HISTOLOGICAL ASSESSMENT FOR THE EFFECT OF CHLOROQUINE IN THE KIDNEY AND LIVER OF ALBINO RAT

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ABSTRACT

This current study was designed to assess expected histological lesions in rats kidney and liver during treated with chloroquine with different concentrations and at different time periods, 35 white rat females (Sprague-Dawley) were used in this study and distributed to 7 groups With 5 rats per group, the first group represented the control group that fed on a normal pilte and water throughout the experiment period, second group was oral administration with chloroquine (1.2) mg/kg for 30 days, the third group was administrated with chloroquine (1.2) Mg/kg for 45 days, fourth group administrated with chloroquine (2.25) mg/kg for 30 days, fifth group administrated with chloroquine (2.25) mg/kg for 45 days, sixth group was administrated with chloroquine (3.6) mg/kg for 30 days, the seventh group was administrated with chloroquine (3.6) mg/kg for 45 days, the administration was done using the Gavage for Oral administration, the behavioral examination of experimental groups was showed anorexia, laziness. The microscopical examination was revealed, segmentation and atrophy in glomeruli, the increased in space of glomeruli and the thickening of the Bowman capsule, detach of some endothelial cells into the renal tube lumen, degeneration of endothelial cells of renal tubes, as well as the breakdown and degeneration of renal glomeruli cells, hemorrhage and hyaline degeneration within the renal tubes. Cellular necrosis, hemorrhage, congestion, many inflammation cell infiltration was seen, detach of endothelial cells that line the central vein of liver.

I. INTRODUCTION

Chloroquine is a drug belonging to the aminoquinoline group, derived from quinine extracted from the bark of the Peruvian eucalyptus tree, and chloroquine is
on the WHO model list of essential drugs. Chloroquine was discovered by German scientist Hans Andersag in 1934 at Bayer L.G Farbenindustrie A.G. Laboratories in Eberflide, Germany, They called it Resochin and used the brand name Aralen (Manzoor et al., 2017), the first used in the early 20th century in malaria treatment, where it works against the asexual phase of malaria within red blood cells and of all kinds except the parasite-resistant strain found in South America and Southeast Asia and despite the introduction of many new antimalarial drugs chloroquine remains the most prescribed drug for malaria prevention and treatment, Chloroquine has been developed and successfully used as an important therapeutic role in various rheumatology diseases, including systemic lupus erythematosus, rheumatoids, arthritis, other infections and skin diseases (and Fishman, 2010 Pasadhika), and is currently used in the treatment of SARS-COV-2 acute respiratory syndrome caused by covid-19 coronary virus (Touret and Lamballerie, 2020). Chloroquine is taken orally (Plowe, 2005), and can be administered to the patient by injection in the event of severe disorders and the drug is given at a reduced dose and takes the drug for a period of one to six weeks (Savarino et al., 2003), The lethal dose is estimated at (30-50 mg/kg), as doses above 40 mg/kg are fatal, and doses higher than 20 mg/kg can also be toxic (Karalis et al.), 2020), chloroquine is rapidly absorbed into the digestive tract and is widely distributed in tissues with a very large distribution volume ranging from (200-800) liters (Ducharme and Farinotti, 1996), bioavailability (75-89%) (Krishna and White, 1996), is associated with (50-65%) plasma proteins and is evenly filtered through the kidneys and liver, and chloroquine is undergo to the first stage of metabolism to form metabolites desethyl-and bisdesethylchloroquine pharmacologically effective, Slowly reduced chloroquine concentrations and main metabolites slowly decrease with half-life bio- availabilities about (20-60) day. Chloroquine and its metabolites competitively inhibited the food inter reactions (Ducharme and Farinotti, 1996). In order to the board clinical using of chloroquine in the treatment of many diseases, but ideas about its mechanism of action remain, that chloroquine are weak alkaline and have a large distribution volume and a half-life of about 50 days, chloroquine interferes with lyzosome activity and self-phagocytosis, interacts with membrane stability and changes signal pathways and transcription activity, which may inhibit cytokine production and modify some common stimulation molecules, which may explain this procedure beside with the chemical properties of the drug, Clinical efficacy and adverse effects knowledge (Schrezenmeier and Dörner, 2020), the chloroquine have many side effects such as blurriness, nausea, vomiting, abdominal spasm and headaches, diarrhea, swelling
of the legs/ankles, shortness of breath, nail/skin pallor, muscle weakness and facilitating ecchymotic /bleeding, and hearing and mental problems (Siqueira, 2020).

