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Selenium-Supplemented Diet Influences Histological Features of Liver and Kidney in Tilapia *(Oreochromis niloticus)*

Sonia Iqbal [1†], Usman Atique [1,2, †*], Muhammad Sharif Mughal [3], Muhammad Younus [4], Muhammad Kamran Rafique [4], Muhammad Sultan Haider [5], Hafiza Sundas Iqbal[6], Shahid Sherzada[1] and Tanveer Ali Khan[7]

[1]*Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan;* [2]*Department of Bioscience and Biotechnology, Chungnam National University, South Korea;*[3] *Department of Zoology, Government College University (GCU), Lahore, Pakistan;* [4]*College of Veterinary and Animal Sciences, Jhang, University of Veterinary and Animal Sciences, Lahore, Pakistan;* [5]*Department of Zoology, University of Lahore,Pakistan;* [6] *Department of Zoology, Lahore College for Women University, Lahore, Pakistan;* [7]*Department of Zoology, University of Punjab, Lahore, Pakistan*

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† Indicates equal author contributions.

Abstract

Selenium is considered as an eco-toxicological paradox owing to its antioxidant and toxic properties. This study aimed at exploring the potential impacts of selenium supplemented in feed on the histology of vital organs of tilapia (*Oreochromis niloticus*). During this study, neither behavioural abnormalities nor any fish mortalities recorded in fish subjected to different selenium levels during this trial. The results revealed significant histological changes in liver and kidney tissues, mainly linked in a dose-dependent manner. The resultant histological changes exhibited mild or no alterations in fish that consumed the diet having 2 mg Se/kg. However, the intensity of histopathological alterations manifested more in the liver and kidney tissues of fish, having fed on a higher dose of selenium (8 mg Se/kg) as compared to control, 2, and 4 mg Se/kg in the feed. In the case of the liver, there were severe cytoplasmic vacuolations of hepatocytes and central vein dilation, erythrocytes haemolyzed, prominent vascular hypertrophy, and fibrosis of perivascular parts conspicuously noticeable leading to loss of characteristic architecture of hepatic tissues. However, in kidney tissues, renal tubules were seen atrophied and degenerative vacuolar changes in the renal tubular epithelial cells, pyknotic nuclei, as well as a thin layer of fibrous connective tissue (FCT), observed which were swiftly proliferating in peritubular parts of the medulla. In conclusion, selenium incorporated in higher concentrations damaged the vital organs in a dose-dependent manner resulting in histological alterations and proved to be harmful to the fish. However, lower level (2 mg/kg) did not influence or have the least affected histological changes in vital organs.

Keywords: Selenium; Liver; Kidney; Histological changes; Tilapia; Hepatocytes

1. Introduction

Tilapia *(Oreochromis niloticus)* has emerged as a model organism gaining attention from researchers for a variety of biological investigations such as immunology, growth, and histological inferences, i.e. the microscopic examination of different vital organs/tissue (Galman and Avtalion, 1989; Coward and Bromage, 1998; Benli and Özkul, 2010; Iqbal *et al.,* 2017; Guerreiro *et al.,* 2018). Exploring the chronic and acute toxic effects of varying selenium levels in the aquatic ecosystem and organisms has recently gained more attention (Lemly, 2004; Han *et al.,* 2011). Histological studies of kidney, liver, and muscles have been performed to investigate the influence of different micro-additives in the tilapia diet (Ramesh *et al.,* 2014; Obirikorang *et al.,* 2018; Kokou *et al.,* 2019; Ismail *et al.,* 2019) when used for beneficial purposes like growth enhancer.

Selenium (Se) is a non-metallic element and occurs in nature in different combinations such as selenite, selenate, and selenomethionine (Takayanagi 2001; Mechora *et al.,* 2013; Iqbal *et al.,* 2017). The primary natural sources of this element for fish are the water bodies and alluvial sediments (Patterson *et al.,* 2010; Younus *et al.,* 2015). It is a well-proven antioxidant as well as an indispensable part of numerous biological molecules like DNA and proteins (Han *et al.,* 2011; Moon *et al.,* 2020; *Bae et al.,* 2020). Therefore, it is recognized for its critical physiological role and is a prerequisite for the standard functioning of various enzymes and the immune system (Patterson *et al.,* 2010; Ramesh *et al.,* 2014; Sarkar *et al.,* 2015; Iqbal *et al.,* 2020). Selenite and selenate are prevailing compounds of selenium existing in the aquatic environment due to higher water solubility. Selenium concentrations (0.1 - 0.5 mg/kg dry weight of feed) recommended for normal physiological activities of fish (Hilton and Hodson, 1983; Gatlin and Wilson, 1984; Han *et al.,* 2011). However, excessive accumulation of

* Corresponding author e-mail: physioatique@gmail.com.

selenium in aquatic organisms caused rare haematological, histopathological, teratogenic, and reproductive disorders (Lemly, 2002) due to its persistent nature, bio-accumulative properties, and toxicity.

