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Article

Diagnosis of Chloroquine Side Effect on the Histological Structure of Rat Spleen

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Abstract: Chloroquine is recognized as an antimalarial agent that exhibit substantial anti-inflammatory and immunomodulatory properties. Chloroquine extended therapeutic application is sometimes constrained by systemic toxicities, necessitating additional exploration of its histopathological impacts; hence, the current work sought to assess how varying dosages and exposure durations of chloroquine effect on splenic tissue architecture of Sprague-Dawley female rat. Histopathological alterations in splenic tissue were evaluated across control and treated groups to determine the time-dependent severity of chloroquine-induced pathological changes. Thirty-five Sprague-Dawley female rats were assigned to seven experimental cohorts (n = 5 per group). Control animals (first cohort) provided with standard diet and water ad libitum throughout the experiment. Chloroquine was administrated orally to the six treatment cohorts according to a dose- and time-dependent protocol (1.6, 2.5, and 3.8 mg/kg) across short term (35 days) and prolonged (50 days) treatment durations. At the end of the experimental period, the rats were euthanized, and their spleens were removed for histological analysis. Histological examination of the chloroquine-treated cohorts demonstrated notable modifications that were dependent on dosage and duration, including vascular and sinusoidal congestion, hyperemia, haemorrhage, and widespread hemolysis in the red pulp. Vacuolar degeneration was noted in both the white and red pulp, along with sinusoidal dilation and fibrous oedema in certain groups. Within the splenic tissue.The intensity of these lesions escalates with increased dosages and prolonged exposure durations. In summary, these results indicate that chloroquine causes splenic toxicity that is dependent on dosage and duration, marked by progressive vascular degradation and structural changes in spleen tissue.

Keywords: Chloroquine, Rat spleen, Spleen toxicity, Chloroquine splenic histopathology, Chloroquine induce splenotoxicity, Chloroquine induced splenopathy, Chloroquine histotoxicity , Chloroquine dose dependent toxicity.

Introduction

Chloroquine is a drug that is used to treat malaria, but recent studies have demonstrated multiple immunological and cellular effects. Chloroquine accumulates in lysosomes of phagocytes and alters the pH of the lysosomes disrupting autophagy, immune responses and suppresses signalling mechanisms governed by Toll-like receptor [1]. Chloroquine has anti-inflammatory potency through suppression of pro-inflammatory cytokine synthesis and modulation of immune cell response, and hence is frequently used for conditions like rheumatoid arthritis and lupus erythematosus (SLE) [2]. However, studies have shown that long-term exposure to high doses of chloroquine can lead to histological and functional impairment, primarily by triggering oxidative stress and membrane disorganisation in cells [3]. In the setting of SARS-CoV2 pandemic, oral chloroquine was added to several therapeutic regimens because of its putative antiviral and anti-inflammatory properties. However, subsequent clinical trials failed to demonstrate definitive therapeutic efficacy and, instead, resulted in considerable adverse events, including cardiac rhythm disorders [4]. Long term treatment or high dose adminsteration of chloroquine may adversely impact multiple organs. It causes hepatic dysfunction with hepatocellular changes and renal impairment with specific histological changes [5], [6]. It also causes cardiac arrhythmias and QT prolongation [7]. Of importance, chronic exposure may also cause retinopathy and progressive loss of vision [3]. It is classically associated with neuromuscular toxicities and presents as generalised weakness [8]. Chloroquine is known to induce vascular and lymphoid changes in the spleen that affect the normal splenic structure and denote its possible splenotoxic effect [9].

The spleen is the largest secondary lymphoid organ in the body and is found in the left hypochondriac region of the abdominal cavity inferior to the diaphragm and posteromedial to the stomach [10]. It is encased in a thick capsule of connective tissue, with trabeculae extending into the splenic parenchyma, providing structural support and passageways for blood vessels [10]. The spleen has two functional regions in histology; the red pulp and the white pulp [10]. The white pulp consists predominantly of organised lymphoid tissue surrounding central arterioles and is important for immune surveillance and lymphocyte activation [10]. In blood Clearing of old or damaged erythrocytes The recycling of iron Blood is carried out to the spleen through the splenic artery that gives rise to smaller arteries that ultimately supply blood to the splenic tissue to support its haematological and immunological functions [10]. Considering the cellular and immune effects of chloroquine and its link to oxidative and histological damage in systemic tissues after long-term exposure or high doses, the present study found the spleen to be a target organ particularly susceptible to inflammatory reactions and oxidative stress. The present investigation attempts to explore the probable histopathological changes following chloroquine treatment and its effect on the microarchitecture of the spleen.

