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Acute Effect of Cadmium Chloride on the Histological Structure of Liver in Japanese Quail *Coturnix coturnix japonica*

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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 research aims to identify the effect of acute exposure to cadmium chloride on the histological structure of the liver in one of the economic birds, which is 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 liver 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 necrosis, vacuolation, hemorrhage, ballooning swelling, congestion, infiltration of inflammatory cells, as well as enlargement of the sinuses and Kupffer cells hyperplasia. 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; Liver

I. INTRODUCTION

Heavy metals are naturally occurring elements throughout the earth's crust.There are 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. From the common heavy metals are Cadmium,Chromium, Lead, Copper, Mercury, 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 heart, liver, 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 will not found in 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 will lead to 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 hepatocytes and also due to damage to the fetus and reproductive organs [6]. The main action in poisoning is that it induces and leads to increase of 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 was extensively used in vertebrate physiology and diseases that affect human health [10]. The short life of the Japanese quail, along with its physiology that was similar to human sphysiology, made it an ideal bird 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 effects of the cadmium chloride ($CdCl_2$) on the tissue structure of the liver 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 this 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. First group was the control group, which was dosed with distilled water for four consecutive days. The second group was dosed with CdCl2 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 liver 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 that attached to an optical microscope.

III. RESULTS AND DISCUSSION

Results
Natural structure of the Liver
Microscopical examination of the liver in quail showed that it is histologically composed of large-sized polygonal cells known as hepatocytes. These cells are separated by spaces known as blood sinusoid, lining cells, especially Kupffer's cells. Hepatocytes are also formed in the form of plates or cords surrounding the central vein or its branches. These cords form with the central vein, that surrounded by what is known as hepatic lobules (Figures1,2).

Histopathological effect of $CdCl_2$ after four days
There were vacuolation and necrosis of hepatocytes and condensation in their nuclei, death in some cells, and intracellular hemorrhage were observed. Irregularity of the hepatic cords and an inability to distinguish the boundaries separating the hepatocytes were also noted. On the other hand, enlargement of blood sinuses and enlargement of Kupffer cells was observed. Also, stain M showed fibrosis lining some sinuses, and fibrous deposits were found inside the sinuses in some areas of the liver. As for the hepatic veins, congestion was observed in some of these veins and separated the vein wall from the surrounding hepatic cells (Figures 3,4).

Histopathological effect of $CdCl_2$ after 15 days
Histopathological changes that observed, were more severe than in the previous period. Severe congestion of blood vessels and fibrosis and hyperplasia of the fibroblasts around the components of the portal area, and partial obstruction of the bile ducts were observed. Infiltration of inflammatory cells has also been observed near the blood vessels. Strong infiltration of these cells was also observed in some areas of the liver, as these cells formed a spherical arrangement with red blood cells in the middle and large phagocytes. Dilated blood sinusoid and Kupffer's cells were also seen. As for hepatocytes, hepatic cords appeared irregular, necrosis, vacuolation, and hemorrhage between the hepatocytes, ballooning swelling, and an increase in the acidity of some cells and condensation of the nuclei, as well as apoptosis in some hepatocytes (Figures 5,6).

Histopathological effect of $CdCl_2$ after 30 days
The changes in the quail liver appeared less severe than in the previous period, but they were still effective. Hepatocyte irregularity appeared, a large swelling in some hepatocytes and the nuclei of some cells, condensation, and an increase in cytoplasmic acidity in other cells. Also, hepatocyte hemorrhage and necrosis and vacuolation and ballooning of some cells were found. The sinuses appeared very wide in some areas and narrow in others, as well as the enlargement of some Kupffer's cells. Congestion appeared in the blood vessels, which was severe in some and weak in others, with the dilaceration of some of these blood vessels (Figures 7,8).

