Evaluation of cytotoxicity, histopathology and organosomatic indices of *Oreochromis niloticus* as biomarker of Industrial effluent toxicity

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ABSTRACT

Aquaculture makes a significant contribution to aquatic goods and dietary protein. It, like the rest of the food industry, is confronted with huge problems such as adverse weather occurrences, environmental stress factors, and industrial pollutants. Sub-lethal exposures of industrial effluents at acute and chronic durations were subjected to *Oreochromis niloticus*. Toxic impacts of effluents on hematological, histological, hepatosomatic and nephrosomatic indices were recorded in exposure groups with reference to unexposed fish. Exposure to industrial effluents damaged the fish tissues irreparably, demonstrated by altered histoarchitecture of liver and kidney, with the common anomalies of necrosis, focal are of necrosis, hemorrhage, edema, hypertrophy, atrophy and toxicant accumulation along with the anomalies specified for both organs. The HAI values of kidney for acute (1.31) and chronic (1.46) exposure groups were found non-significantly different and its values for the liver were 1.46 and 1.62, respectively. The organosomatic index of the liver exhibited highly significant ($p < 0.05$) variations between experimental groups as well as highly significant ($p < 0.001$) variations in the kidney in comparison to the control group specimens. The major hematological findings were significant ($p < 0.05$) reductions in hemoglobin, hematocrit, red blood cells and mean corpuscular hemoglobin concentrations in exposed fish groups as compared to unexposed fish.

Keywords: Nephrotoxic, hepatotoxic, histopathology, hepatic index, renal index, toxicity

INTRODUCTION

Aquaculture is growing rapidly in Pakistan and there is an enormous potential for development of this sector. Fish is a good source of protein, vitamins and minerals, such as selenium, vitamin B12, niacin, phosphorus and potassium. It is a source of cheap and precious animal protein for the human population (Makori *et al*., 2017). Thus, the fish industry plays a major role to the economy of Pakistan as an earner of foreign exchange. Because of the low cost of tilapia production, it is called the "aquatic chicken" of the industry. The yearly value of farmed tilapia output is projected to be US 1.8 billion $. Tilapias are the second most widespread aquaculture species, with a minimum 90 countries on all continents except Antarctica having imported those (Waris *et al*., 2023).

Because of direct outflows or atmospheric deposition, aquatic habitats serve as the final sink for a variety of industrial and residential toxins. Complex polymers in untreated industrial wastewater are not only detrimental to human health and detrimental to people's health, but they have also been discovered to be harmful to aquatic creatures, posing severe environmental concerns. Most of the world's ecosystems are often polluted by xenobiotics as a result of various industrial operations (Athira and Jaya, 2018). Fish is a vertebrate animal that is found all over the world and is an important source of animal protein for both humans and animals. It is one of the foods that preserve human health since it outperforms other animals regarding of food conversion efficiency. It also does not compete with humans for the environment and food, as animals do; it does not consume water and it does not consume water but rather fertilizes it (Elarabany and Bahnasawy, 2019).

Histopathological indicators are connected to stress biomarkers because they function similarly but in distinct ways. Pollutants must be metabolically activated in the presence of stress biomarkers in order to trigger cellular alterations in the affected organism. For example, the action of xenobiotic compounds in many cells causes the creation of many enzymes, changing cellular metabolism and ultimately leading to cell death. Due to the histomorphological biomarkers, the pollutant's influence manifests as necrotic damage and other cell and tissue problems. As a result, histopathological biomarkers, like stress biomarkers, can be effective predictors of the number of certain toxins in the environment (Savari *et al*., 2020).

Histopathological changes in the fish organs have been identified as useful techniques for assessing fish health in polluted water. Histopathology is the examination of abnormalities or deformities in tissues or cells in order to determine the sub-lethal and chronic consequences of contamination levels. The metal buildup causes structural defects in the fish tissues. Numerous histological abnormalities in fish muscles, gills, kidneys , and liver have been documented in reaction to agricultural, industrial, and sewage pollution (Essawy *et al*., 2022). The fish liver is regarded as an essential organ that has been severely harmed by pollution. As the liver is essential for xenobiotic metabolism and detoxification, it may be sensitive to much smaller dosages than the other organs (Mahboob *et al*., 2020). Fish kidneys play an important function in excretion and water balance adjustment in preserving a fish's internal conditions and osmosis (Nkwuda *et al*., 2020). The kidney in freshwater fish is crucial for preventing excessive solute loss since the fish's blood contains more salt than the water around their body (Tavares-Dias, 2022).