Materials and methods

Drug used

The General Company for the Manufacture of Medicines and Medical Supplies in Samarra, Iraq, supplied the absolutely pure chloroquine powder.

Experimental design

The experiment included 35 Sprague Dawley rat female, with an average weight of 150-200 g and an average age of 24 months. The experimental rats were maintained under controlled environmental conditions: 25°C, with a regulated 12 hour light/dark cycle, and provided with continuous access to water and supplements.

Prior to the commencement of the investigation, all feasible consideration was given to the weights of the seven groups into which the rats were randomly assigned: six experimental treatment groups and a non-treated control group. Seven equivalent experimental cohorts were treated according to the following protocols: • For 50 days, rats in Group 1 (G1, control) were given food and water but were not given any further treatments.

Rats were orally administered chloroquine solubilized in one mL of sterile distilled water on a daily basis at dose levels of 1.6 mg/kg for 35 days in Group 2 (G2) and 50 days in Group 3 (G3). 2.5 mg/kg dose of chloroquine solubilized in one mL of sterile distilled water was given to Group 4 (G4)

for 35 days and to Group 5 for 50 days. Chloroquine diluted at 3.8 mg/kg in one mL of sterile distilled water, was adminstreated for 35 days to Group 6 rats (G6) and 50 days to Group 7 rats (G7).

All experimental groups are provided with free food and water during the study durations. The Histology Laboratory at Tikrit University's College of Science performed the histological examinations on the tissue slices. Following the sequential steps outlined by Al-Hajj [11], the splenic tissue sections underwent processing according to the standard histological methods:

After removing the spleen, the specimens placed immediately in 10% neutral buffered formalin fixation solution for 24 hour duration prior to tissue processing. Subsequently, the tissue specimens dehydrated in ascending concentrations of ethanol (70%, 80%, 90%, and 100%), followed by cleaning in xylene and embedded in paraffin wax at 60°C to yield paraffin-embedded tissue blocks. Using rotary microtome, the blocks were sectioned at a 6 um thickness. Before being mounted on slides using D.P.X. and coated with cover slips, the sections were stained with hematoxylin and eosin (H&E). The last step was to take pictures of the prepared slides using a digital camera that had been modified for microscopy and examine them under a light microscope.

Results

Splenic tissue sections of control cohort displayed an unaltered microarchitecture under microscopic examination with clear disitinction between the red and white pulps, the white pulp contained well organised lymphoid follicles and periarteriolar lymphoid sheaths surrounding the central arterioles, while the red pulp showed a normal appearance with no evidence of vascular or cellular abnormalities. In addition, splenic trabeculae extending from the capsule were clearly evident confirming the preservation of the tissues structural integrity as shown in Figure (1). On the other hand, administration of chloroquine induced various histopathological changes in a dose and time dependent manner. In the second group (G2) which for 35 days administrated 1.6 mg/kg chloroquine, the spleen showed early pathological alteration with intravascular hemolysis, partial sinusoidal congestion and intense vacuolar degeneration in the white pulp. Moreover, marked sinusoidal hyperemia and diffuse haemorrhage within the red pulp were observed, indicating vascular and parenchymal injury (Figure 2, 4). Also, prolongation of the treatment duration at the same dose (1.6 mg/kg) in (G3) caused progression of the vascular changes as evidenced by diffuse hyperemia of the red pulp sinusoids accompanied by persistent hemolysis within several vascular space, as shown in figure (5). The splenic lesions in (G4) were even worse after increasing the dose to 2.5 mg/kg for 35 days, with prominent lymphoid aggregation in the white pulp, extensive sinusoidal hemolysis, hemorrhagic areas next to the trabeculae and different degrees of sinusoidal dilatation and congestion. Furthermore, focal and diffuse lymphocytic infiltration and fibrous oedema were seen suggesting a more widespread disturbance of normal splenic architecture (figure (6-8)). Furthermore, prolongation of the exposure for 50 days to 2.5 mg/kg chloroquine was accompanied with marked aggravation of the vascular changes which were mainly represented by severe and generalised hyperemia throughout the red pulp as shown in figure (9). (G6) After 35 days administration of 3.8 mg/kg chloroquine, a similar pattern of progressive tissue damage was observed with extensive congestion of red pulp sinusoids around the lymphoid nodules, diffuse hemolysis, sinusoidal dilatation and fibrous oedema in the white pulp as shown in figures (10–12). Finally, the highest level of exposure, represented by (G7) treated with 3.8 mg/kg for 50 days, induced advanced histopathological alterations characterised by vacuolar changes within the red pulp parenchyma associated with multifocal clusters of sinusoidal hyperemia, reflecting persistent and progressive splenic tissue damage correlated with the increasing dose and duration of chloroquine administration (Figure 13).

