Histopathological changes in some tissues of Tigris river fishes, exposed to petroleum hydrocarbons, Baghdad city

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Summary

During the period between January till December 2012, a total of 119 fish samples were collected and examined from Tigris River between Al-Jadiriya Bridge and Al-Zaafaraniya region in two stations, the first station was located under Al-Jadiriya bridge and the second station was located at Al-Zaafaraniya region before Diayla river in order to know the effects of petroleum hydrocarbons in the tissues of the examined fishes. Fish samples belonged to (16) species (*Acanthobrama marmid*, *Albernus caeruleus*, *Aspius vorax*, *Barbua belayewi*, *B. grypus*, *B. xanthopterus*, *Carassius carassius*, *Carasobarbus luteus*, *Cyprinion macrostomum*, *Chondrostomua regium*, *Gara ruffa*, *Heteropneustes fossilis*, *Leuciscus cephalus*, *Liza abu*, *Mystus pelusius* and *Tilapia zillii*). The histopathological examination included 44 specimens from the internal and external organs of *Chontrostoma regium* in order to examine the histopathological changes in some organs like (gills, muscles, kidney, liver and spleen). The results revealed congestion, edema, separating, severe vacuolation and dilation of gill secondary lamella, while muscle samples showed mild hyalinization, infiltration of mononuclear cells and fragmentation. The kidney showed hydropic degeneration, necrosis changes, hyperplastic of melanomacrophages, severe dilation of blood vessels with cellular swelling of renal tubule epithelial lining and depletion of hemopoiotic tissues. Severe dilation and congestion of blood vessels and sinusoid, nucleopleomorphism were noticed with nucleomegalocytic of hepatocytes, hemorrhagic with mineral deposition in hepatic parenchyma. Lymphoid depletion in white pulp and hemopoitic tissues, congestion of red pulp, severe hyperplasia were noticed with dilation of splenic tissues. Water samples from Tigris river were analyzed for measuring the level of petroleum hydrocarbons in water and appeared that the pollution 0.0048 mg/L in Al-Jadiriya station and 0.0674 mg/L in Al-Zaafaraniya station.

Keywords: Histopathological changes, *Chontrostomua regium*, Tigris River, Petroleum hydrocarbons, Baghdad city.

Introduction

Pollution is a common problem in Iraq due to limited water supplies, especially when much of the available water originates from neighbor countries. Petroleum hydrocarbons are usually present in Iraqi rivers due to manufacturing processes and waste from loading ships (1). Aquatic environment was polluted by oil on a daily basis from oil spills, routine shipping, run-offs and clearance. Oil pollution is a growing problem and cause very localized problem but can be catastrophic to local marine wildlife such as fishes, birds and sea otters (2 and 3). Petroleum hydrocarbons is one of the important environmental pollutants in ecosystem because, they are toxic to most living organisms, carcinogenic to other and have the ability to transport through the food chain (4). Fishes may appear more tolerant to hydrocarbons owing to the mucous membrane surrounding their bodies which prevent absorption of these chemicals, but their early life stages can be severely affected. Adult fish, however, will die when exposed for long time to hydrocarbons (5). There were two pathways by which petroleum hydrocarbons can be obtained by fishes, firstly via the gills (clogs the gills of some fish species and respiration becomes impaired) and secondly via the gut by ingested food (6). Petroleum hydrocarbons have been reported to cause a variety of histopathological alterations in fishes (7). Histological biomarkers were used for assessing the effect of petroleum hydrocarbons in aquatic organisms (8). Histological studies were a rapid method for detection of pollution effects on various tissues of fishes and it has been extensively used to determine the

deleterious effects of hydrocarbons (9). This study was carried out in Tigris River before Al-Durah power plant (DPP) near Al-Jadiriya Bridge till Al-Zaafaraniya region. The present work was aimed to study the effect of petroleum hydrocarbons exposure in the different tissues of fishes (*C. regium*).

