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Original Research Article

Catastrophic consequences of the enormous use of hydroxychloroquine during COVID era on liver and kidney of male albino rats: an *in-vivo* study

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ABSTRACT

Background: Hydroxychloroquine (HCQ) is mainly used for the treatment of malaria but during COVID trial, it was used against coronavirus though no history of the drug is known against SARS COV 2 or any other respiratory ailment. Many case studies showed the adverse effects on liver and kidney in many patients after the exposure of HCQ. The main aim of this study is to know the effect of HCQ drug on the liver and kidney of male albino rat at a range of human equivalent dose that was given during COVID period.
Methods: After institutional animal ethics committee (IAEC) approval, ten male albino rats were obtained and divided into two groups-control and treated. Treated groups receives HCQ through oral gavage for six days and then serum, tissue enzymes and total serum bilirubin were measured. Histopathological study was done from liver and kidney tissue. After that statistical analysis was done.
Results: We found significant increase in enzymes glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (ALP) in the HCQ-treated rats than in control and this signifies that there might be damages that occurred in liver and kidney. Increased level of bilirubin in HCQ-treated rats indicate hyperbilirubinemia and may be a sign of jaundice or any other hepatic disorder. From histopathological identification we also found liver and kidney tissues got damaged due to exposure of HCQ.
Conclusions: From this study, we can conclude that the exposure of this drug might have led to the impaired function of organs that could have potentiated their ill fate.

Keywords: COVID, Hydroxychloroquine, Liver, Kidney, Histopathology

INTRODUCTION

Hydroxychloroquine (HCQ) is a drug of quinoline group having the chemical formula of $C_{18}H_{26}ClN_3O$. It is a very old drug and it was synthesized in the year 1946 by introducing a hydroxyl group into chloroquine, a drug of Quinolone group derived from the bark of cinchona tree in 1600s.[1-3] Both chloroquine and HCQ are used for the treatment of malaria. Not only for malaria, in the past seven decades these drugs are continuously used to treat rheumatoid arthritis and systemic lupus erythematosus.[3,4] After oral administration of HCQ the gastrointestinal absorption occurs very fast and is sufficient to rise at its peak concentration value in blood in 2-3 hours after dosing.[5] The bioavailability of HCQ is 70-80%.[6] HCQ can accumulate and remains in steady concentration in the body tissues for 4-6 months, but the mechanism of HCQ as an antimalarial drug is still not well established.[7,8] HCQ and chloroquine were approved by the U. S. Food and Drug Administration (FDA) not only as treatment and

prophylaxis for malaria, but also as treatment for rheumatic arthritis.[4]

A multitude of distinctive immunomodulatory and anti-inflammatory properties made HCQ a clinically attracted drug. As we all know we have gone through a pandemic situation, COVID 19, without having proper knowledge of its medication and treatment. To treat this a lot of clinical trial has been done even without knowing its efficacy. Various evidence has suggested that during the COVID scenario, medications were used in large quantities and India has emerged as the major consumer of antibiotics.[9]

It has been hypothesised that HCQ may have indirect anti-inflammatory effects through the activation of CD8+ T cells and the suppression of pro-inflammatory cytokine responses, as well as antiviral activity and as a result, it was frequently used in the treatment of COVID-19 as well as in pre- and post-exposure prophylaxis.[9] The national recommendations were subsequently updated on 27 June 2020, restricting the use of HCQ to patients with mild illness who are immunocompromised or younger than 5 years old, as well as moderate to severe COVID-19 instances.[10] But before their restriction were imposed, HCQ was used in a very high doses as well as for long duration than the recommended doses for malaria. Tables 1 and 2 is showing the same.

