International Journal of Life Science Research Archive

ISSN: 0799-6640 (Online)

Journal homepage: https://sciresjournals.com/ijlsra/

(RESEARCH ARTICLE)

Check for updates

Histological comparison of rats lung treated with chloroquine

Anhar Rabeea Hussein [1,*], Ateen Amer Hameed [2] and Khulood Naji Rasheed [3]

[1] *Department of Biology, College of Science, Tikrit University, Iraq.*
[2] *Department of Medical Biotechnology, College of Science, Tikrit University, Iraq*
[3] *Department of Anatomy and Histology, College of Medicine Tikrit University, Iraq.*

International Journal of Life Science Research Archive, 2026, 10(02), 159-166

Publication history: Received on 14 May 2026; revised on 21 June 2026; accepted on 24 June 2026

Article DOI: https://doi.org/10.53771/ijlsra.2026.10.2.0049

Abstract

Chloroquine is an antimalarial drug with immunomodulatory and anti-inflammatory properties commonly used to treat certain autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. The drug functions by disrupting cellular processes and restricting the replication and activity of intracellular parasites. This study aims to evaluate the dose- and duration-dependent effects of chloroquine on the lung tissue of female Sprague-Dawley rats. Histological changes were compared between the treated and control groups to determine the relationship between dosage, exposure duration, and the severity of potential pathological variations. This study included 35 female Sprague-Dawley rats, divided into 7 groups of 5 animals each. The first group served as the control and received a standard diet and water throughout the experiment. The six experimental groups were administered chloroquine at varying concentrations and durations: groups 2 and 3 received 1.6 mg/kg for 35 and 50 days, respectively; groups 4 and 5 received 2.5 mg/kg for 35 and 50 days, respectively; and groups 6 and 7 received 3.8 mg/kg for 35 and 50 days, respectively. The animals were then sacrificed, and their lungs were excised for histological examination.

Histological findings in the lungs of rats treated with chloroquine revealed marked pathological changes, including widespread congestion, interstitial hemorrhage, and intravascular hemolysis. Inflammatory lymphocytic infiltration of varying degrees was also observed, along with degeneration, necrosis, and sloughing of the alveolar epithelium. Correlating with the increase in dose and duration of exposure, the severity of pathological changes increased to include thickening of bronchiolar, alveolar sacs, and air sacs walls, focal fibrosis, as well as dilation and congestion of blood vessels. Furthermore, signs of chronic inflammation emerged, characterized by dense cellular infiltration and the presence of alveolar macrophages within the lung tissue. In conclusion, these findings demonstrate that chloroquine induces dose- and duration-dependent pulmonary toxicity, characterized by progressive structural and inflammatory changes in lung tissue.

Keywords: Chloroquine; Rat lung; Pulmonary toxicity; Pneumotoxicity

1 Introduction

Chloroquine is a drug traditionally used to treat malaria, which recent studies have shown to possesses multiple immunological and cellular effects. It acts by accumulating within the lysosomes of phagocytes and altering their pH, thereby influencing autophagy, the immune response, and the inhibition of Toll-like receptor signaling (Al-Hamadani *et al*., 2025). Furthermore, it exhibits anti-inflammatory properties by reducing the production of inflammatory cytokines and regulating immune cell activity; therefore, it is widely used in certain autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (Rao *et al*., 2023). Studies have indicated that prolonged use or high doses may cause histological and functional changes due to its effects on oxidative stress and cell membranes (Yusuf *et al*., 2023). During the COVID-19 pandemic, chloroquine was incorporated into some treatment protocols due to its proposed

* Corresponding author: Anhar Rabeea Hussein

antiviral and anti-inflammatory effects; however, subsequent clinical studies did not demonstrate clear therapeutic efficacy, in addition to reporting significant side effects such as cardiac arrhythmias (dos Reis Neto *et al*., 2020).

Prolonged administration or high doses of chloroquine can affects several organs; it may cause liver dysfunction and cellular changes, reduced renal function with histological changes, and cardiac arrhythmias and QT prolongation. Retinopathy is also one of the most significant chronic effects that can progress to visual impairment, in addition to neurological and muscular effects such as generalized weakness. In the lungs, the drug induces structural damage including congestion, interstitial hemorrhage, and inflammation, which potentially impact on gas exchange efficiency due to oxidative stress and pulmonary cellular dysfunction (Yusuf *et al*., 2023).

