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Histological and Histochemical Studies on the Liver of Nile Tilapia (*Oreochromisniloticus)* at Different localities of Delta Barrage, Egypt

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THE aquatic environment is particularly suffered from the various contaminants due to industry, urbanization and agriculture activities. The present study was carried out to study histological structural of liver in the freshwater fish, *Oreochromisniloticus*, which collected from different localities and exposed to different pollutants. Histological, histochemical, and Immunohistochemical alterations were examined in liver .Samples from El-Riah El-Menoufy and downstream of El-Rahawy area, indicated that, the Last one is more suffered from many histopathological changes than those collected from El-Qanater El-Khyria.As well as, a decrease in carbohydrates distribution, an elevation of different types of fiber and a strong caspase-3 expression are detected. The histopathological damages were related to the discharge of water loaded with sewage in El-Riah El-Menoufy and El-Rahawy drain, which consequently may affect fish and human health. The present study also investigated the concentrations of some heavy metals in river water (iron, copper, zinc and cadmium) and their effects on Nile Tilapia obtained from the same investigate sites. Accordingly, there is a necessity to treat the sewage effluents before discharging to the Rosetta branch.

Keywords: Liver, Histopathology, Immunohistochemistry, Heavy metals, Nile Tilapia.

Introduction

Water pollution is one of the major crises that the world faces today. Various pollutants can find their way to water bodies through industrial, agricultural and household wastes, as well as water management interventions led to most changes in Nile water quality especially in industrial centers of Cairo and the Delta region [1]. Pollution is the presence of foreign materials that negatively affect the environment. Some of these pollutants have sometimes reached the human system through the food chain. Some of these contaminants resist the chemical and biological transformation of the body and then accumulate and cause toxicity in various body tissues including liver, kidneys and nerves [2]. Water pollution resulted in harmful effects on human health, fish activities and water quality [3].

Three main sources of pollution affect the water quality of Rosetta branch, (a) El-Rahawy drain which receives all sewage from El-Giza governorate in addition to agricultural and domestic wastes of the El-Rahawy village which is few kilometers from El-Qanater El-Khyria [4]., (b) Kafr El-Zayat industrial area, which include the industrial effluents from the factories of super phosphate and sulfur compounds, oil and soap industries and pesticides factories, (c) Several small agricultural drains that discharge their wastes into the Nile branch in addition to sewage discharged from several cities and its neighboring villages that are distributed along the two banks of the Rosetta branch [5].

Several studies were carried out on the effect of different types of pollution on the water characteristics of the River Nile. Water in the

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downstream El-Rahawy drain location is suffering from chemical pollution. The physico-chemical characteristics of water in this location showed; a sharp increase in biological oxygen demand, ammonia and dissolved solids, and great decrease in dissolved oxygen [6]. Unfortunately, more than $5x10^{8}$ cubic meter daily including domestic and agriculture wastes were discharged from El-Rahawy drain into Rosetta branch and lied 30 Km north to Cairo at El-Qanater El-Khyria, Egypt [7].

The contamination by heavy metals is an important concern, which affectsevery living organism. They are non-degradable, persistence pollutants and caused many physiological alterations in high concentration. Also they have bio-accumulative nature [8,9]. The discharge of domestic waste waterwhich contains high concentrations of metals such as: iron, manganese, copper, zinc, and lead (Fe, Mn, Cu, Zn and Pb) are the primary source of heavy metals in water [10]. Heavy metals caused acute disorders in aquatic organisms and caused various pathological disorders. Also, they caused hypertension, sporadic fever, renal damage, cramps in human through food chain [2].

Heavy metals have a potential threat to many organisms which may be attributed to their high toxicity. As well as aquatic organisms including farmed fish, have the ability to accumulate these metals in their tissues, either directly from the ambient water or by ingestion of food, and then become potentially toxic, especially, when this accumulation increases to a considerably high level [11].

Fresh water fish mainly absorbed waterborne metals through their gill epithelia then entered the blood circulation to reach other organs and accumulated most significantly in the kidney, followed by liver and gills [12].

The present study was carried out to study the structural and functional indicators of liver in the freshwater fish *Oreochromis niloticus*, which exposed to different levels of water quality.

