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Histopathological Effects of Cadmium Chloride on the Kidney in Japanese Quail

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Abstract— Cadmium is one of the most dangerous heavy metals for its long life and its acute and chronic effects. The current study aims to identify the effect of exposure to cadmium chloride on the histological structure of the kidneys in one of the Japanese quail, Coturnix coturnix japonica. The study included two groups, a control group, and an experimental group. The birds of the experimental group were dosed to a concentration of 50mg/kg for four continuous days. The birds were sacrificed 4,15,30,60 days after the start of the experiment. The results of the histological examination of the kidney showed the emergence of many lesions in the four periods, to varying degrees between them. Among the most prominent of these is the appearance of the emergence of hydropic degeneration, necrosis, and desquamation in the renal tubules. Deformation, irregularity, and dilaceration of some glomeruli and atrophy of others were observed, expanding Bowman's space. The study concluded that the cadmium chloride causes much tissue damage to the body of the quail bird and that it is necessary to take into account not to be exposed to this element to maintain its economic value.

Keywords— Cadmium chloride; Japanese quail; kidney

I. INTRODUCTION

Although heavy metals are naturally occurring elements throughout the earth's crust, most environmental pollution and human exposure resulting from them are due to human activities such as mining, smelting, production, domestic and agricultural minerals and compounds containing minerals [1]. Excessive exposure to these minerals causes damage to cells and tissues, leading to various harmful effects and human diseases [2]. Heavy metals are inorganic elements with a density greater than 5g /cm3. Common heavy metals are Chromium, Lead, Cadmium, Mercury, Copper, Zinc, Arsenic, and the less common heavy metals are Iron, Cobalt, and Manganese [3]. Heavy metals may accumulate in vital body organs such as the liver, heart, kidneys, and brain due to exposure to them, which leads to disruption of normal biological performance. These heavy metals enter the body after methods such as consumption of food, polluted water, and polluted air. The heavy metals interact with somebody compounds such as chloride and oxygen and exert their toxic effects [4].

Cadmium (Cd) is a heavy element with an atomic number 48. It is not found as a pure metal in the environment. Still, most often in the form of sulfates, oxides, and carbonates complex in lead and copper and zinc [5]. Cd is a Class A carcinogen according to the classification of International Agency for Research on Cancer [6]. The most soluble salts are chloride, acetate, nitrates, and sulfates, while the insoluble salts are sulfides [7]. Animal cadmium contamination leads to significant economic losses as there is increasing concern about environmental pollutants emerging in livestock production systems [8]. Cd produces chemical and biological dysfunction in the body, and the accumulation of Cd in the body causes injury to the renal tubule cells and inflammation of the hepatic endothelial cells and also causes damage to the fetus and reproductive organs [6]. The main action in poisoning is that it induces and leads to an increase in the production of reactive oxygen in the cells causing oxidative lesions in different tissues, which indicates Cd toxicity. Therefore, Cd toxicity leads to changes in the antioxidant defense system by altering lipid peroxide levels [9].

Japanese quail *Coturnix coturnix japonica* belongs to the order Galliformes. It has been extensively used in vertebrate physiology and diseases that affect human health [10]. The short life of the Japanese quail, along with its physiological similarity to humans, made this bird an ideal model for studies dealing with aging, immunology, reproductive biology, toxicology of chemical compounds, and the effects of environmental agents causing endocrine disorders; [11]. Based on the previous, this study came to identify the acute effect of cadmium chloride ($CdCl_2$) on the tissue structure of the kidney in Japanese quail.

II. METHODOLOGY

Study birds

The current study was conducted on Japanese quail *C. c. japonica*. They were (3) weeks old and weighed between (143-143.5g). The birds were obtained from the College of Agriculture and Forestry, Department of the Animal Production, University of Mosul. They were raised in

wooden cages with attention to cleanliness, sterilization, and continuous nutrition. The natural conditions of lighting, temperature and ventilation were adopted during the experiment [12].

Choose the dose used in the study.
The dose of the study was chosen based on the experiment to determine the LD_{50} [13]. The dose used in this study was 50 mg/kg of body weight, as it was dissolved in 0.5ml of distilled water and dosed through the mouth with a Gavage needle.

Experimental design
In this study, 30 quail birds were used from both sexes, divided equally into two groups. The first group was the control group, which was dosed with distilled water for four consecutive days. The second group was dosed with $CdCl_2$ according to the specified concentration for the same period in the control group. Three birds from each group were sacrificed after 4, 15, 30, and 60 days from the start of the experiment.