The lungs are located within the thoracic cavity and are covered by the pleura, which consists of the parietal layer lining the chest wall and the visceral layer covering the surface of the lungs these two layers meet at the hilum, through which blood and lymphatic vessels pass and the bronchi enter and exit. The right lung consists of three lobes, while the left consists of two lobes. Each lobe is connected to a lobar bronchus branching off from the main bronchus, which originates from the trachea, and then branches into secondary and tertiary bronchi, leading to the respiratory bronchioles. These branches terminate in, alveolar ducts, and alveolar sacs containing alveoli, which are the primary site of gas exchange (Abdullah *et al*., 2021).

Given the documented Cellular and immunological effects of chloroquine, along with its association with histological damage and oxidation in systemic tissues after prolonged administration or high doses, the lung was selected in the current study as a sensitive target organ for inflammatory responses and oxidative stress. This study is aimed at investigating the possible histopathological consequences of the drug and assessing its effect on the functional structure of lung tissue.

2 Materials and methods

2.1 Drug used

Pure chloroquine powder (100% purity) was obtained from the General Company for the Manufacture of Medicines and Medical Supplies in Samarra, Iraq.

2.2 Experimental design:

In this study, 35 healthy female Sprague-Dawley rats, weighing (150–200) g and aged (2–4) months, were utilized. The animals were housed under controlled environmental conditions, including a stable temperature of 25°C, and provided with nutrients and water.

The rats were randomly divided into seven groups: six experimental groups and one control group, and the weights of each group were considered as much as possible before the start of the study. The animals were

distributed into seven distinct groups (five animals per group) and treated as follows:

- **Group 1 (G1, control):** rats received nutrients and water for 50 days without any treatment.
- **Groups (G2) and (G3):** rats were orally administered chloroquine at a dose of 1.6 mg/kg body weight, dissolved in 1 mL of distilled water, daily for 35 and 50 days, respectively.
- **Groups (G4) and (G5):** rats were orally administered chloroquine at a dose of 2.5 mg/kg body weight, dissolved in 1 mL of distilled water, daily for 35 and 50 days, respectively.
- **Groups (G6) and (G7):** rats were orally administered chloroquine at a dose of 3.8 mg/kg body weight, dissolved in 1 mL of distilled water, daily for 35 and 50 days, respectively.

All experimental groups had access to food and water throughout their respective study periods.

2.3 Histological Sections Preparation:

The tissue sections were prepared in histology laboratory. College of Science, Tikrit University. Microscopical sections were processed following the methods described by Al-Hajj (2010), which comprised the following steps:

Excised lung segments were immediately immersed in 10% neutral buffered formalin for 24 hours for fixation. Subsequently, the tissues were dehydrated through a graded series of ethanol (70, 80, 90, and 100)% then cleared in

xylene, and embedded in paraffin wax at 60°C, after that paraffin blocks were sectioned at a thickness of 6µm by using a rotary microtome. The obtained sections were stained with hematoxylin and eosin (H&E), mounted onto slides using D.P.X., then covered with cover slips. Finally, the prepared slides were examined using a light microscope and photographed using a digital camera adapted for microscopic imaging.

3 Results

Histological examination of the lungs from the control group (G1) demonstrated a normal histological architecture, characterized by regular and uniform alveolar sacs with well-preserved tissue integrity.

The pulmonary alveoli appeared clear and open, with normal air spaces separated by thin, intact interalveolar septa, as shown in Figure. (1). In the second group extensive hemorrhagic congestion was observed at the periphery of the histological sections, accompanied by diffuse interstitial pulmonary hemorrhage and intravascular hemolysis, severe lymphocytic infiltration was also observed, as shown in Figure (2). In Histological sections from the third group, thickening of the alveolar sacs walls and air sacs was observed, accompanied by exudate, interstitial inflammation and widespread hemorrhage in the pulmonary interstitium, as shown in Figure (3).Histological examination of the fourth group revealed acute focal lymphocytic infiltration around the alveoli, accompanied by thickening of the alveolar walls and inflammatory exudate within the alveolar spaces Additionally, vascular congestion and intravascular hemolysis were noted, as shown in Figure (4).Microscopic investigation of the fifth group demonstrated degenerative changes and desquamation of the respiratory epithelium lining the bronchioles. This was accompanied by thickening of both the bronchiolar and alveolar walls, as shown in Figure (5).Distinct pathological alterations were prominent in the sixth group including degeneration and necrosis of the bronchiolar epithelium, accompanied by vascular congestion, dilation, and wall thickening, alongside with widespread inflammatory cellular infiltration within the pulmonary tissue, as shown in Figure (6). Microscopic assessment of the seventh group revealed vascular congestion accompanied by vascular dilation and wall thickening. Additionally marked thickening walls both of alveolar sacs and alveoli was observed throughout the section, along with both focal and diffuse inflammatory cell infiltration within the pulmonary interstitium, as shown in Figure (7).

