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Biochemical and Histopathological Damages caused by Antimalarial drug, Chloroquine in Liver and Spleen of Wistar Rat

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The aim of this study was to investigate the effects of chloroquine on two vital tissues in wistar rat (liver and spleen). Healthy adult male wistar rat weighing between 100 and 150 g were used for this study. The treated group was given 200 mg/kg body weight/day of chloroquine phosphate orally for 25 days. Control animals were kept on distilled water. Microscopic examination of liver and spleen tissues revealed changes in histology. The results of our study showed that prolonged exposure to the antimalarial drug chloroquine phosphate results in adverse damages on tissues.

Key words: Antimalarial drug, Chloroquine, Liver, Spleen and Wistar rat.

Malaria is a disease that is caused in humans by parasites of the *Plasmodium* species through the bite of infected anopheles mosquitoes. About 3.3 billion people half of the world's populations are at risk of malaria with 1.5 to 2.7 million deaths, predominantly among children, especially children in sub-Saharan Africa[1]. Chloroquine was first synthesized in Germany, but it was not recognized as a potent antimalarial drug until the 1940s during the US World War II military effort. By 1946, it was found to be far superior to other contemporary synthetic antimalarials[2]. Chloroquine became the cornerstone of antimalarial chemotherapy for the next 40 years. Chloroquine quickly became the drug of choice globally to treat uncomplicated *P. falciparum* infections, and it was used as part of the Global Malaria Eradication campaign launched by the WHO in 1955. Chloroquine is one of the least expensive antimalarials available and is still in widespread use.

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Chloroquine [7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline, CQ] is a 4-aminoquinoline derivative antimalarial compound. Apart from being an established antimalarial, CQ also finds use as an anti-inflammatory drug in the treatment of rheumatoid arthritis[3-5] discoid lupus erythematosus [6-7], and amoebic hepatitis[8]. In spite of reports of chloroquine resistance in many parts of the world, it is still used as first line of therapy against malaria in many developing countries owing to its being readily available and cheaper. However, despite being effective in range of diseases, its use was always under scrutiny as it has narrow safety margin and has shown wide range of side effects including cardiac and neurological disorders, retinopathy, and ototoxicity[9-11]. Severe liver injury and hepatitis in the presence or absence of systemic features have also been described in CQ users[12-14]. Liver being the largest gland and major site for drug metabolism has aroused considerable interest among researchers, and some studies were conducted in the past that have addressed the chloroquine-induced hepatotoxicity[15-16]. However, several studies[15-17] advocate that hepatotoxicity caused by CQ is mainly due to its oxidative potential.

However, there have been few other reports[18-19] that consider malaria infection as such a cause for oxidative stress and propose antioxidant action of CQ for its antimalarial property. Chloroquine is a potent autophagic drug that may lead to the cellular degradation of hepatocytes and the concurrent production of vacuoles[20]. Observed increases in the number of lysosomes suggest further cellular degradation. This degradation is accompanied by the fusion of lysosomes with autophagic vacuoles, resulting in the biogenesis of new lysosomes[21] The reported accumulation of chloroquine in lysosomes has an apparent destabilizing effect on lysosomal membranes. Toxic manifestations appear rapidly, within one to three hours after ingestion[22]. Thus, more information is needed about the effects of chloroquine on the organs in which the drug accumulates to gain insight into the impact of the long-term administration of this drug.

MATERIALS AND METHODS

Sixteen adult male wistar rat weighing between 100 and 150 g were selected for this study. The animals were kept in well-ventilated wire mesh cages, exposed to a 12 h light cycle in an air-conditioned atmosphere at a temperature of 26±2 °C and provided with food and water. Two groups of animals were made. Group I marked as the untreated control, and Group II marked as the chloroquine-treated test group.

Chloroquine phosphate (99.3% pure) and other chemicals were obtained from Sigma-Aldrich (UK). Chloroquine phosphate was dissolved in single distilled water. The dose of the drug was selected based on its oral LD_{50}, which is 500 mg/kg body wt. for rat[23]. The drug was administered orally at a dose of 200 mg/kg body wt. for 25 days. A dose of less than 200 mg/kg body wt. did not produce significant results in other tissues, and a higher dose resulted in significant toxicity[24]. Hence, to evaluate the impact of an intermediate dose, 200 mg/kg body wt. was selected in the present study.

The animals were sacrificed at the end of treatment on the 26th day. The liver and spleen of the control and treated animals were removed. Histological studies of the spleen and liver were carried out using standard hematoxylin and eosin staining techniques.