Recent studies confirming higher doses of selenium (8 mg/kg) resulted in inducing histopathological modifications and damaged the characteristic structure of liver cells. Studies in tilapia highlighted haemosiderin pigments, haemolysis of cells, and fatty degenerations, whereas lower concentrations (e.g., 2 mg/kg) induced necrotic changes (Morrison and Wright, 1999; Lemly, 2002) around blood vessels and caused haemolysis of cells. Similarly, lower selenium concentrations enhanced glutamate oxaloacetate transaminase (GOT) level, glutamate pyruvate transaminase (GPT) secretions, and lactate dehydrogenase concentration (LDH) in the target fishes (El-Hammady *et al.,* 2007; Wang *et al.,* 2018).

In most of the studies, using liver tissues as primary reference organs while assessing the selenium effects was due to the reported preferential selenium accumulation in the case of examined exposed fish specimens (Hodson *et al.,* 1980). Selenium compounds were also capable of protecting the internal organs from the toxicity of heavy metals such as cadmium and mercury and palm oil (Watanabe *et al.,* 1997; Zulfahmi *et al.,* 2018). Selenium is reported as an integral component of the enzyme glutathione peroxidase, which assumes the role in catalysis reactions that can protect the cell membranes against potential oxidative damages (Rotruck *et al.,* 1973). The use of different forms of organic selenium, for instance, selenomethionine and seleno-yeast to improve the bioavailability of selenium, also examined due to elevated potentially and higher bioavailability than ordinary inorganic selenium forms (Watanabe *et al.,* 1997; Schram *et al.,* 2008).

Very interestingly, several authors reported a fine line of difference between the edible (positive) and toxic (harmful) role of selenium. The disparity renders it as an existing contradiction in the field of aquatic toxicology since it is well established as an eco-toxicological paradox to act like both as essential micro-nutrient as well as a toxin depending upon its level in the environment (Schram *et al.,* 2008; Iqbal *et al.,* 2017). Therefore, it became critical to ascertain its role in changing the natural terrain of liver and kidney tissues when used as micronutrients to reveal a distinguishable line between constructive nutrient concentration and destructive toxic limits.

Considering the grander importance of selenium, the present study was conducted to investigate the potential role of selenium in the histopathological changes in vital organs in tilapia. We studied kidney and liver to witness the possible effects of different levels of selenium dispensed to tilapia incorporated in fish feed under laboratory conditions.

2. Materials and Methods

2.1. Experimental site

The 90 days long study about the potential effect of selenium on histology of selected vital organs of tilapia *(O. niloticus)* was conducted in Research and Training Facilities at the Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences (Ravi Campus, Pattoki), Lahore, Pakistan.

Table 1. Selected Feed Ingredients (dry weight), inclusion level and chemical composition of experimental and basal diets

Sr. #	Ingredients	Inclusion level (g/100g)			
		Basal diet (Control)	Treatment 1	Treatment 2	Treatment 3
1	Fish meal	8	8	8	8
2	Guar meal	30	29.998	29.996	29.992
3	Soya bean meal	9	9	9	9
4	Wheat bran	18	18	18	18
5	Canola meal	8	8	8	8
6	Rice polish	24	24	24	24
7	Vitamin Premix [a]	2	2	2	2
8	Selenium free mineral premix [b]	1	1	1	1
9	Selenium dose [c]	0.00	0.002	0.004	0.008
	Total	100 g			
Chemical composition					
1	Crude protein	30.2	30.2	30.2	30.1
2	Crude lipid	7.3	7.2	7.3	7.3
3	Dry matter	86.4	86.4	86.3	86.5
4	Ash	6.8	6.7	6.6	6.9

a: Vitamin premix (IU or g/kg diet): vitamin A, 16000 IU; vitamin D, 8000 IU; vitamin K, 14.72; thiamin, 17.8; riboflavin, 48; pyridoxine, 29.52; cyanocobalamin, 0.24, tocopherols acetate, 160; ascorbic acid (35%), 800; niacinamide, 79.2; Calcium-D-pantothenate, 73.6; folic acid, 6.4; biotin, 0.64; inositol, 320; choline chloride, 1500; L-carnitine, 100; **b***: Selenium free mineral premix; (g /kg of diet): calcium, 5.5; phosphorus, 17.5; iron, 10; magnesium, 2.8; copper,1.5; iodine, 0.15; manganese, 9.5; zinc, 25; cobalt, 0.13;***c***: Sodium selenite (Na_2SeO_3) in milligrams*