Histopathological effect of $CdCl_2$ after 60 days
Many histopathological changes appeared, which included enlargement of the sinuses with greater enlargement than the previous period, hepatocyte irregularity, necrosis, and vacuolation in the hepatocyte, with the cytoplasm being intensified significantly, especially with H&E staining, as well as the presence of inter-hepatocellular hemorrhage and balloon swelling in some of them. It also noted severe congestion in some blood vessels, accompanied by infiltration of inflammatory cells and fibrosis and hyperplasia of the fibroblasts. While in other vessels, it was relatively slight, and enlargement of the bile duct wall cells was seen with occlusion of some of them (Figures 9,10).

Discussion
The results showed that after four days from exposure to the $CdCl_2$, many Histopathological effects included vacuolation and necrosis of hepatocytes. Irregularity of the hepatic cords, enlargement of blood sinuses, and fibrosis lining some sinuses was observed. The liver is the target organ in both chronic and acute exposure to Cd and other pathogens [17], as hepatocytes treated with Cd showed decreased mitochondrial membrane potential and a

significant increase in ROS production [18]. It has been suggested that acute hepatotoxicity involves two pathways, one for primary injury resulting from direct effects of Cd and the other for subsequent infection resulting from inflammation. The first results from the binding of

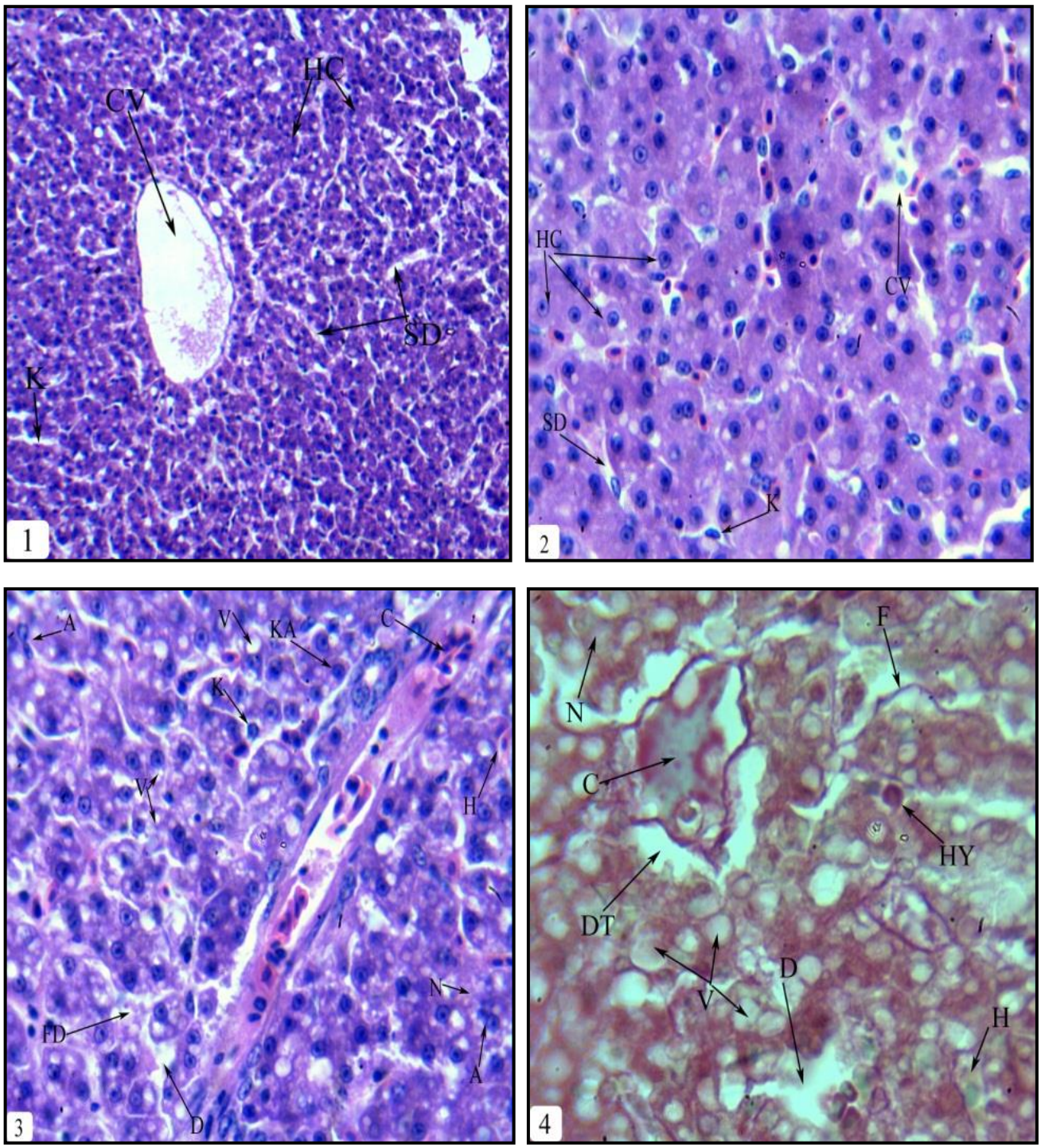

Figure (1): Normal histological structure of the Liver in Japanese quail. H& E stain,10X.; Figure (2): Normal histological structure of the Liver in Japanese quail. H&E stain,40X.; Figure (3): Histological structure of the Liver after 4 days from treated with $CdCl_2$. H&E stain, 40X.; Figure (4): Histological structure of the Liver after 4 days from treated with $CdCl_2$. M stain,40X. Abbreviation: (CV) central vein; (HC) hepatocytes; (SD) blood sinusoid; (K) Kupffer's cells; (A) Apoptosis; (V) Vaculation; (KA) Karyopyknosis; (FD) Fibrous deposits; (C) Congestion; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (DT) Detachment; (F) Fibrosis; (HY) Hypertrophy.

