Chloroquine Induced Lesions in the Visceral Tissues of Albino Mice

Hussain I. Hussain, PhD*, Elham M. Mahmood, PhD*, Saif Kamil, PhD*, Huda A. Hameed, PhD**, Samira AH. Abdulla, PhD*, Entedhar R. Sarhat, PhD

ABSTRACT

Introduction: Many drugs are irritating to the gastric mucosa and induce gastric erosion and when these side effects are coupled with additional parameters, such as bacterial infection, stress, and gastric pH, these together induce gastric ulcer. The present study aimed to evaluate the gastric erosion effects of chloroquine using mice models.

Methods: A total of 20 mice were used for this study, divided into two groups of 10 each; the control group was administered only standard food and water, and the chloroquine group was given standard food with water with additional chloroquine solution of 1.2 mg/kg daily orally for a month. The stomachs were dissected and sliced for histological staining and analysis.

Results: Chloroquine has remarkably induced tissue degeneration and villi sloughing alongside white blood cell infiltration with patchy areas of stomach erosion compared to the normal architecture of the stomach tissue of the control group.

Conclusion: Chloroquine-induced gastric erosion with potential involvement of the many regions of the stomach reaching deep tissue layers.

Keywords: Chloroquine drug, albino mice, visceral tissues

INTRODUCTION

Since ancient times, humans have used drugs to treat many mental and organic diseases, and there is no doubt that scientific development has led to the invention of devices and the manufacture of many types of drugs and medicines from plant sources or chemicals (1), and medications made from chemicals have a vital impact on Human or animal, and they are used to treat, heal, and diagnose diseases, or they are used for specific periods or continuously in the case of chronic diseases, as medicines can affect physiological processes in natural and pathological cases, as medicines are divided depending on their mechanism of action and effect, some of the medicines work It inhibits the building of the cell wall of bacteria, some of which work by inhibiting the synthesis of proteins, and some of them work by inhibiting the construction of deoxygenated DNA (2).

Global consumption of antibiotics increased significantly between 2000 and 2001, so the World Health Organization developed a plan on antimicrobial resistance in 2015 and the optimal use of antibiotics (3,4), as the frequent and incorrect use of antibiotics leads to increased exposure of the body to colonization of pathogenic organisms (5).

Recent publications have drawn attention to the potential benefit of chloroquine in the treatment of severe acute respiratory syndrome (SARS-CoV-2) caused by the coronavirus COVID-19 (6). The ability of chloroquine to bind with glycosylation at the cellular receptors of the virus and also alter the internal pH required for viral cell breakdown (7). Safe in pregnancy, chloroquine is recommended to treat malaria and COVID-19 in pregnancy (8), and there is not enough evidence to determine if chloroquine is safe to give to people 65 years of age or older (9). In addition to being safe, it's cheap (10) and has antiviral effects(11). It's used for malaria and other infectious diseases(12) including treatment of extra-intestinal dysentery when taken orally(13) or in severe cases given systemically(13) and can be used safely for up to 6 weeks(14) at the usual therapeutic dose of (30-50) mg/kg (15).

The side effects of chloroquine are blurred vision, gastric upset, headache, swelling of the legs/ankles, shortness of breath, pale nails and skin, muscle weakness, facilitating bruising and bleeding, hearing and mental problems (16). Chloroquine is rapidly and completely absorbed from the gastrointestinal tract, and chloroquine is deposited in tissues, including the liver (17). Because of the frequent use of chloroquine at present, this study was conducted, which aimed to Assessment of the effect of using chloroquine on the histological structure of the small intestine, and stomach. Assessment of changes in the tissue structures of the small intestine as a result of the effect of chloroquine.

MATERIALS AND METHODS

Study settings: The present study was commenced in the animal facilities of the College of Veterinary Medicine (Tikrit University, Iraq), starting on 06.01.2022 to 06.06.2022.