Blood is a therapeutic reflection of the organism's body, and its properties are crucial in identifying fish's functional and structural state after hazardous agent exposure. Hematological examinations are an essential sign of stressor illness induced by pollutants and environmental conditions (Chaudhary *et al*., 2023). Hematological factors such as hematocrit, hemoglobin, number of RBC, and WBC are useful biomarkers in assessing toxicity in monitoring of environment and marine organisms (Kumar *et al*., 2022; Bojarski and Witeska, 2020). As a result, hematopoietic responses can be used to investigate the sub-lethal impact of contaminants on fish well-being. In various fish species, somatic indicators are additionally employed in environmental risk evaluations (Kroon *et al*., 2017). Formerly, the organ somatic markers in largemouth bass as well as tilapia were evaluated to determine metal pollution in aquatic environments (Girgis *et al*., 2019; Gehringer *et al*., 2013). Therefore, the current study aimed to determine the impact of environmental stress and industrial contaminants on *O. niloticus* by studying the hematological, histological and organ indices as sensitive and reliable indicators that assess the environmental stress and toxicity of contaminants.

MATERIALS AND METHODS

Quaid e Azam Industrial Estate (QIE) is a large industrial hub of Pakistan, situated at Sheikhupura and it is spread over an area of 565 Acres. Effluents of textile, tannery and chemicals industries from QIE, Sheikhupura were collected and brought to the laboratory in ice boxes according to the guidelines of USEPA, 2017 and Steel and Torrie, 1996 and preserved in a refrigerator at 4°C to prevent the degradation of samples. The effluents collected from different industries i.e. textile industry, tanning industry and chemical industry were collected in equal quantities to make a composite for the determination of LC_{50} value (i.e. 24.94%) of exposed fish samples. Sub-lethal exposure trials of industrial effluents on Nile tilapia (*Oreochromis niloticus*) with concentrations of $1/3^{rd}$ and $1/10^{th}$ of LC_{50} were carried out for acute and chronic durations, respectively. The exposure trials were carried out in triplicate groups in a non-static, renewal system by following the WET and USEPA 2002; with regular feeding of fish throughout the trial @3.5% of the body weight.

Hematological analysis

The peripheral blood samples of the fish from the caudal vein were collected by using heparinized syringes and taken in the EDTA tubes to avoid coagulation in blood. Total RBC, WBC and platelets count, estimation of hemoglobin, hematocrit, MCV, MCH and MCHC by using a hematology analyzer by following Shah and Altindag, 2005.

Histological assessment

After the blood procurement, the fish was sacrificed humanely and dissected to observe the histological alterations in the liver and kidney of exposed fish, in comparison to the unexposed fish. Dissected organs were fixed in the formalin and dehydration was carried out in three different grades of alcohol i.e. 50%, 70%, 90%. The samples were incubated in 1:1 solution of alcohol and xylene overnight at about 60˚C. After incubation, fish organs were embedded in pure wax and section cutting of the tissues was done by utilizing microtome and stained at the end by

Hemotoxyline and Eosine to prepare the permanent slides. Slides were observed under a Fluorescent microscope (Euromex BS.3153-PLi) at 40 X magnification and scoring was done by following Bernet *et al*., 1999.

Study of organosomatic indices

The organosomatic index of dissected fish organs (kidney and liver) was measured and recorded at the end of acute and chronic exposure trials by using the following formula:

$$\text{Organosomatic index } = \frac{Organ\ weight}{Fish\ weight} \text{ x } 100$$

Statistical analysis

SPSS-23 (statistical software) and analysis of variance test were applied to examine the mean values of all parameters under investigation at $p<0.001$ and $p<0.05$ significance level, with Waller-Duncan as the post-hoc test and Tukey's Newman Q test. All the data was presented as the means and standard deviation (Mean±S.D) using Descriptive statistics.