Chloroquine is safe for use for women during pregnancy, so the U.S. Centers for Disease Control and Prevention prefers to use the drug to treat chloroquine-sensitive malaria during pregnancy (Melendez and Gonzalez, 2020). The study aimed to show how the drug affects on tissue of certain vital organs such as liver and kidneys.

II. MATERIAL AND METHODS

2-1 Drug used in the study:

Powder of chloroquine drug 100% purity produced from the General Company for the Manufacture of Medicines and Medical Supplies in Samarra.

2-2 Preparation of animals:

In this study, 35 Sprague-Dawley rats, with a weight rate (115-185 g) and 9-6 months old, were in good health and were subjected to appropriate and stable environmental conditions (25°C) and nutrition.

2-3 Study design:

Rats used in the study were randomly divided into six groups of experiments, as well as the control group, and the weights of each group were taken into account as much as possible before the start of the study.

Groups:

Group 1 (G1) control group that included 5 rats who were given food as well as water daily for 45 days.

Second group (G2): This group included 5 rats, who were given food and water daily with chloroquine at a concentration of (1.2) mg/kg per oral administration for 30 days.

Third group G3: This group included 5 rats, who were given food and water daily with chloroquine at a concentration of (1.2) mg/kg per oral administration for 45 days.
Fourth group (G4): This group included 5 rats, who were given food and water per day with chloroquine after dissolving it in 1ml of distilled water with a concentration of (2.25 mg/kg) per oral administration for 30 days.

Fifth group (G5): This group included 5 rats, who were given food and water daily with chloroquine after dissolving it in 1ml of distilled water with a concentration of (2.25 mg/kg) per oral administration for 45 days.

Sixth group (G6): This group included 5 rats, who were given food and water daily with chloroquine after dissolving in 1ml of distilled water with a concentration of (3.6) mg/kg per oral administration for 30 days.

Seventh group G7: This group included 5 rats, who were given food and water daily with chloroquine after dissolving in 1ml of distilled water with a concentration of (3.6) mg/kg per oral administration for 45 days.

Histological Sections Preparation:

The tissue sections was prepared in histology Lab., college of Science, Tikrit University, and prepared the microscopical sections according the method of Al hajj (2010), which include the following processes:

The collected tissues Each segments of kidney and liver was taken and immersed in 10 % formalin foe 24 hours followed by immersion in graded series of alcohol from 70, 80, 90 and 100 %, then clearing with xylene and embedded in paraffin wax at 60 cº. Blocking of the samples were done and the sectioning were performed using a rotary microtome. The thickness of the sections were 6 micrometer. The tissue sections after application of staining with Hematoxylin and Eosin were mounted on the slides using D.P.X and covered by cover slides. The slides were examined using light microscope and photographed by manipulated camera prepared for this purpose.

III. RESULTS

Effect of drug on behavior:

The results of the current study showed that there were not many changes in rat behavior treated with the drug, as the consumption of water and food for animals was normal, but there was a laziness and a tendency to sleep these disorder was minor in low doses and increased with high doses and duration of administration period.
Results of experimental organs

Kidneys:

Control group:

Microscopical examination showed that the cortex of kidney contained partial segmented glomeruli, surrounded by narrowed space. Proximal tubules shows narrowed lumen surrounded by a low pyramidal cells, the distal tubules was lined with simple cuboidal cells as shown in the figure (3-1).

Second group:

The results showed that the whole cortex has hyperplasia of the colloidial collagen fibers bundle with a glassy appearance in most section homogeneous appearance, the convoluted tubules was decreased in number. Atrophied glomeruli were and appeared as dark compact mass surrounded infiltrated inflammatory cells, as shown in the figure (3-2).

