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ABSTRACT

This study aimed to determine the concentration of depleted uranium and the main histopathological changes in local fish and chickens at a selected Iraqi area suspected to be polluted with uranium. Different locations of Al-Tuwaitha region (Southern of Baghdad) were surveyed randomly to collect a total of 15 samples from each animal species. The animals were sacrificed and dissected at the site of collection. Muscle samples were collected to determine uranium concentrations using nuclear fission track analysis with CR-39 detectors. For histopathological changes, sample tissues from fish (liver, gills, and kidney) and chickens (liver, kidney, and lung) were collected and fixed in 10% neutral buffered formalin. The results showed that there was a significant elevation in uranium concentration in both fish and chicken muscles ($1.94\pm0.77 \mu g/Kg$ and $2.19\pm0.82 \mu g/Kg$, respectively) compared with recommended uranium concentration. Histopathological examination showed several effects, included congestion and thickening of blood vessels walls, vacuolation, necrosis, fibrosis and inflammatory cells infiltration in most tissue sections of collected organs. In conclusion, the uranium residues that found in both fish and chicken meats could raise the concerns about consumption of both animal species that had been bred in Al-Tuwaitha, and could be an indicator of environmental pollution with uranium in this region.

Keywords: Fish, Chicken, Histopathological changes, Uranium

Introduction

Fish and chicken meat are considered as basic sources for humans consumptions all over the world (1). With the actual increase in uranium products in the environment that contain uranium oxides can be ingested or inhaled, it is useful to understand its distribution and toxicity for humans and animals (2, 3) and its potential to enter the human food chain. Uranium entering the food chain by ingestion of feed, water, and soil are a major pathway for animal contamination with radionuclides (4). Water contaminated with uranium would be the most influential parameter

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Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad, Iraq. Received, 10 May, Accepted, 15 October 2019, Published: 28 December 2019. contributing to uranium activity concentration in chicken meat (5).

Uranium is considered as one of the most serious pollution concerns due to its radioactivity and heavy metal toxicity (6). Uranium is a natural component of the earth's crust (7). Uranium exists naturally in the oxidative states +2,+3,+4, and +6. Natural uranium is a mixture of three isotopes U234, U235 and U238 which all are similar chemically, but have a different radioactive properties (8, 9). The adverse effect from occupational and experiments on uranium exposures that have been established significantly include lung cancer, from exposure to radon produced from radioactive decay (10, 11) and chemically induced kidney toxicity and bladder damage (12). Moreover, several authors have shown that the kidneys are not the only biological target of acute and chronic exposure to low levels of uranium, but the changes have also been reported in gastrointestinal tracts (13), central nervous system (14, 15), and liver (16). One of the major sites of uranium pollution in Iraq is Al-Tuwaitha nuclear research site, which has been

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destroyed in 1991 Gulf War. The barrels contained radioactive materials and service were stolen from the site in 2003 War (17). This event resulted in considerable radioactive contamination in this site and its neighboring areas. Therefore, this study was performed in order to determine depleted uranium residues and to investigate the histopathological impacts on both fish and chickens bred in Al-Tuwaitha region, Baghdad, Iraq.

Materials and Methods

Samples Collection

Fifteen samples of each of local fish ponds and chickens were collected randomly from the different sites at Al-Tuwaitha region. Animals were captured and weighed (0.8-2 kg, 0.5-1.4 kg), after that the animals were sacrificed. Liver and kidney from both species, gills from fish and lung from chickens were obtained and preserved in universal screw flask containing 10% formaldehyde solution for histopathological study. In addition, about 500 gm muscles from each species were taken for uranium determination.

Detection of Uranium Concentration in Fish and Chicken Muscles

Muscle samples (about 500 gm) were taken from fish and chickens for preparing the required amount of ash for fission track analysis technique. The fish were dried gradually, and then ashes at 600 °C.

The ashes were grinded by using a mortar, and stored in plastic containers. About 0.5 gm of ashes samples was mixed with 0.1 gm of starch, which was used as a binder.

Thereafter, the blend was compressed into a pellet of 1 cm diameter and 1.5 mm thickness.