2.2. Fish management and experimental plan

Healthy tilapia fish were collected from the nursery ponds at the training facilities and were acclimated to laboratory conditions in indoor cemented rectangular-shaped tanks for two weeks duration before the experiment. The given feed compositions were based on selenium-graded inclusion levels along with respective chemical composition given in table 1. Three doses of formulated fish feed were prepared on the basis of selenium supplementation level viz., 2 mg/kg (Treatment-1), 4 mg/kg (Treatment-2) and 8 mg/kg (Treatment-3) of selenium in fish feed and were properly mixed followed by extrusion, drying and finally storage at -20°C while considering each dose as a distinctive treatment. The controlled diet did not receive selenium supplementation. This trial was executed in four fixed cemented rectangular fish tanks constructed with dimensions as 2.896 × 0.762 × 0.914 m (length × width × depth) and with 2.018 cubic meters total water capacity. Tank 1, 2, and 3, were designated as treatment tanks, whereas the fourth one as selenium deficient (control). There were three replicates in each treatment tank, as well as in the selenium-deficient treatment tank. The stocking density fixed to 15 fish per tank having weight ranges 10 – 25 g and fed on 30% crude protein feed dispensed at the rate of 3% body weight thrice per day. The physicochemical water quality was monitored daily to manage the potential water quality stressors on histopathological changes. We ensured continuous supply of fresh and well-oxygenated turbine water while the optimal water levels were maintained by discharges via overflow pipes.

2.3. Histopathological assessments

Samples from the excised tissues of selected vital organs of interest viz., liver, being major detoxifying organ and kidney, being the excretion factory, procured after euthanizing the fishes (by using MS222) followed by anesthesia. The removed organ samples were properly preserved in neutrally buffered formalin solution for 24 hours, followed by dehydration of the tissues as per the method of Lille and Fullmer (1976). The clearing, infiltration and embedding, section cutting (5 µ), and stretching of tissues were performed after that. Hematoxylin was the staining reagent used for tissues nucleic acid staining. On the other hand, eosin used as staining reagent for cytoplasm and extracellular matrix as established by Luna (1968) and Bernet *et al.* (1999). Slides were stained by using the method described by Lille and Fullmer (1976). Then, coverslips were mounted by using DPX followed by coding and stochastic analysis. These slides were analyzed quantitatively to explore the histopathological alterations including degenerated vacuoles, necrosis, apoptosis as well as the general health of cells that are visible by hematoxylin and eosin (H & E). Three views per fish were conducted at 10 × and 40× and analysis was tabulated by using the scoring system as described by Bernet *et al.* (1999). Photographs were accomplished with a trinocular microscope using a Nikon digital camera (Bancroft and Gamble, 2007).

3. Results

The physicochemical quality of the experimental culture environment maintained around the optimal ranges (Table 2). No apparent disease symptoms, slow movements, morbid, or moribund fish samples were noticed during the study duration. Before histological sample processing, systematic macroscopic observation of fish, including internal and external morphology, was performed with the help of lens and naked eye, and it did not reveal any macroscopic abnormalities, attaching parasites on gills and skin and no injuries were noticed. The comparative details of histopathological alterations in liver and kidney of tilapia *(O. niloticus)* fed on selenium graded diets are mentioned in table 1.

Table 2. Records of selected physicochemical parameters in treatment and control Tanks

Water Quality Parameters	Treatments				Permissible limits
	Control	Treatment 1	Treatment 2	Treatment 3	
pH	8.58±0.020	8.56±0.028	8.58±0.017	8.57±0.018	7-9
D.O.	6.20±0.150	6.04±0.167	6.14±0.289	6.26±0.274	>5
Temperature (°C)	30.35±0.022	30.35±0.026	30.33±0.030	30.34±0.022	15-35
TDS	396.92±26.88	378.06±23.378	441.81±37.648	430.19±32.532	500-1200
EC (µS/cm)	649.09±14.776	659.27±34.58	663.29±30.429	697.79±23.835	300-1500
Hardness	18.1±0.012	18.2±0.014	18.03±0.018	17.9±0.015	>20
Nitrates	0.83±0.13	0.84±0.15	0.83±0.14	0.84±0.20	0-100
Chlorides	6.5±0.11	6.9±0.19	7.0±0.13	7.0±0.18	4-160
Salinity	0.8±0	0.8±0.001	0.8±0.01	0.8±0.02	--
Ammonia	N.D.	0.011±0.0034	0.012±0.0051	0.010±0.0032	0-0.05