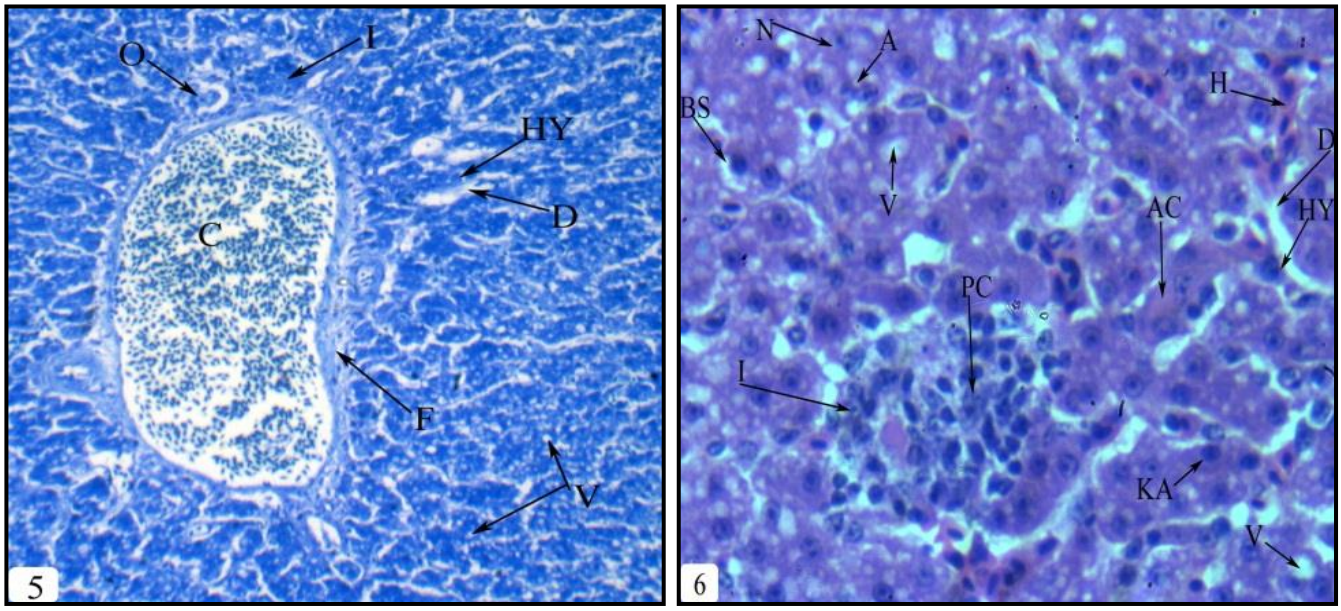

Figure (5): Histological structure of the Liver after 15 days from treated with $CdCl_2$. TB stain, 10X.; Figure (6): Histological structure of the Liver after 15 days from treated with $CdCl_2$. H&E stain, 40X. Abbreviation: (A) Apoptosis; (V) Vaculation; (KA) Karyopyknosis; (C) Congestion; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (F) Fibrosis; (HY) Hypertrophy; (O) Obstruction; (I) Infiltration; (BS) Ballooning swelling ; (PC) Phagocytes; (AC) Acidity.