Materials and Methods

Sampling Sites

About 25 Nile Tilapia were collected from three stations in the River Nile at summer 2018; El-Qanater El-Khyria before bifurcation, El-Riah El-Menoufy which receives the sewage wastes of women's dungeon and downstream of El-Rahawy drain after 5 km at El-Rahawy drain which receives sewage from El-Giza governorate, as well as, mixture of agricultural and domestic wastes. The experimental procedures were approved by the animal ethical committee in accordance with the guide for care and use of laboratory animals (Approval No. MUFS / F / HI / 2/ 19).

Determination of Heavy metals in water

Fifty water samples were collected in the same time of the season with histological fish samples to avoid any other environmental factors, the effect of the external factors on fish so that the effect that was determined was mainly due to the changes in the water quality. Sampling was performed in summer, because it is the most active season in the rate of growth in fish. Water samples for the determination of heavy metals were collected in cleaned plastic bottles of 1L capacity from the three sites under this study, preserved with 5 ml concentrated nitric acid, on the spots and stored in refrigerator. Iron, copper, zinc and cadmium ions (Fe, Cu, Zn and Cd) were determined in water by using atomic absorption model (Perkin Elmer, 3110, USA) with graphite atomizer (HGA-600), according to the method described by APHA [10].

Histological preparation and examination

Liver samples were collected from *Oreochromis niloticus* from El-Qanater El-Khyria, El-Riah El-Menoufy and downstream of El-Rahawy drain immediately after fish dissection. Liver was fixed in 10 % formalin for 24 hours. The fixed samples were washed, dehydrated, and finally embedded in paraffin wax. Sections were cut at 5 µm thickness using rotary microtome. Sections were mounted on clean slides, and stained with hematoxylin and eosin according to Bancroft and Stevens [13].

Histochemical Studies

Special stains were applied for chemical localization of carbohydrate and collagen in the liver. Paraffin sections were treated with Periodic acid-Schiff's reaction (PAS) for demonstration of carbohydrates [14]. As well as, Masson›s Trichrome stain [15] stain was used for demonstration of collagen, and Alcian Blue stain with Periodic acid–Schiff (PAS) for acid polysaccharide substances according to Bancroft and Stevens [15].

Immunohistochemical Study

Caspase-3 (Cysteine-aspartic proteases) is an indicator for the occurrence of apoptosis. Immunohistochemical variations were observed in liver sections by using suitable antibodies of anti-caspasestain. Immunohistochemical detection of

caspase-3 was performed using 5-µm, formalin-fixed, paraffin-embedded sections using caspase 3 antibodies at 1:50 dilution (DAKO, Carpinteria, CA). The avidin–biotin complex technique was used. Antigen retrieval was performed in all cases by steam heating the slides in a 1-mmol/L solution of EDTA (pH 8.0) for 30 minutes. After blocking of endogenous biotin, staining was performed using an automated immunostainer (DAKO) followed by detection by using a streptavidin-biotin detection system (DAKO). Positive reaction for Caspase-3 was visualized as brown coloration of the cytoplasm of the in testicular cells, Negative controls were done using the same steps except that phosphate buffered saline was applied instead of the primary antibodies [16].

Statistical analysis

Using one-way analysis of variance (ANOVA) test for the present data analysis.All values were expressed as mean ± standard deviation (Mean ± SD). Differences were regarded as significant if probability value $p< 0.05$ and highly significant if $p<0.001$.

Results

The present results showed that the heavy metal element concentrations in River Nile were mainly within the permissible limits according to e.g. Egyptian law (48/1982) and criteria by FAO (1985) [17]. At the same time, agricultural and industrial activities are mainly responsible for elevated concentrations of the measured elements (Fe, Cu, Zn and Cd) in the water. The highest concentrations were at discharge point of El-Rahawy drain and lowest values were at El-Qanater El-Khyria, the data were expressed in mg/l(Fig.1 and Tab.1).