Animals: A total of 20 Albino mice (healthy, weight 25-35g, age 3-4 months) were used. The mice were housed under standard conditions (weekly changed and sterilized plastic polyacrylic cages, bedding with sawdust, room ventilated, Temperature 22±1°C, 12h-light-dark cycle) with free access to water and standard food (wheat 34%, barley 20%, corn 25%, animal protein 10%, powdered milk 10%, table salt 1%, and water to make the mixture cohesive paste to be dried),

* College of Medicine, Tikrit University, Tikrit, Iraq.
Email: entedharr@tu.edu.iq
** College of Veterinary Medicine, Tikrit University, Tikrit, Iraq

Drugs: Chloroquine 100% pure powder was kindly provided by a State Company for the Pharmaceutical Industry and Medical Appliances in Samarra (Iraq). The powder was dissolved in DW to be administered to the mice. Hence, the mice were divided into two groups

G1: control group: 10 mice on standard feeding with free access to food and water for 30 days.
G2: 10 mice on standard feeding with free access to food and water with chloroquine after dissolving it in 1 ml of distilled water at a concentration of 1.2 mg/kg for each animal orally for 30 days (15).

Sample processing: After the end of the experiment, the animal is killed by separating the cervical vertebrae and fixing the mice on the dorsal side above the autopsy plate, and their front and hind limbs will be fixed with pins. The animals will be dissected from the beginning of the abdominal cavity with sharp scissors. The small intestine, liver, and stomach organs were removed and these tissues were washed with a physiological solution of normal saline (18).

Histological study: The target tissues were handled to construct microscopic slides. Each tissue was washed with buffered saline. The tissue was then dissected and fixed in 10% formalin. The next day, the fixed tissue was cleaned under running tap water. Following that samples were dehydrated and cleared in successive steps to be infiltrated with liquefied wax and embedded in liquid paraffin to be ready for trimming. The formed tissue blocks were sliced by microtome. The formed slices were then stained by hematoxylin and eosin stain. The slides were examined under a light microscope and diagnosed

RESULTS

The results of the microscopic examination showed the intestinal mucosa of the normal group contained intestinal villi, each villus was lined by simple columnar epithelium with microvilli on its apical or luminal surface. The core of each villus was formed by loose connective tissue infiltrated with fibroblasts and white blood cells (WBCS) which extended to the lamina propria which have intestinal glands (Figure 1). The lamina propria of the small intestine was infiltrated with WBCs among the intestinal glands and these glands continue at the base of villi via crypts of Lieberkuhn (Figure 3).

The submucosa was a narrow zone formed by loose connective tissue and had a certain number of WBCs and blood capillaries. The tunica muscularis was formed by inner circular and outer longitudinal smooth muscle fibres (Figure 3). The mucosal tissue of the stomach was formed by gastric pits, lined by simple columnar epithelium and beneath the epithelium, there was lamina propria which was engorged with gastric gland, each gland was lined by mucus neck cells. Parietal cells, chief cells and in between the gastric gland, there was infiltration of WBCs (Figure 2).

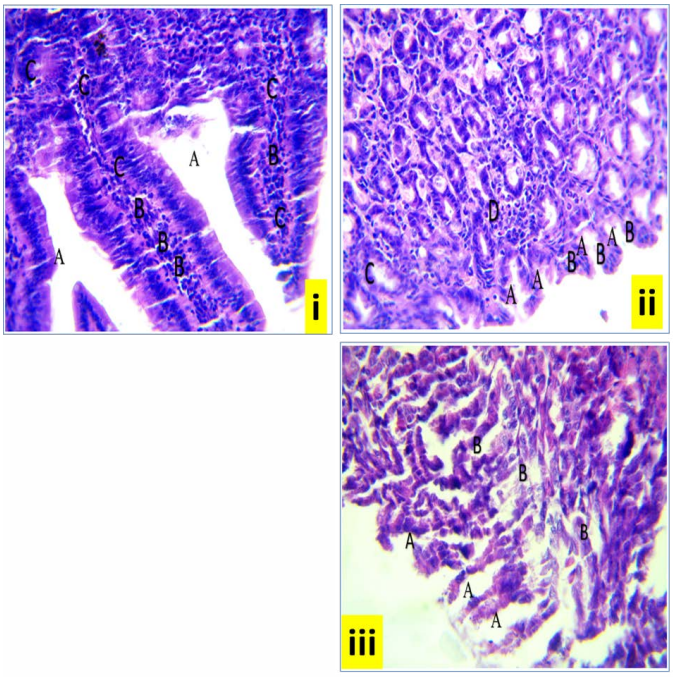

Figure 1. A representative images of the control group versus chloroquine of the intestinal villi and gastric pits (i) Intestinal villi (A), lined by simple columnar epi. with microvilli on its apical surface, core of villus (B) have WBCs lamina propria with intestinal glands (C). (ii) Gastric pits of stomach (A), simple columnar epi. (B), gastric glands (C) WBCs (D) in the interstitial connective tissue. (iii) Stomach, degenerated cells of gastric pits (A), lamina propria with degenerated cells of gastric glands (B) (H&E X40).