Prepare histological sections
The birds were sacrificed after being anesthetized with chloroform. Then, after the abdominal area opened, the kidney was removed for each bird. The histological sections were prepared for the study using the method [14]. The tissue sections were colored with five stains: Delafield's Haematoxylin and Eosin stain (H&E) [16], Masson's stain (M) [16], Toluidine blue (TB) [15], Alcian blue pH 2.5 (AB) [15], Alcian blue & Periodic Acid – Schiff (AB&PAS) [15]. The examined and imaged sections were doing with a digital camera attached to an optical microscope.

III. Results and Discussion

Results
Natural structure of the kidney
The microscopic examination of the kidneys in quail showed that the normal tissue of the kidney consists of building units known as nephrons. Each nephron consists of several parts: Bowman's capsule, which contains a network of blood vessels inside it known as the glomerulus. The proximal convoluted tubule, the distal convoluted tubule. A tube connects between these tubules, known as the Henle's loop, and the distal convoluted tubule connects to the last part, the collecting tubule. The components of the nephron on the one hand and the total nephron, on the other hand, are connected by an interstitial connective tissue (Figures 11,12).

Histopathological effect of $CdCl_2$ after four days
The results showed that were deformation appeared in some glomeruli and other glomerular irregularities. It also appeared necrosis within the glomerulus and infiltration of inflammatory cells within it. Enlargement of Bowman's capsule was observed in some renal units, and Bowman's space appeared wide in some units and narrow in others. Hydropic degeneration appeared in the urinary tubules, necrosis in the walls of these tubules, and an increase in the acidity of the cells of some of these tubules. Thickening of the basement membrane of the cells of the urinary tubules was observed, degeneration and necrosis of the interstitial tissue appeared, and hemorrhage and infiltration of inflammatory cells near the glomeruli. Hyperplasia of fibroblasts near some urinary tubules was noted. It was also observed that severe congestion in some blood vessels and slight in others and the appearance of fibrosis in the walls of some blood vessels (Figures 13,14).

Histopathological effect of $CdCl_2$ after 15 days
Congestion appeared in the blood vessels of the kidney and necrosis of its walls. There was also hemorrhage, necrosis, and degeneration of the interstitial tissue of the kidneys, infiltration of inflammatory cells, and the emergence of phagocytic inflammatory cells between the urinary tubules. Also, degeneration and necrosis of the cells of the urinary tubules, especially the proximal convoluted tubules noted, and the separation of cells in some urinary tubules from the basement membrane, which appeared relatively thick and increased acidity of some of these cells. An expansion of some urinary tubules has also been observed. As for the glomeruli, there was a anastole of the glomeruli and an irregularity in the shape of each other. Also, degeneration and necrosis of the glomerulus, thickening, and hyperplasia of Bowman's capsule, as well as wide Bowman's space (Figures 15,16).

Histopathological effect of $CdCl_2$ after 30 days
Deformation appeared in some glomeruli and condensation in other glomerular components, as well as degeneration and necrosis of some of these glomerular components and infiltration of inflammatory cells inside the glomeruli. Bowman's space appeared relatively wide compared to the previous period. As for Bowman's capsule, hyperplasia appeared in some glomeruli, as well as dilaceration of some parts of Bowman's capsule in some glomeruli. For the urinary tubules, there was expansion in some of them, necrosis and degeneration in some of their cells, as well as a separation in the cells of some of these tubules from their basement membrane. The cavities of these tubules also showed a moderately positive response to the AB-PAS stain, indicating mucosal carbohydrates in the cell tops and cavities of these tubules. It also appeared in the acidic cytoplasm of the cells of these tubules. The interstitial tissue disintegrated among the other components. Infiltration of inflammatory cells, degeneration, and necrosis of the components of this tissue is observed. Hemorrhage and the emergence of inflammatory cells in this tissue between the urinary tubules appeared. Congestion appeared in some blood vessels, and hyperplasia of the walls of some blood vessels was observed (Figures 17,18).