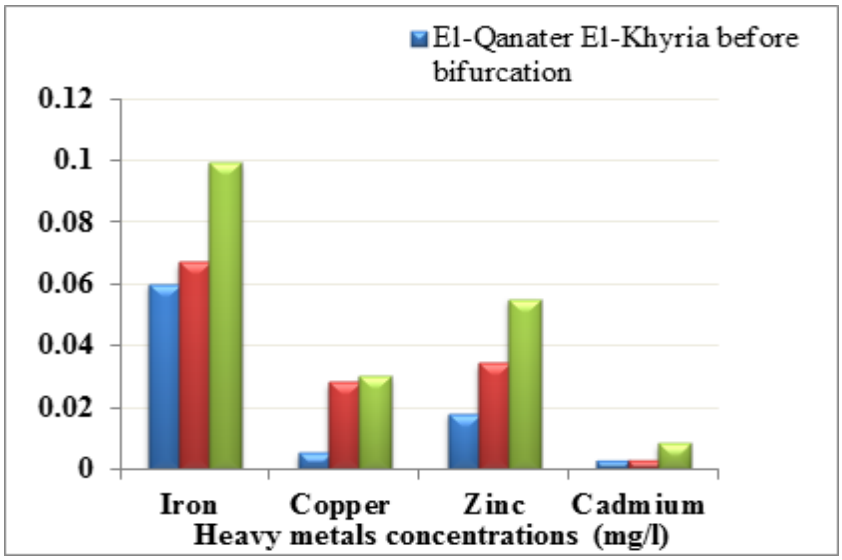

Fig.1. Different heavy metals concentrationsin water samples collected from three different locations along delta barrage, Egypt.

TABLE 1. Statistical analysis (ANOVA) for heavy metals tests (mg/l) in the different sites

Heavy metals / Sites	Iron concentration	Copper concentration	Zinc concentration	Cadmium concentration
El-Qanater El-Khyria before bifurcation	0.0602	0.0054	0.0179	0.0028
El-Riah El-Menoufy	0.0671	0.0283	0.0345	0.0029
Downstream of El-Rahawydrain	0.0992	0.0302	0.0551	0.0085

ANOVA: analysis of variance; Each value is represented as means ± SD: standard deviation.

Histopathological findings of Liver

The microscopical examinations of Tilapia livers revealed several histopathological changes in hepatic tissue as shown in our study in different water quality.

Liver of healthy Nile tilapia (*Oreochromis niloticus*) consists of hepatocytes that arranged in branched laminae and separated by sinusoids. Hepatocytes are polygonal cells with a homogenous cytoplasm and a central spherical nucleus with a densely stained nucleolus. They were located among sinusoids forming cord like structure known as hepatic cell cords. Blood flows from branches of hepatic portal vein and hepatic artery through the sinusoids to central veins which empty into the hepatic vein. The pancreatic structure was observed in association with venous vessels. Nile tilapia collected from El-Qanater El-Khyria before bifurcation showed a liver architecture with slight pathological abnormalities (Fig.2a). Nile tilapia fish collected from El-Riah El-Menoufy showed degeneration and hemorrhage in both hepatic and pancreatic structures, necrosis and cytoplasmic vacuolation in hepatocytes (Fig.2b). While, the hepatic parenchyma of Nile tilapia fish collected from downstream of El-Rahawy area, which suffered from the highest pollution degrees of water in recent study, showed severe histopathological changes than those obtained from El-Riah El-Menoufy such as; mild eosinophilia, diffuse fatty change, congestion of hepatic sinusoids, hemorrhages in pancreatic structures, severe necrosis including pyknosis of nuclei, patchy degeneration with edema and leucocytic infiltration (Figs.2c&d).