Materials and Methods

During the period from January till December 2012, a total of 119 fish samples were collected from Tigris River between Al-Jadiriya Bridge and Al-Zaafaraniya region in two stations, the first station was located under Al-Jadiriya Bridge and the second station was located at Al-Zaafaraniya region before Diyla River. Fish samples belonged to 16 species, they were classified according to (1 and 10). According to fishermen, the samples were caught by gill nets and cast nets. Five to ten fishes were collected weekly, these samples were transferred alive or freshly dead to the research laboratory in (Fish and Animal Resource Center/ Agriculture Research Director/ Ministry of Science and Technology) by plastic containers. Fishes were examined as soon as possible after killing them by pithing method. Total and standard lengths were taken and fishes were weighted by balance, from these samples 44 fishes of Balout Al-Maloky *C. regium* for histological preparation were taken.

Preparation of histological sections: A piece of tissues from organs of fishes exposured to hydrocarbons (gills, muscles, kidney, liver and spleen) were taken for preparation of histological sections according to (11). Formalin solution 10% was used for fixation for 24-48 hours. The fixed samples were washed by water for 30 seconds. The samples were placed in ethyl alcohol (70, 80, 90 and 100%) for two hours for each concentration except for 100% which was left over night. The samples were cleared by xylene A and xylene B for 0.5 hours to each one. Paraffin wax with a melting point of 54-56 °C was used by putting them in the oven 60 °C for three hours. The samples were embedded in the blocks with few amount of glycerin. The liquid wax was poured in these blocks and left in a cold climate until become solid. Afterwards, the blocks were put in the ice box. The samples were sectioned with the Rotary microtome with a thickness of 4-6 µm. The tissue slices were put in water bath, then on the slide. The slide was put in the oven 70 °C to remove the additional wax. Then the slide was left in front of an air-conditioner for 24-48 hours. The staining was carried out by using the haematoxylin and eosin stain technique (12). Stained mounted sections were examined under light microscope and Photographs were taken at x40 magnification of microscope using the camera at 50mm focal length.

Determination of total petroleum hydrocarbonate: The method by (13) was used and the procedure is: One hundred ml of sample was extracted with 45 ml Diethyl ether 99% (C4H10O) made by Fluka Guarantee, after shaking. The upper solution was collected and put in a dry and weighted conical flask. To evaporate all solvents the mixture to stand for one hour, then prepare blank composed of all the materials used except the sample. The results obtained by using the equation below. The unit used is mg/L.

$$\boldsymbol{T.H.C.mg/L} = (\boldsymbol{Remeaning\ weight\ of\ sample} \times \mathbf{1000}) - (\boldsymbol{Remeaning\ weight\ of\ blank} \times \mathbf{1000}) / (\boldsymbol{Sample}\ (\mathbf{100}\ \boldsymbol{ml})$$

Results and Discussion

Throughout the period from January till December 2012, a total of 119 fish samples were collected from Tigris River between Al-Jadiriya Bridge and Al-Zaafaraniya region, Baghdad city. The range of total length was 16-41 cm. the range of weight was 62-1300 gm. The water samples from Tigris River were analyzed and the pollution was 0.0048 mg/L in Al-Jadiriya station and 0.0674 mg/L in Al-Zaafaraniya station.

Grossly the skin of exposed fishes showed hemorrhagic foci with increase of mucous secretion. Also, redness and round areas on the base of fins were noticed. Gills of exposed fishes presented hemorrhagic areas with increase in mucous. Microscopically the examination of the different organs of exposed fishes to petroleum hydrocarbons were:

Gills showed normal cells shape (Fig. 1-A), congestion of secondary lamella capillary with edema together with moderate

proliferative changes of secondary lamella filament's (Fig. 1-B). Also, epithelial lifting (separating of secondary lamella epithelium) from supporting lamella cells (Fig. 1-C). The microscopic lesion showed severe vacuolation of secondary lamella epithelia together with severe dilation and congestion of secondary lamellae capillary and intravascular dilation of central venous sinus (Fig. 1-D), other section showed severe lamellae fusion due to severe cellular proliferation and infiltration, together with deformation of supporting cartilage and congestion (Fig. 1-E).

