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Histopathological Effect of Petroleum Hydrocarbons in some Internal Organ Tissues of the Common carp (*Cyprinus carpio* L. 1758) in Tigris River- Baghdad City

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Abstract

During the period of one year 2012, a total of 150 fish samples were collected and examined from Tigris River in Al-Zaafaraniya Region before Diayla River in order to know the effects of petroleum hydrocarbons in some internal organ tissues of the examined fish. These fish belonged to (16) species, it all comes back to the cyprinidae. The histopathological examination included 55 samples from Common carp, the samplings of internal organs like (brain, intestine and ovary) from *C. carpio* were taken in order to examine histopathological changes and that appear there were many histopathological changes in the examine organs included neuronal shrinkage of neurons with chromatolysis of other, severe congestion and edema in cerebral tissues and severe mineralization in cerebral blood vessel walls. Also, severe destruction of epithelium muscular of intestinal villi, severe necrosis with congestion and mononuclear cells infiltration in submucosa layer and goblet cells hyperplasia. In ovaries, severe necrotic and degenerative changes, congestion between follicles. Some ovarian follicles showed thickness became abnormality irregular in shape and congestion in secondary follicles. The sampling of water from Tigris river in Al-Zaafaraniya region were analyzed and appear that the pollution 0.0674 mg/L.

Key words: Petroleum hydrocarbons, Histopathological effects, Internal tissues, *Cyprinus carpio*, Tigris River.

Introduction

Aquatic pollution caused by petroleum hydrocarbons is commonly known and increased in recent years in Tigris River due to discharge of domestic wastes, industrial waste, boats, car wasting, lubrication station and agriculture irrigation pumps. Aquatic pollution is a growing problem while the cleanup efforts are weak, this type of pollution killing many species and destroying the ecosystem (1). Wherever polluted water exists, there is always the danger that an individual will drink it and cause disease (2). Petroleum hydrocarbons is known as a complex mixture of many chemical compounds that are soluble in water. Therefore, they are toxic to most aquatic organisms, carcinogenic to other and have fatal effect on aquatic organisms through the food chain. In addition, they have toxic effect to human health (3). Histopathological changes using as biomarker and use of aquatic organisms as bio indicators of pollution (2). The present study is a description the histopathological changes of petroleum hydrocarbon in some of internal tissue of fishes *Cyprinus carpio* L.

Materials and Methods

The study was conducted throughout the year 2012. A total of 150 fish samples were collected from Tigris River in Al-Zaafaraniya Region before Diyala River. These fishes belonged to 16 species, it all comes back to the cyprinidae. These fishes were classified according to (4). These samples were caught by gill nets and cast nets and transferred alive or freshly dead to the fish department laboratory (fish and animal resource center-agriculture research director) by cold plastic containers. Fishes were examined as soon as possible after killing them by pithing method. Total and standard lengths were taken. From these samples 55 fishes of common carp (*Cyprinus carpio*) were taken. The range of total length was 13-24 cm.

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Preparation of histological sections: A piece of exposed organ tissues to hydrocarbons (brain, intestine and ovary) were taken for preparation of histological sections according to the (5). 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 (before that the slide dip on the xylene). The slide was put in the oven 70 °C to remove the additional wax. 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. Stained mounted sections were examined under light microscope. Photographs were taken at x40 magnification using the camera at 50mm focal length.

Water analysis for petroleum hydrocarbons determination: The procedure of water analysis according to the method of (6).

Results and discussion

Histopathological changes due to hydrocarbons:

According to references **(**7), the histological structure of normal tissues appears:

Brain: The fish's brain is similar in its basic component to the brain of higher animals, but with many differences in form and complexity. It is usually divided into five divisions comprising, from the anterior: the telencephalon, the diencepholon, the mesencephalon, the metencephalon or cerebellum and medulla oblongata. The telencephalon or forebrain is responsible for olfaction and for aspects of colour vision, memory and reproductive and feeding behavior. The diencephalon is very variable in form but is usually small and subdivides into three distinct components, the epithalamus, the thalamus and hypothalamus.

Intestine: intestine of most fish is a simple tube which does not increase in diameter to form a colon posteriorly. This tube in the herbivorous fish being very much longer than that of carnivorous species, intestine may be straight, sigmoid or coiled. It has a simple, mucoid, columnar epithelium, sub mucosa often richly with eosinophilic granule cells and limited by a dense muscular is mucosa and fibro elastic layer.