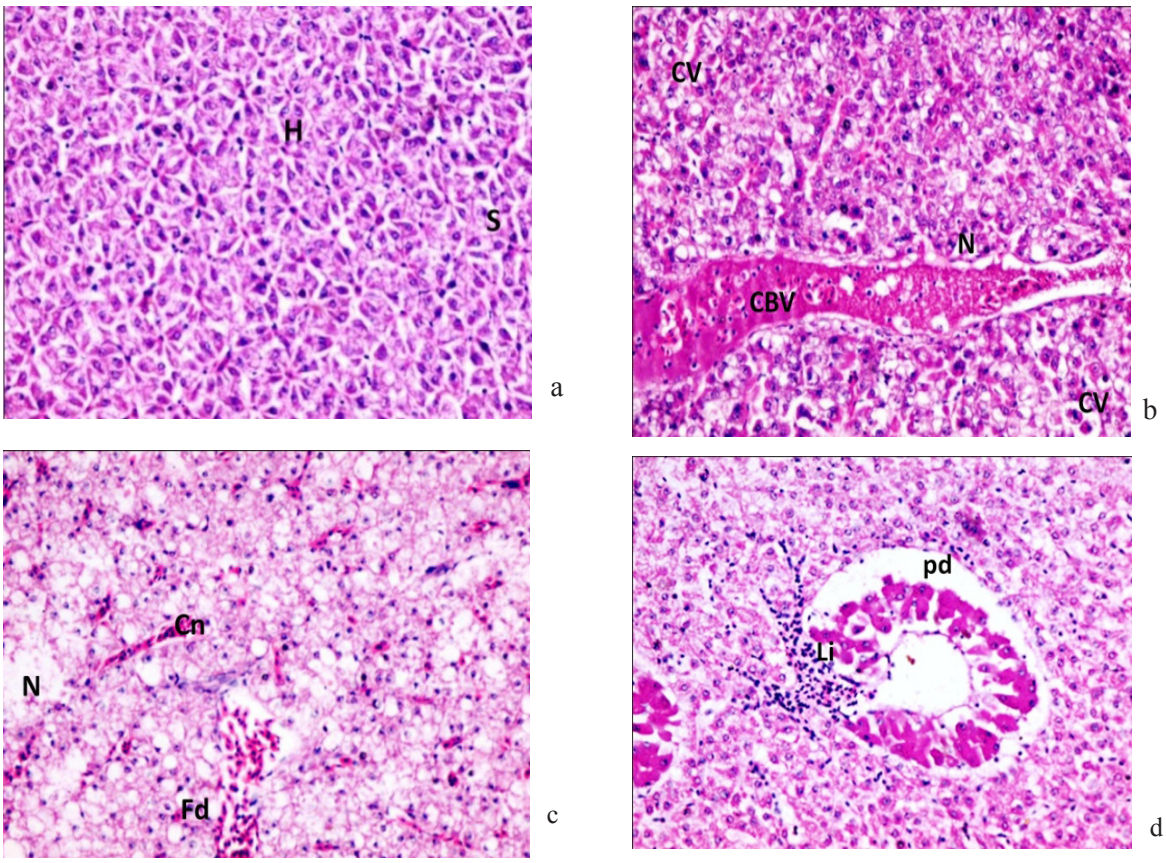

Fig. 2 (a-d). Liver sections of *Oreochromisniloticus* obtained from (a) El-Qanater El-Khyria showing hepatocytes (H), blood sinusoid (S); (b) El-Riah El-Menoufy showing severe congested blood vessel (CBV), necrosis (N) and cytoplasmic vacuolation in hepatocytes (CV); (c&d) downstream of El-Rahawy area showing mild eosinophilia, diffuse fatty change (Fd) and congestion of hepatic sinusoids(Cn) in blood sinusoids, severe necrosis (N), pyknosis of nuclei, patchy degeneration and edema (pd) and leucocytic infiltration (Li), H&E. × 400.

Histochemical observations

The present study indicated reduction of carbohydrates in liver and an increase of different types of fibers in blood sinusoids and around blood vessels of fishliver from downstream of El-Rahawy area more than the other two sites.

Reddish or magenta granules were observed in the cytoplasm and basement membrane of hepatic cells of Nile Tilapia when liver sections were reacted with Periodic acid Schiff's, which indicated the presence of carbohydrate. However,they appeared as a large number of different sizes reddish or magenta granules with PAS-negative stained nuclei indicating absolute lack of glycogen (Fig. 3).

Masson›s Trichrome Stain was used to differentiate between collagen and smooth muscle in liver, nuclei stained black, Cytoplasm, muscle and erythrocytes stained red, Collagen stained blue (Fig. 4).

Alcian Blue with PAS indicated the presence of acid polysaccharides substances in blood vessel walls. Alcian blue reacts with both sulfated and carboxylated acid polysaccharides and glycoproteins and produces blue colorin cytoplasm and reddish pink nuclei (Fig. 5).

Immunohistochemical observations

Immunohistochemical examination showed nearly negative caspase-3 expression in fish obtained from El-Qanater. Expression of caspase-3 is appeared as brownish color in the cytoplasm. The highest expression of caspase-3 was in the cytoplasm of hepatic cells of fishes obtained from El-Rahawy drain (Fig. 6).