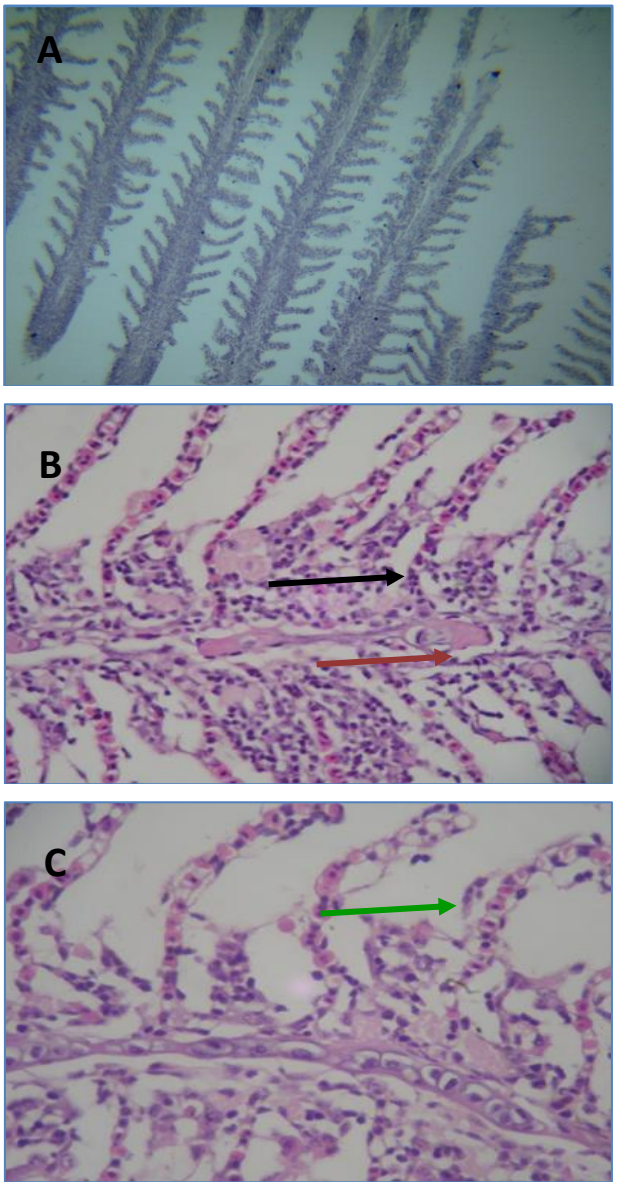
Muscles showed normal muscle bundles (Fig. 2-A), mild hyalinization of muscle fibers, appeared more eosinophilic associated with necrotic changes of some muscle bundles with congestion of blood vessels (Fig. 2-B), in addition showed slight infiltration of mononuclear cells between muscle fibers with fragmentation of muscle bundles (Fig. 2-C).

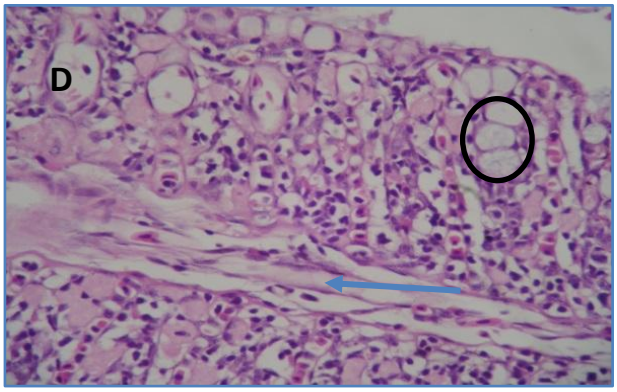
Kidney showed normal glomeruli and tubules (Fig. 3-A), variable degree of degenerative and necrotic changes in epithelial lining of renal tubules varied between severe hydropic degeneration to tubular necrosis (Fig. 3-B). In other sections it was showed hyperplasia of melanomacrophages as cluster with acute cellular swelling (Fig. 3-C). In figure (Fig. 3-D) showed severe depletion of hemopoiotic tissues, severe hydropic degeneration with acute cellular swelling of tubular epithelial lining.