Histopathological effect of $CdCl_2$ after 60 days
An expansion of Bowman's space was observed, and thickening of Bowman's capsule wall and hypoplasia of cells in it. There was also severe congestion in the capillary vessels inside the glomerulus and infiltration of

inflammatory cells in them, and necrosis of some of their components. Congestion appeared in the blood vessels in the kidney with the appearance of fibrosis in the walls of some of them. The interstitial tissue surrounding the components of the kidneys appeared disassembled in some areas and condensed in others. Diffuse hemorrhage appeared between the components of the interstitial tissue, as well as necrosis and degeneration. Fibrosis and proliferation of fibroblasts were observed, and the appearance of inflammatory cells and fibrous deposits. There was an increase in the acidity of the cytoplasm of some of its cells for the urinary tubules, especially the proximal convoluted tubules. Also, necrosis and degeneration of some cells of its walls and expansion and Hydropic degeneration were observed in some of them. Separation of some cells is also seen from the basement membrane on which they are based, which in turn appeared to be relatively thicker than what appeared on it in previous periods (Figures 19,20).

Discussion

The results showed that after four days from exposure to the $CdCl_2$, many Histopathological lesions in the kidney appeared, including deformation appearing in some glomeruli and other glomerular irregularities. It also appeared necrosis within the glomerulus and infiltration of inflammatory cells within it. Hydropic degeneration appeared in the urinary tubules, necrosis in the walls of these tubules. Hyperplasia of fibroblasts near some urinary tubules was noted. These changes were also shown in a study conducted on mice treated with a dose of $CdCl_2$ when aqueous swelling and enlargement of the tubular cells near the renal cortex were observed [17-24]. Cd poisoning is associated with severe damage to different organs and tissues of mammals, as Cd poisoning has been linked to deterioration of the condition of birds' bodies, and it has been proven that exposure to Cd causes toxicity of the liver, testicles, and ovarian toxicity in chickens [23,25]. Exposure to Cd also leads to abnormal indicators of kidney function, such as increased urea nitrogen and creatinine levels in the blood.

After 15 days, congestion appeared in the blood vessels of the kidney and necrosis of its walls—the emergence of phagocytic inflammatory cells between the urinary tubules. As for the glomeruli, there was an Anatole of the glomeruli and an irregularity in the shape of each other. Also, degeneration and necrosis of the glomerulus, thickening, and hyperplasia of Bowman's capsule. The kidney is the main organ affected by chronic exposure to Cd toxicity and accumulation as a result of preferential absorption through receptor-mediated endocytosis through metallothionine-cadmium filtering in the proximal renal tubules, which leads to the release of Cd into the cytosol, where it can generate ROS and activate programmed death [25].

Whereas, after 30 days, deformation appeared in some glomeruli and condensation in other glomerular components. For the urinary tubules, there was expansion in some of them, necrosis and degeneration in some of their cells, as well as a separation of the cells of some of these tubules from their basement membrane. The cavities of these tubules also showed a moderately positive response to the AB-PAS stain, indicating mucosal carbohydrates in these tubules' cell tops and cavities. These findings appeared in previous studies when treated with $CdCl_2$, as their results showed degeneration, hemorrhagic foci, and central venous congestion. Also, the renal cortex appeared in several hemorrhagic areas, moderate renal failure, and expansion of Bowman's capsule [26]. It has been found that cadmium directly damages mitochondrial functions in the proximal tubular epithelium [27], which increases mitochondrial permeability and swelling and thus impedes respiration. These changes are responsible for ROS production, leading to apoptosis of renal cells [27-28].

Whereas, after 60 days, An expansion of Bowman's space was observed. There was also severe congestion in the capillary vessels inside the glomerulus and infiltration of the inflammatory cells in them, and necrosis of some of their components. Diffuse hemorrhage appeared between the components of the interstitial tissue. There was an increase in the acidity of the cytoplasm of some of its cells for the urinary tubules, especially the proximal convoluted tubules. Also, necrosis and degeneration of some cells of its walls and expansion and Hydropic degeneration were observed in some of them. Exposure of mice to Cd resulted in tissue damage in the renal cortex, decreased glutathione peroxidase activity, superoxide, catalase, and glutathione, and damage to mitochondria and nuclei. It activated the apoptotic pathway in mitochondria. Cd induces nephrotoxicity accompanied by histopathological alterations, inhibition of endogenous enzyme activity, and mitochondrial programmed death pathway [29]. Cd also disrupted the balance of trace elements and promoted oxidative damage [22].

IV. Conclusion and Future Scope

Acute exposure to $CdCl_2$ greatly affects the tissue structure of vital organs in quail birds in particular and economic animals in general, and causes many tissue lesions that may lead to the death of these birds. Therefore, attention must be paid to the food, drink and housing of these birds to ensure that they are not exposed to pathogens and in order to maintain their economic value.

