHg by shaping edifices containing the two elements present in equimolar proportion. It is well documented that the metallic element mercury selenide (HgSe) is found within the liver of well-evolved marine 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 current study is to determine Hg toxicity and its effect on *Labeo rohita* species as well as the combined effect of Hg and Se on this specie. The current study helps to understand how the *L. rohita* species respond to different levels of heavy metal (Hg) contamination/presence.

2. Methodology

2.1. Sample Collection and Maintenance

The fingerlings of *Labeo rohita* were obtained from a fish farm settled at a distance of 40 kilometres from the city (latitude 31˚58 N, longitude 74˚13'E), on the north towards the GT road, Manawan. The collected fish were raised in a polyculture of major carps following well-established procedures and practices. They 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 performed to keep the fish safe from any harmful infection caused by the bacteria. 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 glass aquaria filled with 15 liters of water, about ¾ of the capacity of each aquarium. The water of aquaria was replaced with fresh and clean water once a day. Fish was weighed in grams and measured in centimetres. Afterwards, they were randomly distributed in each aquarium at the rate of 25 fishes per aquarium. Fish was then exposed to three sub-lethal concentrations of mercuric chloride, that is, 0.125µg/g, 0.250µg/g, and 0.500µg/g, respectively with a commercial diet plan for 24, 48, 72, and 96 hours. Then, commercial diet was mixed with both $HgCl_2$ and Se, that is, (0.125+6 µg/g, 0.250+6 µg/g and 0.500+6 µg/g). Test concentrations were restored after every 24 hours by cleaning and replacement of the de-chlorinated water. The above protocol was repeated after every 24 hours. The fish in 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 specimens were anesthetized immediately to reduce stress. Clove oil (National Chemicals, Pakistan) was used to induce anaesthesia [16]. The fish was put in the solution for 3 to 5 minutes. Nonetheless, the time period of anaesthesia can be altered 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 was removed and weighed on an electric balance. The examination of the microanatomy of the tissue was started with the surgery of animal brain, kidney, gills and liver. Tissues were preserved in one hundred millilitres of 100% unbiased buffered formol, which comprises four percent aldehyde in phosphate-buffered brackish mixture until processed for histological examination. Embedding was

performed to the pieces of fish sample approximately 1 cm^3 before loading the cassettes for histological processing. The sample-loaded cassettes were passed for the dehydration of the tissues in the following pattern of alcohol (30% → 50% → 70% → 90%). The samples were cleared after two applications of xylene, followed by the application of molten wax (58˚C) for impregnation. The resultant tissues were then placed in a mould containing additional liquefied wax (embedded) and allowed to cool down until the wax hardened. Tissues were then removed from the cassettes and moulded 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 a thickness of 3-4 micrometers by a rotating mechanized microtome (Shandon, Thermo, CD-2235) fixed with a 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 them. They were then placed under warm water, allowing the wax to melt and fix tissue on the slide.

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

2.5. Estimation of Lethal Concentration (LC_{50})

Keeping in view the scope of the study, it was necessary to estimate LC_{50} for the selection of the different dosages of Hg before taking further initiatives. LC_{50} test (24, 48, 72, and 96 hours) was conducted based on fish mortalities (when they were exposed to a known toxicant concentration sequence) and its comparison was made 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 $HgCl_2$. An appropriate quantity of $HgCl_2$ was added to the required lethal concentration based on the understanding that there would be no mortality up to 0.125 µg/g. However, almost all the fish died within 96 hours at 0.500 µg/g exposure to Hg [19].

2.6. Statistical Analysis

Statistical analysis was performed using SPSS (Ver.19). LC_{50} was detected by probit analysis using Statplus 5.

3. Results

Percentage survival rate and lethal concentration were assessed. No mortality was observed in control group; however, due to the increase in the amount of Hg survival rate decreased from 96 h to 24 h. When Se was added to all Hg concentrations, survival rate increased from 24 h to 96 h. LC_{50} and LC_{90} were also high in Hg groups. However, when Se was added lethal concentration decreased. This shows that the addition of Se reduces the effect of Hg in feed (Table 1).