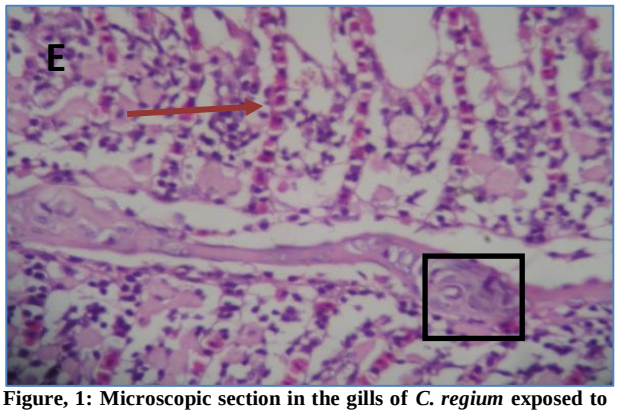
Liver showed normal hepatic cells (Fig. 4-A), severe dilation and congestion of blood vessels and sinusoid associated with degeneration and necrotic changes of hepatic cells (Fig. 4-B). In addition the hepatic cells showed nucleopleomorphism with nucleo -megalocytic (Fig. 4-C), other liver sections showed hemorrhagic and congestion with mineral deposition in hepatic parenchyma associated with severe necrosis of hepatic cells (Fig. 4-D).

Spleen showed normal splenic cells (Fig. 5-A), the main characteristic lesion was severe hyperplasia as cluster in splenic tissues with severe dilation of splenic sinuses (Fig. 5-B), severe lymphoid depletion in white pulp and hemopiotic tissues, together with congestion of red pulp (Fig. 5-C). All these changes in these tissues were not observed in the fish tissues from Al-Jadiriya Bridge.

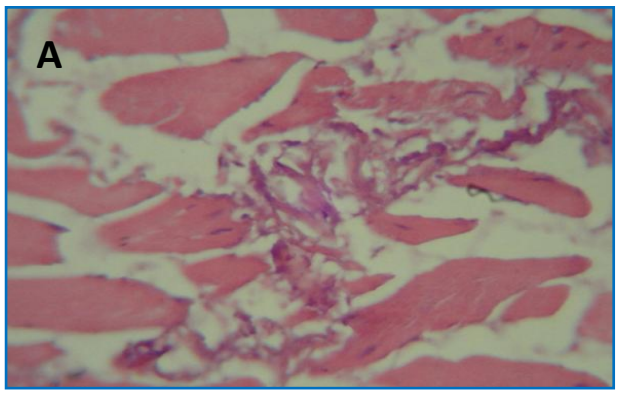
The present study was carried out in Tigris River between Al-Jadiriya Bridge and Al-Zaafaraniya region after Al-Durah power plant, this plant is located on the west set of the river, south-west of Baghdad and to the west of Durah refinery by 5.5 km (14), it is one of the most important and largest industrial facilities in Iraq. This region was contaminated by discharge of domestic wastes, industrial and housed waste, boats, car wasting, lubrication station, agriculture irrigation pumps, Durah power plant effluents, Al-Rashed electronic plant and Duarh refinery (15).