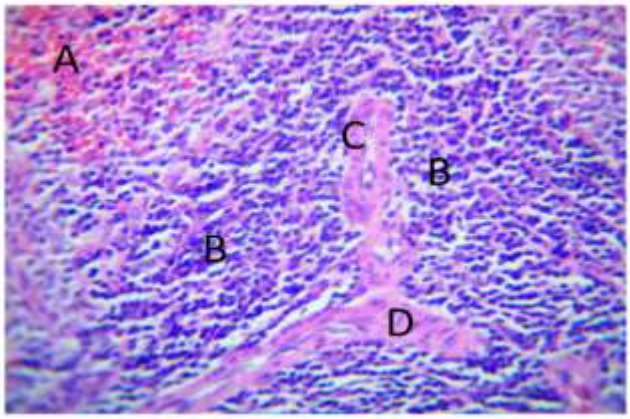

Figure 1. *Photomicrograph of a splenic section from a control rat (G1) showing the normal architecture of the red pulp (A) surrounding the white pulp, which consists of lymphoid tissue in the form of lymphoid follicles composed of B lymphocytes and a periarteriolar lymphoid sheath (PALS) (B) surrounding a small central arteriole (C). A splenic trabecula (D) extending from the surrounding capsule is also evident. (H&E, 40X).*

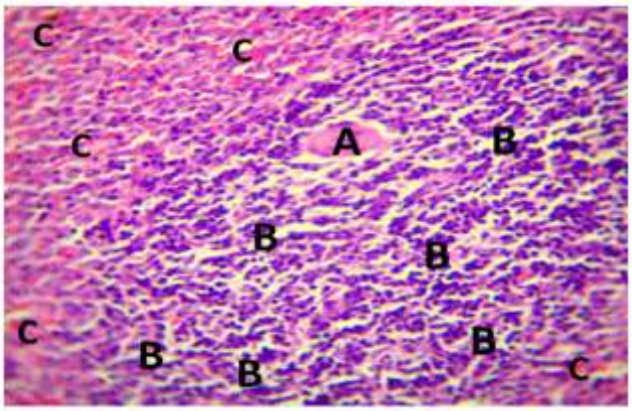

Figure 2. *Photomicrograph of a rat spleen from G2 (receiving 1.6 mg/kg of chloroquine daily across 35 exposure days) demonstrating evidence of hemolysis within a splenic arteriole (A), extensive parenchymal vacuolation of the white pulp (B), and partial sinusoidal congestion within the red pulp parenchyma (C). (H&E, 40X).*

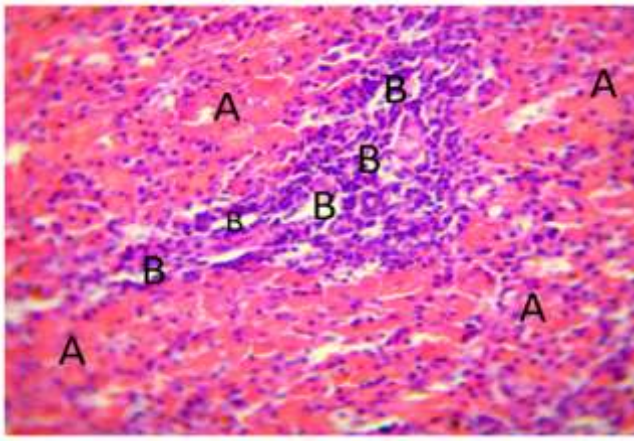

Figure 3. *Photomicrograph of a splenic section from G2 rats (at a dose of 1.6 mg/kg daily for 35 days), demonstrating marked sinusoidal hyperemia throughout the red pulp (A) and vacuolation of the white pulp parenchyma (B). (H&E, 40X).*

