Comparative Histological Changes in the Liver of Nile Tilapia (*Oreochromis niloticus*) from Different Aquatic Habitats in Kerbala Governorate, Iraq

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Abstract The present work was aimed to evaluate the histopathological changes in hepatic tissue of Nile tilapia (Oreochromis niloticus) isolated from various sources of water in the Kerbala Governorate, Iraq. A total of 40 fish specimens were collected from several sites in the Kerbala Governorate representing a variety of environmental conditions. Liver samples were carefully dissected and fixed in 10 % neutral buffered formalin and processed by routine histological techniques used for microscopic examination. Tissue specimens were histologically diagnosed with Harris haematoxylin with eosin (H&E) and Periodic Acid–Schiff (PAS) stained. The results of the histological comparison concluded that all experimental groups presented histologically abnormal liver tissues, highlighting the great influence of the environment on liver integrity and consequently on fishes' health. The most significant changes were in portal and parenchymal fibrosis with an increase in connective tissue growth. The distinct hepatocellular alterations recorded were disruption of hepatic cord architecture with vacuolar and hydropic degeneration. Vascular changes were there as well with either constriction or dilation of hepatic sinusoids and infiltration of proteinaceous material. Histochemical evaluation demonstrated a higher concentration of glycosaminoglycans (GAGs) in the hepatic parenchyma. Likewise, inflammatory responses were observed with an increased infiltration of fibroblasts and melano-macrophage cells (MMCs). The degree of liver changes was different among the studied groups from moderate degenerative and fibrotic changes to severe cellular damage and necrosis, suggesting a progressive pattern of liver damage depending on the environmental conditions. These results emphasise the relevance of hepatic histological alterations as reliable biomarkers in the assessment of aquatic pollution and general environmental health status.

***Keywords* —** Histology, Liver, Tilapia, Bio-indicator

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the most farmed fish species in the world due to its rapid growth performance, strong adaptability to a wide range of environmental conditions and its high value in aquaculture production systems (1,2). This species is quite resilient to alterations in the environment like as temperature, salinity and water quality and its adaptability to different aquaculture systems has helped in its extensive dissemination (3). The Nile tilapia (*Oreochromis niloticus*) is known as a 'aquatic chicken' because it is cheap to produce and provides important low-cost protein from easily accessible animals, particularly in poorer countries (4,5). It is worth mentioning that the introduction of tilapia farming has significantly contributed to the growth of rural development, poverty reduction and increased food security through improved availability of protein and creation of jobs in the aquaculture industry (1,5**)**. The organism has a high degree of physiological and ecological adaptability as evidenced by the fast growth rate, wide tolerance to environmental conditions (including temperature and salinity changes), stress tolerance and disease resistance, varied foraging strategies, and effective reproduction in captivity (2,6).Fish health and their performances in aquatics environment are influenced by the major determinants like the quality of water ;Temperature, dissolved oxygen, pH, ammonia, turbidity and total dissolved solids are vital parameters for the metabolic and physiological activities of fish(7,8). Although the organism is somewhat resistant, long-term exposure to harsh environments can cause biological stress which eventually will lead to changes in structure and function in vital organs such as the liver, a critical organ for metabolic functions (9,10). Degradation of water quality, often caused by man-made contamination from chemicals, heavy metals and organic matter, can cause serious disturbance in the aquatic habitat. It creates physiological stress, disorders physiological processes and can, finally, jeopardise the existence of fish stocks. (9,11).

The liver has an important function in biotransformation, detoxification and elimination of xenobiotics. However, the consequent accumulation of contaminants in the liver can induce structural and functional alterations and reveal the failure of detoxification. The most documented histopathological alterations include hepatocyte vacuolation, melanomacrophage centers, necrosis, inflammation, fibrosis and bile duct thickening (12). The liver is therefore a very important organ for fish such as Nile Tilapia (Oreochromis niloticus), as it is responsible for metabolic regulation, detoxification and storage of needed substances. The liver is involved in many biochemical processes, including protein synthesis, lipid metabolism, and biotransformation of endogenous and exogenous compounds. Owing to its multifunctional nature and direct exposure to blood-borne substances, the liver is considered a primary target organ for assessing physiological status and environmental impacts in fish (13,9).