HCQ and chloroquine have been suggested as treatment for SARS-COV-2 on the ground of both anti-inflammatory and anti-viral properties. The drug may interfere with the entry of virus into the cell and corona virus highjack the ACE-2 receptor for its entry into the cell and is known to interfere in the glycosylation of ACE-2, which result spike protein-ACE-2 binding less efficient and hamper the entry of virus.[17] It also inhibits the viral release into the intracellular space by disrupting lysosome-endosome fusion and inhibit the release of proinflammatory cytokines.[18] It was observed in various studies that HCQ acts by the disruption of the interaction of the S protein of SARS-COV-2 with the host cell membrane.[19]

Though HCQ is relatively safe to use in treating malaria and auto immune disease but covid-19 patients were more susceptible to its adverse reaction because of the compromised function of vital organs secondary SARS-COV-2 infection, as HCQ is cleared by the kidney and liver it may cause impairment of renal or hepatic function, besides drug-drug interaction are major cause of sum of HCQ's adverse effect including cardiovascular, neuropsychiatric and gastrointestinal side effects.[20]

Table 1: The dosage used in malaria.

Diseases	Recommended doses and durations
Malaria (acute)	800 mg followed by 400 mg at 6, 24, and 48 h (CDC guidelines).[11]
Malaria (prophylaxis)	400 mg once weekly on the same day of the week beginning two weeks before visiting a malaria-prone region and continuing for four weeks after leaving the region.[12]

Table 2: The doses and duration of HCQ used during COVID-19.

Doses and duration of HCQ used during clinical trial of COVID	Related references
HCQ (600 mg/d, 10 d) + AZ (500 mg/d, d 1 and 250 mg/d, d 2-5)	Molina et al.[13]
HCQ (1200 mg/d for 3 d followed by 800 mg/d; total duration: 2 weeks [mild/moderate] or 3 weeks [severe])	Tang et al.[14]
HCQ (200 mg×3/d, 10 d) + AZ (500 mg on d 1 followed by 250 mg/d, 4 d)	Million et al.[15]
HCQ (200 mg×2/d, 5 d)	Chen et al.[16]

The patient with acute respiratory distress syndrome due to COVID-19 presented with a rapid increase in transaminases after the introduction of HCQ followed by rapid reduction after the drug was discontinued, hepatic dysfunction and elevation of liver enzymes have been reported in 30-60 % cases of COVID-19 more frequently in the patient in the ICU and the side effects experienced by the COVID-19 patient as well as the incidence of these adverse effects among the treated patients depends largely on the administered dose of HCQ and the co-existence of cardiac, hepatic and renal diseases which might potentiate the toxicity of this drug.[21] Administration of HCQ has been banned prescribing for COVID and an exhaustive literature survey regarding HCQ reveals that not much work is reported on control release studies of HCQ drugs.[22]

This present study is aimed to examine the effect of HCQ exposure on human health with the objective to analyse different biological parameters viz. certain enzymatic levels and associated histopathological studies.

METHODS

Animal treatment and housing

Animal experiments were performed with the approval of animal ethics committee, Raja N.L. Khan Women's College, Autonomous, Midnapore, West Bengal. Male albino rats weighing about 150-160 g were obtained. Prior to the experiment, all animals were acclimatized for 7 days. Animals were housed in plastic cages under the facility with a regular day–night cycle at room

temperature. Animals were grouped into two- control and HCQ-treated and in each group five rats were present. Total tenure of this study was from mid of June 2023 to first week of July 2023.

Dose selection

HCQ sulfate (HCQ) was purchased from Sigma Aldrich, and it was given 33 mg/kg/day orally through gavage for 6 consecutive days by considering its lethal dose 1240 mg/kg body weight of rat.[23] The dose and the duration were selected in accordance with the doses and duration of HCQ used in COVID in adult human and by calculating its equivalent doses for rat according to FDA dose calculation formulae.[24] The dose was adjusted by the body weight of each rat day by day.

Tissue collection

After euthanizing rats, the liver, kidney, were isolated and rinsed with cold isotonic saline. 10% w/v tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000g for 15 minutes and the supernatants were collected for further experiments. Procedure of euthanasia was strictly followed by CPCSEA guidelines.