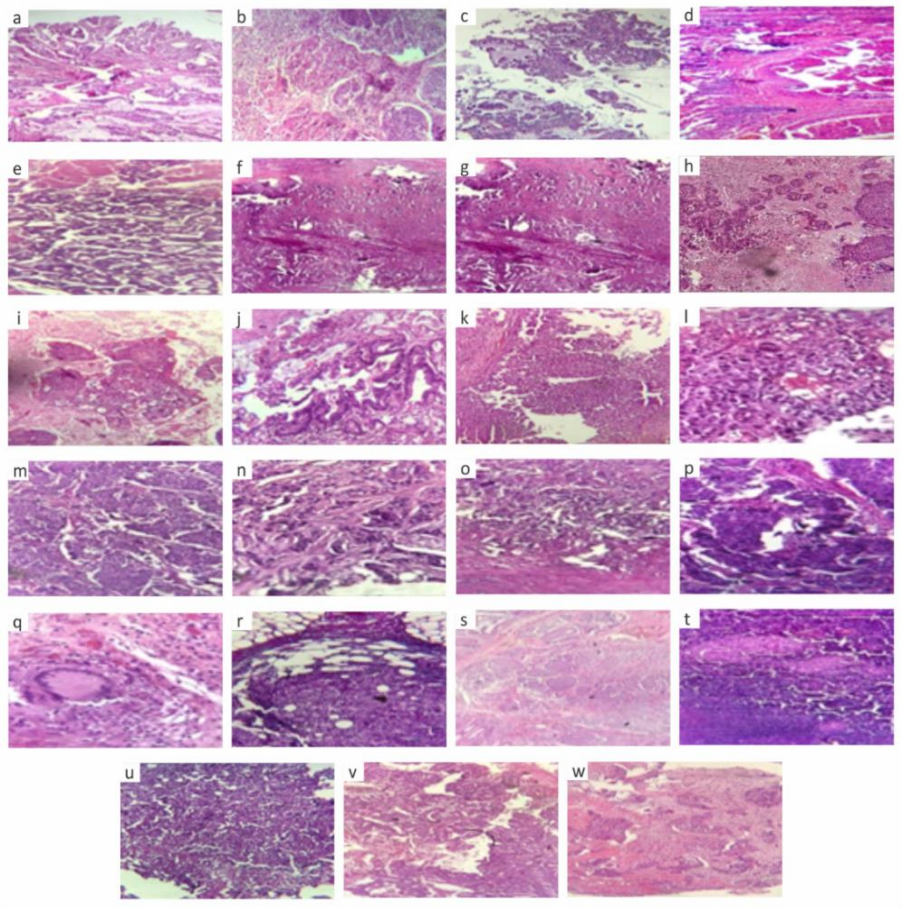
Table 1. Survival rate and effect of lethal concentrations on *Labeo rohita* fingerling in the presence of Hg and Se

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		

In the brain, no change in the cells was observed in all time durations in control. Whereas, in the experimental group, after 24 h exposure, granular cell loss, neutrophil cells loss, and aggregation of purkinje cells was observed.. In 48 h, the presence of neural cells, pyramidal cells, and nissl substances were observed. In 72 h, delicate chronic changes were observed in neural cells. Gangrene of neurons, living hydrops, and congestion of neural cells were also noticed. In 92 h, the degeneration of neural cells was initiated and protoplasm vacuolation occurred. Severity was observed in brains cells when Hg concentration increased. When Se was added to Hg, improvement was observed in all brain cells (Fig 1a).