Third group:

The results of this showed atrophied and complete degeneration of glomeruli with an expansion of capsular space that surrounded it, as well as the little thickening of parietal wall Bowman's capsule. Other sections shows dilated lumen of both proximal and distal tubules, which filled by leakiest glomeruli and fibrin inflammatory filaments, as shown in figure (3-3).

Fourth group:

The results showed hypertrophy of renal glomeruli in the cortex and the spread of white blood cells on the surface of the glomeruli, in addition to the presence of hypertrophy of epithelial cells lining the both proximal and distal tubules with narrowing in their lumen, as shown in the figure (3-4).

Fifth group:

The results showed that the kidney cortex contained atrophied and segmented glomeruli, surrounded by space, with dilated lumen of both proximal and distal tubules, which filled by leakiest glomeruli, as well as desquamation of some epithelial cells lining this tubules. Spot of congestion and hemorrhage in between tubules and around superficial glomeruli, as shown in figure (3-5).
Sixth group:

The results showed that the kidney cortex contains partial segmented glomeruli in some area and complete segmented in other area, with dilated lumen of both proximal and distal tubules, which filled by leakiest glomeruli, as well as desquamation of some epithelial cells lining this tubules, as shown in figure (3-6).

Seventh group:

It showed hypertrophy of epithelial cells lining renal tubes with pyknotic there nuclei, as well as a blood congestion in interstitial tissue between the tubules, with degeneration and desquamation of the numbers of cells lining tubules in their lumen, as shown in the figure (3-7).

Liver

Control group:

Microscopic examination showed hepatic lobules in the hepatic tissue, each lobules contained in the middle the central vein which empty from blood, blood drainage in its sinusoids which contains on Kupffer cells, and those sinuses are surrounded by rows of polygonal hepatic cells and each cell has a dark central spherical nucleus with a number of nucleoli, as shown in figure (3-8).

Second group:

The results of this group was showed degeneration in hepatic cells in the hepatic lobule with pyknosis of nucleus and others karyolysis nuclei, as well as the presence of other liver cells was hypertrophy, necrotic cells which appears as vacuoles. Central vein was appeared empty in which blood sinusoid sinusoids open at its rim, as shown in figure (3-9).

Third group:

The results of present study was conduct, the central vein was contained a blood clot, surrounded by rows of polygonal hepatocyte, in other spot revealed hepatocyte hyperplasia, with congested small blood vessels by hemolytic RBCs, as well as spread of Kupffer cells in many blood sinuses, as shown in the figure (3-10).
Fourth group:

The results of this sections showed acute hypertrophy with the pyknotic nuclei in the marginal hepatic cells of liver, other liver cells was showed karyolitic nucleus, with pale vacuolated cytoplasm, as shown in figure (3-11).

Fifth group:

The microscopical examination was showed hypertrophy of hepatic cells with vacuolation there cytoplasm and karyomegaly of several hepatic cells, as well as an increased in central vein lumen, decreased the number of endothelial cells of central vein, rupture their basement membrane and surrounded by infiltrated WBCs from outside. Blood vessels was contain a Kupffer cells in their lumen with a number of red blood cells, as shown in figure (3-12).

Sixth group:

The results showed acute hypertrophy of hepatic cells, with large spherical nuclei, these cells appearance as aggregate groups. In between those cells a network of blood sinuses that contained Kupffer cells. In other spot we found congested thickened wall portal hepatic vein, as shown in figure (3-13).

Seventh group:

The results of this group was found, hypertrophy in some hepatic cells, and the central vein was surrounded by rows of hepatomegaly cells with pale nuclei surrounded by hepatic sinuses in which spread by kupffer cells, other spot was reveal some sinuses lumen have a homogenous inflammatory edema (colioed), as shown in figure (3-14).
Figure (3-1): Rat kidney of the I group (control group), The cortex of the kidney – the renal glomerulus with tiny capillaries (A) The capsular space (B) Bowman's capsule (C) The proximal convoluted tubules (D) The distal convoluted tubules (E)(X40,H&E).

Figure (3-2): Rat kidney of the II group, vitreous transformation in the colloidal fibers in the cortex of the kidney (A) Atrophy of the convoluted tubules (B) Atrophy of the glomerulus and infiltration of numbers of white blood cells on its surface (C) (X40,H&E).