The efficiency of a fish is to a great extent dependent on a complex relationship between several environmental variables and its biology. Factors such as water temperature, oxygen content, quality and a fish's ability to adapt to different environments greatly affect physiological and behavioural adaptation (14,8). In environments characterized by poor water quality, the liver is especially vulnerable to damage caused by pollutants including heavy metals, ammonia, and organic contaminants**.** In such habitats where the water quality is poor, the liver is prone to damage from toxic substances such as heavy metals, ammonia and other organics. If such stressors are prolonged for a long period of time, they may induce several pathologic changes such as liver cell damage, necrosis, vacuolation and disorganisation of tissue structure. Hence, liver histology has now become a well-established tool to evaluate the effects of environmental stressors and pollutants on fish, particularly those living in fluctuating aquatic habitats such as the Nile tilapia (15,16).

Histology is known as a sensitive and accurate marker in assessment of effects of environmental stressors on fish physiology, The first sign of toxicological changes may be changes at the microscopic level before any physiological problems develop (17,18). Cell structural changes such as cell destruction and degeneration are important for the study of non-lethal effects of water pollution. Thus, the histopathological examination is regarded as one of the main techniques in the aquatic toxicology used to study and evaluate the effect of pollution on the aquatic ecosystems and the biological effects of the water pollution on fish (17,9). Interestingly, the present study was attempted to determine histopathological alterations taking place in the liver tissues of Nile tilapia (*Oreochromis niloticus*) in relation to different water qualities in order to explain the working of environmental stress on their wellbeing

MATERIALS AND MTHODS

A total of forty (40) Nile tilapia (Oreochromis niloticus) specimens were collected in August 2025, during a period characterized by unusually high ambient temperatures of approximately 50 °C. The samples were divided into four groups, with each group consisting of ten (10) specimens. Water samples were collected from four main regions in Kerbala governorate, namely the north, south, west, and east sectors, corresponding to the first, second, third and fourth groups, respectively. Physicochemical water quality parameters such as temperature, pH value, salinity and total dissolved solids were analysed for all water sampling stations. Moreover, the water systems under study exhibited high ammonia concentration, variation in dissolved oxygen and turbidity, all indicating physicochemical stress in the examined habitats (see Table 1).

At each sampling site, water samples were collected in sterile glass jars, 500 mL each. The samples were promptly conserved in insulated buckets with ice to maintain their physicochemical properties. The water samples were then taken to the laboratory for physical and chemical examination. At the same time, after sampling, the ventral side of the fishes was orientated upwards and the liver was carefully dissected.

The obtained liver samples were washed in physiological saline solution to remove any blood and fixed by soaking in 10% neutral buffered formalin for sufficient amount of time.**.** All samples were subsequently transferred into properly labelled containers and processed following standard histological techniques. Paraffin-embedded sections were stained using Harris hematoxylin and eosin (H&E) and Periodic Acid–Schiff (PAS) also Masson's trichrome staining was applied. Histological slides were examined and photographed using a light microscope; the examine included a histological alterations and structural features.

Ethical approval

Under the reference number UOK.VET.AN.2025.138, this research was carried out in the anatomical laboratory of the College of Veterinary Medicine at the University of Kerbala – Iraq.

Table 1. Spatial variation pf physicochemical water parameters measured in different areas of Kerbala governorate

Sample location	North Kerbala governorate	South Kerbala governorate	West Kerbala governorate	East Kerbala governorate
Temperature (°C)	24	24	24	24
Dissolve oxygen (mg/L)	9.2	7.2	7.0	3.8
Ammonia (mg/L)	0.09	0.25	0.08	0.16
Turbidity (NTU)	9	6.4	18.8	9
pH	7.61	7.47	9.14	7.39
T.D.S (mg/L)	834	830	5772	842