RESULTS

Histopathological assessment

In comparison to normal kidneys of control fish (Figure 1A), the histological analysis of fish exposed to industrial toxicants exhibited morphological and structural anomalies like glomerulus and tubular degeneration, tubular necrosis, focal area of necrosis, hemorrhage, edema, hypertrophy and atrophy. Lost cellular integrity of renal tubules and toxicant accumulation sites in the renal structure of exposed fish were observed, these accumulation sites are resided by the heavy metals prevalent in the textile, tannery and chemical industry effluents (Figure 1B-F).

Frequencies of toxicant accumulation sites, edema, atrophy, hypertrophy, tubular necrosis and lost cellular integrity were higher in the acute ($1/3^{rd}$ of LC_{50}) effluents exposed group with the number of 14, 21, 22, 9, 42 and 45, respectively. Whereas, the tubular degeneration and focal area of necrosis were more prevalent in the chronic ($1/10^{th}$ of LC_{50}) exposure concentration group (Figure 3). The HAI values of the kidney for acute (1.31) and chronic (1.46) exposure groups were found non-significantly different when compared with one another.

The liver of treated group samples showed loss of cellular architecture, nuclear alterations, clustering of nuclei, hemorrhage, edema and hepatic cell inflammation resulting by acute ($1/3^{rd}$ of LC_{50}) and chronic ($1/10^{th}$ of LC_{50}) effluents exposure (Figure 2). The liver of *Oreochromis niloticus* exposed to industrial wastewater exhibited other degenerative changes like cytoplasmic vacuolation, hepatocyte hypertrophy and atrophy, hepatic necrosis and focal area of necrosis. Damaged bile duct, hepatocytes with increased sinusoidal space/enlarged sinusoids, hydropic changes, toxicant accumulation, fatty degeneration and accumulation of hemocytes in hepatic portal vein hepatic cells were also recorded. The prevalence of heavy metals in the environmental toxins may have caused the vacuolar deterioration, hemorrhagic and necrotic changes seen in this investigation.

Frequencies of hemorrhage, hyperemia, and hepatocyte atrophy were highest (15, 9 and 43, respectively) in the chronic exposure group, whereas cytoplasmic vacuolation, hypertrophy, inflammation and infiltration were highest in acute group fish with the values of 174, 16, 5 and 23, respectively (Figure 4). The HAI values of acute and chronic exposure groups were recorded and found higher in the chronic group with a value of 1.62 whereas 1.46 was HAI value of acute exposure group.

Hematological analysis

The Hb concentration in blood samples was (12.2 ± 0.14 g/dl) in the blood of the control group fish, whereas the acute and chronic exposure groups exhibited values of 4.5 ± 0.14 and 2.25 ± 0.07 g/dl, respectively with a highly significant decline. The highest red blood cells count (12.25 ± 0.35 $x10^6$/µL) was recorded in the blood of control fish, a highly significant decline at $p<0.001$ was noted in the acute exposure group (1.95 ± 0.21 $x10^6$/µL) and chronic group with the value of 0.85 ± 0.07 $x10^6$/µL. A similar pattern was observed in the hematocrit levels of control (52.5 ± 0.14 L/L), acute (10.4 ± 0.3 L/L) and chronic group (7 ± 0.28 L/L) fish. TLC and MCV were found lowest in the acute group, slightly elevated in the chronic group and the highest values were recorded in the control group. Platelet count was recorded in the pattern of chronic < acute < control, where exposed group values were non-significantly declined (Figure 5). MCH and MCHC were found highest in the control group (78.5 ± 3.53 and 87 ± 1.41 %) and lowest in chronic exposure group with the figures 35 ± 14.4 % and 29.65 ± 0.5 %, respectively.

Organosomatic Index

In fishes exposed to acute and chronic concentrations of industrial effluents, both hepatosomatic index, as well as renal somatic index, were calculated. A discernable decline at a significant level ($p<0.05$) was observed in hepato somatic index of acute and chronic exposure groups fish with the figures of 1.14 ± 0.28, 0.85 ± 0.29, respectively, in contrast to the fish in the control group (2.93 ± 1.10). On the other hand, a highly significant reduction in the renal somatic index of fish exposed to acute (0.31 ± 0.27) and chronic (0.16 ± 0.05) exposure conditions was calculated at $p\leq0.001$, when compared to its value in unexposed fish.