D.O.: Dissolved oxygen, TDS: Total dissolved solids, EC: Electrical conductivity, T.A. Total alkalinity, N.D.: Not detected. All values are mentioned in mg/L (ppm) except pH, temperature, and electrical conductivity.Our results displayed that the central vein was dilated in the liver of control treatment fish. Moreover, mild vacuolation was also seen in hepatocytes cytoplasm. However, portal areas were not distinctly observed. In the case of kidney tissues from control group fish, tubules were seen with the empty lumen. Haematopoietic tissues were also present (Figure 1 a,b).

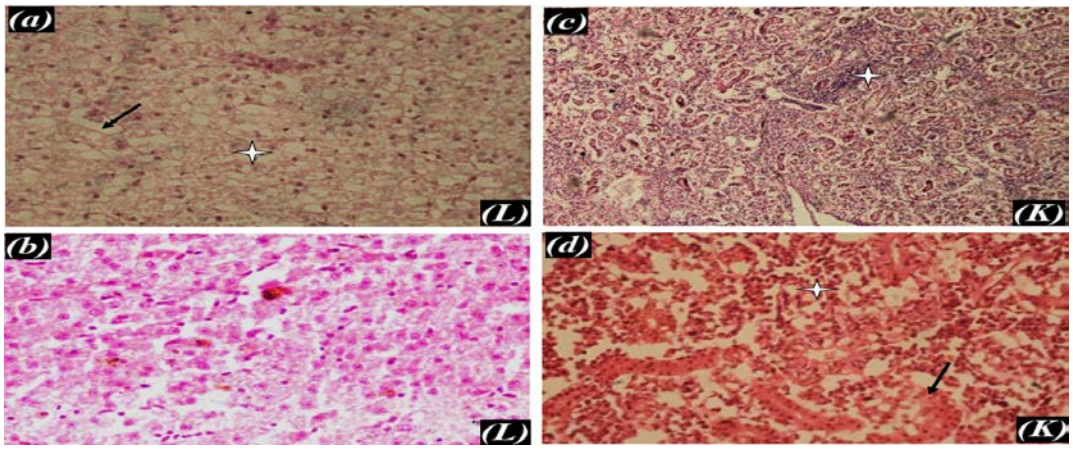

Figure 1. Comparison between Liver (L) and Kidney (K) histological changes in control and 2 mg/kg treatments. (a) T. S. of liver control group (Central vein seems to be dilated (represented by a thin black arrow). Mild vacuolation (indicated by a star) is seen in hepatocytes cytoplasm. Portal areas are not seen distinctly here (H&E 40X); (b) T. S. of Liver Se 2mg/ kg feed (Treatment-1) Mild degenerative changes are seen (Less histological alterations seen); (c) T. S. of kidney control group (Tubules are seen with empty lumen (indicated by thin arrow). Hematopoietic tissue is also present (represented by a star) (H&E 10x); (d) T.S. of kidney treated with Se 2mg/ kg feed (Treatment-1) (Mild infiltration of mononuclear cells, vacuolation in tubular epithelial cells seen with pyknotic nuclei (represented by an arrow). Some renal tubules are completely collapsed with their obliterated lumen (designated by a star) (H&E 40x).

Mild degenerative changes were seen in the liver of tilapia (*O. niloticus*) fish exposed to selenium (2 mg Se/kg). Slight histopathological changes were observed in the liver and kidney of fish exposed to 2 mg Se/kg. We observed mild infiltration of mononuclear cells found in the kidney of fish exposed to selenium supplemented feed (2 mg Se/kg). Vacuolation in tubular epithelial cells seen with pyknotic nuclei. Some renal tubules completely collapsed with their obliterated lumen (Figure 1 c,d).

In the present study, in the liver of fish exposed to 4 mg Se/kg, the blood vessels extremely dilated with thickening in the wall. Fatty degeneration was also present in parenchymal cells with peripherally displaced nuclei seen clearly. The kidney of the fish exposed to 4 mg Se/kg showed degeneration and fibrosis. The lumen of renal tubules filled with eosinophilic proteinaceous mass. Degenerative changes were evident in the linings of epithelial cells (Figure 2 a-d).