In the kidney, no significant change was observed in the tissue after 24, 48, 72, and 92 h with any treatment in the control group. In experimental group, slight inflation was observed in the area between the capillary vessel and capsule glomeruli after 24 h and shrinkage was observed after 48 h exposure. After 72 h, numerous degenerative changes were observed in some areas, that is, 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 occurred. When both metals were exposed to the fish, some minor changes were observed. Although improvement was observed in low Hg concentrations, yet in high concentrations Hg caused extreme shrinkage of capillary, and capillary and membrane were conjointly exaggerated. Gangrene and pycnosis were ascertained in animal tissue lining and the epithelium of kidney tubules was 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, change in gill histology was not very conspicuous. In 48 h, *Eubacteria cholerae* showed deformities within the gills. Degeneration and sphacelus of the gill filament were observed. In 72 h, cellular damage was observed within the gills in the form of animal tissue propagation. In 92 h, gill abnormalities increased and these incorporated dysplasia of animal tissue

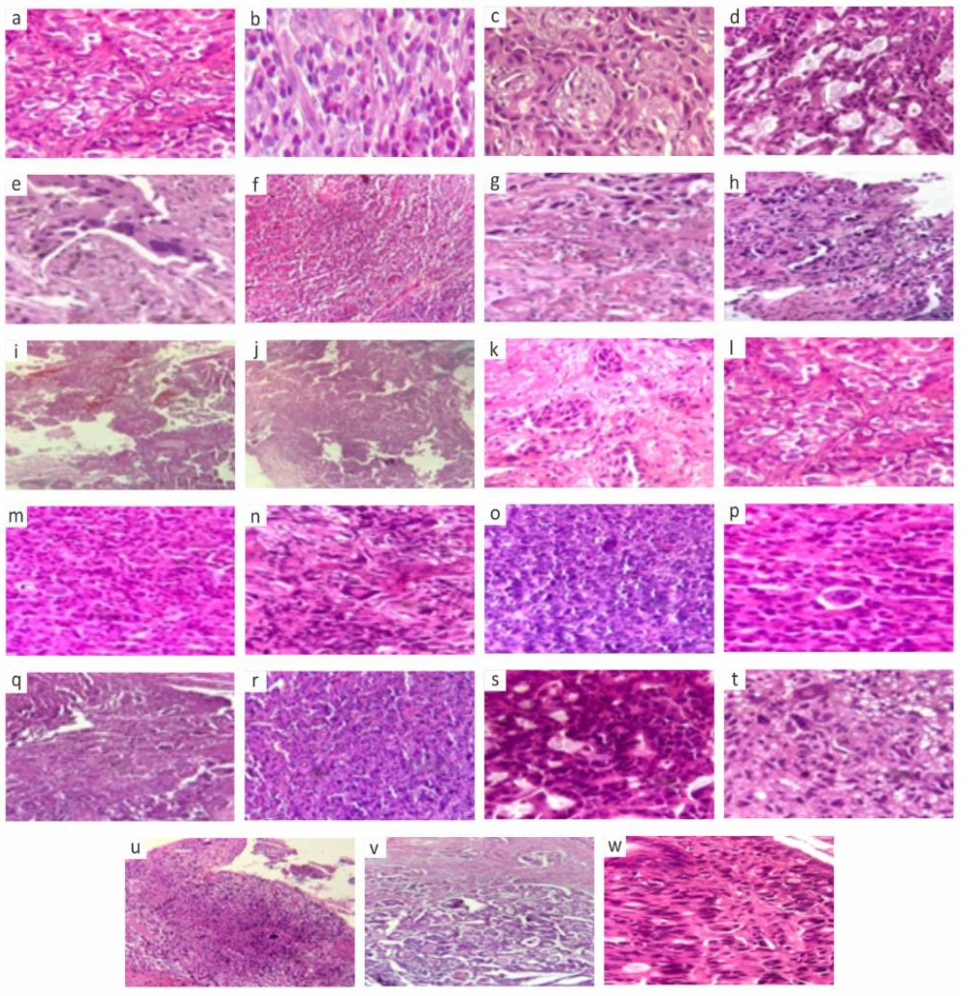


(1a)