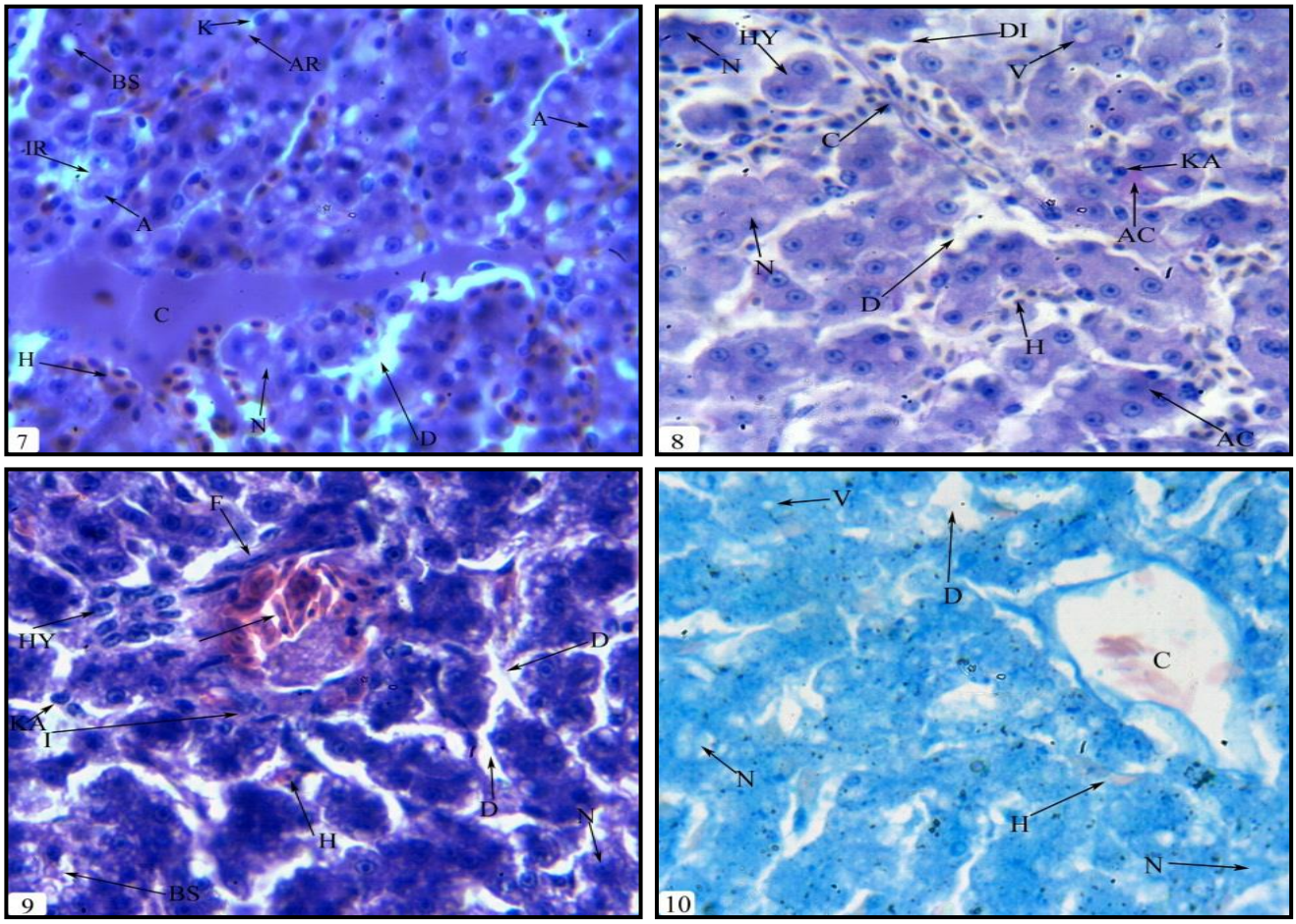

Figure (7): Histological structure of the Liver after 30 days from treated with $CdCl_2$. H&E stain, 40X.; Figure (8): Histological structure of the Liver after 30 days from treated with $CdCl_2$. AB&PAS stain, 40X. Figure (9): Histological structure of the Liver after 60 days from treated with $CdCl_2$. H&E stain, 40X.; Figure (8): Histological structure of the Liver after 30 days from treated with $CdCl_2$. AB stain, 40X. Abbreviation: (A) Apoptosis; (V) Vaculation; (KA) Karyopyknosis; (C) Congestion; (D) Dilatation; (H) Hemorrhage; (N) Necrosis; (HY) Hypertrophy; (I) Infiltration; (BS) Ballooning swelling ; (AC) Acidity; (IR) Irregularity; (AR) Arctation; (DI) Dilaceration ; (F) Fibrosis.

cadmium to sulfhydryl groups on critical molecules in mitochondria, Mitochondrial dysfunction. Although Cd directly affect liver cells, there are compelling reasons to think that injury to hepatocytes results in the body's perfusion resulting from damage to endothelial cells. Secondary injury due to acute exposure to Cd is believed to occur because the activation of Kupffer's cells and a series of events involving several kinds of the liver cells and a large number of the inflammatory and cytotoxic mediators [19].

After 15 days, More severe histopathological changes observed than in the previous period. Strong infiltration of the inflammatory cells has been observed near the blood vessels, as these cells formed a spherical arrangement with red blood cells in the middle and large phagocytes. Dilated blood sinusoid and Kupffer's cells were also seen. The hepatic cords appeared irregular, hemorrhage between the hepatocytes, and ballooning swelling. It's founded that the chickens were given feed contaminated with $CdCl_2$ showed damage in the liver, included multifocal vasculitis, perivascular lymphocytes, hepatic fibrosis, pronounced vasculitis, thick-walled blood vessels, a large number of lymphocytes, granulocyte infiltration, hepatocyte enlargement, enlarged hepatic sinuses, and mild congestion [18]. Rabbit livers treated with Cd showed focal granulomas and peripheral bile duct damage as well as perineal lymphocyte infiltration [20].