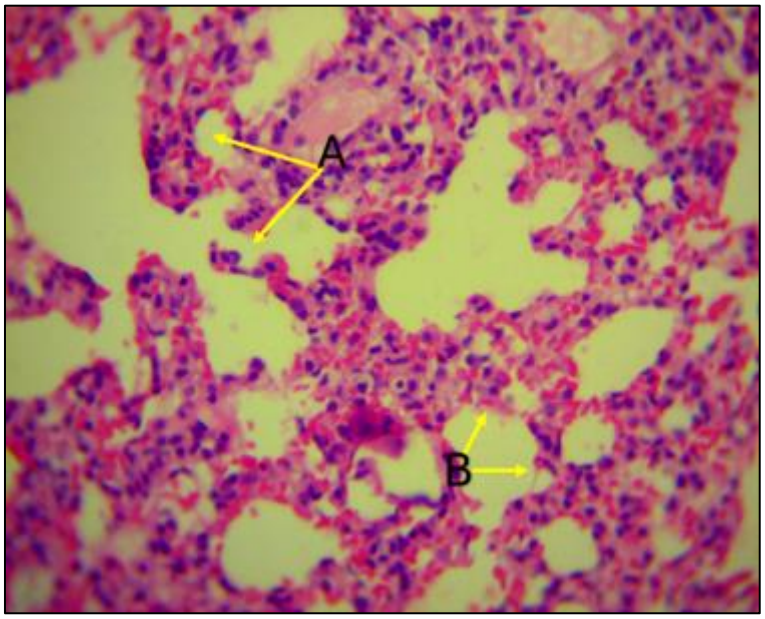

Figure 1 Photomicrograph of a G1 control rat lung, showing the normal histological appearance of both the alveolar sacs (A) and the pulmonary alveoli (B). (H&E, 40×)

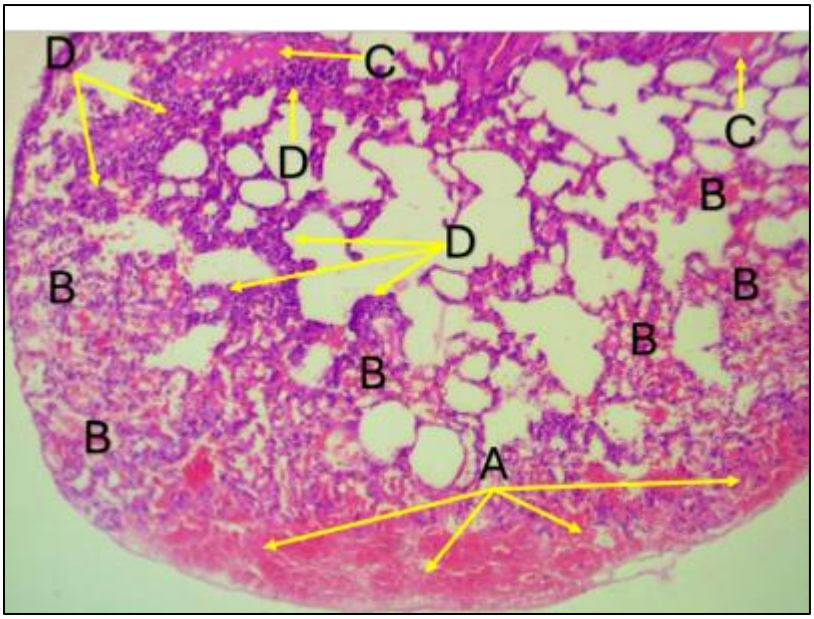

Figure 2 Photomicrograph of a rat lung from G2 (1.6 mg/kg chloroquine for 35 days), showing extensive congestion at the periphery of the tissue section (A), widespread interstitial hemorrhage (B), intravascular hemolysis within the blood vessels (C), and dense lymphocytic infiltration (D). (H&E, 40×)