Figure (3-3): Rat kidney of the III group, Atrophy and degeneration of the renal glomeruli (A) Capsular dilatation (B) Dilatation of the cavities of the convoluted tubules (C) Glomerular infiltrate and fibroinflammatory filaments (D) (X40,H&E).
Figure (3-4): Rat kidney of the IV group, glomerular hypertrophy (A) Hypertrophy of cells lining the proximal and distal convoluted tubules (B) (X40, H&E).

Figure (3-5): Rat kidney of the V group, Glomeruli with atrophy (A) Glomerular infiltrate into the lumen of the convoluted renal tubule (B) Congestion and hemorrhage (C) (X40, H&E).

Figure (3-6): Rat kidney of the VI group, Renal cortex with lobulated glomeruli (A) Glomerular infiltrate into the lumen of the convoluted urinary tubules (B) Degeneration of epithelial cells in the lumen of the tubules (C) (X40,H&E).
Figure (3-7): Rat kidney of the VII group. Pulp of renal tubule epithelial hypertrophy. (A) Epithelial cell debris in tubule lumen (B) (X40, H&E).

Figure (3-8): Rat liver of the I group (control group). The hepatic lobule contains the central vein (A) The rows of polygonal hepatocytes with spherical nuclei (B) The blood sinusoids, and the Kupffer cells (C) (X40, H&E).

Figure (3-9): Rat liver of the II group. Hepatic lobule - central vein (A) Enlarged hepatocytes devoid of nuclei and transformed into vacuole-like (B) Nuclei pyknosis (C) sinusoids and containing kupffer cells (D) (X40, H&E).
Figure (3-10): Rat liver of the III group, The central vein with a blood clot (A) Rows of hepatocytes (B) Sinusoidal with degenerated liver cells (C) Clot in the sinusoids (E) (X40, H&E).

Figure (3-11): Rat liver of the IV group, periphery of liver tissue - hepatocyte hypertrophy (A) Pyknotic nuclei (B) Vacuolated hepatocytes with ruptured cytoplasm (C) (X40, H&E).

Figure (3-12): Rat liver of the V group, the central vein with sloughing and loss of endothelial cells (A) Rupture of parts of the basement membrane (B) White blood cells (C) Hypertrophy of hepatocytes (D) kupffer cells and red blood cells (E) (X40, H&E).
IV. DISCUSSION

Kidneys:

The results of present study showed, that the kidney is affected by the different doses of chloroquine, through the amount of damage to its tissues, desquamation, atrophy, degeneration and distention was found in urinary tubules and glomeruli in different degrees and bleeding and many congestion in there tissue, this may be due to its work on mineral balance as well as to the harmful effects of the drug, where some reports noted that 5-20% of cases that can be attributed to acute kidney failure directly to medications and chemicals (Mahmoudi et al., 2020), the results of current study agreed in terms of the degeneration of distant, proximal convoluted tubules, with the results of wang (2020) when treating rats at a dose (50 mg/kg) for 4 weeks, can caused glomeruli atrophy and congestion.
of blood vessels. And also agreed with the results of the Elshishtawy (2014) when the rats were oral administration at doses (250 mg/kg) with chloroquine concentration for 6 weeks.

The results of the microscopical examination showed degeneration, with multiple hemorrhage hotspots, and it was also found that some hypertrophied cells that lined renal tubules compared with normal kidneys these results agreed with the study of Pari and Murugan (2006) when treating mice with chloroquine at doses (970 mg/kg) for 8 days orally. This result is also agreed with Morrissey and his group (2001) that showed the toxic substances lead to the edematous of renal tubules, enlarge cells and edematous lead to the constructive the lumen of tubules and finally lead blocked its and necrotic the lining cells, a result of diminish of oxygen that role the functioning of mitochondria to produce energy as ATP to work to balance the osmotic pressure, and a defect in the presence of sodium that leads to the disruption of pressure and fluid accumulate within cells.