RESULT

The current study of group (1) showed that the portal vein was encapsulated by thickened connective tissue involving fibroblasts infiltration (Fig. 1). Within the hepatic parenchyma, hepatocytes appeared disorganized and characterized by vacuolar degeneration (Fig. 2). Also further confirmed an expansion of parenchymal fibrosis (Fig. 3). Additional vascular changes involved the thickening of the portal vein wall (Fig. 4), which was accompanied by the interstitial deposition of glycosaminoglycans (GAGs) throughout the parenchyma (Fig. 5). However the notes of group 2) mentioned that the portal vein was surrounded by a prominent thickening of the portal connective tissue, which was accompanied by presence of fibroblasts (Fig. 6). Additionally, an infiltration of melano-macrophage cells (MMCs) was observed (Fig. 7). Histochemical examination revealed an elevated deposition of glycosaminoglycans (GAGs) within the hepatic parenchyma (Fig. 8). This was further characterized by proteinaceous infiltration in sinusoids and a notable expansion of parenchymal fibrosis (Fig. 9). Furthermore the current investigations of group (3) noticed that the hepatic parenchyma exhibited widespread hydropic degeneration (Fig. 10). This cellular swelling led to significant mechanical pressure on the sinusoids, effectively narrowing their lumen. Furthermore, necrotic changes were evident characterized by hepatocyte nuclear pyknosis (Fig. 11).

These alterations were accompanied by proteinaceous infiltration within the sinusoids (Fig. 12) and a marked parenchymal fibrosis (Fig. 13). Finally our research realized that the group (4) showed the liver architecture displayed a disorganization of hepatocytes accompanied with a marked dilation of the hepatic sinusoids (Fig. 14). Moreover, significant fibrotic changes were observed within the parenchyma, alongside an evident proliferation of the peritubular connective tissue (Fig. 15).

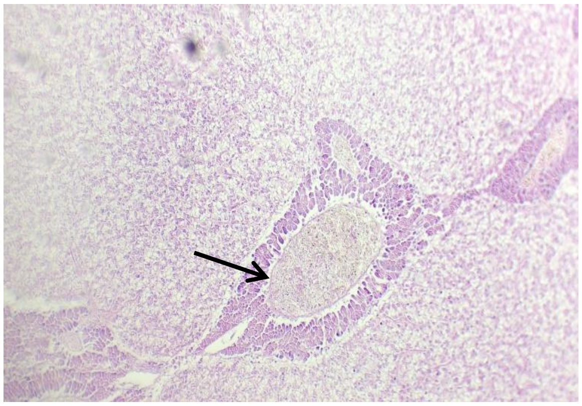

Figure 1. histological cross section of Nile tilapia liver from north Kerbala showed portal vein surrounded by thickening portal connective tissue with fibroblasts (→), (H&E, 100x).

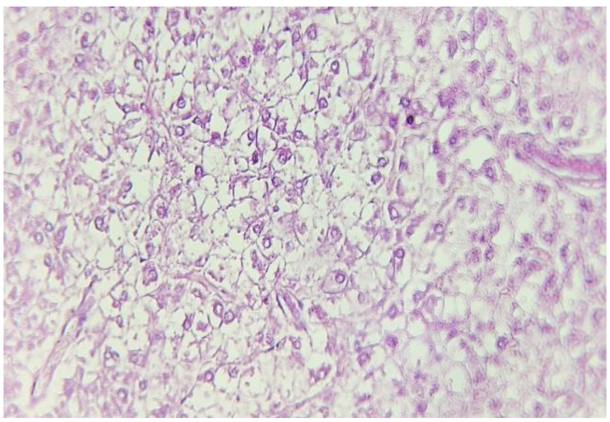

Figure 2. histological cross section of Nile tilapia liver from north Kerbala showed disorganized hepatocytes with vacuoles, (H&E, 100x).