Whereas, after 30 days, The changes in the quail liver appeared less severe than in the previous period. However, they were still effective and included hepatocyte irregularity, a large swelling in some hepatocytes. The sinuses appeared very wide in some areas and narrow in others. Congestion appeared in the blood vessels, which was severe in some and weak in others. It has been found that Cd causes in hepatocytes swelling of both the rough endoplasmic reticulum and the mitochondria, which lose their cristae. It also causes nuclear chromatin compression, the increase of lipid droplets, the separation of liver cells from each other, and an increase in the production of the mineral protein metallothionine in the hepatocytes [21].

While, after 60 days, Many histopathological changes appeared, which included enlargement of the sinuses with greater enlargement than the previous periods, hepatocyte irregularity, and the presence of inter-hepatocellular hemorrhage and balloon swelling in some of them. It also noted severe congestion in some blood vessels, accompanied by infiltration of inflammatory cells and fibrosis and hyperplasia of the fibroblasts. The histopathological effects of Cd on the liver have been primarily associated with lipid peroxidation enhancement and DNA damage, and low doses of Cd can lead to inherited metabolic disturbances associated with fatty liver [22]. Exposure to Cd also leads to a significant decrease in the activities of glutathione peroxidase, with an increase in Malone dihydrate, damage to the infrastructure of the liver, and liver damage, as shown by a study conducted on chickens [23].

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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