The pellet was covered with CR-39 track detector on both sides and was put in a plate of paraffin wax at a distance of 5 cm from (Am–Be) neutron source with a thermal flounce equal to $(3.024 \times 10^9 \text{ n cm}^{-2})$ for 7 days, to cause latent damage to the CR-39 detector.

After the irradiation process, the CR-39 detectors were etched in NaOH solution with normality (N= 6.25) at temperature of 60 °C for 5 h. Olympus optical microscope with magnification of 400 × was used to record the induced fission tracks densities. The fission track densities were measured on the surfaces, showing uniform distribution of uranium samples, and were measured by comparison between the tracks density registered on CR-39 detectors around the samples pellet and that of standard samples pellet by using the following equation $U_X = U_X(P_X/P_S)$ (18).

Histopathological Study

The collected tissue samples from fish and chicken were kept in 10% formaldehyde solution for fixation, and then processed routinely by using the histokinete. Tissue sections were embedded in paraffin blocks, sectioned by microtome, stained with hematoxylin and eosin, and then examined by using light microscope to record the histopathological changes (19).

Statistical Analysis

Data were subjected to confident limit analysis using SAS (Statistical Analysis System, version 9.1) (20). P < 0.01 was considered statistically significant (21).

Results and Discussion

Oxidative - Antioxidative stress Biomarkers

The results of detected uranium concentrations in tissue muscles of both fish and chickens are presented in Table 1. The results showed that there was a significant increase (P \leq 0.05) in uranium concentration in muscle tissues of both animal species compared to the acceptable uranium concentration estimated by (22).

Table 1. The mean \pm SD of uranium concentration in fish and chicken muscles ($\mu g/kg$)

Type of samples	No. of samples	Minimum acceptable concentration	Uranium concentration
Fishes muscles	15	0.03	1.94±0.77*
Chicken muscles	15	0.09	2.19±0.82*

Depleted uranium (DU) in soil and waters can lead to an accumulation in the food chain when

ingested by humans and animals. Moreover, the presence of DU in the Iraqi food chain was documented by measuring the uranium in animals' organs in different Iraqi cities in its highest concentration (2). This is in correlation the significant elevation of uranium with concentration in the fish and chicken muscles reported in this study (Table 1). This result agrees with Jeambrun et al. (5) who reported that natural radionuclides, uranium, thorium and their decay products, are present in the environment and can be transferred to animals and animal products and ultimately to humans. Also, the study of the activity concentrations in grain mixture, drinking water and soil particles ingested by poultry permits a better understanding of the main sources of each radionuclide and the calculation of concentration ratios and transfer coefficients for chicken meat and egg contents. Branislava et al. (23) concluded that uranium in broilers primarily accumulates in kidney and liver compared with brain and muscles. Another study by Hassan (24) reported the elevation of uranium concentration in fish tissues ranging from 1.78 \pm 0.19 µg/ kg⁻¹ to 8.31 \pm 0.51 µg/kg⁻¹ when he compared between six common commercial species of Iraqi river fish. The suggested reason for the elevation of uranium concentration in animals' tissues may be due to the ability of uranium compounds to accumulate leading to toxicity, in addition to the persistence in the environment high and propensity for bioaccumulation (25).

Histopathological Findings

A-Fish Organs

Kidneys. Kidney sections showed congestion and thickening of blood vessel walls and shrinking of epithelial cells lining of the renal tubules (Figure 1). Atrophy of glomerular tufts with thickening in bowman's capsules and vacuolation in some tubular epithelia were observed (Figure 2). Many sections showed depletion of hemopoietic significantly increase at level ($P \le 0.01$), tissue with an increase in melano-macrophage deposition (Figure 3).

Liver. Histopathological changes in liver were present as fibrin network with inflammatory cells infiltration between the parenchyma and hepatopancreas (Figure 4). There was wall thickening and congestion of central vein with focal aggregation of melamo-macrophages beside the central vein (Figure 5). Other sections showed severe destruction on hepatic parenchyma with oozing of blood and inflammatory cells into the necrotic area (Figure 6).