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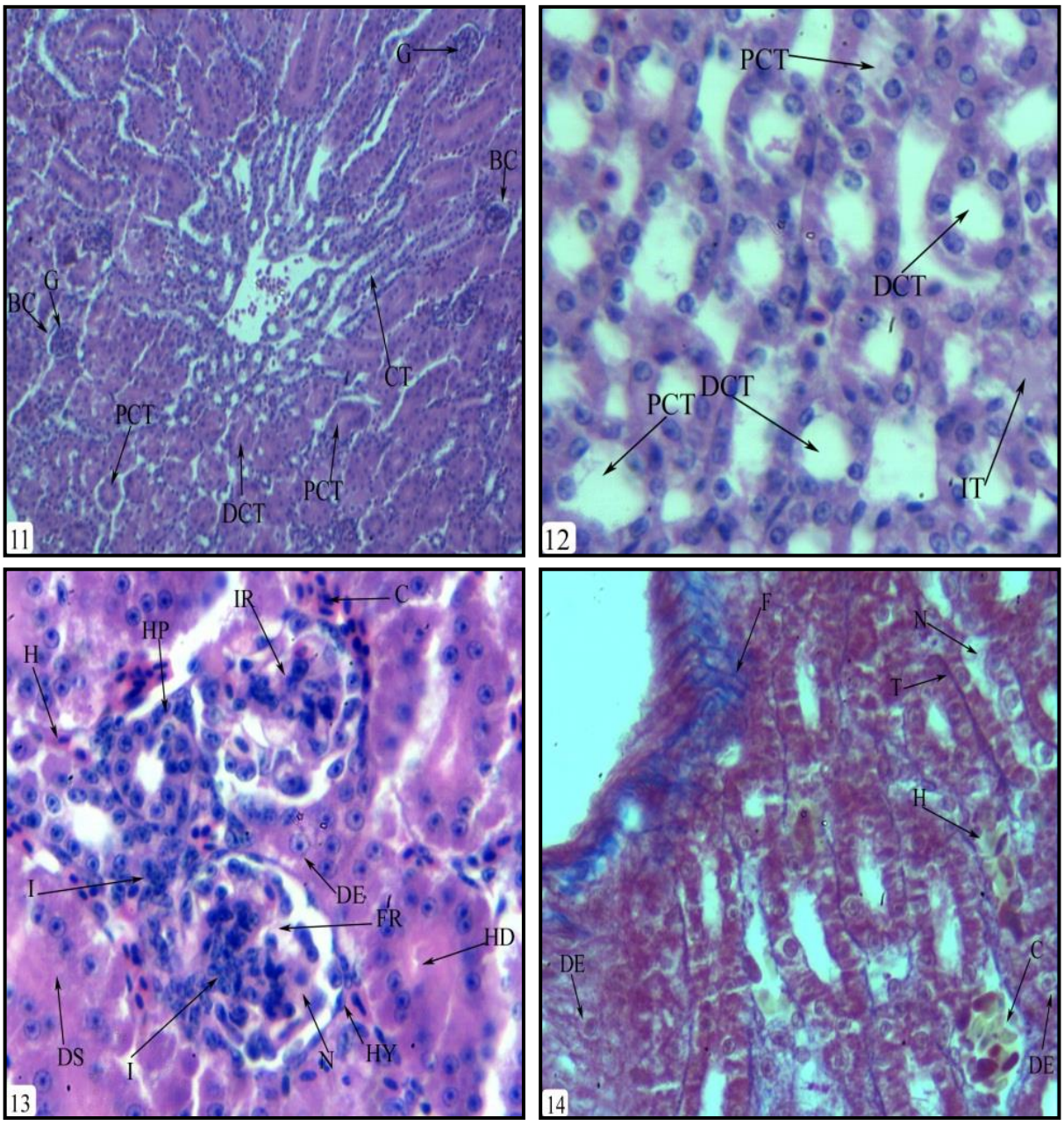