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. Increased effects on these cells were observed with respect to the increase in Hg concentrations. When Hg was mixed with Se, cell growth was observed as normal in low Hg concentrations. On the contrary, high Hgconcentrations prevented cell damage to a much lesser extent (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, some minor changes were revealed in the experimental group, such as the degradation of viscous parenchyma cells and the fact that viscous cells did not create distinct lobules. In 48 h, there was structural harm done to the central vein, the occurrence of busted and irregular viscous plate, granulation in living substance, necrosis, vacuolization, and the disruption of hepatocytes. In 72 h,

deterioration, mortification of hepatocytes, vacuole degeneration in hepatocytes, occlusion formation in central veins, and dilation occurred. In 92 h, tissue displayed several alterations such as mild necrosis, pyknosis, cytoplasmic degeneration, and the infiltration of leukocytes. In high concentrations of Hg, extreme alterations were observed in nuclear structures causing nuclear degradation. The structure of the liver was completely denatured and 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).



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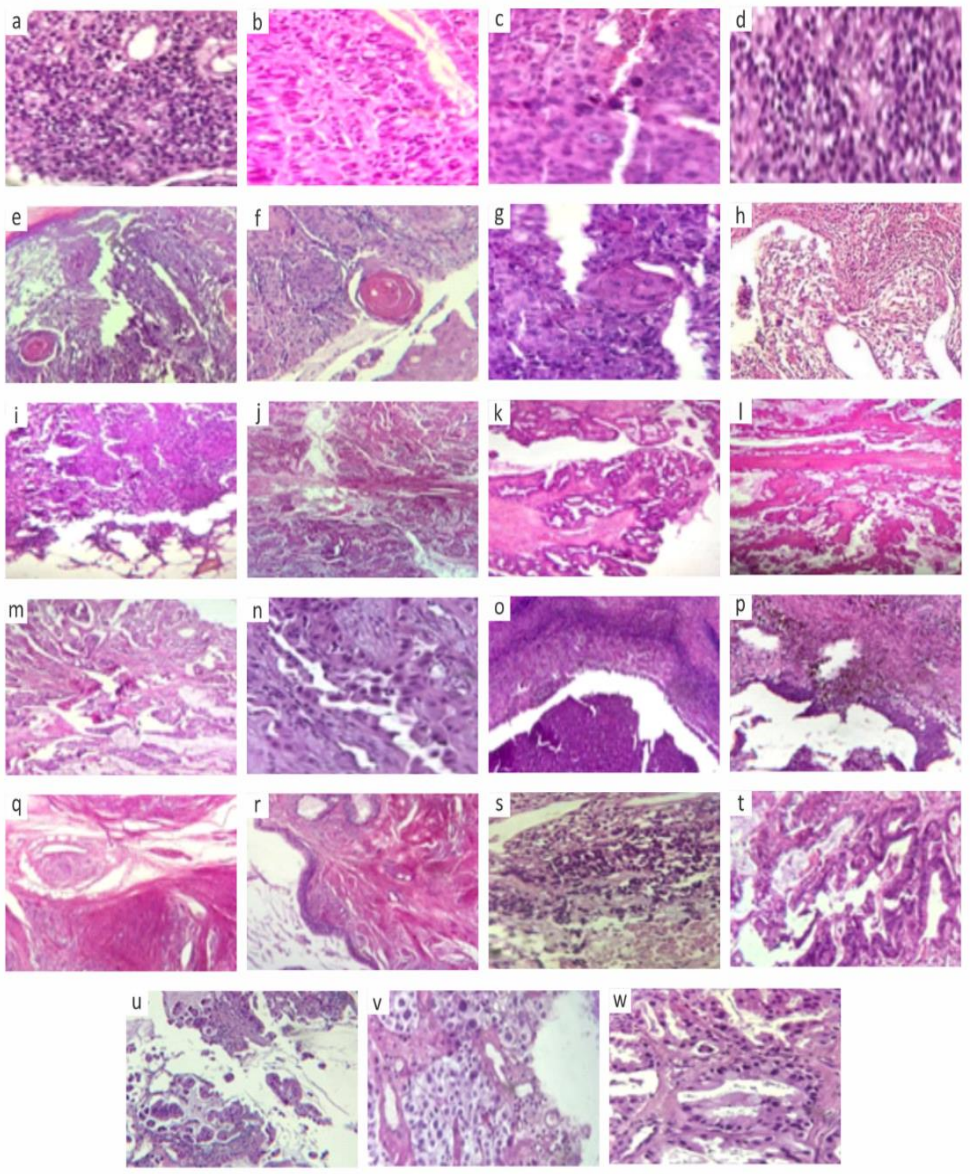