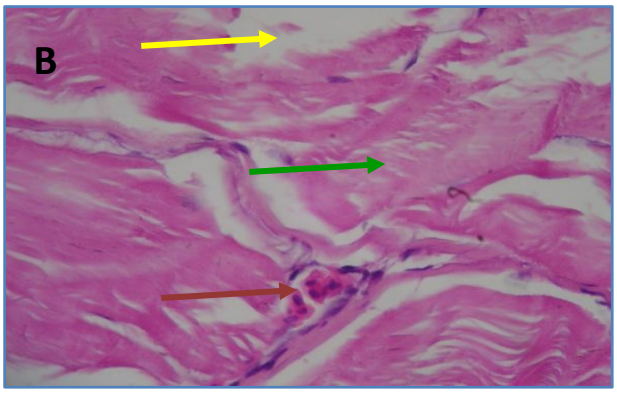


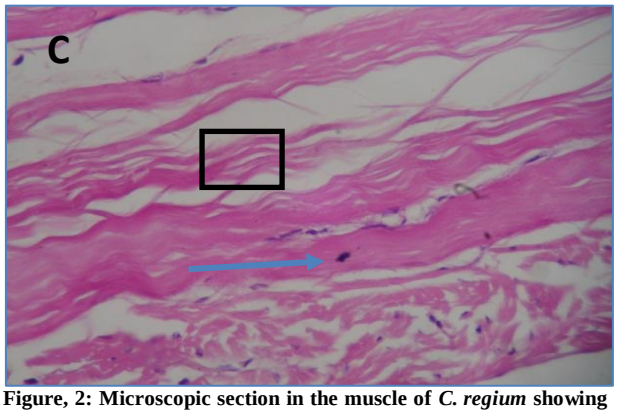




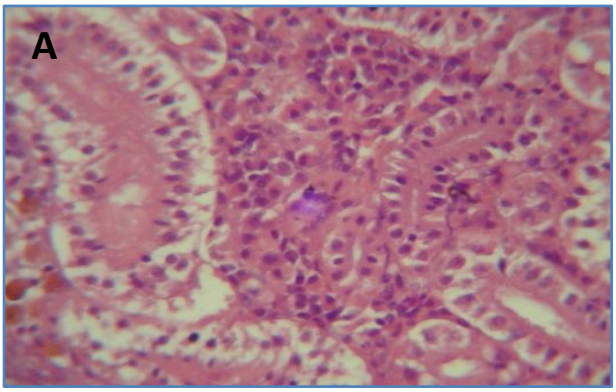
Figure, 1: Microscopic section in the gills of *C. regium* exposed to petroleum hydrocarbons showing normal cells shape (A), congestion (Red arrow), edema (Blak arrow) (B), with epithelial lifting (Green arrow) from supporting lamella cells (C), severe vacuolation (circle), severe dilation (Blue arrow) and congestion of secondary lamellae capillary and intravascular, dilation of central venous sinus (D), severe lamellae fusion, cellular proliferation and infiltration and deformation of supporting cartilage (square) and congestion (E). X40 (H and E stain).

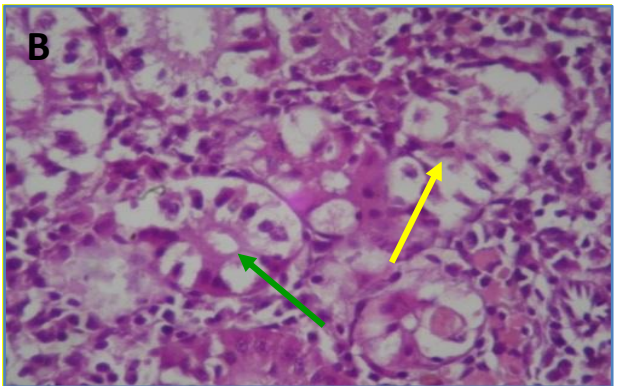


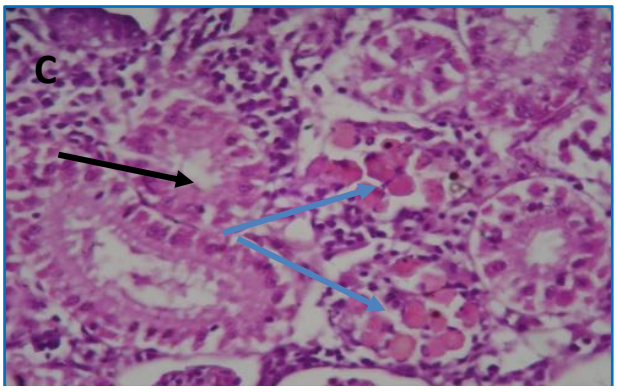


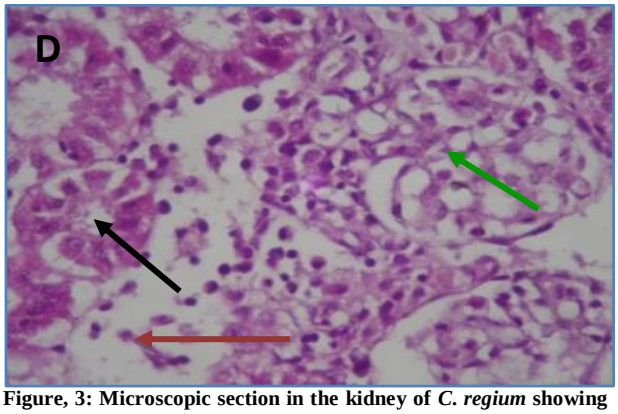


Figure, 2: Microscopic section in the muscle of *C. regium* showing normal muscle bundles (A), mild hyalinization (Green arrow) of muscle fibers, appear more eosinophilic and associated with necrotic changes (Yellow arrow) and congestion (Red arrow) (B), mild infiltration of mononuclear cells (Blue arrow) with fragmentation (square) of muscle bundles (C) X40 (H and E).