Serum biochemical parameters

Blood was collected after 6days of treatment. The blood was then allowed to clot and then it was centrifuged at 5000 rpm for 10 minutes then the serum was collected and biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) were estimated from serum by the Reitman and Frankel method.[25] The level of alkaline phosphatase (ALP) was measured by Kind and Kings method.[26] Total serum bilirubin was estimated by Malloy and Evelyn method.[27]

Tissue biochemical parameters

The GOT, GPT, and ALP levels were estimated from the collected supernatant of the liver and kidney by the same procedure as that of serum. Calculation was done after measuring the total protein per milligram of tissue by Biuret method.[28]

Histological identification of liver and kidney tissues

After sacrifice the kidney and liver tissues were isolated and fixed in Bouins fixative. The section of 5 µ width was preferred and stained with haematoxylin and eosin.

Statistical analysis

Statistical analysis was done by using two tail t-test method through t-test calculator available at socsscistatistics.com. Mean and SD values are represented in each bar diagrams. $p<0.05$ considered as significant.

RESULTS

After statistical analysis of the collected data of enzymes quantity we found significant increase in SGOT, SGPT and ALP and total serum bilirubin in the HCQ-treated rats as showed in Figures 1 and 2 with the mean±standard deviation value for control versus treated rats, SGOT 46.64±2.72 versus 127.77±6.08, SGPT 53.33±16.33 versus 153.33±26.67 and ALP 44.46±8.96 versus 150.87±7.94 Total serum bilirubin 0.68±0.04 versus 1.88±0.16 and these are statistically significant at $p<0.05$.

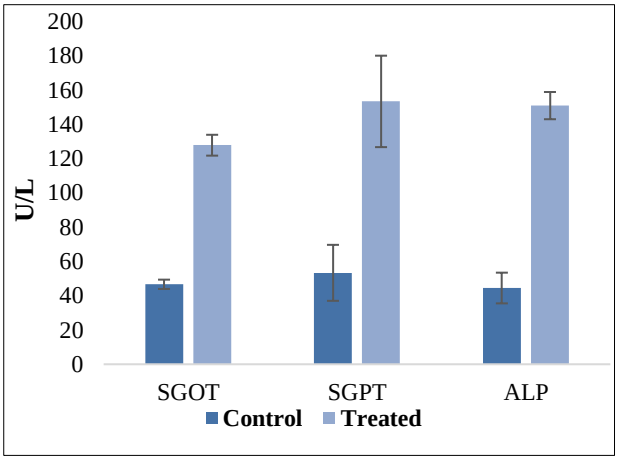

Figure 1: Bar diagram represents the differences in the level of serum enzymes in between control and treated rats p<0.05.

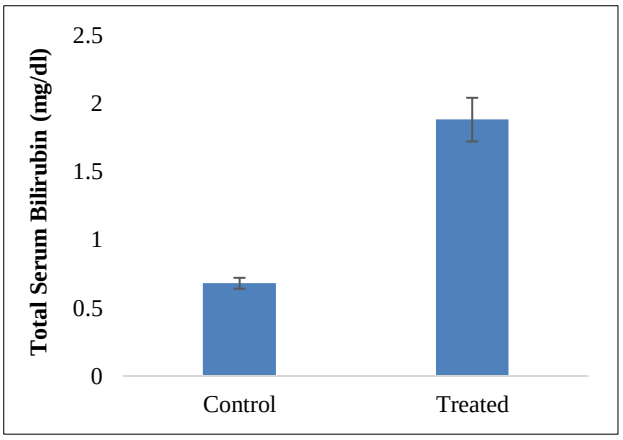

Figure 2: Bar diagram represents the differences in the level of total serum bilirubin in between control and treated rats (p<0.05).

Increased level of SGOT, SGPT and ALP indicates there might be some adverse effect occurred in the HCQ-treated rats. Increasing total serum bilirubin also indicates it might be the sign of jaundice or any other hepatic disorders. To ensure this we evaluated GOT, GPT and ALP and similar results were seen in liver tissue as depicted in Figure 3, showing the mean±SD values of the above-mentioned parameters as control versus treated rats, GOT 0.51±0.03 versus 0.89±0.09, GPT 0.95±0.22 versus 4.7±0.54, ALP

1.74±0.11 versus 4.9±0.25 and all are statistically significant at p<0.05. Thus these can be considered as the indicating marker of the occurring damages in liver.