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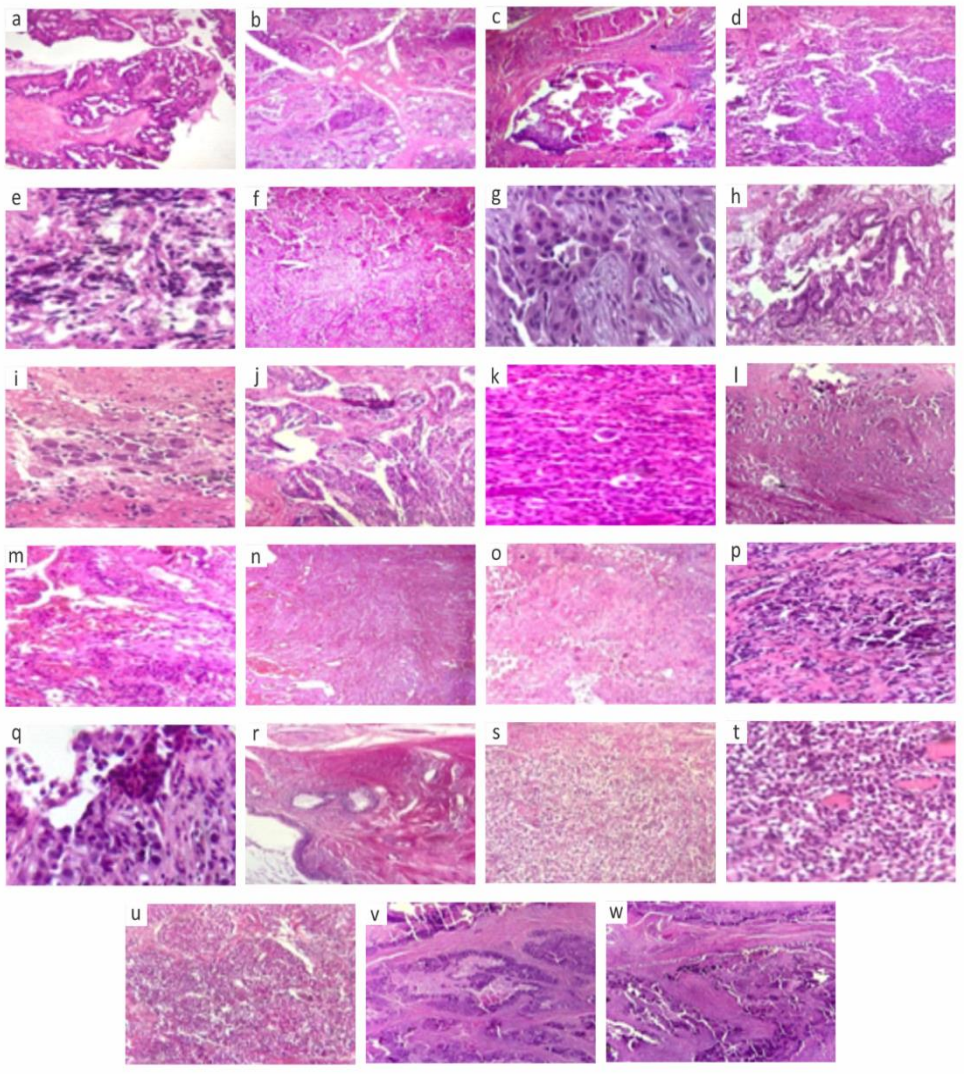

(1d)

Figure 1. Effect of Mercuary, Selenium and the 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.