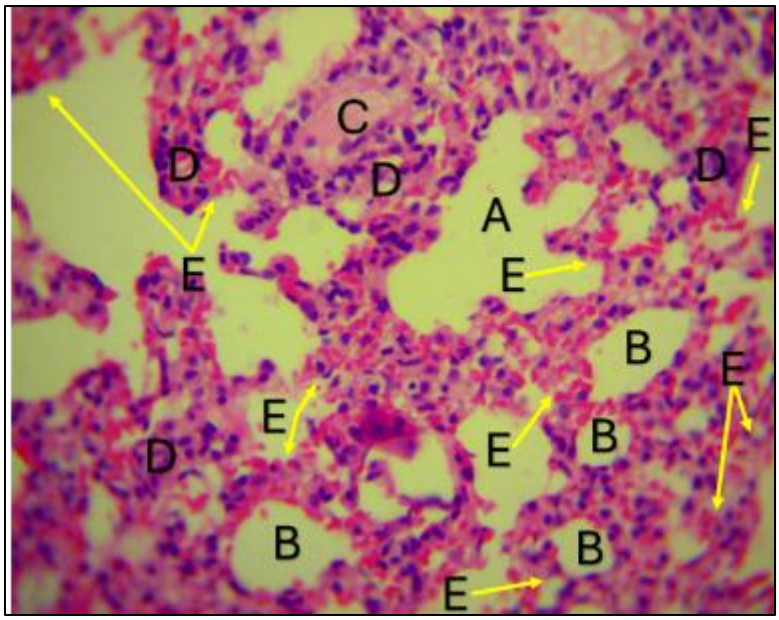

Figure 3 Photomicrograph of a rat lung from G3 (1.6 mg/kg chloroquine for 50 days), showing thickening of the walls of the alveolar sacs (A) and alveoli (B) across the tissue section inflammatory exudate Additionally, a prominent (C) and inflammatory cellular infiltration (D), with widespread interstitial hemorrhage (E). (H&E, 40×)

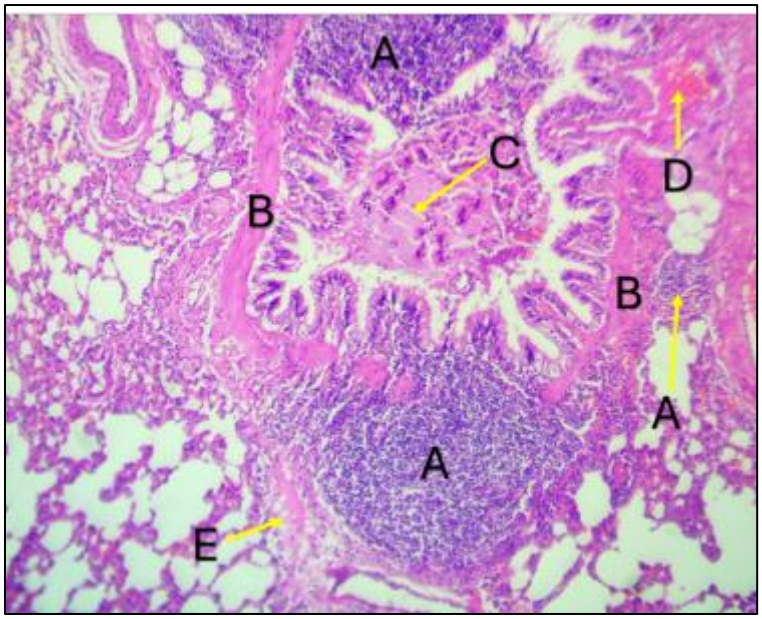

Figure 4 Photomicrograph of a rat lung from G4 (2.5 mg/kg chloroquine for 35 days), showing focal acute lymphocytic infiltration at the periphery of the alveoli (A) with thickening of their walls (B) and accumulation of inflammatory exudate within their lumens (C), vascular congestion also notable (D), and hemolysis at the other vessel (E). (H&E, 40×)