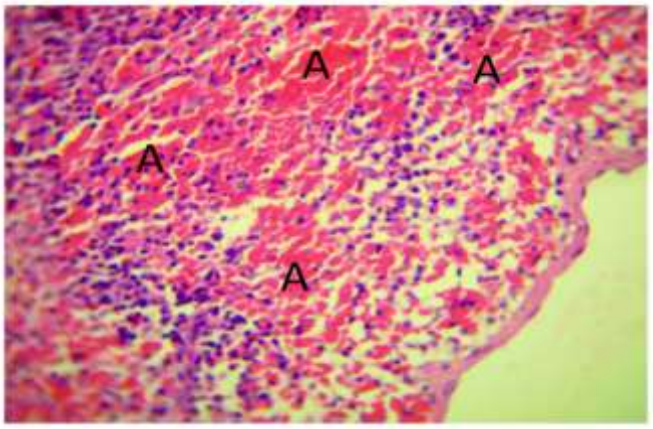

Figure 4. *Photomicrograph of a splenic section from G2 rats (receiving 1.6 mg/kg of chloroquine daily across 35 exposure days), demonstrating evidence of diffuse hemorrhage throughout the red pulp (A). (H&E, 40X).*

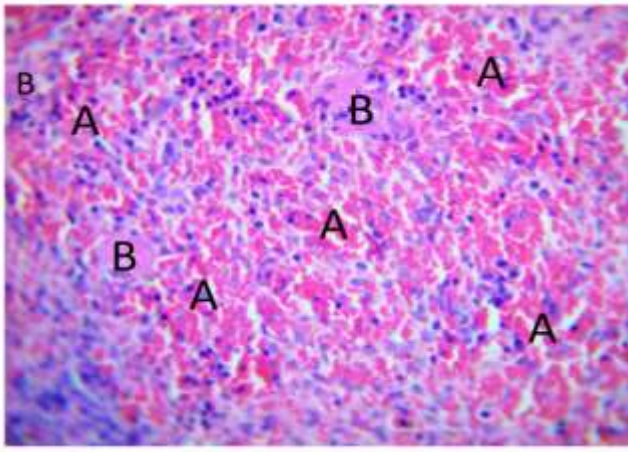

Figure 5. *Photomicrograph of a splenic section from G3 rats (receiving 1.6 mg/kg of chloroquine daily across 50 exposure days), demonstrating diffuse hyperemia throughout the red pulp sinusoids (A), accompanied by hemolysis in several of them (B). (H&E, 40X).*

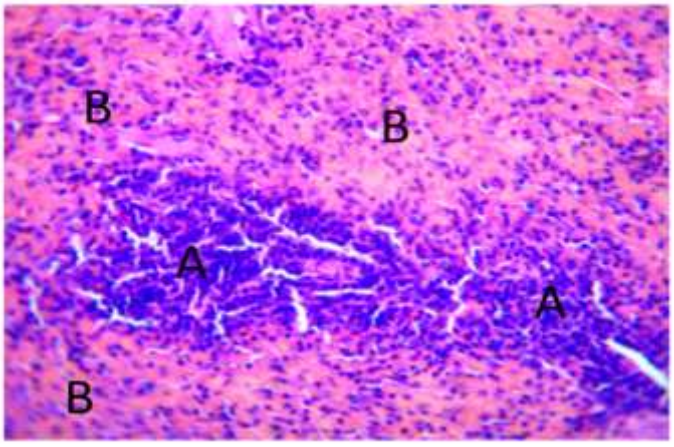

Figure 6. *Photomicrograph of a splenic section from G4 rats (at a dose of 2.5 mg/kg chloroquine daily for 35 days), demonstrating densely aggregated nodular lymphocytes (A) and sinusoidal hemolysis throughout the red pulp (B). (H&E, 40X).*