DISCUSSION

Renal tissue absorbs enormous amounts of blood and is an essential part of toxicological investigations since it serves as a significant route of disposal for xenobiotic compounds. The fish kidneys are crucial in maintaining osmotic equilibrium in fish. Moreover, renal tissues absorb a large volume of blood and serve as a significant route of biochemical product excretion. As a result, changes in renal tissues may be a useful indication of pollution. A normal histoarchitecture of fish kidneys in the control group showed the normal appearance of renal tissue and Bowman's capsule (Figure 1A), whereas severe changes in kidney tissues of effluent-exposed fish were observed, such as vacuolation, glomerular deterioration, tubular decay, necrosis, hemorrhage, toxicant accumulation areas, atrophy and hypertrophy, and loss of cellular integrity, as a result of acute (Figure 1B-D) and chronic effluents exposure (Figure 1E-F). Roughly identical alterations in kidney tubule diameter, decreased lumen, glomerular degeneration, and hypertrophy were observed by Chaudhary *et al*., 2023 in *L. rohita* subjected to toxicants. The findings of the current investigation were similarly consistent with those of Saini (2013), who discovered diseases such as blood vessel congestion, tubule degeneration, and vacuole degeneration in the kidneys of fish obtained from freshwaters in Punjab.

Fish liver is the primary location of numerous biotransformation activities and is responsible for toxicant elimination. Heavy metal buildup can impair their activities and limit the circulation of blood to all areas of the body. The liver parenchyma seems to be formed up of giant cells crossed by a network of sinusoids and emptied by the network of minute bile canals, as illustrated in the anatomy of the control group fish (Figure 2A). Polygonal hepatocytes have a consistent size and polarity, with a thin apical surface and big spherical nuclei. Microscopic examination of the healthy liver of fish, *Oreochromis niloticus* revealed a clear histological structure of hepatocytes, liver cells along with hepatic portal vein.

Sub-lethal exposure of toxins to *G. affinis* by Alkshab and Taha, 2021 resulted in degeneration, vacuolar degradation, necrosis, congestion, edema and hemorrhage as well as the structural disintegration between various cerebral tissues of the fish. Navaraj and Yasmin, 2012 reported that acute exposure of *O. niloticus* to tannery factory waste resulted in severe necrosis and vacuolar changes with empty spaces at 1.2 and 1.4% exposures, respectively and the results obtained by the current study were quite similar. Kaur *et al*. (2018) explored the clinic pathological changes in the liver of a freshwater cyprinid, Rohu, exposed to toxicants, and histopathology revealed several hepatic lesions, including leukocyte infiltration, cytoskeletal decay, pyknosis, severe necrosis, and nuclear degeneration.

Histopathological findings by Pérez *et al*., 2018 demonstrated that the effects of untreated effluents were more severe on the liver of such fish than on their gills. Histopathological effects due to textile industry toxicity in a freshwater fish *Channa punctatus* were studied by Dyk *et al*., 2012 and light microscopy divulged several anomalies including infiltration of lymphocytes, sinusoidal dilations, necrosis and vacuolation in the liver. The toxicopathic, non-neoplastic, and pre-neoplastic modifications were more prevalent in *C. gariepinus* from contaminated regions, according to histological examination. Topal *et al*., 2014 discovered similar alterations in brown trout subjected to environmental toxins, which is corroborated by the findings of Suchana *et al*., 2021, Deepak *et al*., 2021, and Martnez-Durazo *et al*., 2023.

Alterations in its hematopoietic parameters are early indicators of pathophysiological abnormalities that emerge in the body as a result of various toxicants since blood is a sensitive tissue that is highly impacted by environmental influences. As blood is supposed to be a reflection of the entire organism, blood parameters are extremely important in evaluating the changed physiological condition of the fish after exposure to hazardous chemicals (Praveena *et al*., 2013). In present study, the sub-lethal acute and chronic exposure concentration of industrial effluents caused evident changes in the hematological parameters of the freshwater fish, *Oreochromis niloticus*.

According to the results, the values of red blood cells, hemoglobin, hematocrit and MCHC displayed a highly significant decrease at $p<0.001$, in experimental groups compared with the control. Dysfunction of the hemopoietic systems of fish was indicated by this depletion along with a non-significant decline in total leukocytes. Additionally, a substantial drop ($p<0.05$) in MCH and MCV values could be attributed to defense against effluent toxicity, which could be attributed to a decline in Hb, PCV, and RBC values, as well as disturbances in the cellular metabolism of fish. Many researchers have recorded similar changes in the hematological parameters of fish, depending on the effluent exposure period and intensity of toxicity concerning LC_{50} (Olusola *et al*., 2023; Singh and Pandey, 2021; Andaleeb *et al*., 2020; Chaudhary *et al*., 2023; Muthukumaravel *et al*., 2022; Alimba *et al*., 2019 and Ahmed, 2013).