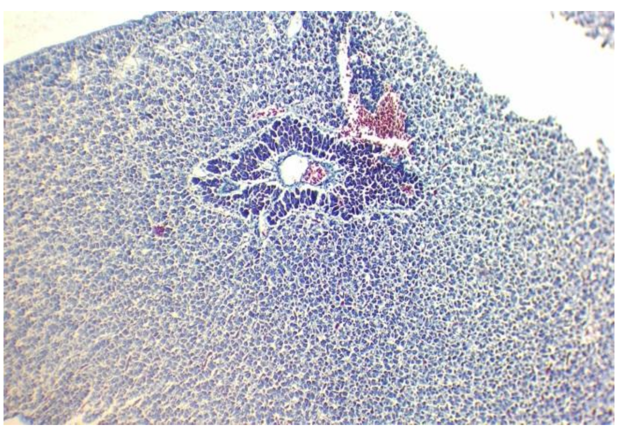

Figure 3. histological cross section of Nile tilapia liver from north Kerbala showed increase fibrosis in parenchyma of liver, (Masson's trichrome, 100x).

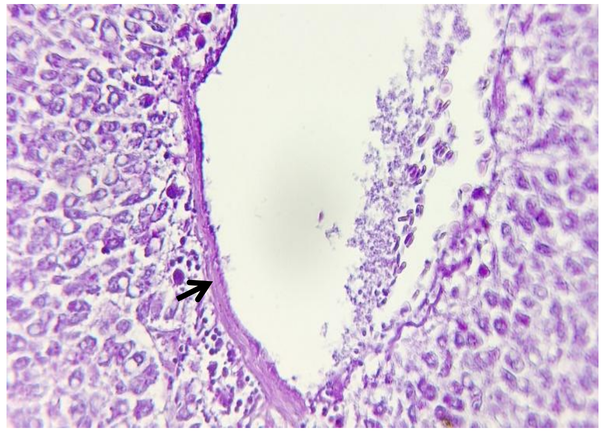

Figure 4. histological cross section of Nile tilapia liver from north Kerbala showed thickening of wall of portal vein (→), (PAS, 100x)

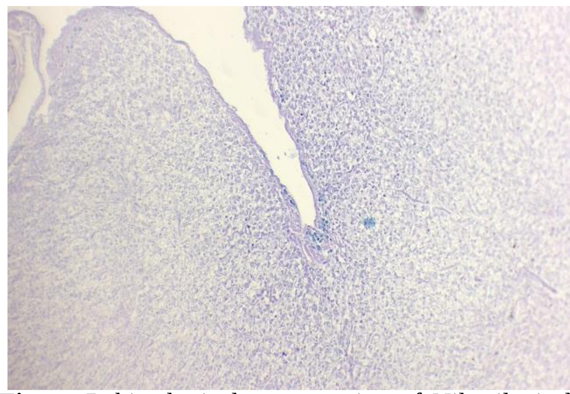

Figure 5. histological cross section of Nile tilapia liver from north Kerbala showed glycosaminoglycans (GAGs) deposition in parenchyma, (Alcian blue, 40x).

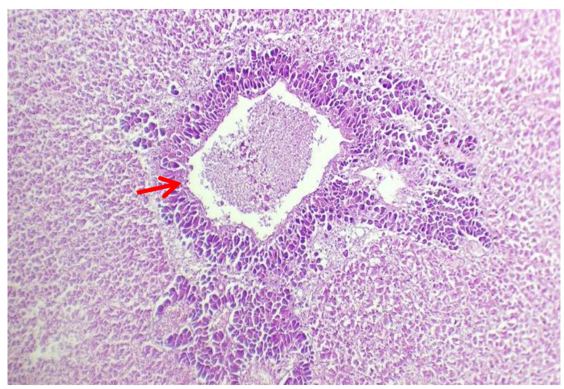

Figure 6. histological cross section of Nile tilapia liver from south Kerbala showed portal vein surrounded by thicker portal connective tissue with fibroblasts (→), (H&E, 100x)

