Berkala **PENELITIAN HAYATI**

ISSN: 08526834 | E-ISSN:2337-389X

Volume 30 | No. 2 | **August** | 2024

Original Article

Possible hazards on gastrointestinal system and total gut bacterial colony due to boundless use of Hydroxychloroquine during COVID era: a six days exposure study on male albino rats

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Abstract

Hydroxychloroquine (HCQ), a drug of quinolone group, used in clinical settings to treat malaria. Lately, there has been interest in its potential efficacy against coronavirus, with several proposed mechanisms. It has been demonstrated to limit the replication of sars-cov-2 virus in vitro but clinical trials found ineffective for this purpose and a possible risk being that dangerous side effects may arise. Therefore, this study is designed to give a light on its possible side effects on smooth muscle movement, gut bacterial composition. Ten male eight weeks old albino rats were obtained and randomly divided into two groups control and treated with five animals each. Rats of treated group received HCQ 33mg/kg/day for six consecutive days and after that faecal parameters, smooth muscle contractility test, total bacterial colony count were estimated from both groups. From the analysis decreased faecal count and increased faecal water content were found in the treated rats than in control. Colonic and Gastro-intestinal transit time significantly increased in the treated rats and abnormalities were seen in small intestinal muscle contractility. Faecal and small intestinal total bacterial count was significantly lowered in HCQ-treated rats. In conclusion, it can be said that HCQ might be related to adverse consequences on normal gut movements and its bacterial community. In addition, we can conclude that HCQ might induce decreased intestinal motility led to the resulting detrimental effects on normal gut bacterial composition.

Keywords: hydroxychloroquine, gastrointestinal motility, gut bacteria, aerobic, COVID

Received: April 24, 2024 Revised: July 14, 2024 Accepted: August 19, 2024

Introduction

In COVID era it was hypothesized that HCQ may prevent the movement of sars-cov-2 from early endosomes to early lysosomes that are important for viral genome release (Mauthe, M et al.,2018). The production of autophagosomes, which break the S protein preventing the membrane fusion, is further triggered by the rise in pH of lysosomes and endosomes caused by HCQ (Zhou, D et al.,2020).

Hydroxychloroquine, was initially used as an antimalarial, an immunomodulatory and anti-inflammatory agent for the management of autoimmune and rheumatic diseases such as systemic lupus erythematosus (Schrezenmeier, E., & Dörner, T. 2020). The absorption of HCQ occurs in upper intestinal tract having renal clearance of 21% and bioavailability of 0.7-0.8. (McChesney, E. W. 1983, Fanouriakis, A.et al.,2019).Apart from its beneficial effect it also possess some side effects, in a study by Mahmoudi, J. et al.,2021 found that due to Hydroxychloroquine and Chloroquine exposure, nephrotoxicity was observed in COVID patients. Falcão MB et al.,2020,in a case report showed that after using HCQ 400mg daily for 2 weeks patients required liver transplantation and even died. In 2019, a study revealed that higher doses of HCQ usually greater than 100mg twice a week may lead acute liver injury in patient with history of porphyria cuteana tarda (Cheema, B et al.,2019).In a study in 2023,found that due HCQ exposure at the dose of 400mg/kg followed by 200mg/kg for six days might lead to enhance the liver enzymes and urea level (Aldraji, A et al.,2023). In a previous study, researchers found that liver and kidney might be damaged due to consecutive HCQ exposure at a dose of 33mg/kg/day on male albino rats for six days. Enhancement of liver enzymes, bilirubin level and histopathological changes were also found in their study (Samanta, T et al.,2023).

Overdoses of Hydroxychloroquine are extremely rare, but extremely toxic (Juurlink, D. N. 2020). Eight people are known to have overdosed since the drug's introduction in the mid-1950s, of which three have died (Marquardt, K., & Albertson, T. E. 2001). Narrow therapeutic index defined as there is little difference between toxic and therapeutic doses and Hydroxychloroquine belongs to this group . Within an hour of ingestion with overdose of HCQ shows serious signs and symptoms and most seen side effects of CQ/HCQ are gastrointestinal (GI) abnormality along with nausea and vomiting (Srinivasa, A et al.,2017). These signs and symptoms in GI tract occur may be due to the gut microbial dysbiosis.