The hepatosomatic index and renal somatic index are the indicators of hepatomegaly and effluent toxicity. In a separate investigation, using tilapia challenged to heavy metals and persistent organic pollutants exposure, Ibor *et al*., 2017 discovered that HSI values were poorer in biota than in the control group. In contrast, another research using *Tilapia mossambica*, Al-Ghais, 2013, found that fish exposed to sewage effluents (with greater levels of pollutants including metals) had higher HSI values than non-exposed organisms. El-Serafy *et al*. (2013) found that the CF, HSI, and GSI were considerably lower in exposed specimens compared to control fish values. These results were then classified as unfavorable impacts of heavy metals exposure. All these findings were in accordance with the results obtained by the current investigation, which prove that industrial effluents impose substantial impacts on the somatic indices of fish when exposed at sub-lethal toxicity levels. Similar results have been reported by Singh *et al*., 2022; Martínez-Durazo *et al*., 2023; Amachree and Idam, 2022 and Handa and Jindal, 2023.

Conclusion

In conclusion, the exposure of industrial toxicants to *O. niloticus* resulted in their accumulation and induction of stress in the renal and hepatic tissues of the fish, which led to lowered organosomatic indices and irreversible damage to the organ functioning due to their altered histoarchitecture. The alteration in hematological parameters also indicated that the ability of fish to fight disease diminishes with dose-dependent exposure hence its chances of survival are reduced.

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Consent to Publish

The authors have no conflict and agree to publish.

Authors Contributions

I.M. and G.J. wrote the main manuscript and carried out the investigation and data analysis; G.J. designed the research proposal and wrote the methodology and edited it; I.M. prepared all the figures; All authors reviewed the manuscript.