Figure (11): Normal histological structure of the Kidney in Japanese quail. H&E stain, 10X.; Figure (12): Normal histological structure of the Kidney in Japanese quail. H&E stain, 40X.; Figure (13): Histological structure of the Kidney after 4 days from treated with $CdCl_2$. H& E stain, 40X.; Figure (14): Histological structure of the Kidney after 4 days from treated with $CdCl_2$. M stain, 40X. Abbreviation: (G) Glomerulus; (BC) Bowman's capsule; (CT) Collecting tube; (PCT) Proximal convoluted tubule; (DCT) Distal convoluted tubule; (IT) Interstitial tissue; (C) Congestion; (H) Hemorrhage; (N) Necrosis; (HY) Hypertrophy; (I) Infiltration; (IR) Irregularity; (F) Fibrosis; (HP) Hyperplasia; (DS) Desquamation; (DE) Degeneration; (FR) Fragmentation; (HD) Hydropic degeneration; (T)

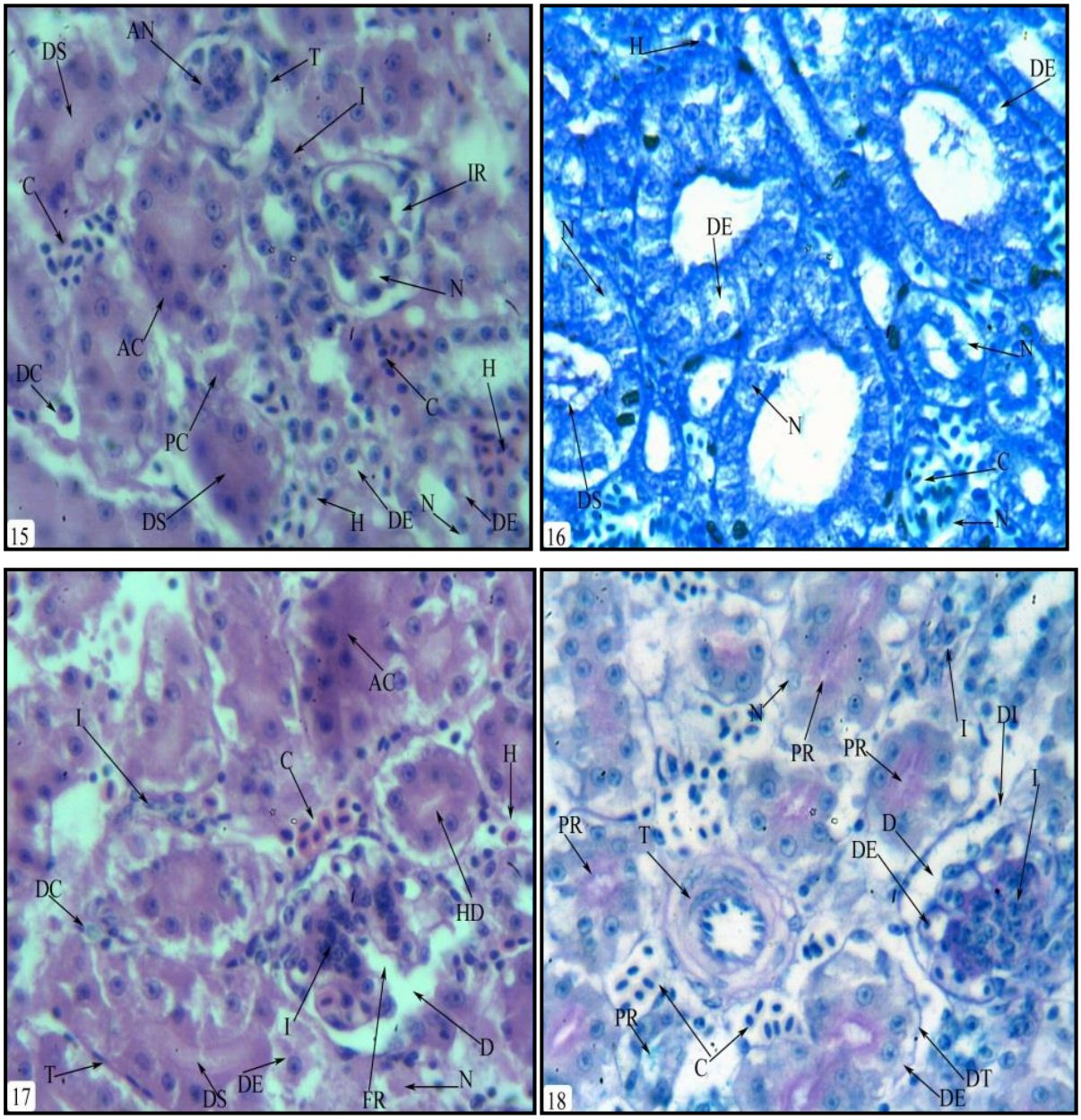

Figure (15): Histological structure of the Kidney after 15 days from treated with $CdCl_2$. H&E stain, 40X. Figure (16): Histological structure of the Kidney after 15 days from treated with $CdCl_2$. TB stain, 