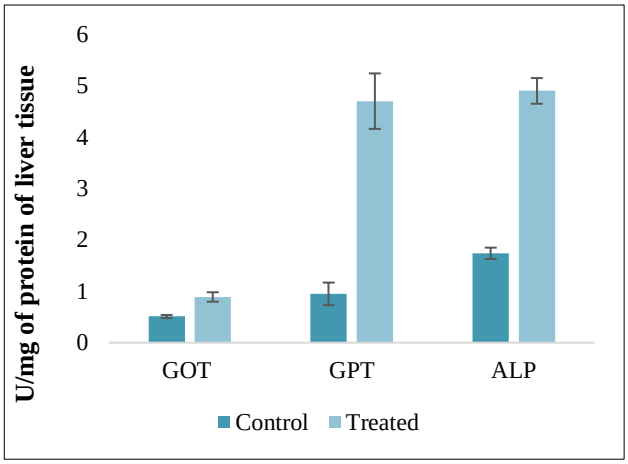

Figure 3: Bar diagram represents the differences in the level of liver tissue enzymes in between control and treated rats, p<0.05.

GOT, GPT and ALP also present in kidney and to know the impact of HCQ on the concerned tissue, we measure these enzyme quantities in kidney tissue and found significant increase in HCQ-treated rats as in Figure 4, showing the mean±SD values of the previously mentioned parameters as control versus treated rats, GOT 0.68 ±0.14 versus 2.76±0.08, GPT 1.77±0.26 versus 6.94±0.71, ALP 1.65±0.12 versus 7.78±0.29 and all are statistically significant at p<0.05.

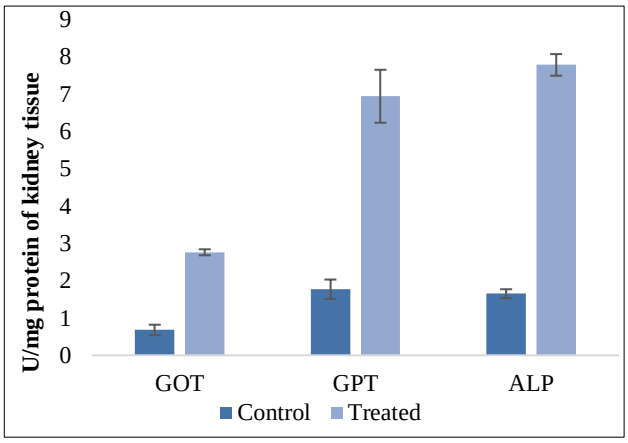

Figure 4: Bar diagram represents the differences in the level of kidney tissue enzymes in between control and treated rats, p<0.05.

Histopathological study of liver and kidney tissues was done to know whether any changes occur in after HCQ exposure and we found prominent changes in the histological structure of liver and kidney as described with photomicrographs below.

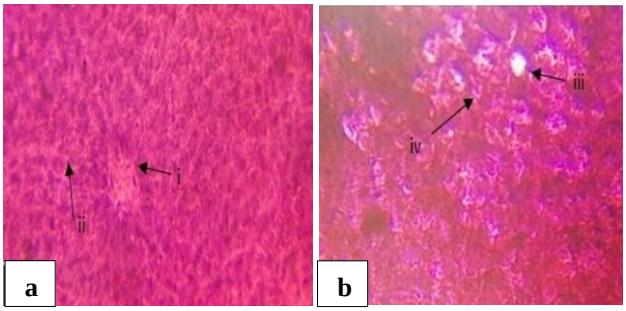

Figure 5: Photomicrographs of liver tissue sections of (a) control and (b) HCQ-treated rats (HE stain, magnification 400) showing control rats having i. normal central vein ii. separated and prominent hepatocytes and in contrast HCQ- treated rats having iii. decreased diameter of central vein and iv. clump hepatocytes).