Table 3. Comparative details of histopathological alterations in liver and kidney of tilapia (*Oreochromis niloticus*) fed on control and selenium graded diets

Dose Range	Histopathological changes in selected vital organs	
	Liver	Kidney
Control	Central vein dilated Mild vacuolations in hepatocytes cytoplasm.	No distinct portal areas Tubules with the empty lumen Hematopoietic tissues present
Treatment 1 (2 mg Se/Kg)	Mildly degenerated	Mild infiltration of mononuclear cells Pyknotic nuclei Vacuolations in tubular epithelial cells Renal tubules collapsed with the obliterated lumen
Treatment 2 (4 mg Se/Kg)	Infiltration of fat in the vacuoles of the hepatocyte cytoplasm Thickening of blood vessels leading to dilation Fatty degeneration in parenchymal cells Peripherally displaced nuclei Hemosiderin pigments in blood vessels Vascular congestion in blood vessels	Degeneration Fibrosis The lumen of renal tubules filled with an eosinophilic proteinaceous mass Degenerative changes in epithelial cells Congestion of blood vessels
Treatment 3 (8 mg Se/Kg)	Fibrosis in the perivascular area Severe vacuolations in hepatocytes cytoplasm Fibrous connective tissue tracts indicate fibrosis Vascular hypertrophy Central vein dilated Haemolysed erythrocytes Dilated central vein with the empty lumen Hemosiderin pigment in a central vein Nuclei elongated and pushed towards periphery	Renal tubules undergoing atrophy Degenerative vacuolar changes in renal tubules Pyknotic changes in epithelial cells nuclei Hyaline casts in the tubular lumen Sloughing of tubular epithelial cells The thin layer of fibrous connective tissue in peritubular areas

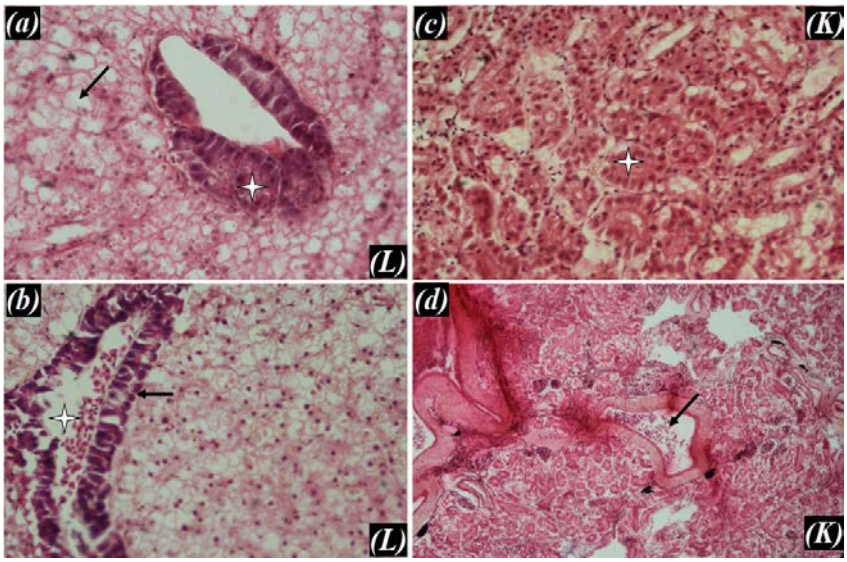

Figure 2. Comparison between Liver (L) and Kidney (K) histological changes treated by Se 4 mg.kg. (a) T. S of Liver treated with Se 4mg/kg (Treatment-1) (Blood vessel is extremely dilated with thickening in its wall (represented by a star). Fatty degeneration is also present in parenchymal cells with peripherally displaced nuclei seen clearly (represented by arrow) 40x H&E Stain); (b) T.S of Liver treated with Se 4mg/kg (Treatment-2) (Vascular congestion is seen with degenerative changes in blood vessel wall (represented by arrow). At some places haemosiderin pigment is also visible, 40x H&E.); (c) T. S. of Kidney treated with Se 4mg/kg (treatment-2) (Lumen of renal tubules is filled with eosinophilic proteinaceous mass (represented by star). Degenerative changes are evident in lining epithelial cells are also seen. 40x H&E); (d) T. S. of Kidney treated with 4mg/kg (Treatment-2) (Lumen of blood vessel is dilated with congestion (represented by arrow) H&E 40x).