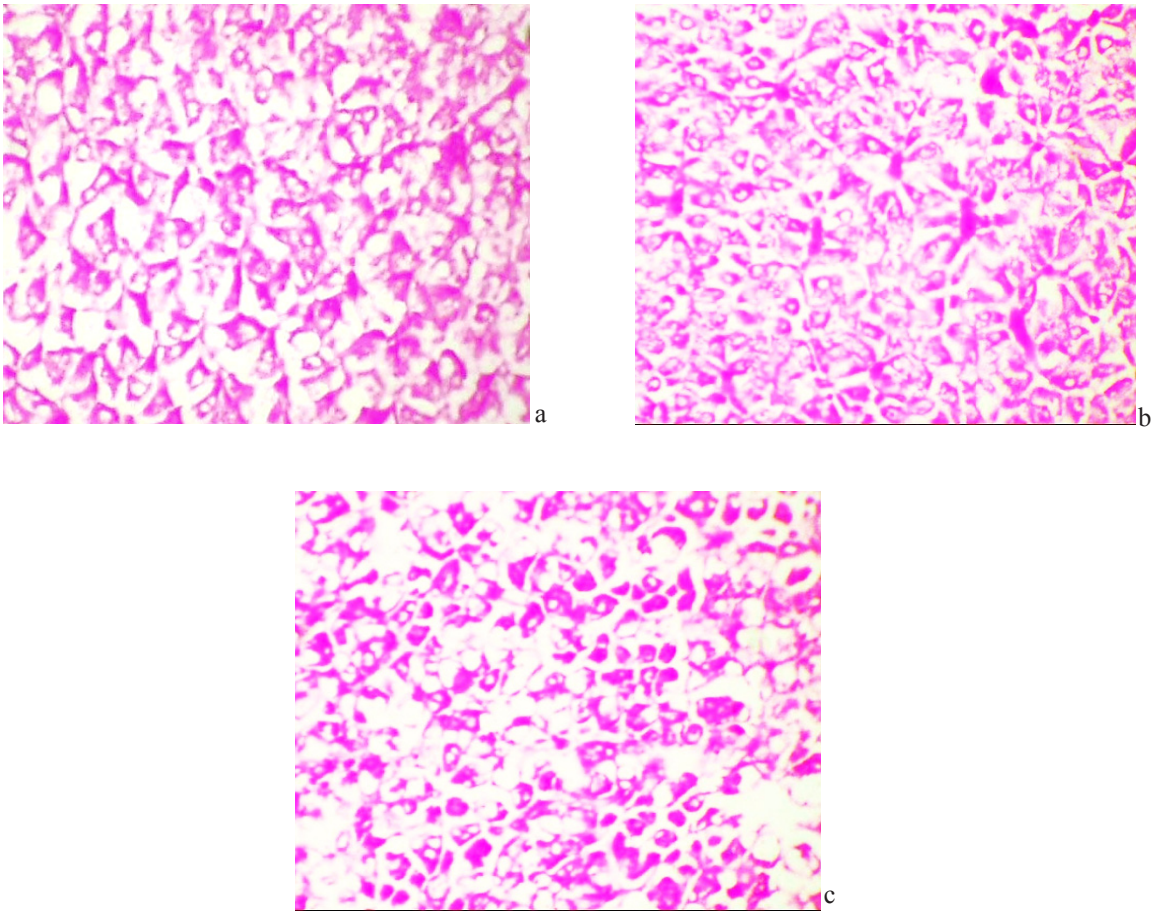

Fig. 3 (a-c). Liver sections of *Oreochromisniloticus* obtained from (a) El-Qanater El-Khyria, (b) El-Riah El-Menoufy and (c) downstream of El-Rahawy area showing the distribution of carbohydrates in cells cytoplasm, Periodic acid Schiff's × 100.