40X.; Figure (17): Histological structure of the Kidney after 30 days from treated with $CdCl_2$. H&E stain, 40X.; Figure (18): Histological structure of the Kidney after 30 days from treated with $CdCl_2$. AB&PAS stain, 40X. Abbreviation: (C) Congestion; (H) Hemorrhage; (N) Necrosis; (I) Infiltration; (DS) Desquamation; (DE) Degeneration; (T) Thickness; (AC) Acidity; (DC) Devorative cell; (FR) Fragmentation; (HD) Hydropic degeneration; (D) Dilatation; (DI) Dilaceration; (DT)

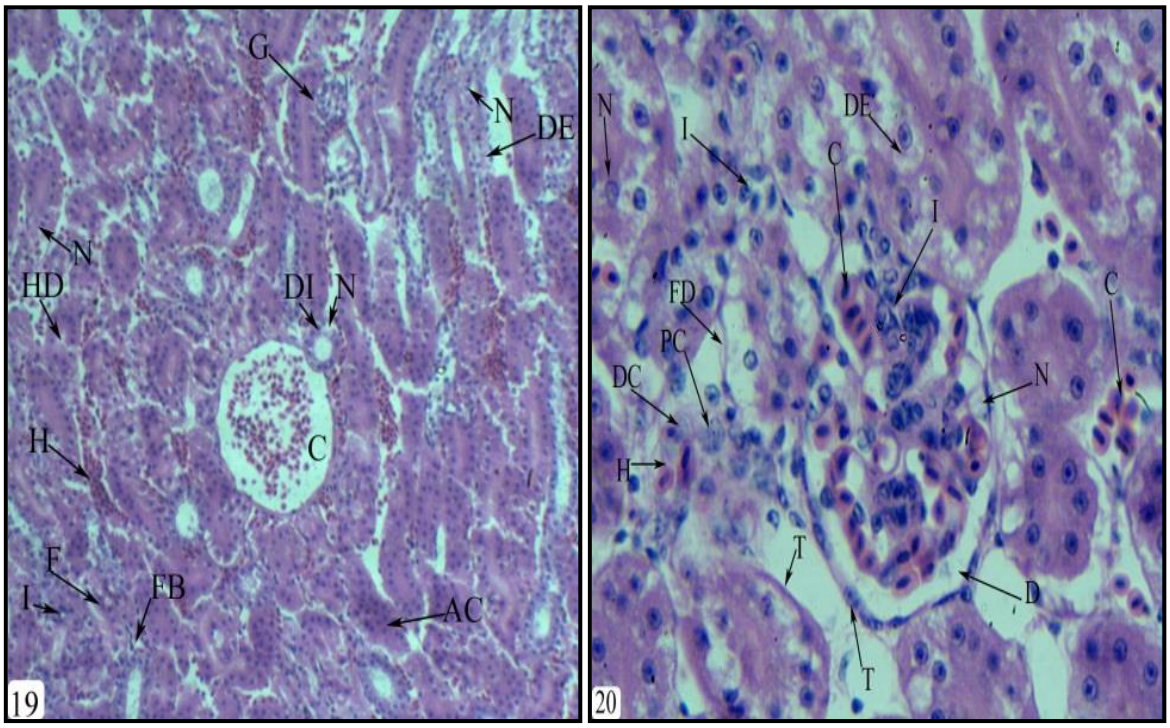

Figure (19): Histological structure of the Kidney after 60 days from treated with $CdCl_2$. H&E stain, 10X.; Figure (20): Histological structure of the Kidney after 60 days from treated with $CdCl_2$. H&E stain, 40X. Abbreviation: (C) Congestion; (H) Hemorrhage; (N) Necrosis; (I) Infiltration; (DE) Degeneration; (T) Thickness; (AC) Acidity; (DC) Devorative cell; (HD) Hydropic degeneration; (D) Dilatation; (DI) Dilaceration; (G) Glomerulus; (F) Fibrosis; (FB) Fibroblast; (FD) Fibrous deposits; (PC) Phagocyte.