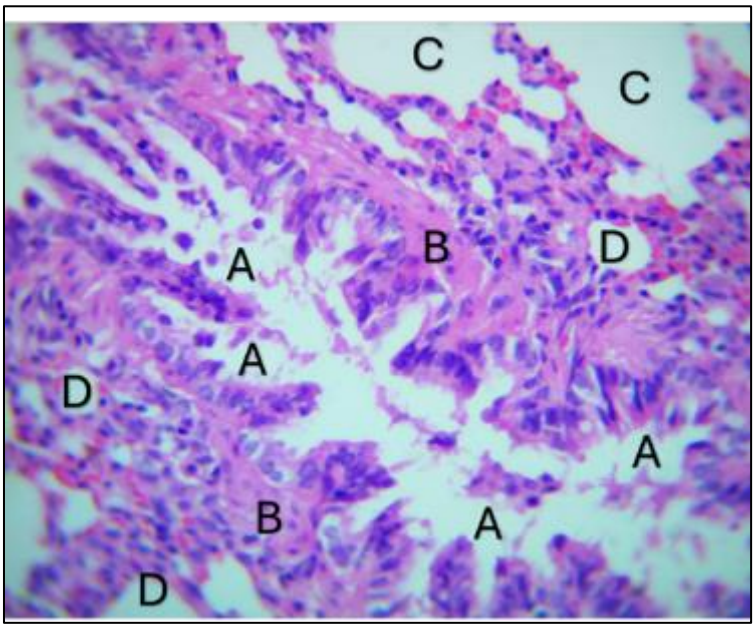

Figure 5 Photomicrograph of a rat lung from G5 (2.5 mg/kg chloroquine for 50 days), showing degeneration and sloughing of the respiratory epithelium lining the air bronchioles (A) Furthermore, marked thickening is observed across the bronchiolar walls (B), thickening of the alveolar walls (C), and thickening of alveoli (D). (H&E, 40×)

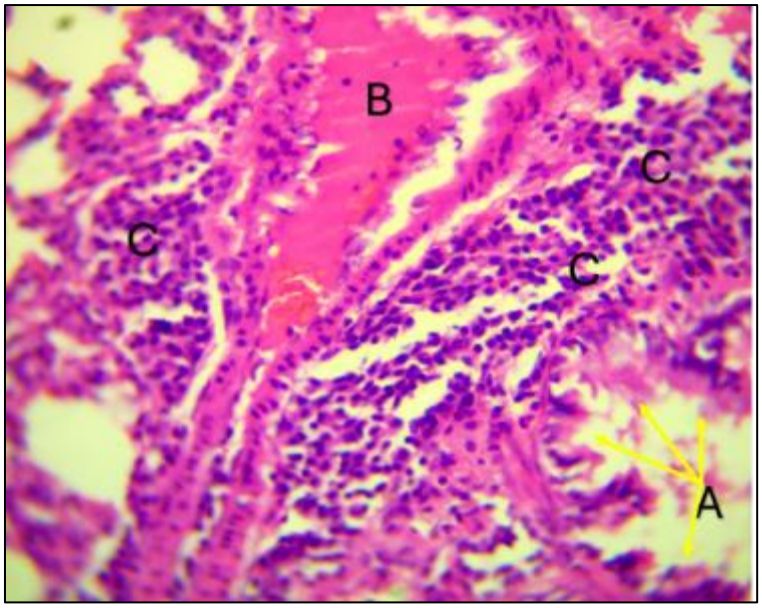

Figure 6 Photomicrograph of a rat lung from G6 (3.8 mg/kg chloroquine for 35 days), showing degeneration and necrosis of the bronchiolar epithelium (A), a dilated, congested blood vessel with thickened walls (B), and widespread dense inflammatory cellular infiltration (C). (H&E, 40×)