Gills revealed congestion of central venous sinus vessels with interstitial edema and inflammatory cells infiltration (Figure 7) with fusion and hyperplasia of primary and secondary lamella (Figure 8). Many sections showed inflammatory cells infiltration and blood congestion of blood vessels (Figure 9), necrosis of the gills filaments with extensive sloughing in gills filaments (Figure 10).

B-Chickens Organs

Kidney. Many section showed vacuolation in glomerular tuft and dilation in bowman's space as well as thickening of bowman's capsule with sloughing of tubular epithelia (Figure 11). Occurrence of chronic interstitial nephritis characterized by presence of inflammatory cells and fibrosis in renal parenchyma (Figure 12), with atrophy of glomerular tufts (Figure 13).

Liver. Histopathological sections showed extensive area of necrosis with inflammatory zone (Figure 14). Other sections showed mononuclear cells infiltration with fibrosis (Figure 15), congestion and thickening in blood vessel wall with inflammatory cells inside their lumen and between the hepatocytes (Figure 16).

Lung. The main pathological findings were congestion of blood vessels with deposition of hemosiderin and thickening in Para bronchial wall due to inflammatory cells infiltration (Figure 17). Also, congestion of atrium with inflammatory cells infiltration was very clear (Figure 18).



Figure 1. Histopathological section of kidney shows congestion and thickening of blood vessels wall (\rightarrow) and shrinking of epithelial cells lining the renal tubules (\rightarrow) (H&E, 100X)



Figure 2. Histopathological section of kidney shows atrophy of glomerular tufts (\longrightarrow) with thickening in bowman's capsules also vacuolation in some tubular epithelia (\longrightarrow) (H&H. (H&E, 400X)



Figure 3. Histopathological section of kidney shows depletion of hemopoietic tissue () with increase in melanomacrophage deposition () (.H&E, 200X)



Figure 5. Histopathological section of liver shows thickening and congestion of central vein (\longrightarrow) with focal aggregation of melanomacrophages beside the central beside the central vein (\longrightarrow) (H&E, 400X)



Figure 6. Histopathological section of liver shows severe destruction on hepatic parenchyma with oozing of blood and inflammatory cells into the necrotic area (\rightarrow) (H&E, 400X)



Figure 4. Histopathological section of liver shows presences of fibrin network with inflammatory cells infiltration between the liver parenchyma and hepatopancreas (\longrightarrow) (H&E, 100X)



Figure 7. Histopathological section of gills shows congestion of central venous sinus vessels (\rightarrow) with interstitial edema and inflammatory cells infiltration (\rightarrow) (H&E, 200X)



Figure 8. Histopathological section of gills shows fusion and hyperplasia of primary and secondary lamella (\longrightarrow) (H&E, 400X)



Figure 11. Histopathological section of kidney shows vacuolation in glomerular tuft (\rightarrow) and dilation in bowman's space as well as thickening of bowman's capsule (\rightarrow) with desquamation of tubular epithelia (\rightarrow) (H&E, 400X)



Figure 9. Histopathological section of gills shows necrosis of the gills filaments with inflammatory cells infiltration (\longrightarrow) (H&E, 400X)



Figure 12. Histopathological section of kidney showing chronic interstitial nephritis characterized by presence of inflammatory cells (->) and fibrosis (->) in renal parenchyma (H&E, 400X)



Figure 10. Histopathological section of gills shows extensive sloughing in gills filaments (\rightarrow) with inflammatory cells infiltration and blood congestion of vessels (\rightarrow) (H&E, 200X)



Figure 13. Histopathological section of kidney of chicken shows atrophy of glomerular tufts (\rightarrow) with vacualation of tubular epithelia (\rightarrow) (H& E, 400X)



Figure 14. Histopathological section of liver shows extensive area of necrosis (\rightarrow) with focal inflammatory cells aggregation (\rightarrow) (H&E, 400X)



Figure 15. Histopathological section of liver shows mononuclear cells infiltration (→) with fibrosis (→) (H&E, 400X)



Figure 17. Histopathological section of lung of chicken shows congestion of blood vessels () with deposition of hemosiderin () and thickening in Para bronchial wall due to inflammatory cells infiltration () (H&E, 200X)



Figure 18. Histopathological section of lung of chicken shows congestion of atrium with inflammatory cells infiltration (→) (H&E, 200X)