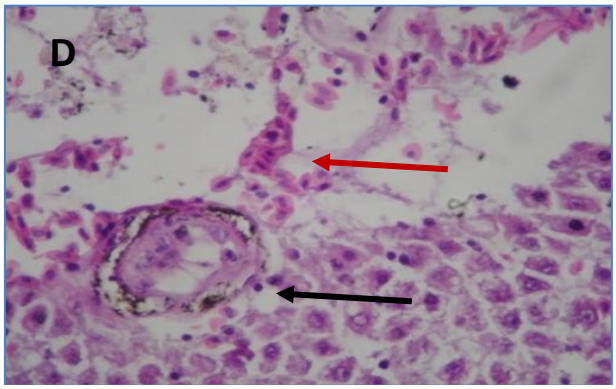




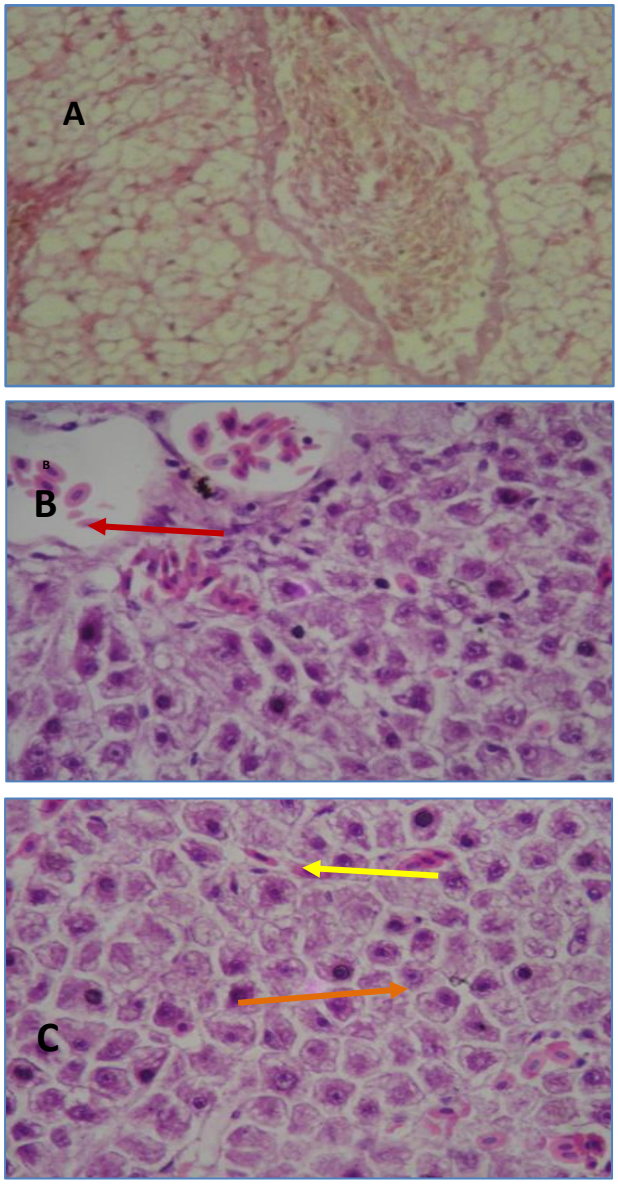


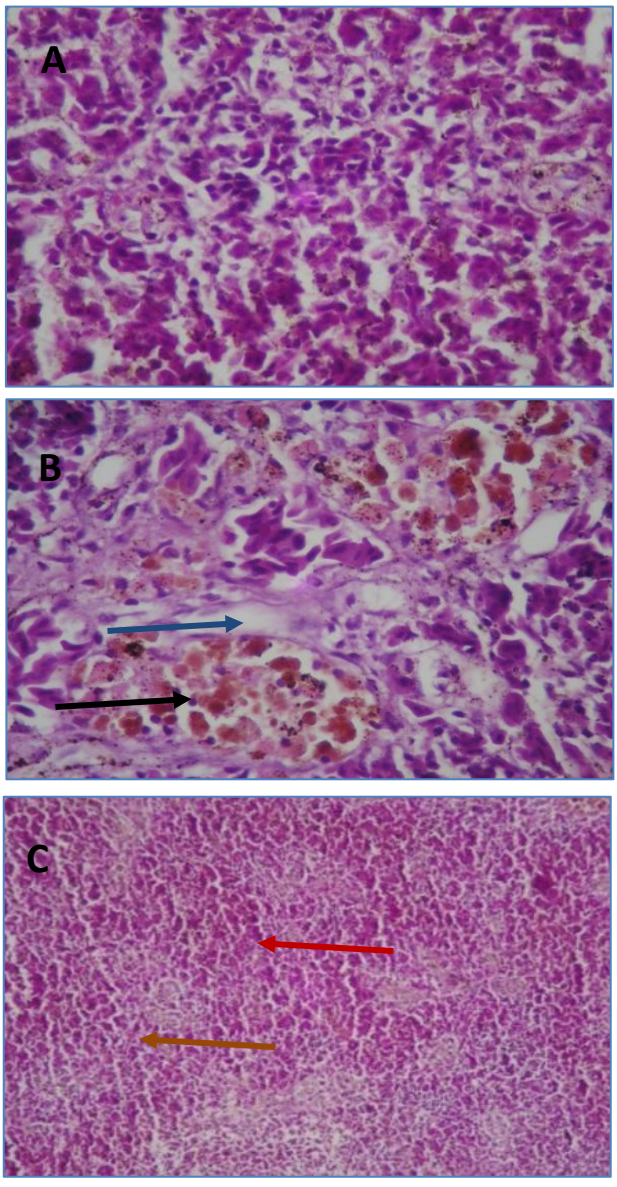


Figure, 3: Microscopic section in the kidney of *C. regium* showing normal glomeruli and tubules (A), hydropic degeneration (Green arrow) and necrotic changes (Yellow arrow) in renal tubular (B). hyperplasia of melanomacrophages (Blue arrow), cellular swelling (Black arrow) (C), severe hydropic degeneration, acute cellular swelling of tubular epithelial, severe depletion (Red arrow) of hemopoiotic tissues (D). X40 (H and E stain).



Figure, 4: Microscopic section in the Liver of C. regium showing normal hepatic cells (A), severe dilation and congestion of blood vessels and sinusoid (Red arrow), degeneration and necrotic (Yellow arrow) (B). nucleopleomorphism (Blue arrow) with nucleomegalocytic (Orang arrow) of hepatocytes (C), hemorrhagic with mineral deposition (Black arrow) in hepatic parenchyma (D). X40 (H and E stain).





Figure, 5: Microscopic section in the spleen of C. regium showing normal splenic cells (A), severe hyperplasia (Black arrow) in splenic tissues with severe dilation (Blue arrow) of splenic sinuses (B), severe lymphoid depletion (Brown arrow) in white pulp and hemopoiotic tissues, congestion (Red arrow) of red pulp (C). X40 (H and E stain).