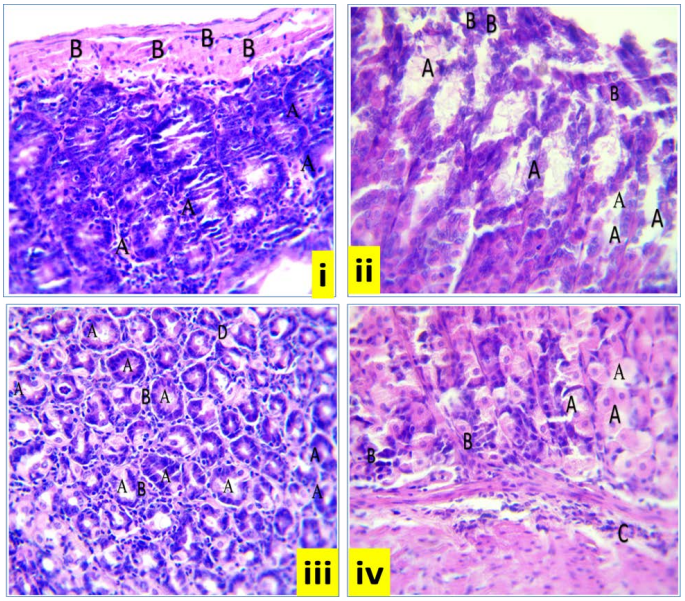

Figure 2. A representative images of the control group versus chloroquine of the Lamina propria (i) Lamina propria of small intestinal (A), with intestinal glands (crypts of lieberkuhn) submucosa (B) had blood vessel. Lamina propria of stomach, gastric glands (A), WBCs infiltration (B), in the interstitial

connective tissue. (ii) The lamina propria of small intestine, degeneration of cells of intestinal glands (A), sloughing of cells inside lumens of glands (B). (iii) Lamina propria of stomach, gastric glands (A), WBCs infiltration (B), in the interstitial connective tissue. (iv) The lamina propria of small intestine, engorged with intestinal mucosal glands (A), WBCs infiltration (B) in between the glands submucosa with WBCs infiltration (C). (H&E X40).

The lamina propria of the stomach was crowded with gastric glands, and surrounded by infiltration of WBCs in the interstitial connective tissue (Figure 2). The deeper layers of lamina propria of the stomach contain crowded glands of the stomach which are mostly mucus cells and parietal cells which are extended to the submucosa (Figure 2).

The intestinal villi were lined by simple columnar epithelium, the core of the villi contained a great number of lymphocytic aggregations, and the nuclei of epithelial cells were present at the base of columnar cells in the form of blue-line (Figure 3). The lamina propria of the small intestine was engorged with intestinal mucosal glands. The epithelial cells of those glands are degenerated and certain cells are sloughed into the lumen of glands (Figure 3).

The lamina propria of the small intestine contained intestinal glands, which were engorged cells and in between the glands, there was lymphocytic diffusion, particularly at the base of glands near the muscularis mucosa and submucosa, the submucosa have also lymphocytic infiltration adjacent to the tunica muscularis (Figure 3).

The loose connective tissue (tunica serosa) of the small intestine around the muscular coat was infiltration by inflammatory WBCs, especially the lymphocytes and macrophages and these cells are present around the blood vessels and nerve plexus, macrophages are infiltration in the connective tissue of muscular perimysium (Figure 1).

The gastric mucosa contained degeneration of epithelial cells covering the stomach surface and gastric pits also the lamina propria of gastric mucosa contained dissociated glands from each other with mostly degeneration of its glandular cells (Figure 3).

The deepest layers of lamina propria of the stomach have the gastric glands which have chief cells which secrete the zymogen granules of pepsinogen its cytoplasm was bluish-pinkish color and parietal cells of light cytoplasm and those cells were larger than chief cells. The submucosa has aggregation of WBCs and macrophages (Figure 3).