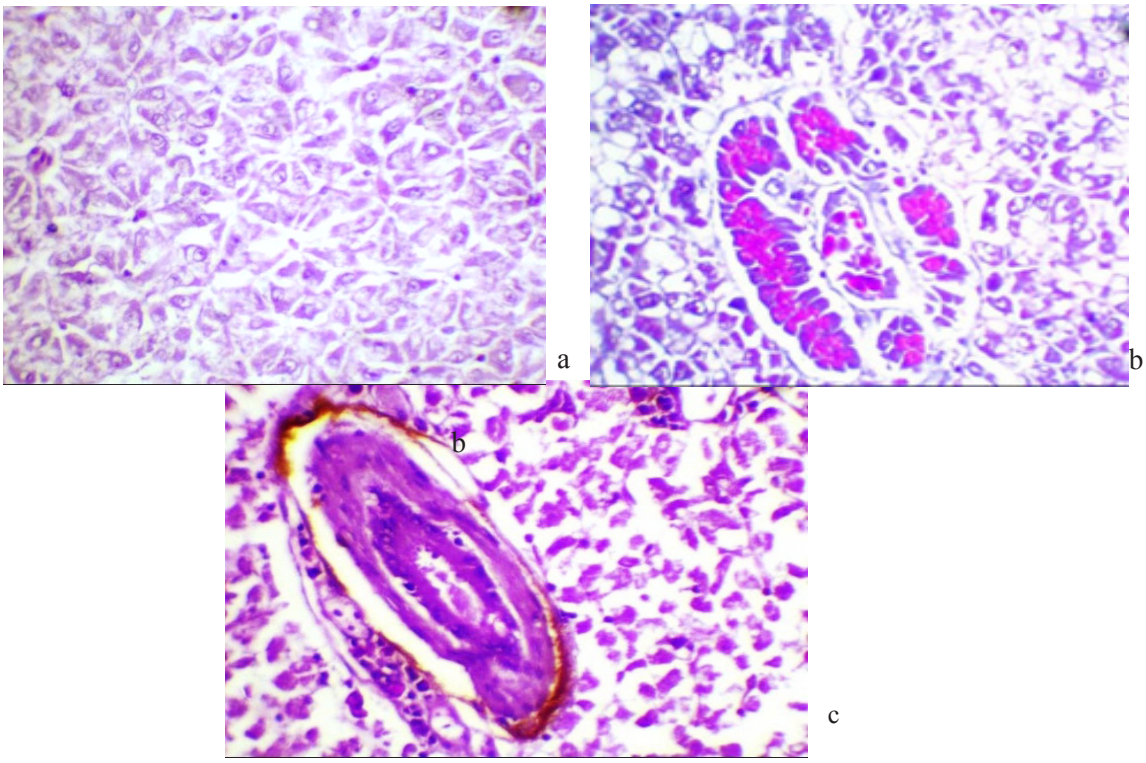

Fig. 4 (a-c). Liver sections of *Oreochromisniloticus* obtained from (a) El-Qanater El-Khyria, (b) El-Riah El-Menoufy and (c) downstream of El-Rahawy area showing the distribution of collagen fibers, Masson's Trichrome × 100.

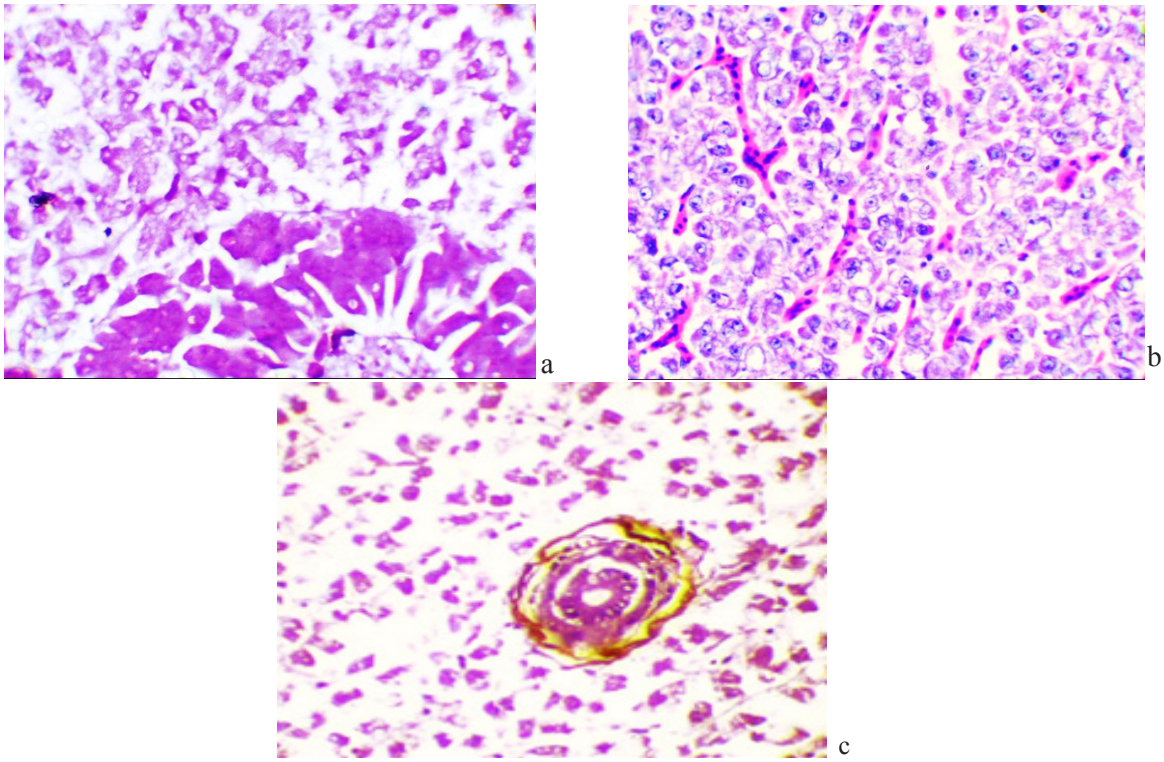

Fig. 5 (a-c). Liver sections of *Oreochromisniloticus* obtained from (a) El-Qanater El-Khyria, (b) El-Riah El-Menoufy and (c) downstream of El-Rahawy area showing the distribution of acid polysaccharides substances, Alcian Blue and Periodic acid Schiff's × 100.