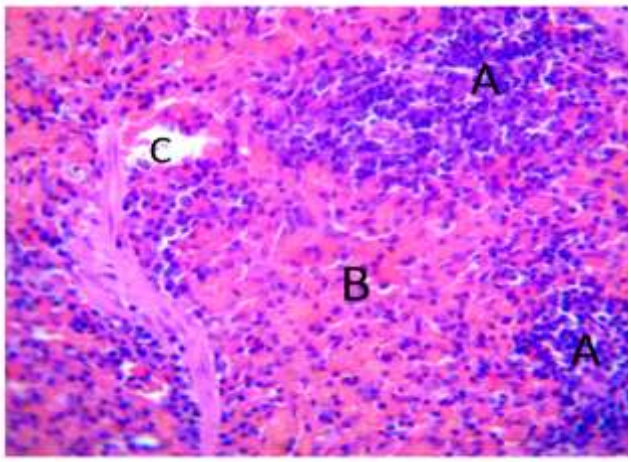

Figure 7. *Photomicrograph of a splenic section from G4 rats (receiving 2.5 mg/kg of chloroquine daily across 35 exposure days), demonstrating splenic nodules (A) surrounded by red pulp sinusoids exhibiting extensive hemolysis (B), and hemorrhage (C) adjacent to one side of a trabecula. (H&E, 40X).*

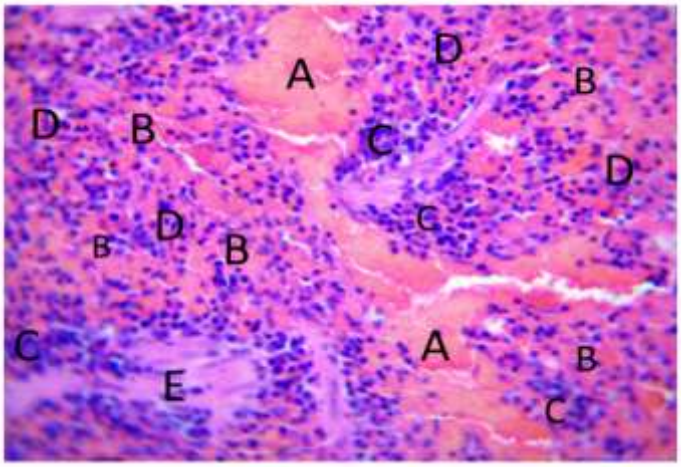

Figure 8. *Photomicrograph of a splenic section from G4 rats (receiving 2.5 mg/kg of chloroquine daily across 35 exposure days), demonstrating dilation of several blood sinusoids with extensive hemolysis (A), complete congestion of other sinusoids (B), focal (C) and diffuse (D) accumulations of lymphocytes, together with fibrous edema (E). (H&E, 40X).*

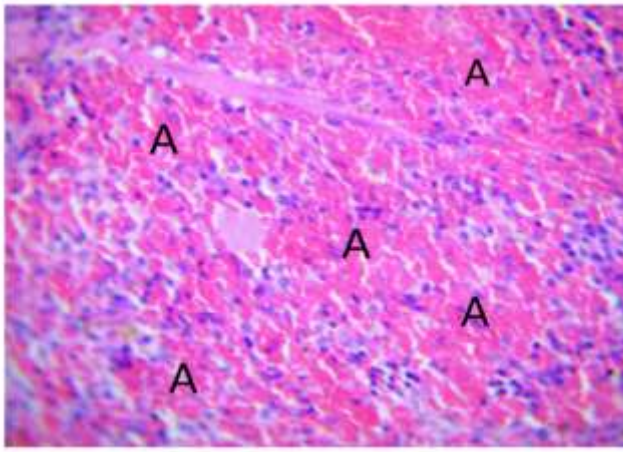

Figure 9. *Photomicrograph of a splenic section from G5 rats (receiving 2.5 mg/kg of chloroquine daily across 50 exposure days), demonstrating evidence of severe and widespread hyperemia throughout the red pulp (A). (H&E, 40X).*

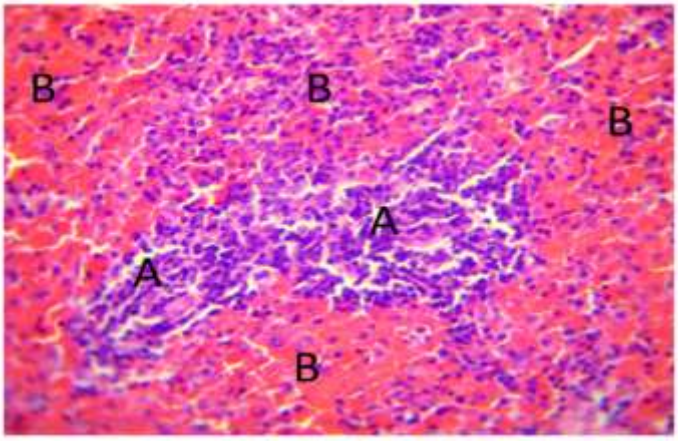

Figure 10. *Photomicrograph of a splenic section from G6 rats (receiving 3.8 mg/kg of chloroquine daily across 35 exposure days), demonstrating lymphoid nodules of the white pulp (A) surrounded by red pulp sinusoids exhibiting extensive congestion (B). (H&E, 40X).*