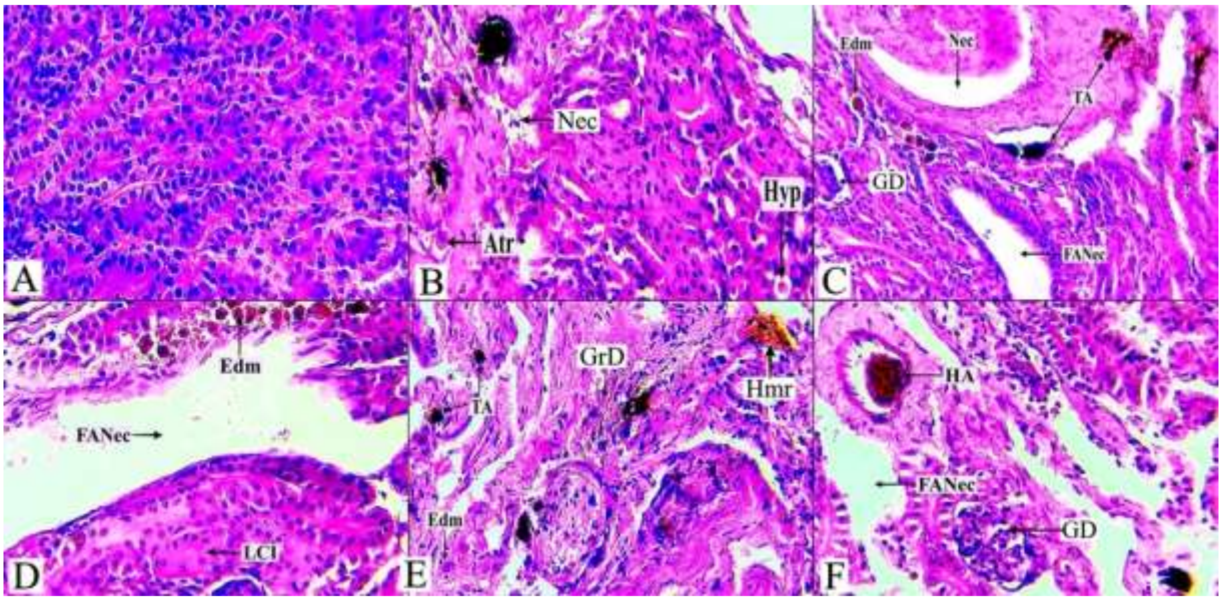

Figure 1. Photomorphographs of sections of kidney showing (A): Unexposed group, (B-D): Acute exposure group and (E-F): Chronic exposure group showing; Necrosis (Nec), Focal area of necrosis (FANec), Edema (Edm), Hypertrophy (Hyp), Hemorrhage (Hmr), Lost cellular integrity (LCI), Toxicants accumulation (TA) and Nuclear abnormalities. H & E stain, 40 X.

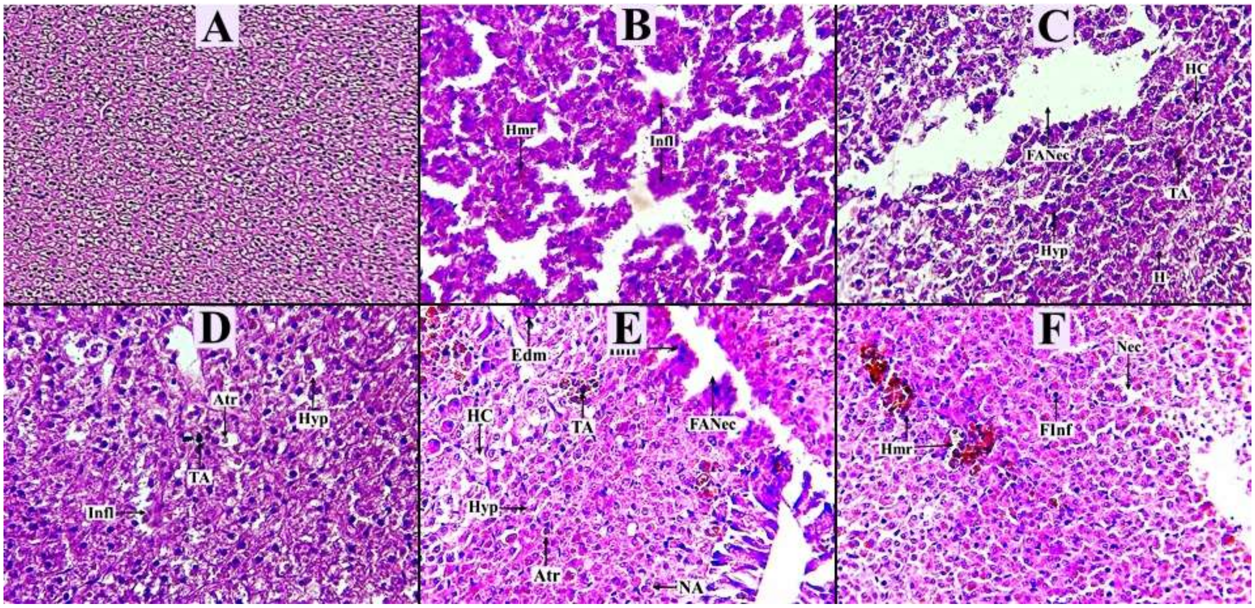

Figure 2. Photomorphographs of sections of liver showing (A): Unexposed group and (B-D): Acute exposure group and (E-F): Chronic exposure group showing; Necrosis (Nec), Focal area of necrosis (FANec), Edema (Edm), Hypertrophy (Hyp), Atrophy (Atr), Hemorrhage (Hmr), Inflammation (Infl), Toxicants accumulation (TA) and Nuclear abnormalities (NA). H & E stain, 40 X.