Results indicated that petroleum hydrocarbons values were higher at Al-Zaafaraniya region than Al-Jadiriya Bridge due to contamination in this region. The present results revealed that exposing of Balout Al-Maloky (*C. regium*) from Al-Zafaraniya region to petroleum hydrocarbons induced histopathological changes in the different organs like (gills, muscles, kidney, liver and spleen), while not observing these changes on the fishes from Al-Jadiriya bridge. According to the results in the gills, these changes may be due to continuous irritation and toxic effect of petroleum hydrocarbons. These results were similar to the observation in the gills of pink salmon exposed to oil contaminated gravel by (16, 17 and 18). The muscles of exposed fish to the petroleum hydrocarbons revealed changes included mild hyalinization, fragmentation of muscle fibers and infiltration of mononuclear cells between muscle fibers, these changes were similar to the showing by (18). Petroleum hydrocarbons have been reported to affect the external surface of fishes that contact with the aquatic environment. The toxic nature of petroleum has been caused severe damage in all organ's tissue and the accumulation of these compounds over time in the bodies of animals can cause serious illness and may be carcinogenic to other (19). Also, exposure of *Pacific herring* larvae to crude oil caused erosion of pectoral fins, irregular, degeneration and non-membrane bound intracellular spaces in muscle tissues (20). These effects have been related to a stress response which might result from exposure to toxic compounds (21). Petroleum hydrocarbon is one of stress factors in fish health, therefore, its increase the secretion of Glucocorticoid that inhibits the immune system (22). Histopathological changes of fish kidney were similar to the observation by (18 and 23). The histopathological changes in the liver tissue, depends on the concentration of toxic compounds and period of exposure (6). The pathological changes in the hepatocytes which belong to the accumulation of toxic effect of petroleum hydrocarbons in the cytoplasm of the cells that indicates marked to hepatotoxic effects and deformation of the hepatocytes, these results were agree to observation by (24 and 2). The results revealed changes in the spleen, these changes belong to the toxic effect of petroleum which caused damage in RBCs that appear as congestion and hemorrhage in tissues. These changes were similar to results by (25 and 26).

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التغيرات النسجية المرضية لبعض أعضاء أسماك نهر دجلة المعرضة للهيدروكاربونات النفطية، مدينة بغداد

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الخلاصة

خلال المدة بين كانون الثاني إلى كانون الأول 2012، جمعت وفحصت 119 نموذجا من أسماك نهر دجلة في الجزء بين جسر الجادرية ومنطقة الزعفرانية وبواقع محطتين ألاولى عند جسر الجادرية وألاخرى عند منطقة الزعفرانية قبل نهر ديالى لمعرفة مدى تأثير الهيدروكاربونات النفطية في أنسجة أعضاء الأسماك المفحوصة. تعود الأسماك المفحوصة الى (16) نوعا (السمنان العريض *Acanthobrama marmid*، اللصاف *Albernus caeruleus*، الشلك *Aspius vorax*، طويني *Barbus belayewi*، الشبوط *B. grypus*، الكطان *B. xanthopterus*، الكرسين *Carassius carassius*، الحمري *Carasobarbus luteus*، بنيني كبيرالفم *Cyprinion macrostoma*، بلعوط ملوكي *Chodrostoma regium*، الكركور الأحمر *Gara ruffa*، أبو الحكم *Heteropneustes fossilis*، برعان سفالس *Leuciscus cephalus*، الخشني *Liza abu*، أبو الزمير *Mystus pelusius* و تلابيا *Tilapia zillii*). تضمن الفحص المرضي النسجي 44 عينة من أسماك البلعوط الملوكي *C. regium* أخذت عينات من الأعضاء الداخلية والخارجية مثل (الغلاصم، العضلات، الكلية، الكبد والطحال) وتبين من نتيجة الفحص وجود احتقان، وذمة، انفصال وتوسع وتفجي شديد في الصفائح الغلصمية الثانوية. في المقاطع النسجية للعضلات تميزت بوجود تزجج خفيف، إرتشاح للخلايا وحيدة النواة وتكسر الحزم العضلية. تنكس مائي وتنخر، فرط تنسج في الخلايا البلاعم الكبيرة الميلانية، توسع الأوعية الدموية، وتورم خلوي في بطانة النبيبات الكلوية ونفاذ في النسيج المكون للدم في الكلية. توسع شديد واحتقان الأوعية الدموية والجيوب الكبدية، تضخم وتوسع أنوية الخلايا الكبدية، نزف مع نفاذ معدني في متن الكبد. نفاذ لمفي في منطقة اللب الأبيض والنسيج المكون للدم في الطحال واحتقان اللب الأحمر، فرط تنسج شديد وتوسع نسيج الطحال. حُلِّلَتْ عينات من ماء نهر دجلة عند محطتي الجادرية والزعفرانية لقياس مستوى الهيدروكاربونات النفطية في الماء وتبين إن نسبة التلوث 0.0048 ملغم/ لتر في محطة الجادرية و 0.0674 ملغم/ لتر في محطة الزعفرانية.

الكلمات المفتاحية: التغيرات النسجية المرضية، سمكة البلعوط الملوكي، نهر دجلة، الهيدروكاربونات النفطية، مدينة بغداد.