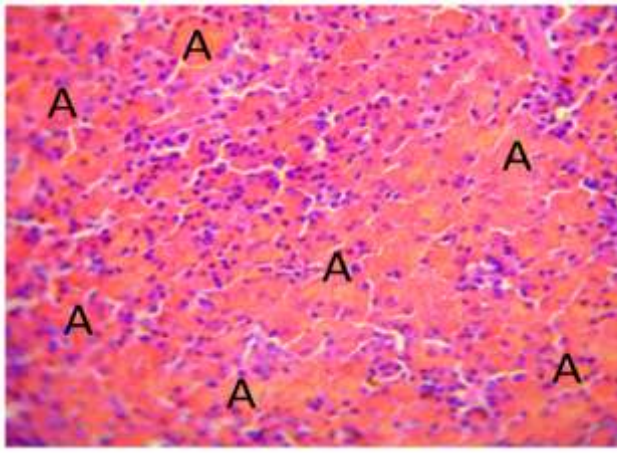

Figure 11. *Photomicrograph of a splenic tissue from G6 rats (receiving 3.8 mg/kg of chloroquine daily across 35 exposure days), demonstrating diffuse hemolysis throughout the histological section (A). (H&E, 40X).*

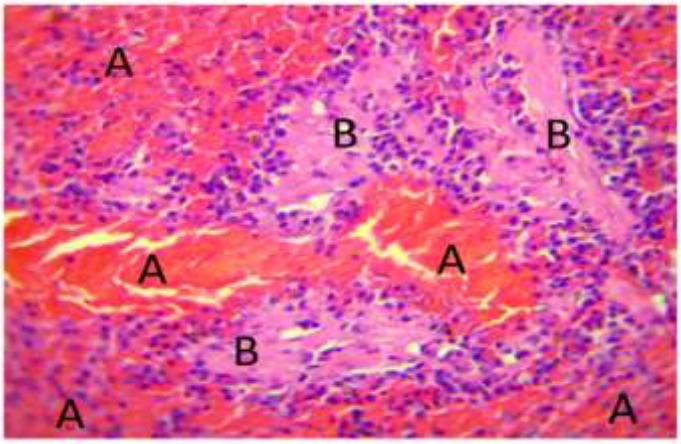

Figure 12. *Photomicrograph of a splenic tissue from G6 rats (at a dose of 3.8 mg/kg chloroquine daily for 35 days), showing sinusoidal dilation and diffuse hemolysis within the red pulp (A), accompanied by fibrous edema in the white pulp (B). (H&E, 40X).*

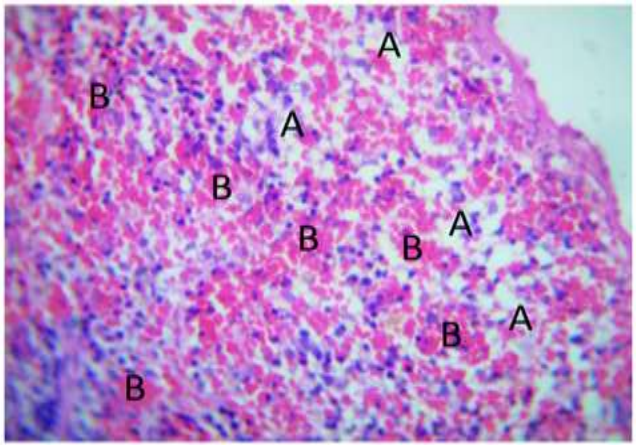

Figure 13. *Photomicrograph of a splenic tissue from G7 rats (receiving 3.8 mg/kg of chloroquine daily across 50 exposure days), demonstrating vacuolation within the red pulp parenchyma (A), associated with multifocal clusters of sinusoidal hyperemia (B). (H&E, 40X)*