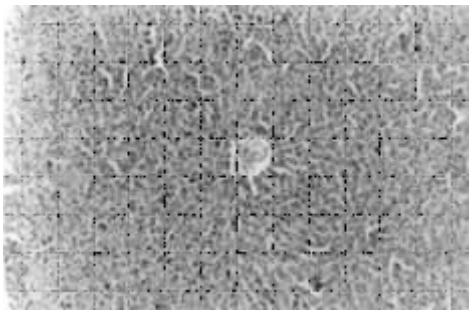
RESULTS

Histological studies revealed some damages in the liver caused by administered drug, the accumulation of iron in the liver and spleen (haemosiderosis), death of cells of the liver (hepatic necrosis), sinusoidal dilatation and atrophy of hepatocytes and multifocal areas of coagulative necrosis.It also shows significant liver damage as exhibited by pronounced eosinophilic bodies and lymphocyte infilteration. (Fig. 1. A-B)

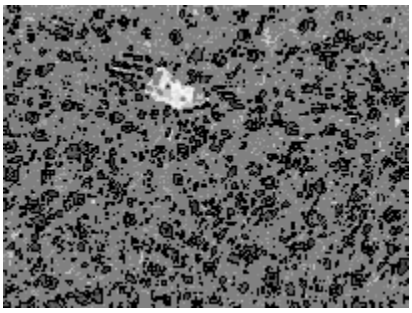
The spleen tissue of treated animals exhibited disorganization of the megakaryocytes, with cells around the trabeculae, disorganization of the red pulp and an elevated number of mast cells under high magnification. Corpuscles were also spread out uniformly (Fig. 2. A-B).

DISCUSSION

Malaria is a disease that was once on the verge of eradication but has recently returned with

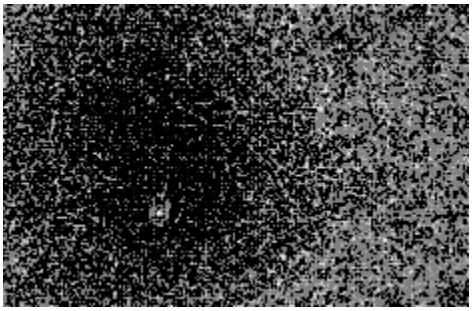


1A. Normal rat liver tissue. Hepatic cord well arranged

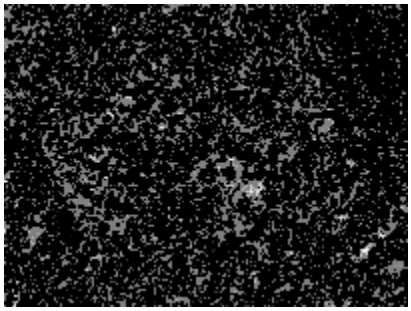


1B Treated Liver showing sinusoidal dilatation and atrophy of hepatocytes and multifocal areas of coagulative necrosis.

Fig. 1. L.S. of Control liver and treated liver under high magnification (40×)



2A. Control spleen showing normal organization of the red pulp with megakaryocytes.



2B. Treated spleen chronic myloproliferative disorder with progressive bone marrow fibrosis and extra medullary hematopoiesis with mast cells

Fig. 2. L.S. of control spleen and treated spleen under high magnification (40×)

greater vigor. This return calls for better preventive and curative treatments and for improved disease control methods. The widespread use of antimalarial drugs further demands the critical evaluation of drug toxicity and damage to tissues. In this study, the administration of chloroquine for 45 days resulted in anomalies that could clearly be attributed to the toxicity of this drug.

In response to the damage caused, macrophages (Kupffer cells) in the liver actively proliferate (kupffer cell hyperplasia) breaking down ruptured red blood cells by phagocytic action and splitting the haemoglobin molecules. This results into pathological effects like accumulation of iron in the liver (haemosiderosis), which is usually linked with anaemia and could sometimes lead to liver cirrhosis, a condition of decreased liver function if not treated effectively. Extensive

and rapid death of parenchyma cells of the liver (hepatic necrosis) also occurs. Hemozoin (malaria pigment) is a disposal product formed from the digestion of red blood cells by malaria

parasites, and it is observed in either the cytoplasm or outside hepatocyte and kupffer cells as black or brownish granules. On the other hand, haemosiderin is a granular brown substance composed of ferric oxide left from the breakdown of haemoglobin. It is usually observed in the cytoplasm of kupffer cells. Haemosiderosis is a form of iron overload disorder resulting in the accumulation of haemosiderin. Both haemosiderosis and hemozoin were observed in the liver, but hemozoin was more in the spleen.

In the spleen, haemosiderosis was also observed. This organ is the site for the breakdown of worn-out red blood cells and stores the iron they contain. Histologically, corpuscle degradation around the sinusoids, the scattering of cells and the degradation of the red pulp were observed in the spleen. The increased numbers of megakaryocytes, blast cells and mast cells suggest a possible change in hematopoiesis [25].

In conclusion, the results of our study suggest that prolonged exposure to the antimalarial drug chloroquine phosphate results in adverse effects on vital tissues. Given the risks to humans due to the widespread use of this quinoline derivative, proper instruction and careful monitoring by doctors are needed when chloroquine is to be taken for longer durations. This work also identified the need for more studies in the future to shed light on other aspects of antimalarial drug toxicity and therapeutic treatments.

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