The lamina propria of the stomach revealed to be demonstrated of a great number of glands which appeared with cellular degeneration, and desquamation of those cells in the lumen of its glands, with the presence of thickening of the basement membrane of the glands (Figure 3).

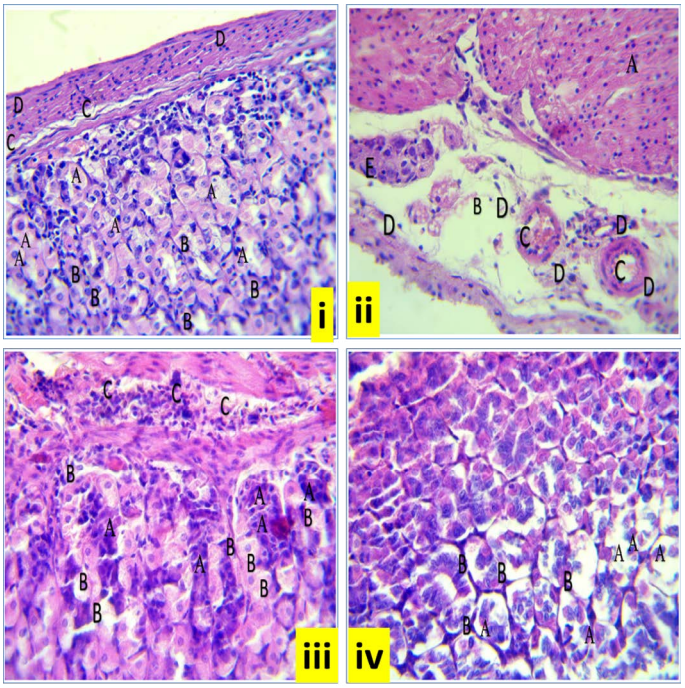

Figure 3. A representative images of the control group versus chloroquine of the gastic layers. (i) Gastric glands, parietal cells (A), mucus cells (B), submucosa (C), muscular coat (D). **(ii)** Muscular coat of small intestine (A), tunica serosa (B), have blood vessels (C) WBCs (D) nerve plexus (E). **(iii)** Gastric mucosa, chief cells (A) parietal cells (B), lymphatic aggregation (C). **(iv)** Gastric mucosa, degeneration of cells of gastric glands (A) thickening of basement membrane of glands (B) (H&E X40).

DISCUSSION

The present findings of the present study revealed a correlation between the degenerative changes of the visceral organs and the administration of chloroquine, the light microscopic observation in the present study revealed the desquamation of the surface epithelium of the stomach and small intestine with degeneration of gastric glands, like chief cells, and parietal cells, also lymphocytic aggregation in the lamina propria of stomach and intestine, and this result is in agreement with (20,21), who demonstrated that Chloroquine when given to the Albino Rat one dose weekly for six weeks led to damage to gastric mucosal cells (mucus cells, Zymogen cells, parietal cells and Argentaffin cells) when examined after the arrest of the drug.

Chloroquine has also been associated with uplifted gastric ulcer score and encouraged postponement of gastric tissue healing, perhaps due to potential chloroquine impact on gastric ulcer healing stages or suppresses the downstream signalling that stimulates ulcer healing (38), including potentiating gastric acidity through the competition on histamine H-receptors binding. In addition to this histamine receptor stimulatory activity, the potentiation of muscarinic receptors reported by Ajeigbe *et al* 2008a and 2008b (39,40) leads to reduced gastric pH and volume during gastric ulceration.

Chloroquine has also been drawn backed by their potential lipid peroxidation capacity via increasing free radical generation, suppressed the ulceration healing process due to modulating tissue quasi-equilibrium and further enhancing tissue injury which was further supported by earlier findings by Ajeigbe *et al* 2012 (41). Free radicals have been documented to exert an adverse effect on the stomach (39,40).

Also, colonal mucosa injury was indicated by administration of anti-malarial drug (chloroquine) for male albino Rats at 8mg/ kg by (21), infiltration of inflammatory cells with ulceration of colonic mucosal epithelial cells and this result is not a way from data recorded in the present study for intestinal mucosa, so the use of chloroquine as malarian treatment in colitis patients may not be advisable (42).