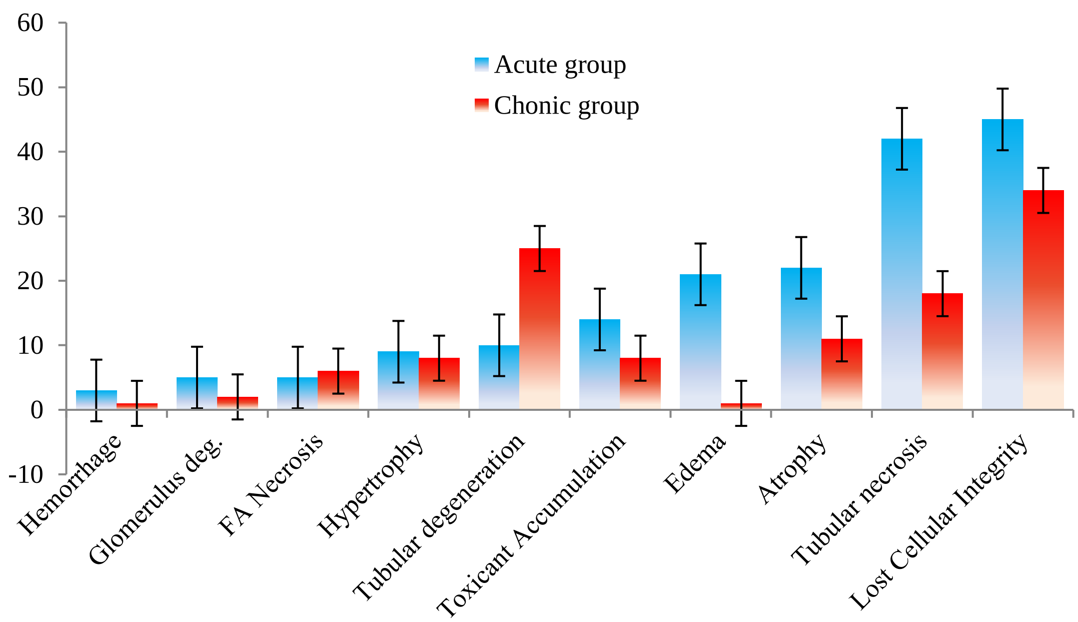

Figure 3. Graphical representation of the frequency of histological alterations recorded in kidneys of fish exposed to industrial effluents for acute and chronic exposure durations.

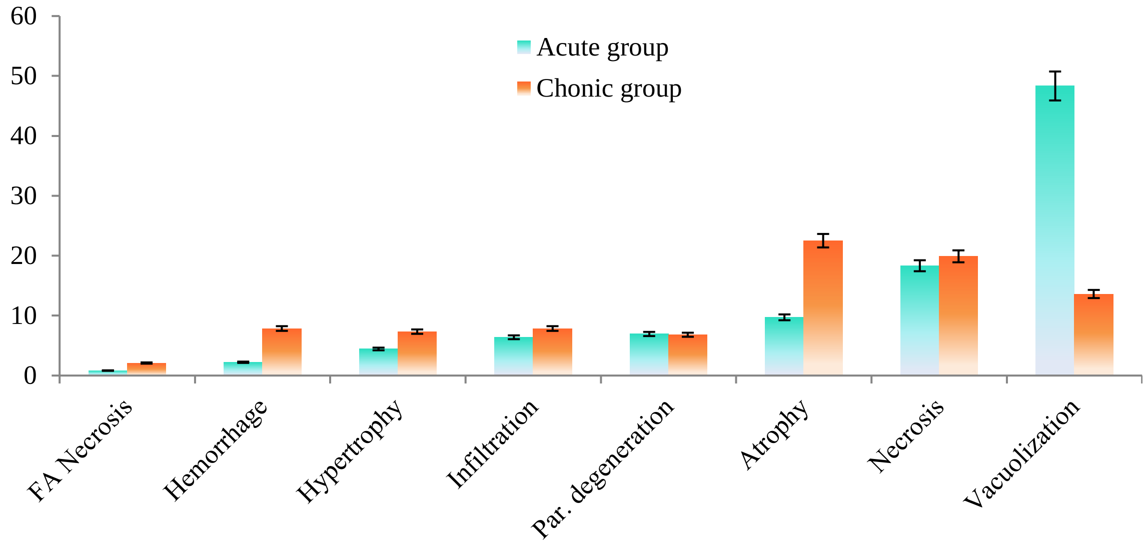

Figure 4. Graphical representation of the frequency of hepatic histological alterations recorded in tilapia exposed to industrial effluents for acute and chronic exposure durations.