Figure 16. Histopathological section of liver of chicken shows congestion and thickening in blood vessel wall with inflammatory cells inside their lumen (-->) and between the hepatocyte (-->) (H&E, 400X)

The histopathological study in chickens and fish showed particularly similar lesion in kidney and liver. These results were severe congestion and thickening in blood vessels, vacuolation, fibrosis, inflammatory cells infiltrations, and necrosis in some organs. This finding occurred due to the toxicity of uranium exposure in these organs could effect endothelial cells and stimulate the inflammatory process, although the ability to produce ROS in many organs resulting in tissue damages (tissue atrophy, necrosis, exfoliation, and inflammation). This result is in agreement with those of previous toxicological studies (26-29).

Also, it may be due to the ability of uranium to accumulate in these organs. Keith et al. (30) investigated that the very young animals can absorb greater quantities of uranium than older animals. In chickens, many studies coincided our results. Previous investigations by (31) performed broilers showed that after uranium on contamination, most uranium levels accumulated in kidney and liver. muscles and the histopathological changes in broilers contaminated with a dose of 25 mg ²³⁸U daily. Lesions were found in kidney, liver, and small intestine, in the form of dystrophic changes in the kidney tubules epithelium, edema, and vacuolization of the cytoplasm of hepatocytes, and necrosis of intestinal villi. Many authors (32-34) concluded that the histopathological changes observed in kidneys are the result of their physiological ability to reabsorb and accumulate divalent metals. Uranium specifically accumulates in the proximal tubules in the inner cortex and the outer stripe of the outer medulla, where it causes apoptosis and renal lesions. Fish are more sensitive to stress specially that induced by heavy metal toxicity; however, uranium enters the fish body through the intestine, gills and skin, and accumulates chiefly in the bones and to a smaller extent in the viscera, gills, and muscles (35).

Our histopathological findings in fish kidneys were demonstrated as congestion and thickening of blood vessels, shrinking of epithelial cells lining the renal tubules, and vacuolation in some tubular epithelia.

Also, fibrosis and interstitial inflammatory cells infiltration, with atrophy of glomerular tufts were markedly observed. This finding may be due to the oxidative stress and lipid peroxidation that result from exposure to uranium present in contaminated water (36). The present findings are in agreement with many other studies that reported the most common alterations occur in the kidney of fish exposed to contaminated water, and manifested as tubule degeneration (vacuolation and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of bowman space with swollen Bowman capsule cells and melanomacrophages in the kidney (37, 38). The depletion of hematopoietic tissue found in most sections in the present investigation can be attributed to malfunctioning of the hematopoietic organs caused by uranium that caused more rapid destruction of cells (39, 40). The increased melano-macrophages in liver and kidneys could be attributed to a response to environmental or chemical stress or to the increased necrosis areas rather than the disturbance of the immune system. Moreover, it has been suggested that the increase in MNCs could be related to humoral and inflammatory responses and to the detoxification of exogenous and endogenous substances (41).

The gills, which participate in many important functions in fish, such as respiration, osmo-regulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (42, 43).

The histopathological results of gills revealed congestion of central venous sinus vessels with interstitial edema and inflammatory cells infiltration. This lesion may occur due to damage of pillar cells, which result in an increased blood flow inside the lamellae causing dilation of the marginal channel, blood congestion or even an aneurysm. Also, the most obvious signs of gill tissue impairment included alterations of epithelial cell, evidenced by either extensive edema or breakdown (desquamation or extensive sloughing of gills epithelium), which was observed in fish exposed to DU (44, 45).