Table 4. A histopathological score of the liver of tilapia fed on control and selenium graded diets (n=5)

Histopathological Change	Control	Treatment 1	Treatment 2	Treatment 3
Focal necrosis	-	-	+	+
Vacuolation	+	-	++	+++
Hemorrhage	-	-	++	+
Pyknotic hepatocytes	-	-	+	+++
Hypertrophy	-	-	-	++
Congested blood cells	-	-	++	+++
Fibrosis	+	-	+	+++
Inflammatory cell infiltration	+	-	++	+
Tumor (Benign/malignant)	-	-	-	-

Where - Symbolizes no significant histopathological alterations; + Mild alterations; ++ Moderate alterations; +++ Severe alterations

Table 5. A histopathological score of the kidney of tilapia fed on control and selenium graded diets (n=5)

Histopathological Change	Control	Treatment 1	Treatment 2	Treatment 3
General necrosis	-	-	++	++
Pyknotic nuclei	+	+	-	+++
Vacuolations	-	+	+	+++
Fibrosis	-	-	+++	+++
Congested blood cells	+	-	+++	++
Atrophy	-	-	+	+++
Hemorrhage	-	-	++	++
Tumor (benign/malignant)	-	-	-	-

Where - Symbolizes no significant histopathological alterations; + Mild alterations; ++ Moderate alterations; +++ Severe alterations

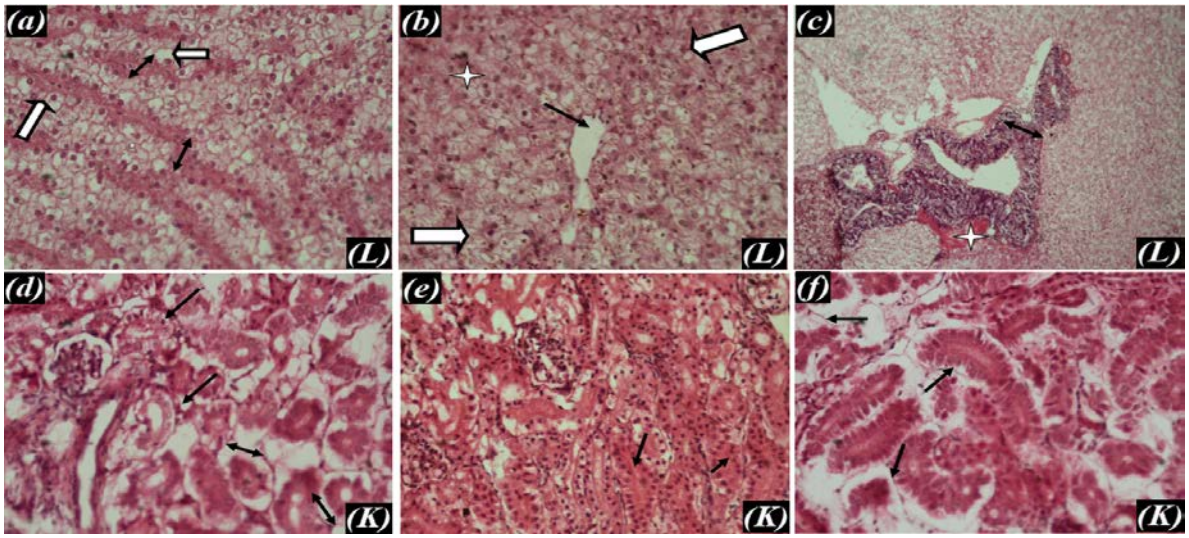

Figure 3. Comparison between Liver (L) and Kidney (K) histological changes under Se 8 mg/kg treatment. (a) T. S. of Liver treated with Se 8mg/kg (Treatment-3) (Severe vacuolation is present in hepatocytes cytoplasm indicate fatty change (represented by arrowhead). Fibrous connective tissue tracts are also present indicates fibrosis (black arrow) 40x; H&E); (b) T. S. of Liver treated with Se 8mg/kg (Treatment-3) (Dilated central vein with empty lumen is seen (indicated through black arrow). Haemosiderin pigment is also present (represented by arrowhead). Unstained fat vacuoles seen in hepatocyte cytoplasm (represented through star) (H&E 40x); (c) T. S. of Liver treated with Se 8mg/kg (Treatment-3) (Vascular hypertrophy (represented through double arrow) with thickened wall is seen. In perivascular area fibrosis is also seen) (H&E 40x); (d) T. S. of Kidney treated with Se 8mg/kg (Treatment-3) (Renal tubules are undergoing atrophy (represented by double arrow). Degenerative vacuolar changes are seen in many renal tubules (indicated through thin arrow) (H&E; 40x); (e) T. S. of Kidney treated with Se 8mg/kg (Treatment-3) (Hyaline casts are present in tubular lumen (indicated by thin arrow). Pyknotic changes are seen in epithelial cells nuclei) (H&E; 40x); (f) T. S. of Kidney treated with Se 8mg/kg (Treatment-3) (Thin layer of fibrous connective tissue (FCT) is seen in peritubular areas at many places (indicated through thin arrow). Tubular epithelial cells are also undergoing sloughing) (H&E; 40x).