When the foreign body or toxic substances enter the body, the process of inflammation begins with the first step of identifying the foreign substance, and then the blood vessels begin to distention, fluids are accumulate and inflammatory cells filtered around the tissues as they are attracted to the site of infection to treat inflammation (Johnson et al., 2002) the inflammation is divided into acute and chronic inflammation depending on the time in which symptoms occur (Stevens et al., 2002). Inflammation is the initial response to the entry of the pathogen and its achieved by increasing the movement of plasma and WBCs from the blood to the infected tissues, which leads to the congestion of these blood vessels due to increased blood flow and vasodilation, these changes can appear in tissue sections in the form of congestion or hemorrhage (Kumar et al., 2007), and Kumar (1997) also indicated that the causes of hemolysis RBCs due to local bleeding and this finally engulfed by phagocyte cells.

Cooper and Magwere (2008) noted that chloroquine accumulates in tissues where inhibit the metabolism in kidney cells and increased the activities of lysosomes within cytoplasm, and also deposits in the adrenal glands and directly affects Kidney function by modifying the secretion patterns of aldosterone which affect on Na ions balance. Chloroquine deposition in the epithelial cells of the kidneys may result in possible interference of ion movements, and several studies have shown that cellular phagocyte process become inhibited, in Many age-related diseases, where cellular phagocyte process is necessary to maintain cellular balance.
and has an important role to play in ensuring the normal function of organs (Elshishtawy et al., 2014).

**Liver:**

The liver is a complex organ because it has enzyme pathways that play a major role in physiological functions, so many drugs and toxic substances can change these processes pathways (Chavez-Tapia et al., 2006), and the metabolism of chemicals substances in the liver can induce damage in liver cells through oxidative effort and the loss of mitochondrial function and finally induce liver damage, and these damage can cause toxicity directly through cellular toxicity or self-sensitivity to this substance (Amacher, 2012).

The results of the present study showed the presence of cellular tissue damage in liver of rats which treated with the drug, represented by hypertrophy of some hepatic cells, pyknosis nuclei, vacuolation there cytoplasm, nucleus fragmentation and cell necrosis. This is in line with what Akin (2021) found when rats were injected with chloroquine at a concentration of 50 mg/kg for 50 days, represented by necrosis, degeneration and death of hepatic cells.

The results of the current study in terms of hepatic cell degeneration and focal lymphocyte infiltration were agreed with the study of Niknahad (2016) when rats were injected with (20 mg/kg) KG of chloroquine for 48 hours, as well as agreed with the results of Elshishtawy (2014) when rats were orally administration with a dose of chloroquine (250 mg/kg) for 6 weeks.

Hepatocytes undergo, hypertrophy and degeneration may result from a disturbance in metabolic processes of these cells which based on the concentration of the drug dose in the bloodstream, and the exposure of cell nuclei to lysis at progress stages indicates that hepatic cells are first line to treat with toxic substances because they are the first cells to be reached by gastrointestinal tract (Kumar, 2003). This makes them constantly susceptible to the damage that appears in cells when cell treating with toxicants, all these defect occurs increased with high doses of toxic substance and the time to which the organ is exposed, or the cause of cell hypertrophy may be due to the effect of the drug on cellular membranes and ion balance.

The results of presence study showed, aggregate of infiltrated inflammatory cells and kupffer cells in abundance as well as hemorrhage and congestion of blood vessels, so the degenerative hepatocytes which produce of chemical attractions that
act as chemotactic to inflammatory cells such as neutrophil and other inflammatory cells (Jaeschke et al., 1999).

The mechanism of chloroquine toxic effects is still unknown, but some studies have shown that it may be due to the formation of some oxidized metabolites, which increase the production of reactive oxygen species, and that chloroquine accumulates particularly in cells such as Kupffer cell in the liver, resulting in cellular damage as a whole, including accumulate of non-digestible substances in lysosomes of hepatocytes with and increasing their size and number (Alkadi, 2007). Xu (2018) have indicated that antimalarials inhibit cellular damage to the liver, leading to hepatic cell degeneration.

V. CONCLUSIONS

The results of the current study can be concluded as follows:

1. The dose of treatment chloroquine, although as a specific dose for uses ends with a side effect on the tissues of vital organs.

2. All organ tissue used in the current study had a detrimental effect by chloroquine, where degeneration, hypertrophy, necrosis, infiltration of W BCs and congestion of blood vessels and occurred of hemolysis within some lumen of other vessels.

VI. REFERENCES