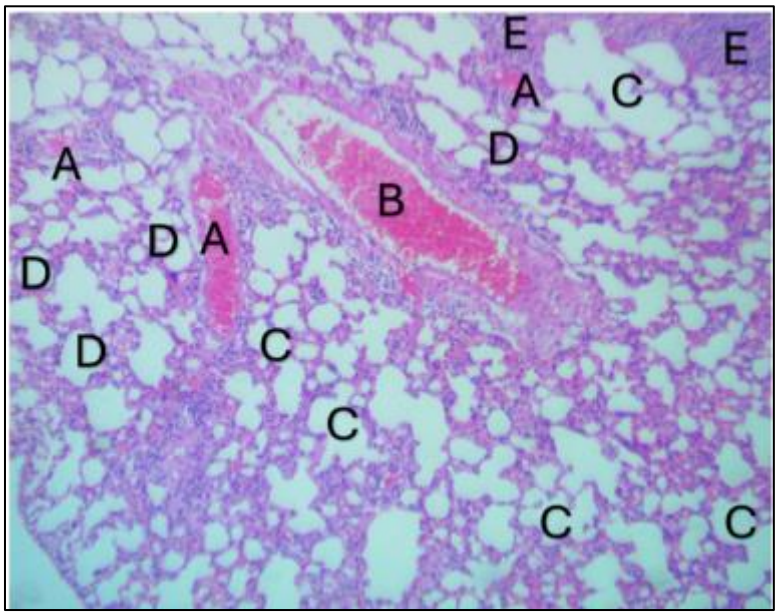

Figure 7 Photomicrograph of a rat lung from G7 (3.8 mg/kg chloroquine for 50 days), showing congested blood vessels (A), one of which is dilated and has a thickened wall (B) Additionally, extensive thickening is observed across the walls of the alveolar sacs (C) and alveoli (D) throughout the tissue section accompanied by both focal (E) and diffuse inflammatory cellular infiltration within the interstitial tissue. (H&E, 40×)

4 Discussion

In the current study, chloroquine exposure induced pulmonary pathological changes of varying severity in rats. These histopathological manifestations were dose and duration dependent.

The second experimental group showed severe pathological histological changes that may be attributed to endothelial damage mediated by oxidative stress and increased pulmonary vascular permeability. It is possible that the formation

of reactive oxygen species (ROS) induced by chloroquine contributed to the occurrence of vascular dysfunction and disruption of tissue integrity, results consistent with those indicated by Shen *et al*. (2017) and Wang *et al*. (2022). The third experimental group showed advanced structural and inflammatory damage as a result of prolonged exposure to chloroquine. Persistent oxidative stress and persistent inflammatory activation may accelerate the remodeling of pulmonary tissue and impair the function of pulmonary alveoli. Chronic cellular stress also promotes infiltration of inflammatory cells and a gradual change in the architectural structure of the lung. Similar observations were discussed by Ercan *et al*. (2024) and Skeoch *et al*. (2018), who linked prolonged drug exposure to the occurrence of cumulative lung damage. The fourth experimental group showed pronounced histopathological changes that were associated with the enhancement of inflammatory signals and vascular dysfunction. Increasing the dose of chloroquine seems to aggravate endothelial dysfunction and increase the release of inflammatory media, amplifying the damage to the pulmonary tissue even during short exposure periods. Wang *et al*. (2022) and Shen *et al*. (2017) have documented similar results, confirming the role of chloroquine in promoting inflammatory and pulmonary vascular changes. The fifth experimental group revealed advanced structural degeneration that may be associated with mitochondrial dysfunction resulting from chronic exposure to chloroquine. Disruption of the mitochondrial electron transport chain leads to ATP depletion and overproduction of reactive oxygen species, which subsequently activates atrophic pathways and contributes to the gradual degeneration of the epithelium. These mechanisms were supported by studies by Savarino *et al*. (2003) and Wang *et al*. (2022), who confirmed that mitochondrial injury is a major factor in chloroquine-induced histotoxicity. The sixth experimental group highlighted acute and severe pathological histological lesions, confirmed the presence of severe chronic histological manifestations following the administration of the high dose of chloroquine. Excessive oxidative stress and profound mitochondrial dysfunction can lead to irreversible cellular damage by disrupting membrane stability and ion homeostasis. Moreover, the drug caused severe cellular and architectural disorders through the massive generation of reactive oxygen species and the activation of mediated inflammatory chains by molecules of molecular patterns associated with damage (DAMPs). These processes have led to the occurrence of large-scale pathological histological anomalies and enhanced tissue irregularity and damage within the pulmonary tissue. In addition, severe mitochondrial dysfunction caused pronounced microscopic changes in the integrity of the tissue and provoked a severe inflammatory response in the affected tissue. Similar explanations were given in the studies of Subbiah and Tiwari (2021) and Wang *et al*. (2022). The seventh experimental group showed severe pulmonary, vascular and inflammatory changes that may be associated with persistent endothelial dysfunction and chronic inflammatory activation caused by prolonged exposure to chloroquine. Constant oxidative stress can impair the integrity of blood vessels and promote circulatory disorders within the pulmonary tissue, which leads to a gradual remodeling of the tissue and depolarization of inflammatory cells. Moreover, chronic stimulation of inflammatory media may contribute to the occurrence of interstitial inflammatory reactions and structural changes in the architecture of the pulmonary alveoli. These results support the concept that continuous exposure to chloroquine exacerbates pulmonary damage through interrelated vascular, inflammatory and oxidative mechanisms. Wang *et al*. (2022) and Skeoch *et al*. (2018) have proposed similar explanations, suggesting that chronic oxidative stress and inflammatory activation play a pivotal role in the development of pulmonary histopathological damage.