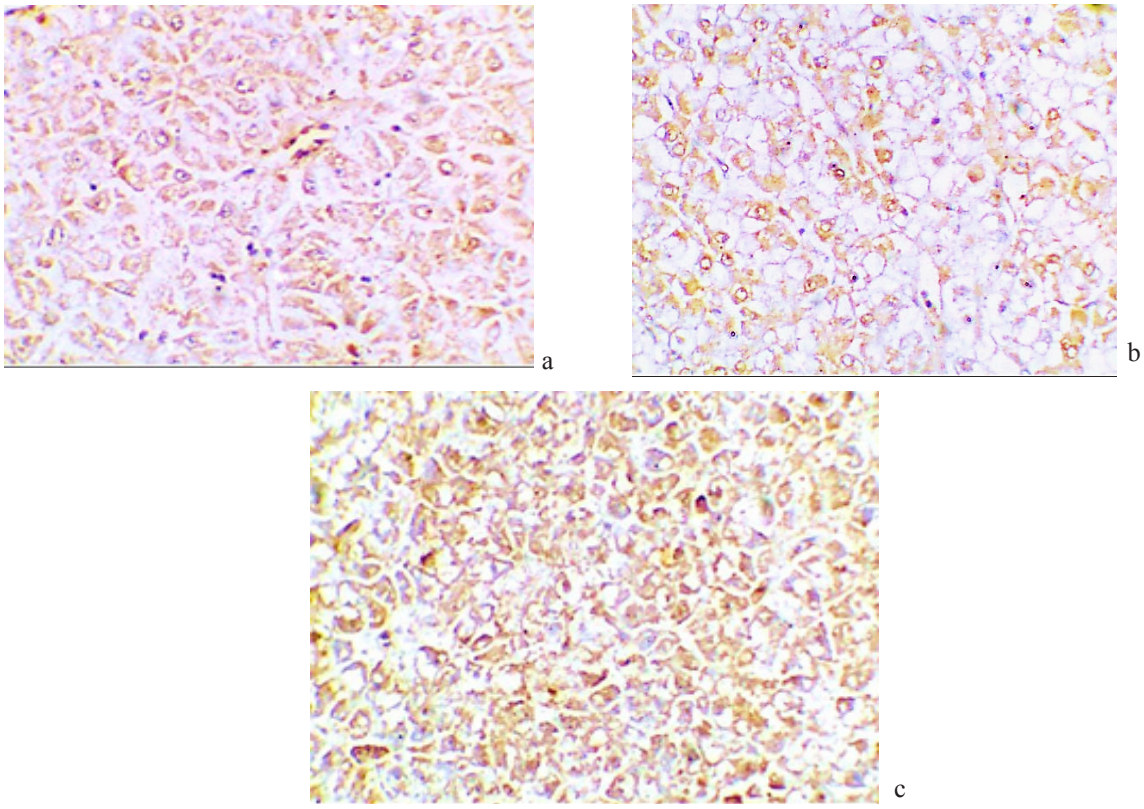

Fig. 6 (a-c). Liver sections of *Oreochromisniloticus* obtained from (a) El-Qanater El-Khyria, (b) El-Riah El-Menoufy and (c) downstream of El-Rahawy area showing the expression of caspase-3 as brownish color in cells cytoplasm, IHC × 100.

Discussion

Fish represents an important target for magnifications of heavy metals as they are at top of the food chain and act as a possible transfer media of many pollutants to human. Heavy metals are accumulated mainly in fish liver as the main metabolic organ;it stores metals for detoxification through producing metallothioneins[18]. The histological studies are used as biomarkers for evaluating toxic effects of different water pollutants on fish and useful for establishing water quality criteria [4, 19].