The occurrence of histopathological modifications was much evident in the kidney of the fish exposed to selenium dose of 8 mg/kg supplemented in the feed as compared to the control, 2, and 4 mg Se/kg. However, the liver of fish exposed to 8 mg/kg Se, vascular hypertrophy with thickened wall was also seen. In the perivascular area, fibrosis was also seen. In the kidney of fish exposed to 8 mg Se/kg, renal tubules were undergoing atrophy. Degenerative vacuolar changes were also seen in many renal tubules (Figure 3 a-f). The histopathological scores of liver and kidney of tilapia fed on control and selenium graded diets are presented in Tables 4 and 5. The results indicated moderate to severe alterations in the liver and kidney of *O. niloticus* in the 2 mg/Kg and 8 mg/Kg selenium treatments. Critical changes in pyknotic hepatocytes, blood cells, and Fibrosis were predominant in the liver in 8 mg/Kg selenium treatment while severe vacuolations, fibrosis, and atrophy were observed in the kidney in the same treatment.

4. Discussion

Histopathological studies are accomplished to explore the preliminary effects or responses as well as acute exposure results to environmental chemical stressors because of the ability of fish to respond to the direct impact as well as secondary effects caused by the mounting stress (Atique *et al.,* 2020a; Khanom *et al.,* 2020; Saeed *et al.,* 2020). The liver and kidney are the vital organs that can respond to such changes (Bernet *et al.,* 2004; El-Hammady *et al.,* 2007). The liver is also one of the essential digestive glands in fish and is the largest extramural organ. Liver in fish is supposed to carry out the essential physiological activities including but not limited to homeostatic maintenance, plasma protein synthesis, storage (e.g., energy, vitamins, and trace metals), nutrient assimilation, bile production, and detoxification. The fish liver may or may not contain pancreatic tissues. The kidney is the principal excretory organ for water elimination, particularly vital to freshwater fishes due to its reabsorption mechanisms for water-salt maintenance. The water quality holds the key of the balanced regulatory functions in fish in controlled (Haider *et al.,* 2016; Batool *et al.,* 2018; Haider *et al.,* 2018; Khan *et al.,* 2018) and natural waters (Atique and An, 2018; Atique *et al.,* 2019; Atique *et al.,* 2020b; Atique and An, 2020; HaRa *et al.,* 2020). The teleost kidney is partly comprised of haematopoietic, excretory parts, phagocytic, and endocrine tissues. The fish kidney may or may not be fused in the structural arrangement. During response generation to the uncomplimentary developments in or outside of the body, they can be studied as indicative organs as they undergo various histological changes.

Mild degenerative changes were observed in liver and kidney tissues of tilapia (*O. niloticus*) fed on 2 mg/kg diet. Our results of the present study are corroborating with El-Hammady *et al.* (2007), who revealed less or negligible histopathological modifications in fish exposed to a lower dose of selenium, i.e., 2 mg /kg in the feed. Noticeably, the colour of the liver in fish exposed to 2 mg/kg was of dull grey-red when matched to other selenium graded treatment tilapias, i.e. 4 mg/kg and a higher dose of 8mg/kg of feed. It may have been caused by the mild degenerative alterations linked to selenium dose (Gatlin and Wilson, 1984). It is imperative to consider that a healthy fish is not only categorized based on absence or presence of histopathological changes because it may exhibit mild or moderate histological alterations or inflammatory responses owing to physical reactions (Bernet *et al.,* 2004). In this study, slight infiltrations of the mononuclear cells in the kidney of fish were witnessed in the lower level of selenium-supplemented treatment, i.e., 2 mg/kg of feed. Our results conform with Peebua *et al.* (2008), who discovered vacuolations in many tubules as well as nuclear pyknosis in the fish kidney.