4. Discussion

The current study is very useful in understanding how to reduce the level of Hg in the organs of fish through the use of Se. During the investigation of the protective effect of Se against Hg in *Labeo rohita*, the groups treated with a combination of Hg and Se showed minor changes in organs as compared to those groups which were solely exposed to Hg.

It has been conjointly ascertained that if the concentration of Hg is very high in the tissue, it should cause severe structural harm [20]. The current study revealed that the presence of Hg changes the structure of the brain, gills, kidney, and liver, even when it is found in low concentrations. Denaturation occurs when it is found in a high quantity. Similar findings with dilation, oedema, and enlarged nuclei of nephritic tubules were additionally reported in the excretory organ of *Mugil auratus* [21]. A study [22] also reported that the toxicity of $HgCl_2$ in fish excretory organ varies from slight disruption of cannular cells to swelling in cells, with different doses in major carps. The authors also reported a similar effect of $HgCl_2$ on the liver of *Cirrhinus mrigala.* Another study [23] reported the effect of Hg on *Danio rerio* and observed a negative impact on growth and survival. All the fishes died within 96 hours after a high exposure to Hg [19]. LC_{50} determined that the population of fish 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. The authors in [24] 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 have shown considerable differences among fish species. The authors in [25] reported that when total Hg and MeHg levels increase, 40% accumulation of Hg increases in the liver of *Brosme brosme* as compared to normal fish. The authors in [26] reported that when fish are exposed to Hg for 96 h, it causes tissue anomalies including blood congestion in gills, swelling of liver, lesion in kidney, vacuolation and exfolitation. Many studies have been conducted to estimate the effect of Hg on different organs of fish [27], including gills [28-30], liver [31-34], kidney [35-37], and brain [38-40].

The authors in [41] reported that the kidney of *Trichomycterus brasiliensis* treated with Se remained in an inappropriate shape, although there was an increase in the number of tubule cells which ultimately reshaped, normally. Se reduced the negative impact on *Danio rerio* in the presence of Hg [23]. The authors in [42] reported that Se improves bioavailability and intercepts the selenium-dependent enzymes. This is why Se can be used to minimize the toxicity of Hg. The authors in [43] also reported that the high concentration of Hg in the weekly food intake exceeds in *Hippoglossus hippoglossus* and causes severe damage but Se reduces the toxic effect of MeHg. Our study indicated that Se, when mixed with Hg, reduces LC_{50} and LC_{90} 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. The authors in [44] reported that the ratio of Hg and Se in the commercial diet plan of fish is crucial to ensure the safety of the ecosystem. Similar studies were conducted to overcome Hg toxicity with the help of Se [45-50].

5. Conclusion

In view of the findings, it was concluded that the presence of mercury at sub-lethal concentrations causes considerable histological damage in different organs in *L. rohita*. It caused severe injury to the cellular structural integrity in the organs of *L. rohita*. However, once the fish were exposed to Se, the tissue recovered and the damage to organ cells decreased, gradually. Our findings revealed that Se plays an important role in reducing toxic metals 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 Se in the fisheries sector. Current histopathological investigations suggest that Se can help to reduce the toxic level of Hg and other heavy metals. Nonetheless, further studies need to be undertaken to clarify exactly the toxicity of Hg to fish and the counteracting effects of Se in neutralizing the toxicity of heavy metals.

Conflict of Interest

The authors declare no conflict of interest.

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