CONCLUSIONS

From the results of the current study, we conclude that dosing with chloroquine, when passing through the treatment lethargy in the movement of animals treated with chloroquine. Dosing with chloroquine, even if it is a therapeutic dose, affects the histological structure, as there was degeneration, inflammation, infiltration of blood cells with congestion of blood vessels and hemolysis in several cavities of blood vessels. An increase in the number of lymphocyte aggregations was indicated in the studied organs by microscopic examination.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes

Potential Conflicts of Interest: None

Competing Interest: None

Acceptance Date: 10-09-2024

REFERENCES

1. More B. Overview of medicine importance and impact. DJ Int J Med Res 2016;1(1):1-8.
2. Karaman R. Commonly used drugs-uses, side effects, bioavailability & approaches to improve it. Nova Science Pub Inc. 2015.
3. Mathur S, Jackson C, Urus H, et al. A comparison of five paediatric dosing guidelines for antibiotics. Bull. World Health Organ 2020;98(6):406-9.
4. World Health Organization. Consideration of antibacterial medicines as part of the revisions to the 2019 WHO Model List of Essential Medicines for Adults (EML) and Model List of Essential Medicines for Children (EMLc): Section 6.2 Antibacterials including Access, Watch and Reserve Lists of antibiotics. World Health Organization. Regional Office for Europe; 2020.
5. Zafar H, Bukhari KT, Noor N, et al. Meta-analysis for searching efficacy of tinidazole and metronidazole. Ulutas Med J 2017;3(2):32-8.
6. Sarhat ER, Zbaar SA, Ahmed SE, et al. Salivary Biochemical Variables of Liver Function in among Individuals with COVID-19 in Thi-Qar Province. Egypt J Chem 2022;65(6):305-10.
7. Manivannan E, Karthikeyan C, Moorthy NH, et al. The rise and fall of chloroquine/hydroxychloroquine as compassionate therapy of COVID-19. Front pharmacol 2021;12(1):584940-5.
8. Manivannan E, Karthikeyan C, Moorthy NH, et al. The rise and fall of chloroquine/hydroxychloroquine as compassionate therapy of COVID-19. Front pharmacol 2021;12(1):584940-5.
9. Filler S, Causer LM, Newman RD, et al. Malaria surveillance-United States, 2001. Morbidity and Mortality Weekly Report CDC Surveillance Summaries. 2003;52(5):1-10.
10. Cortegiani A, Ingoglia G, Ippolito M, et al. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care 2020;57(1):279-83.
11. Delvecchio R, Higa LM, Pezzuto P, et al. Chloroquine, an endocytosis blocking agent, inhibits Zika virus infection in different cell models. Viruses 2016;8(12):322-8.
12. Babayeva M, Loewy Z. Repurposing drugs for COVID-19: Pharmacokinetics and pharmacogenomics of chloroquine and hydroxychloroquine. Pharmgenomics Pers Med 2020; 1(1):531-42.
13. Plowe CV. Antimalarial drug resistance in Africa: strategies for monitoring and deterrence. Malaria: drugs, dis post-gen bio 2005; 1(1):55-79.
14. Savarino A, Boelaert JR, Cassone A, et al. Effects of chloroquine on viral infections: an old drug against today's diseases. Lancet inf dis 2003;3(11):722-7.
15. Karalis V, Ismailos G, Karatza E. Chloroquine dosage regimens in patients with COVID-19: Safety risks and optimization using simulations. Saf sci2020;129(1):104842-8.
16. Carvalho AA. Side effects of chloroquine and hydroxychloroquine on skeletal muscle: a narrative review. Curr pharm rep 2020;6(1):364-72.
17. Akinribido FA, Noronha CC, Okanlawon OA. Effects of Chloroquine on the Morphology and Stereology of Some Tissues in Sprague-Dawley Rats. Int J Biomed Health Sci 2021;8(1):1-10.
18. Saleh NI, Wadee SA, Sarhat ER. Minimizing the side effects of Doxorubicin Induced Hepatotoxicity by using alcoholic extract of Date Palm in adult rats. Bionatura 2023; 8(1): 1-10.
19. Young A, Newton V, Valley P. The experience of deafness–psychosocial effects. VE Newton 2006; 14(1):279-285.
20. Iskander FA, Ahmed YY, Kamel ZM, et al. Experimental studies on the effect of chloroquine (antimalarial drug) on the liver of albino rat. Sci Med J 1990;2(3):63-86.
21. Varga F, Fischer E, Szily T. Effect of gastric emptying rate on the intestinal absorption of chloroquine in rats. Pharmacology. 1975 May 29;13(5):401-8.