Ovary: The mature ovaries can represent as much as 70% of the total body weight. They are suspended from the abdominal wall by a mesentery and usually appear as a small cluster. The primary ovarian cells are the ovarian follicles which line a potential cavity which has a very complex series of folds in its lining. Ova are passed into this cavity as they mature; the ova are passed directly to the outside via an oviduct. Oogonia, the cells which are beginning to mature, are surrounded by a single layer of small epithelial cells and it is this aggregate of ova and epithelial cells which are known as the ovarian follicle. The epithelial cells grow as the ovum grows and are separated from it by a gradually thickening hyaline capsule, the zona pellucida.

The microscopic examination of organ tissues appeared:

Brain: the characteristic feature showed neuronal shrinkage of neurons with chromatolysis of other together, with severe congestion and edema in cerebral tissues and increase proliferation of astrocytes (Figure 1). In addition, other section showed severe mineralization in cerebral blood vessels wall, together with microglial proliferation (Figure 2).

Intestine: The histopathological changes of intestine showed severe destruction of epithelium muscular of intestinal villi, severe necrosis with congestion and mononuclear cells infiltration in submucosa layer (Figure 3), other section showed slight goblet cells hyperplasia with submucosa mononuclear cells aggregate and blood vessels congestion (Figure 4).

Ovary: The histopathological changes of ovaries showed severe alteration in ovarian follicles

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including severe necrotic and degenerate changes together with congestion between follicles (Figure 5). Some ovarian follicles showed thickening and separation of other, separation of oocytes and became abnormality irregular in shape and congestion in secondary follicles (Figure 6). Other section of growing mature follicle showed degeneration and proliferation of granulosa cells result in adhesion of cellular crater of oocytes (Figure 7).

The explanation of the toxic effect of petroleum hydrocarbons on the organs examined, it's back to irritation of hydrocarbon chemicals on epithelial cells of organs examined. Also, hypoxia due to this chemical material that leads to injury to the cells of organs examined (8).

Water analysis: According to the results, the sample of water from Tigris River in Al-Zaafraraniya Region were analyzed and appeared that the measurement of total hydrocarbones was 0.0674 mg/L.

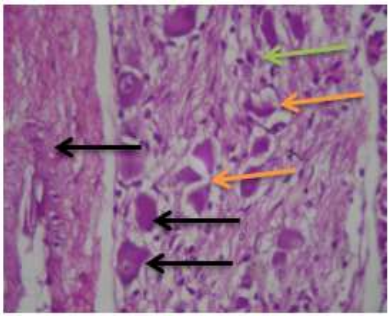

Figure. 1. Section through brain of common carp showed neural shrinkage of neuron chromotolysis of neuron, severe congestion, edema in cerebral tissues and proliferation of astrocytes . (H & E X 40).

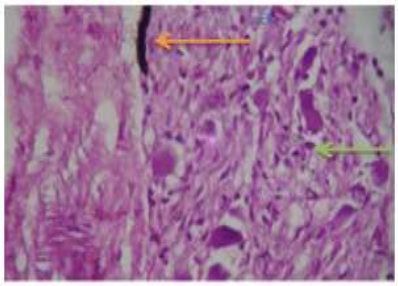

Figure. 2. Section through brain showed severe mineralization in cerebral blood vessels wall together with microglial proliferation . (H & E X 40).

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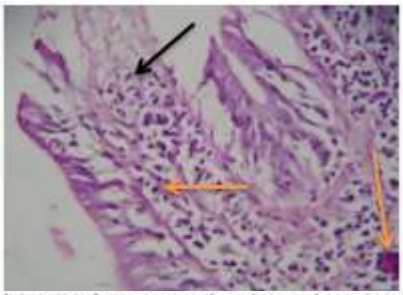

Figure. 3. Section through intestine of common carp showed severe destruction of epithelium muscular of intestinal villi ← infiltration in submucosa layer ←

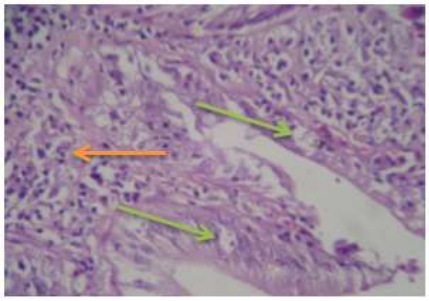

Figure. 4. Section through intestine of common carp showed slight goblet cells hyperplasia ← with submucosa mononuclear cells aggregate and blood vessels congestion ← (H & E X 40).

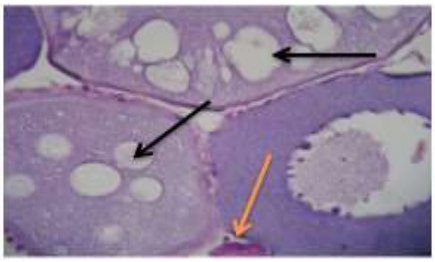

Figure. 5. Section through ovary of Cyrinus *carpio* showed severe alteration in ovarian follicles including severe necrotic and degenerate changes ←, congestion between follicles ← (H & E X40).