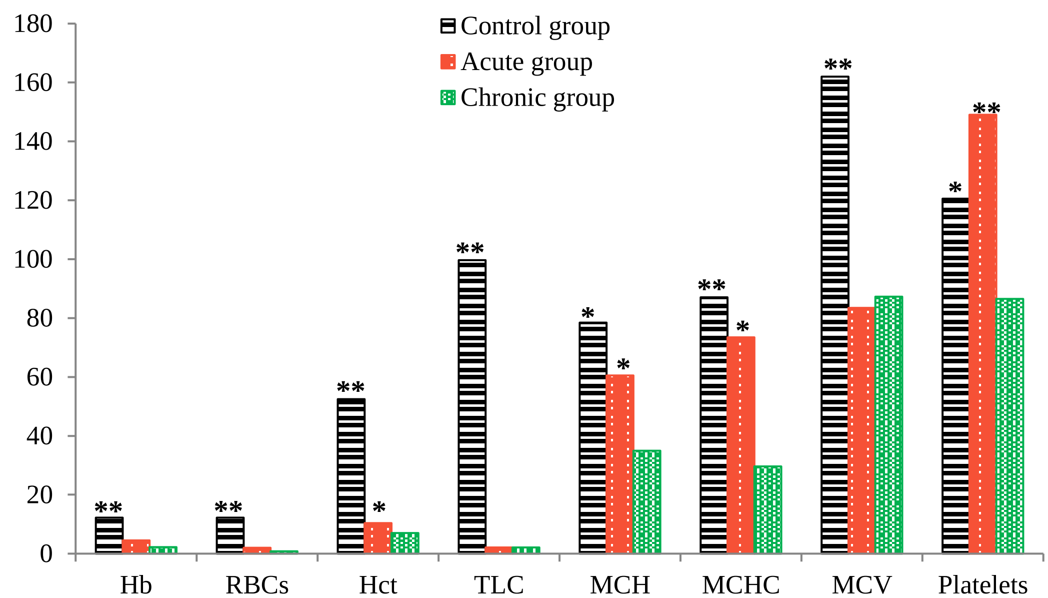

Figure 5. Graphical comparison of the hematological parameters between control group and fish exposed to acute and chronic concentrations of industrial effluents (Single steric on the bars representing the fish groups indicate statistically significant differences at $p<0.05$ whereas double steric show highly-significant differences at $p<0.001$ for different treatment groups).

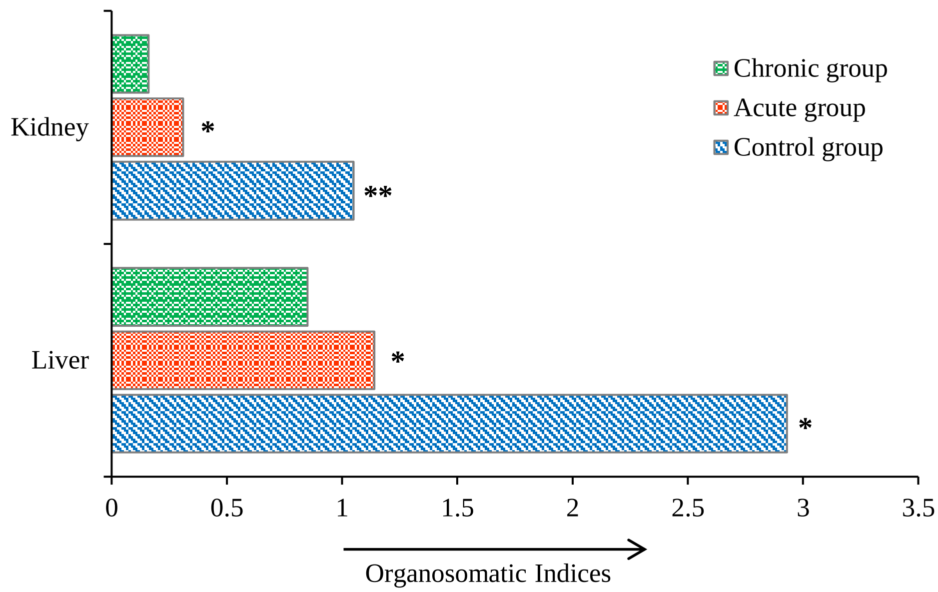

Figure 6. Comparison of organosomatic indices of kidney and liver between control group and fish exposed to acute and chronic exposure durations (Single steric on the bars representing the fish groups indicate statistically significant differences at $p<0.05$ whereas double steric show highly-significant differences at $p<0.001$ for different treatment groups).

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