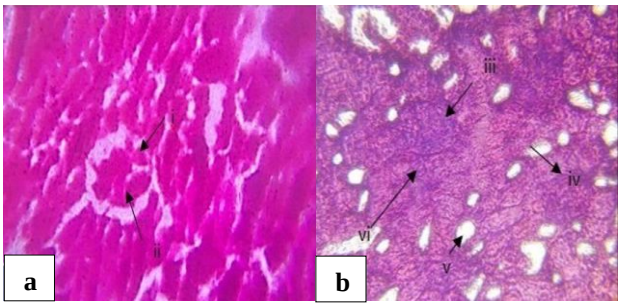

Figure 6: Photomicrographs of kidney tissue sections of (a) control and (b) HCQ-treated rats (HE stains, magnification 400) showing control rats having i. normal Bowman's capsule with prominent Bowman's space and ii. proper sized glomeruli in contrast to HCQ- treated rats having iii, iv. Bowman's capsule without Bowman's space, and v. congested renal cells and vi. swollen glomeruli).

DISCUSSION

Previously, side effects of many medications were well established specially for several antibiotics. In a study in 2019, revealed that higher doses of HCQ usually greater than 100mg twice a week can trigger acute liver injury in patient with history of porphyria cuteana tarda.[29] From our study we found that the GOT, GPT and ALP level is significantly higher in serum as well as in liver and kidney tissues after HCQ exposure, Sekar et al in a study also found a similar effect that a high dose of an insulin mimic medicine sodium orthovanadate led to the increase in GOT, GPT levels in liver tissue.[30] A case reported that a patient with severe COVID-19 after being administrated HCQ, showed a tenfold increase in the level of serum transaminases followed by a rapid decrease after HCQ was withdrawn, the potentiality of HCQ is dose dependent and be associated with hepatotoxicity and the need to monitor liver function during the HCQ therapy.[21] According to Galil, Lupus, acute toxic hepatitis was diagnosed in HCQ treated SLE patient which return to normal level after

cessation of medicine.[31] In the year 2020, in a study by Hussain et al, showed that in HCQ treated patients' mortality rate is 2.5 times greater than compared with control groups and had higher rates of adverse clinical outcomes and side effects.[32]

As similar to our study, Singh et al also found an increase in total serum bilirubin level in male Wistar albino rats after paracetamol and azithromycin exposure.[33] Younis et al proposed that the therapeutic effects of HCQ are not without side effects range from mild gastrointestinal effects to life threatening cardiovascular neurological effects.[34] HCQ is generally well tolerated medicine but have short term toxicity including gastrointestinal effects, dermatology reaction and neuropsychiatric events and long term toxicity includes retinopathy, neuro-myotoxicity and cardiac toxicity, deaths from overdoses most often result from cardiovascular collapse.[35]

After histopathological analysis we found that control liver shows the normal histological structure of liver having the prominent central vein and hepatocytes are separated with blood sinusoids but in case of treated liver it is clearly visible that the diameter of central vein decreased and the clumpy hepatocytes are present. Following the histopathological study of kidney tissues, we found that the glomeruli became swollen in case of HCQ treated rats and due to this the Bowman's capsule have no Bowman's space. Apart from this the renal cells were congested in case of treated rats. In a previous work on doxorubicin (DOX), a broad-spectrum chemotherapeutic agent, used for the treatment of several types of cancer, Chen *et al* found that DOX leads to tissue injuries, including degeneration of the hepatocytes, focal necrosis, and haemorrhage and they showed the hepatorenal toxicity induced by DOX in rats by assessing histopathological changes of liver and kidney tissues through haematoxylin and eosin staining.[36] Studies showed different side effects of HCQ, Misra et al showed that the use of HCQ with cumulative doses are related to retinopathy after years of use and when HCQ is combined with azithromycin cause cardiac toxicity including QT prolongation.[37]

Our study is constrained to the hepatic and renal system but further study on the detrimental effect of this drug on different physiological systems *viz.* cardiovascular, gastro-intestinal etc. is required. Histochemistry of liver and kidney with differential staining of protein, lipid and carbohydrate contents are needed to evaluate more accurate histopathology after exposure to this drug.

CONCLUSION

Higher enzymatic levels and adverse changes in the histology of liver and kidney has made a conclusion that 6 days exposure of HCQ leads to the concussion of liver and kidney in male albino rats. Impairment of these organs might lead to serious illness of the individual. Precautions and some remedial strategy should be implemented in future to get emancipate from these types of damages.

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