Barillet et al. (47) reported aneurism (blood congestion) in some areas of secondary lamellae of gills exposed to ²³⁸U due to the breakdown of both epithelial and pillar cell systems (48), these alterations might be associated with inflammatory processes. Lerebours et al. (47) reported that in gills of zebrafish exposed to waterborne DU, the genes encoding antioxidant defenses or involved in inflammatory processes were up-regulated after

3 and 4 weeks of exposure. Our study noted hyperplasia of primary and secondary lamellae due to toxic effects of uranium that cause chronic irritation or sloughing of the lamellae. This result agrees with other studies that reported the exposure to environmental pollutants (heavy metals pesticides, etc.) resulted in hyperplasia and complete fusion of two neighboring secondary lamellae (48-50). Such symptoms (edema, hyperplasia) result in an enlargement of the distance between blood and water. They, therefore. be considered defense can as mechanisms against surrounding toxicants, and in the same time causing insufficient oxygen supply of the blood (hypoxia) leading to the formation of reactive oxygen species (ROS) and oxidative damage to biological molecules (51-53). Data regarding the genotoxic effects in fish are scarce. We can conclude that the uranium residues found in both fish and chicken meats could raise the concerns about consumption of both animal species that had been bred in Al-Tuwaitha, and could be an indicator of environmental pollution with uranium in this region.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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دراسة التغيرات المرضية النسجية لتاثير التلوث باليورانيوم المنضب على انسجة الأسماك والدجاج المحلي في من المنفي في منطقة التويثة

غصون عبد الكريم نعمة وايمان هاشم يوسف

جامعة بغداد/كلية الطب البيطري/فرع الامراض وامراض الدواجن

الخلاصة

صممت هذه تهدف الدراسة الحالية الى البحث عن التغيرات المرضية النسجية الرئيسية في الأسماك والدجاج المحلي في منطقة عراقية (التويثه) ملوثة باليورانيوم تضمن المسح جمع عشوائي لـ 15 عينة من الأسماك و 15 عينة من الدجاج والتي جمعت من مواقع مختلفة لمنطقة التويثة، إذ تم التضحية بالحيوانات في موقع الجمع وتشريحها لغرض استخراج عينات الانسجة من الأسماك (الكبد والغلاصم و الكلى) و الدجاج (الكبد والله والخلاصم و الكلى) و الدجاج (الكبد والكلى والرئة) وحفظت في موقع الجمع وتشريحها لغرض استخراج عينات الانسجة من الأسماك (الكبد والغلاصم و الكلى) و الدجاج (الكبد والكلى والرئة) وحفظت في محلول الفور مالدهايد بتركيز 10% لغرض در اسة التغيرات المرضية النسجية والعضلات لغرض الكب والكلى والرئة) وحفظت في محلول الفور مالدهايد بتركيز 10% لغرض در اسة التغيرات المرضية النسجية والعضلات لغرض الكشف عن تركيز اليورانيوم باستخدام كاشف 30-30. اظهرت نتائج الدر اسة ارتفاع معنوي في تراكيز اليورانيوم في عضلات الأسماك والدجاج (الكبد والكلى والرئة) وحفظت في محلول الفور مالدهايد بتركيز 10% لغرض در اسة التغيرات المرضية النسجية والعضلات لغرض الكشف 30-50. الفهر مالي الدر اسة ارتفاع معنوي في تراكيز اليورانيوم في عضلات الأسماك والدجاج (200 اليور انيوم باستخدام كاشف 30-30. اظهرت نتائج الدر اسة التوالي معار أيةً باقل التراكيز المقبولة لليورانيوم الأسماك والأسماك والدجاج (200 الدور اليور انيوم في عضلات الأسماك والدجاج (200 الدور اليور انيوم الغرض مالأسماك والدجاج (200 المادي والتيام وارتشاح الأسماك والدجاج (200 المادي والتيام وارتشاح ولما ملي النور اليور اليور اليور اليور اليوم الهر الأسماك والدجاج والدور المار المادي والتيام وارتشاح والماماك والدواي معار ألمان المراضي والتيام وارتشاح الفت ما مراضي المادي المقاطع النسجية. نستنتج من در استنا هذه إن متبقيات اليور اليور التي وجدت في معظم المواجن والأسماك والدور ما وارديم المواجي والتيام والتيام واليور اليور اليور اليور اليور اليور والمو مالغوي وارم المواجي وارلة المراضي وحدت في معظم المواجي و معنون الامر اضي المعامية المعامية المادي المادي والي متبقيات اليور اليور اليوم الدور والأسماك والدجل. والنصم وال

كلمات مفتاحية: اسماك، دجاج، التغيرات المرضية النسجية،اليورانيوم