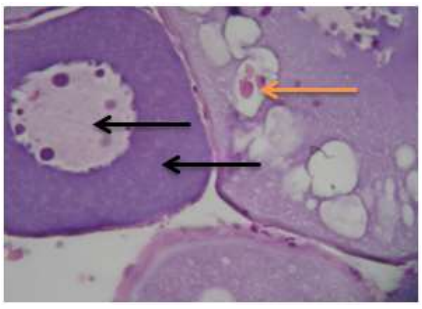

Figure. 6. Section through ovary of *Cyprinus carpio* showed thickening and separation of oocytes and became abnormality irregular in shape ← and congestion in secondary follicles ←

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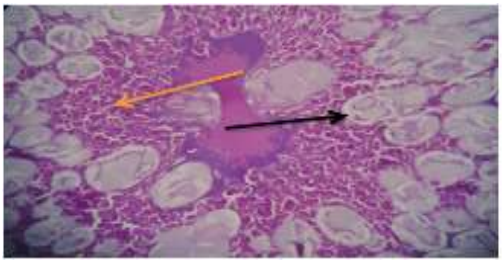

Figure. 7. Section of growing mature follicle showed degeneration ← and proliferation of granulosa cells resulting in adhesion of cellular crater of oocytes ← (H & E X40).

Petroleum hydrocarbon are the commonest sources of pollution in Iraqi water in the recent years, there are toxic if present in sufficient quantity and destruction the aquatic environment. Also, there are cause a reduction in the quality of the aquatic environment. Oil pollution is a growing problem, small quantities of oil spread rapidly across long distances to form deadly oil slicks (8).

The present results revealed that exposing fishes *Cyprinus carpio* L. to petroleum hydrocarbons caused histopathological changes in the brain, intestine and ovary. Toxicity or 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 carcinogenic to other (9). Exposure of the common carp to petroleum induced histopathological changes in the internal tissues. These include neuronal shrinkage of neurons with chromatolysis of other, congestion and edema in cerebral tissues and increase proliferation of astrocytes, severe mineralization in cerebral blood vessels wall and microglial proliferation, these results were similar to those observed by (10 and 11). The results were showed in the intestine of common carp in the present work were agreement to those by (12 and 13). The histopathological changes of ovaries showed severe alteration in ovarian follicles with congestion between follicles. Some ovarian follicles showed thickening and separation of other, oocytes became abnormality irregular in shape. Growing mature follicle showing degeneration and proliferation of granulosa cells resulting in adhesion of cellular crater of oocytes, these results were similar to those by (14 and 13).

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التأثيرالمرضي النسجي للهيدروكاربونات النفطية في أنسجة بعض الأعضاء الداخلية لسمكة الكارب الإعتيادي، نهر دجلة ـ مدينة بغداد

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الخلاصة

خلال مدة عام واحد 2012 ، جمع وفحص 150 نموذجا من أسماك نهر دجلة في منطقة الزعفرانية قبل نهر ديالى لمعرفة مدى تأثير الهيدروكاربونات النفطية في أنسجة بعض الأعضاء الداخلية للأسماك المفحوصة. تعود الأسماك المفحوصة الى (16) نوعا تعود جميعها الى عائلة الشبوطيات. تضمن الفحص المرضي النسجي 55 عينة من أسماك الكارب الإعتيادي، أخذت عينات من الأعضاء الداخلية مثل (الدماغ، الأمعاء والمبايض) وتبين من نتيجة الفحص وجود الكثير من التغيرات المرضية النسجية في الأعضاء المفحوصة شملت إنكماش الليف العصبي وتحلل صبغته الكروماتيه مع وجود النزف والخزب في النسيج الدماغي وترسب المعادن في جدران الأوعية الدموية الدماغية. كذلك لوحظت تبدلات شديدة في الطبقة العضلية للزغابات المعوية ، فرط تنسج في الخلايا الكأسية ، وإرتشاح شديد للخلايا وحيدة النواة. أما في أنسجة المبايض فتبين وجود تبدلات شديدة في الجريبات المبيضية شملت تنكس ونخر هذة الجريبات ، زيادة في سمك جدرانها بحيث أصبحت غير منتظمة الشكل مع نزف في الجريبات الثانوية. تم تحليل عينات من ماء نهر دجلة عند منطقة الزعفرانية وتبين إن إجمالي قيم الهيدروكاربونات النفطية يساوي 0.0674 ملغم/ لتر.

الكلمات المفتاحية: الهيدروكاربونات النفطية، التأثيرات المرضية النسجية، الأنسجة الداخلية، سمكة الكارب الإعتيادي، نهر دجلة.