Discussion

The present study showed that the chronic administration of chloroquine resulted in progressive dose and duration dependent histopathological changes in the spleen. Lesions were primarily vascular and lymphoid in location and increased in severity with increasing dose and duration of treatment indicating a cumulative toxic effect of chloroquine on splenic tissue. This observation may be related to the ability of chloroquine to accumulate in highly perfused organs including the spleen, where it interferes with lysosomal function, cellular metabolism and tissue homeostasis. The early histopathological changes observed in the second group (1.6 mg/kg for 35 days) are in accordance with early stage of chloroquine induced splenic toxicity. Such changes probably relate to lysosomal accumulation of chloroquine leading to lysosomal dysfunction, excess generation of reactive oxygen species and endothelial integrity damage. Therefore, disruption of splenic microcirculation could be an early manifestation of chloroquine induced cytotoxicity. These mechanisms are well supported by the evidence that chloroquine induces lysosomal dysfunction and oxidative stress by inhibition of autophagic flux and lysosomal acidification [12].

Moreover, the extension of the treatment period with the same dose in the third group (1.6 mg/kg daily for 50 days) highlights vascular impairment, indicating that the exposure time is a key factor in tissue injury. Vascular changes progression may reflect cumulative oxidative damage and persistent disturbances of splenic blood flow. It has been shown that chronic exposure to chloroquine increases the generation of reactive oxygen species and compromises microvascular stability in a time-dependent manner [13]. Dose was increased further to 2.5 mg/kg in the fourth group and this was associated predominantly with lymphoid changes, suggesting disruption of the normal homeostasis of immune tissues. Chloroquine is known to perturb lysosomal processing, antigen presentation, and immune-cell regulation, and thus affect the organisation and function of lymphoid compartments. These immunomodulatory effects have been well described, including their capacity to hinder antigen processing and lymphocyte activation in secondary lymphoid organs [14]. Consistent with this pattern, the prolongation of the treatment period in the fifth group (2.5 mg/kg for 50 days) underlined the vascular component of splenic injury, supporting the concept of cumulative vascular toxicity. Persistent microcirculatory dysfunction may impair tissue oxygenation and may be involved in secondary cellular injury. This is consistent with other studies demonstrating progressive endothelial dysfunction and vascular congestion as a result of chronic oxidative stress in experimental toxicological models [15].

In addition, the administration of the higher dose (3.8 mg/kg) in the sixth group seemed to have a marked effect on the integrity of erythrocytes as indicated by the preponderance of hemolytic injury. The main reason for this finding is increased oxidative stress and erythrocyte membrane destabilisation resulting in increased susceptibility of erythrocytes to destruction. Oxidative membrane injury and increased erythrocyte fragility have been reported to be caused by overproduction of ROS and toxic exposure [4]. In the seventh group (3.8 mg/kg for 50 days) this toxic effect was more evident, with degenerative changes being the predominant pathological feature. Development of vacuolar degeneration is indicative of marked impairment of cellular homeostasis and reflects intracellular organelle disruption, alteration of membrane permeability and defective autophagic processes. Chloroquine is a classic inhibitor of lysosomal acidification and autophagy flux, and chronic exposure has been shown to cause profound vacuolar degeneration and cellular collapse in a variety of tissues [5]. In conclusion, the present findings provide an obvious evidence of dose and time dependent progression of chloroquine induced splenic toxicity. Early stages were mainly associated with vascular dysfunction while increasing dose and duration favoured immune tissue remodelling, hemolytic injury and eventually advanced cellular degeneration. These findings align with the established mechanisms underlying chloroquine toxicity, such as oxidative stress, lysosomal dysfunction, microvascular injury, erythrocyte damage, and disruption of immune homeostasis [13].

Conclusion

Chloroquine causes histopathological alterations of spleen in a dose and duration dependent manner. The vascular, lymphoid and degenerative changes occur progressively and compromise the structural integrity of the tissue. The increasing severity of congestion, hemorrhagic lesions, lymphoid

alterations and parenchyma degeneration with increasing dose and treatment duration indicates a cumulative cytotoxic effect on splenic tissue. These findings collectively point to the spleen as a major target organ of chloroquine toxicity, which requires further exploration of the molecular mechanisms and functional consequences of these alterations.

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