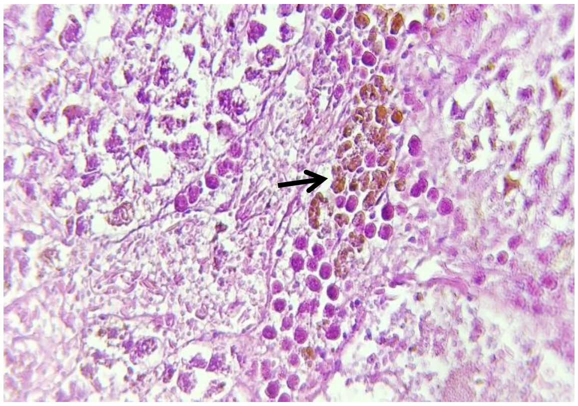

Figure 7. histological cross section of Nile tilapia liver from south Kerbala showed melano-macrophage cells (MMCs) (→), (H&E,400x)

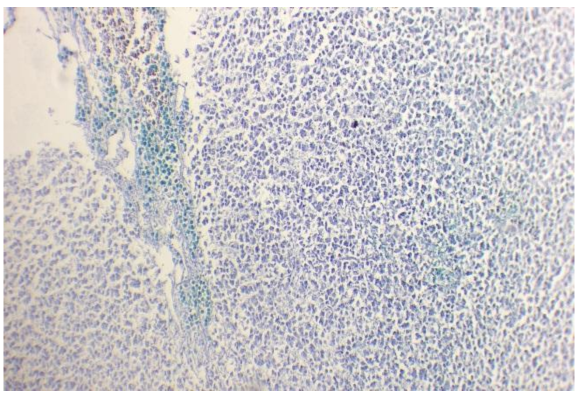

Figure 8. histological cross section of Nile tilapia liver from south Kerbala showed increase glycosaminoglycans (GAGs) deposition in parenchyma, (Alcian blue, 100x)

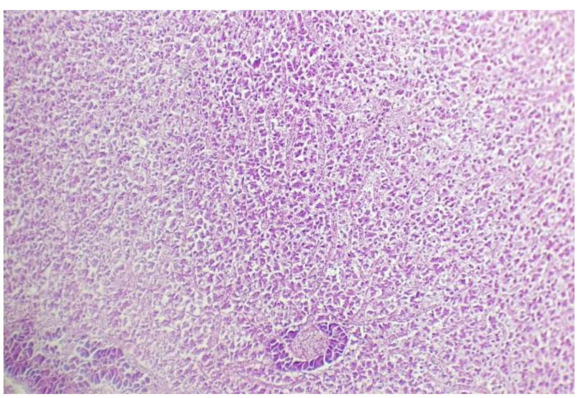

Figure 9. histological cross section of Nile tilapia liver from south Kerbala showed increase protein infiltration in sinusoids also fibrosis in parenchyma, (H&E, 100x).

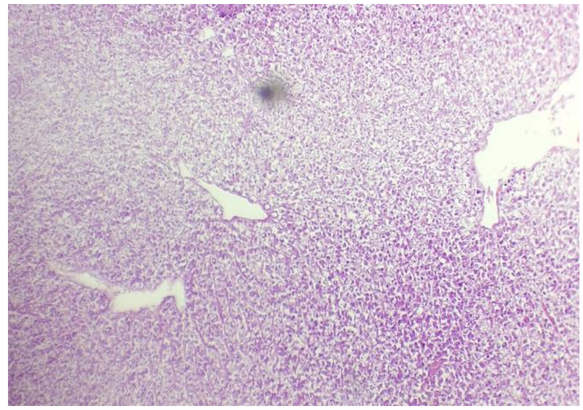

Figure 10. histological cross section of Nile tilapia liver from west Kerbala showed hydropic degeneration of parenchyma, (H&E, 100x).

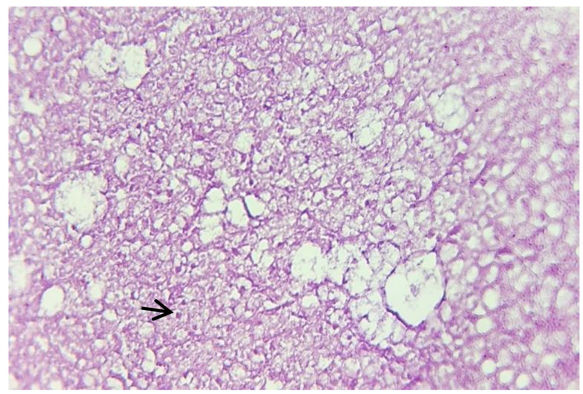

Figure 11. histological cross section of Nile tilapia liver from west Kerbala showed hydropic degeneration of hepatocytes with significant pressure on the sinusoids due to cells swelling, and necrosis appear by nucleus pyknosis of hepatocytes (→) (H&E, 100x)