In the present study, histological alterations of liver were more evident in fish obtained from downstream of El-Rahawy area, which were exposed to the highest heavy metals concentrations.The histological examinations of liver of Nile tilapia (*Oreochromisniloticus*) showed that the changes in water quality at El-Riah El-Menoufy and downstream of El-Rahawy drain had negative impact on histology of the collected fishas a result of pollution stress in these areas. Several histopathological alterations of liver were found including; degeneration,necrosis, hemorrhage, mild eosinophilia, edema and leucocytic infiltration. These damages were related to the discharge of water loaded with sewage in water, which may effect on fish production and consequently on human health. So, it is necessary to treat the sewage effluents before discharging to the river. Also, the highest concentrations of heavy metals (Fe, Cu, Zn and Cd) were detected at discharge point of El-Rahawy drain and lowest values were at El-Qanater El-Khyria as a result due to the presence of agriculture and domestic sewage into the River Nile in that location [20].

Several studies had investigated the effects of pollutants on the histological structure of kidney, liver, muscle and spleen of fish [21-24].

This work confirmed that, quality of water has been presented many changes; some of heavy metals are essential elements in the bodies of living organisms when it present in trace amounts and became toxic with high concentration, this work indicated an elevation of heavy metals concentrations in water, which caused many

alterations on the fish tissues. Similarly, Elewa [7] reported that, the breakdown of organic matter and dead microorganisms increased the concentration of heavy metal in the water. The effect of heavy metals depends on metal concentration, time of exposure, way of metal uptake and environmental conditions such as; water temperature, pH, hardness and salinity[3,19]. Most of these metals accumulated mainly in liver, kidney and gills. While, fish muscles, compared to the other tissues; usually contained the lowest levels of metals [25].

Heavy metals caused many pathological conditions of the fish tissues including kidney, spleen and muscle (26]. Thus, heavy metals induced oxidative stress and damage to living tissues in animals and humans, furthermore, their accumulation caused intensive lesion to mucus tissues, affecting the intestine and skeleton [27].

In the current study, histochemical observations revealed a reduction of carbohydrate contents and an increase of collagen acid polysaccharides substances in liver of fish obtained from El-Riah El-Menoufy and downstream of El-Rahawy drain. Similarly, many histopathological alterations were induced by pollutants and heavy metals caused intensive lesion to mucus tissues in both animals and human [27]. Similarly, a reduction in the distribution of carbohydrates and an increase of collagen amount in the gill and liver in one of the most important fish in Egypt due to different degrees of water salinity [28].

Increased expression of caspase-3 in most of the hepatic cells of samples obtained from El-Riah El-Menoufy and downstream of El-Rahawy drain were observed in the present study, which is an immune-marker of apoptosis [29]. The histochemical, immunohistochemical results were in accordance with the histological one.

The present study investigated the concentrations of some heavy metals in river water (iron, copper, Zinc and cadmium) and their effects on Nile Tilapia obtained from the same investigates sites. So, it is necessary to treat the sewage effluents before discharging to the Rosetta branch.

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Conflict of interest: None

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التغيرات النسيجية والكيميانسيجية في كبد البلطي النيليOreochromisniloticus من منـاطق مختـلفة من دلتـا القنـاطر (مـصر)

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تعاني البيئة المائية بشكل خاص من الملوثات بسبب الأنشطة الصناعية والزراعية المختلفة. أجريت هذه الدراسة لدراسة المؤشرات التركيبية والوظيفية للكبد في أسماك بلطي المياه العذبة، والتي تتعرض لمستويات مختلفة من جودة المياه. حيث أظهرت الدراسة أن الأسماك من منطقة الرياح المنوفي ومجرى منطقة الرحاوي تعاني من العديد من التغيرات النسيجية المرضية أكثر من تلك الأسماك التى تم تجميعها من منطقة القناطر الخيرية وكذلك اظهرت هذه الأسماك انخفاض في توزيع الكربوهيدرات وزيادة أنواع مختلفة من الألياف، وظهور قوي للكاسباز3. وكانت الأضرار النسيجية الظاهرةتتعلق بتصريف المياه المملوءة بمياه المجاري في مصرف الرياح المنوفي والرحاوي ، مما قد يؤثر بالتالي على الأسماك وصحة الإنسان. كما تم دراسة التركيزات لبعض المعادن الثقيلة في مياه النهر(الحديد والنحاس والزنك والكادميوم) وتأثيرها على أسماك البلطي النيلي التي تم الحصول عليها من مواقع الدراسة نفسها. لذلك، من الضروري معالجة مياه الصرف الصحي قبل التصريف إلى فرع رشيد.