22. Saleh SS, Sarhat ER. Effects of ethanolic Moringa oleifera extract on melatonin, liver and kidney function tests in alloxan-induced diabetic rats. Indian J Forensic Med Toxicol 2019;13(4):1015-9.
23. Sarhat ER, Abbas MQ, Ali NH, et al. Evalution of ceruloplasmin, sialic acid and liver function for women with breast cancer. InAIP Conf Proc 2022; 2394 (1):1-10.
24. Aziz Z, Sarhat E, Zaidan Z. Estimation of serum ferroportin and liver enzymes in breast cancer patients. Georgian med news 2023 339(7):37-41.
25. Akin AT, Kaymak E, Öztürk E, et al. Investigation of the therapeutic effects of chloroquine in adriamycin-induced hepatotoxicity. EuroBiotech J 2021;5(1):8-14.
26. Niknahad H, Heidari R, Firuzi R, et al. Concurrent inflammation augments antimalarial drugs-induced liver injury in rats. Adv Pharm Bull 2016;6(4):617-620.
27. El Shishtawy MA, Hassan KH, Ramzy R, et al. Comparative toxicity study of chloroquine and hydroxychloroquine on adult albino rats. Eur Sci J 2015;1:399-407.
28. Mahmood DA, Sarhat ER, Sulaiman YA, et al. Evalution of liver function tests in patients with psoriasis. Revista Latinoamer Hipert 2022;17(6):1-10.
29. Jaeschke H, Ho YS, Fisher MA, et al. Glutathione peroxidase–deficient mice are more susceptible to neutrophil-mediated hepatic parenchymal cell injury during endotoxemia: importance of an intracellular oxidant stress. Hepat 1999;29(2):443-50.
30. Ferrero-Miliani L, Nielsen OH, Andersen PS, et al. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clinical & Experimental Immunology. 2007 Feb;147(2):227-35.
31. Mohammed IJ, Sarha ER, Wadee SA, et al. Histological and biochemical evaluation of the effect of desloratadine drug in parotid gland tissues. Al-Anbar Med J 2021;17(2):72-7.
32. Stevens A, Lowe JS, Young B. Wheater's basic histopathology: a colour atlas and text. 3rd Edition 2002; 26(1):1-10.
33. Kumar KD, Jayaveera KN, Kumar GS. Anti-inflammatory and anti-nociceptive properties of Tephrosia falciformis root extract. Pharmonline 2007;2:371-84.
34. Kumar V, Cotran RS, Robbins SL. Basic pathology. Philad 1997;5:25-40.
35. Rubin E, Reisner HM, editors. Essentials of Rubin's pathology. Lippincott Williams & Wilkins; 2009.
36. AlKadi HO. Antimalarial drug toxicity: a review. Chemother 2007;53(6):385-91.
37. Xu J, Jiao K, Liu X, et al. Omi/HtrA2 participates in age-related autophagic deficiency in rat liver. Aging dis 2018;9(6):1031-1036.
38. Chen N, Wilson DW, Pasay C, et al. Origin and dissemination of chloroquine-resistant Plasmodium falciparum with mutant pfcrt alleles in the Philippines. Antimicrob Agents Chemother 2005;49(5):2102-5.
39. Sarhata ER, Al Anzyb MM, Ahmedb TS. Study of oxidant-antioxidant status in cerebrospinal fluid of children with meningitis. Eurasian Chem Commun. 2022;4(9):863-9.
40. Ajeigbe KO, Nwobodo EO, Oyesola TO, et al. Chloroquine phosphate potentiates indomethacin and HCl/ethanol-induced gastric mucosa injury in rats. Int J Pharma 2008; 4(6): 482-486.
41. Ajeigbe KO, Emikpe BO, Olaleye SB. Augmentation of gastric acid secretion by chloroquine and amodiaquine in the rat stomach. Niger J Physiol Sci 2012;27(June):89-94.
42. Mahmood DA, Sarhat ER, Sulaiman YA, et al. Relationship between Paraoxonase and Malondialdehyde as a marker of oxidative stress in patients with psoriasis. Revista Latinoamer Hipert 2022;17(6):1-10.