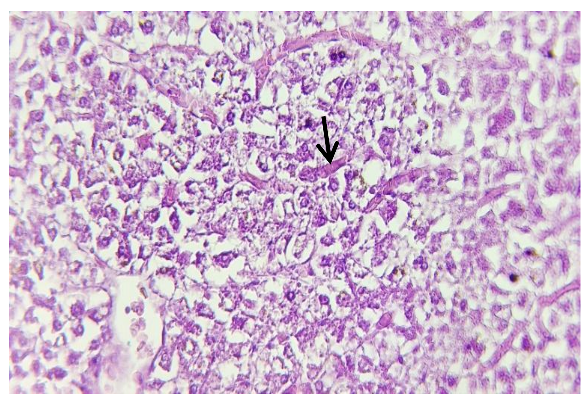

Figure 12. histological cross section of Nile tilapia liver from west Kerbala showed protein infiltration in sinusoids (→), (PAS, 100x)

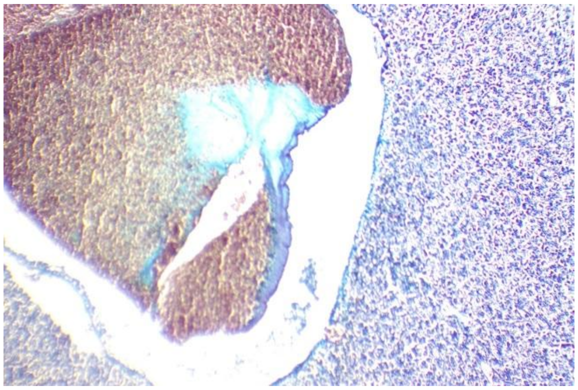

Figure 13. histological cross section of Nile tilapia liver from west Kerbala showed fibrosis in parenchyma, (Masson's trichrome, 100x).

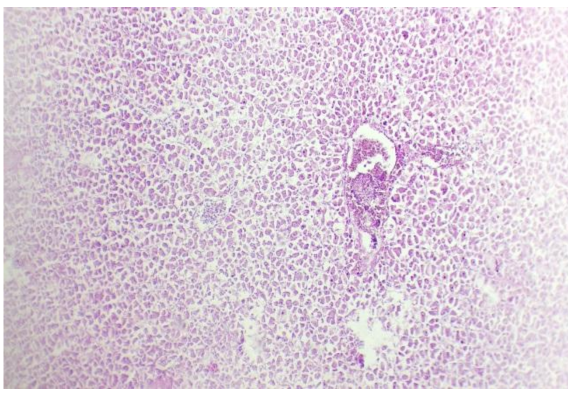

Figure 14. histological cross section of Nile tilapia liver from east Kerbala showed hepatocytes disorganization and dilation of hepatic sinusoids, (H&E, 100x).

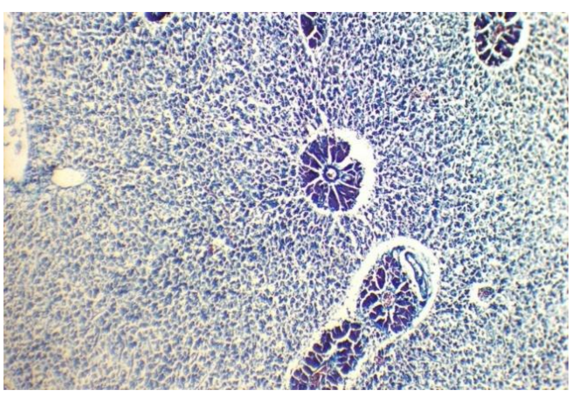

Figure 15. histological cross section of Nile tilapia liver from east Kerbala showed fibrosis in parenchyma, and increase peritubular connective tissue, (Masson's trichrome, 100x)