To the next level of selenium-graded diet effects, the liver in fish devouring the 4 mg/kg feed resulted in the dilation of the lumen of blood vessels and thickening in their walls. Fatty degeneration was also noticed in the parenchymal cells with clearly displaced peripheral nuclei. The kidney of the same treated group denoted degeneration and fibrosis. The lumen in renal tubules was observed to be filled with eosinophilic proteinaceous mass. Also, several degenerative changes were marked in the lining of epithelial cells. Similar results were corroborated by the application of Bernet *et al.* (1999) protocol that supported our conclusions. The outcomes were in a match with the findings of El-Hammady *et al.* (2007), who also recorded the degenerative variations as well as fibrosis in fish kidney fed on similar diet regimes.

Moreover, hepatic cells degeneration and hemorrhages were also distinguishable. The blood vessels dilation was noticed very prominently (Hilton *et al.,* 1980). In the previous studies, renal tubular degeneration as well as the perturbed circulatory mechanism like hemorrhages observed in the cultured fish have been concomitant of an antibiotic treatment (Smith *et al.,* 1973; Roberts, 2012), whereas, the necrosis of renal haematopoietic tissues, which are indeed very sensitive, may arise due to various biotic and toxic situations or medications (Roberts, 2012).

Frequency and histopathological alterations score recorded in our study were very conspicuous in the liver and kidney tissues processed from 8 mg/kg selenium incorporated feed. Tilapia (*O. niloticus*), when fed on a high dose of selenium (8 mg/kg), different sections of liver exhibited hemosiderin pigments along with hemolysis of cells and fatty degenerations. Such abnormal deposition of fat in the fish liver from treatment 3 resulted due to the excessive fat production as well as its utilization (Lemly, 2002). Desai *et al.* (1984) put forth similar observations having said that the fatty degenerative changes in the liver of tilapia linked with the reduction of an energy consumption level or on the contrary, argued at the enhanced amount of fat synthesis.

In the same pattern, uptake and increase of selenium levels in trout (*Salmo gairdneri*) tissues inhabited for a prolonged duration while feeding at supplemented selenium dose (3µg/kg) diet may result in toxicity (Hilton *et al.,* 1980). If trout are exposed to selenium added feed (4.29 and 15.00 µg/g), its detoxification into methyl derivatives and seleno-proteins inside the liver.

Our findings also corroborate with Peebua *et al.* (2008), who confirmed that hepatocytes indicate necrosis, accumulated lipid vacuoles, hydropic swelling as well as the vacuolation present in the liver. It further argued that tubular cells go through hydropic blisters, the lipid vacuoles accumulate in the cytoplasm of the tubular epithelial cells along with pyknotic nuclear alterations (Morrison and Wright, 1999). Therefore, such histopathological changes of hepatocytes indicated towards the hydropic degeneration, as well as the accumulation of lipid vacuoles and necrotic changes in the liver, are essential. On the other hand, the histopathological changes in kidney tissues included the mass of vacuoles in tubular cells and stark necrotic areas and tubular degeneration. Our findings corroborate with the observations of Oulmi *et al.* (1995) in the case of *O. mykiss* and Gupta and Kumar (2006) in *C. mrigala.* Both groups observed small granules in the cytoplasmic region, haemolysis of cells in addition to nuclear deformations of the epithelium in the proximal tubules.

The overall findings of our study corroborate with the investigations of Hilton *et al.* (1980), who published his observations conveying that when selenium level exceeded the limit of 0.38 µg/g, escalated uptake of selenium in liver witnessed. Ramesh *et al.* (2014) and El-Hammady *et al.* (2007) both identified that the liver damages could be seen in case of excessive accumulation of sodium selenite that

ultimately leads to liver toxicity (Lemly, 2002). The liver is so definitely prone to selenium toxicity being the main selenium storage organ, as well as for detoxification (Hodson *et al.,* 1980). Also, degenerative structural changes in tissues of the liver occur when the fish is exposed to an increased concentration of selenium (Sorensen *et al.*, 1984). Besides, toxicants lead to liver cell necrosis and vascular degeneration (Malarvizhi *et al.*, 2012).

5. Conclusion

It is concluded that dietary selenium could inflict tissue damages if fed to juvenile tilapia in considerably higher concentrations. However, it inflicted minimal tissue damages when fed at lower levels incorporated in fish feed. Such significant variations in selenium toxicity in vital organ tissues suggested a higher degree of complexity in the selenium toxicity mechanism. However, the present study paved the way towards the recommendation of lower levels of selenium supplemented in the fish feed for tilapia, which could be very useful for fish health.

Author Contributions

SI and UA equally contributed to this study and conducted the experiment, collected the samples, and analyzed the data and prepared the manuscript under the supervision of MSM and MY. MKR helped in image processing while MSH, HSI, SS, and TAK helped in manuscript preparation.

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Conflict of Interests

The authors declare no conflict of interest.

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