5 Conclusion

In all groups treated with chloroquine histopathological changes of varying severity, depending on the dose and duration of exposure were exhibit. These alterations consisting primarily of vascular abnormalities including congestion, hemorrhage, and vasodilation with intravascular hemolysis, as well as inflammatory responses were prominent, characterized by acute, focal, and diffuse lymphocytic infiltration extending into the pulmonary interstitium. Structural changes in lung tissue were also observed, including thickening walls of the alveolar sacs, air sacs, and alveoli, presence of inflammatory exudate within the alveolar spaces in certain groups. Furthermore, signs of degeneration, necrosis, and desquamation of the respiratory epithelium were noted. Taken together, these findings summarized pulmonary injury which characterized by inflammation, vascular disruption, and degenerative changes, which progressively escalate with increasing dose and duration to chloroquine exposure reflecting a clear toxic effect on the histological structure of the lung.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Abdullah, H., Rasheed, K., & Mezher, M. (2021). A Study of The Histopathological Effect of White Albino Mice Lung Infected with Aspergillus Fumigatus, Treated with Amphotericin B, And Trametes Spp. *Journal of Life and Bio Sciences Research, 2(01), 07-12*.

[2] Al-Hajj, Hamid Ahmed (2010). Optical microscopy preparations, theory and application. Dar march publishing, distribution and printing. First Edition, Amman - Jordan, p. 238.

[3] Al-Hamadani, M., Darweesh, M., Mohammadi, S., & Al-Harrasi, A. (2025). Chloroquine and hydroxychloroquine: Immunomodulatory effects in autoimmune diseases. *World Journal of Biological Chemistry*, 16(2), 107042.

[4] dos Reis Neto, E. T., Kakehasi, A. M., de Medeiros Pinheiro, M., Ferreira, G. A., Marques, C. D. L., da Mota, L. M. H., & Provenza, J. R. (2020). Revisiting hydroxychloroquine and chloroquine for patients with chronic immunity-mediated inflammatory rheumatic diseases. *Advances in Rheumatology*, 60(1), 32.

[5] Ercan, G., Bora, E. S., Çınaroğlu, O. S., Karaali, R., & Erbas, O. (2024). Hydroxychloroquine attenuates sepsis-induced acute respiratory distress syndrome in rats. Turkish Journal of Trauma & Emergency Surgery, 30(7), 465.

[6] Rao, I. R., Kolakemar, A., Shenoy, S. V., Prabhu, R. A., Nagaraju, S. P., Rangaswamy, D., & Bhojaraja, M. V. (2023). Hydroxychloroquine in nephrology: current status and future directions. *Journal of Nephrology*, 36(8), 2191-2208.

[7] Savarino, A., Boelaert, J. R., Cassone, A., Majori, G., & Cauda, R. (2003). Effects of chloroquine on viral infections: an old drug against today's diseases. The Lancet infectious diseases, 3(11), 722-727.

[8] Shen, H., Wu, N., Wang, Y., Zhao, H., Zhang, L., Li, T., & Zhao, M. (2017). Chloroquine attenuates paraquat-induced lung injury in mice by altering inflammation, oxidative stress and fibrosis. International immunopharmacology, 46, 16-22.

[9] Skeoch, S., Weatherley, N., Swift, A. J., Oldroyd, A., Johns, C., Hayton, C., & Chaudhuri, N. (2018). Drug-induced interstitial lung disease: a systematic review. Journal of clinical medicine, 7(10), 356.

[10] Subbiah, R., & Tiwari, R. R. (2021). The herbicide paraquat-induced molecular mechanisms in the development of acute lung injury and lung fibrosis. Critical reviews in Toxicology, 51(1), 36-64.

[11] Wang, J., Jing, X., Hua, L., Zheng, Y., Hu, S., Xiao, J., & Gao, X. (2022). Hypertension related toxicity of chloroquine explains its failure against COVID-19: Based on rat model. Frontiers in Pharmacology, 13, 1051694.

[12] Yusuf, I. H., Charbel Issa, P., & Ahn, S. J. (2023). Hydroxychloroquine-induced retinal toxicity. *Frontiers in Pharmacology*, 14, 1196783.