DISCUSSION

Group (1) exhibited disorganised hepatocytes and vacuolar degeneration with increased parenchymal fibrosis. These changes may be attributed to mild osmotic stress resulting from fluctuations in the total dissolved solids (TDS) concentration which could affect the osmoregulatory functions and cause an imbalance in ions and water content in the bodies of aquatic organisms. A similar observation has been reported by (19), where differences in TDS induce physiological stress responses in fish through the disruption of osmoregulation in fish. Furthermore, this finding is consistent with (20) where multiple stresses lead to severe changes in fish tissue (21) showed that hepatocytes degeneration, fibrosis, and disturbances in liver structure are signs of liver dysfunction in fish exposed to wastewater effluent.

While in group (2), the liver noticed key hepatic alterations in tilapia fish, including portal connective tissue thickening with increased fibroblast activity, infiltration of melano-macrophage cells (MMCs) and expansion of parenchymal fibrosis; these changes were mainly associated with elevated ammonia levels suggesting the induction of oxidative stress and chronic inflammatory responses which promote progressive tissue remodeling in liver; These results were consistent with the findings of (22) who reported that exposure of tilapia fish to polluted water caused hepatic fibrosis, immune cell stimulation and degeneration of liver tissue. These studies indicated that the histopathology changes observed in the liver tissues were consistent markers of environmental stress and pollution of the water body.

In addition, in group (3) water pH variability, high turbidity, and high total dissolved solids (TDS) were important factors affecting fish well-being. These environmental conditions cause ionic and metabolic imbalances and induce physiological stress that causes increased sensitivity of cells to injury. In this case, stress induced liver disorders such as hepatocellular necrosis and protein infiltration in the sinuses The above findings are in accordance with the work of (23) who reported that changes in pH and turbidity negatively affect fish physiology, resulting in stress.

These hepatic changes have also been shown in studies assessing the effect of environmental stress on fish liver. This study experiment observations indicated the occurrence of hepatocyte swelling, hydropic degeneration and sinusoidal narrowing. These observations were often explained as the result of osmotic and metabolic stress due to increased total dissolved solids (TDS) resulting in energy depletion and ionic regulation changes of hepatic cells. These changes are indicative of progressive cellular damage and decreased hepatic blood flow. These results were in agreement with (24) who reported that sublethal environmental stress in fish lead to hepatocellular degeneration, hydropic (vacuolar) changes and vascular disturbances and confirmed that environmental stress causes cellular and structural damage in the liver tissue.

Moreover, group (4) demonstrated severe structural alterations in the liver architecture with disorder of the hepatocytes and marked dilatation of the hepatic sinusoids indicating serious impairment of the hepatic microcirculation. Additionally, the extensive fibrotic alterations and growth of peritubular connective tissue suggested a late stage of chronic hepatic insult. These changes may be due to chronic exposure to environmental stressors, especially persistent hypoxia and poor water quality, resulting in continual tissue damage and irreversible structural remodeling of the liver. This observation was in agreement with (25) who reported that hypoxia induces physiological, tissue and cellular responses in fish, leading to structural and functional changes in vital organs including the liver, The results were also in agreement with the findings of (20,21), who demonstrated that prolonged environmental stress induces severe hepatic architectural derangement and fibrosis in fish.

CONCLUSION

In the liver, signs of progressive destruction of tissue were observed. It was manifested by destruction of hepatocytes, fibrosis, necrosis and activation of melanomacrophages. This means an interference with metabolism and detoxification. Ammonia was identified as one of the main factors in terms of tissue injury when high temperature and low levels of dissolved oxygen occurred.

The variations in pH and high levels of TDS seemed to increase osmotic disturbances and to amplify the negative effect of ammonia on the liver tissues.

The histopathology results indicated that the liver reactions were adaptive or degenerative depending on the severity and duration of the environmental conditions.

Generally, poor water quality has a significant negative effect on the structure and function of liver tissue.

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