It is well documented that the cumulative toxicities after long-term use of HCQ include retinopathy, cardiomyopathy, and rhythm disorder, in fact, up to 20% of patients with COVID-19 had GI symptoms (Wong S.H et al.,2020), and a part of whom with active SARS-CoV-2 GI infection were tightly associated with alterations of gut microbiota (Zuo, T.,et al.,2021). Recently, it has bee hypothesized that gut microbiota dysbiosis is one of the leading mechanisms responsible for the poor outcomes in elderly COVID-19 patients with pre-existing age-related diseases (Zuo, T et al.,2020).

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http://dx.doi.org/10.23869/bphjbr.30.2.20247
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Though, it is obvious that HCQ might have some side effects due to consecutive exposure on the physiological system, but research on its impact on gastrointestinal health remains limited. Hence, this study focuses on identifying the impacts of HCQ on Gastrointestinal system and gut bacterial composition by simple colony count method.

Methods

Ethics statement

All animals are approved by IAEC of Raja N.L Khan Women's College (Autonomous), Midnapore, West Bengal, India in accordance to CPCSEA guidelines with the approval no: 04/IAEC (1)/S/ RNLKWC/2023 of Raja N.L Khan Women's College (Autonomous) Midnapore.

Animal treatment and housing

Ten male eight weeks old albino rats weighing about 150-160 g were obtained and they were acclimatized for 7 days. Animals were grouped into two (n=5 animals each), control and treated. From the 8th day, animals of treated group received single dose of HCQ orally through gavage with drinking water as vehicle, meanwhile, control group received equal amount of plain drinking water through gavage. Animals were housed in plastic cages under the facility with a regular day–night cycle at room temperature.

Dose selection

Hydroxychloroquine sulfate (HCQ) was purchased from Sigma Aldrich, and was given at a single dose of 33mg/kg/day orally through gavage for six constitutive days. The dose was adjusted by the body weight of each rat day by day. This dose has been prepared according to the doses used in malarial condition but the duration is selected on the basis of the duration, used during COVID (Lei, Z et al., 2020) and then it was converted human to rat doses as per FDA dose conversion guidelines (Nair, A. B., & Jacob, S. 2016) by considering its lethal dose 1240mg/kg body weight of rat (El Shishtawy et al.,2015).

Faecal parameter measurement

Freely feeding rats are observed for 8 h, and the number of pellets was counted every 2 h. Faecal water content was measured by comparing the wet weight of the pellets and after drying (24 h at 60 °C) (Ge, X et al.,2017).

Gastrointestinal & colonic transit

After fasting overnight with free access to water, rats were administrated by gavage with a semiliquid solution (0.1 ml) containing Evans blue and methylcellulose (Li, Z., et al.,2011). Then time was recorded at the presence of the first blue pellet and colonic transit of rats was measured with a bead expulsion test (Nezami, B. G et al.,2014).

Smooth muscle contractility

It was done by Dale's method as described by HUKUHARA, T., & FUKUDA, H. 1965, from the duodenum, ileum, and jejunum portions of the intestine of rats of both control and treated groups. Amplitude of each graph were calculated considering ten amplitude levels. Calculated amplitude was expressed as mean± standard deviation.

Total bacterial colony count of anaerobic bacteria from faecal and different segments of small intestine

Bacteria from faecal sample and tissue homogenates were cultured on nutrient agar plates using dilutions ranging from 10^{-1} to 10^{-8} . Total bacterial colony count was determined from the first countable plate after incubating bacteria for 48 hours in anaerobic condition.

Total count of aerobic or facultative anaerobic bacteria from faecal and different segments of small intestine

Bacteria were cultured in aerobic condition by using nutrient agar media and total bacterial count was done.

Statistical analysis

Statistical analysis was done by using two tail t test method by using online t test calculator (https://www.socscistatistics.com/tests/studentttest/default.aspx).$p<0.05$ considered as significant, mean and standard deviation (SD) were expressed in bar diagrams.

Results and Discussion

No significant differences were observed in total body weight between the control and treated groups at both the beginning and the end of the experiment.

Analysis of total anaerobic bacterial count

After analyzing the total bacterial colony count of anaerobic bacteria, we found there was significantly lower colonies in both fecal and tissue samples in case of HCQ treated rats than that of control as shown in Figure 1 & 2.

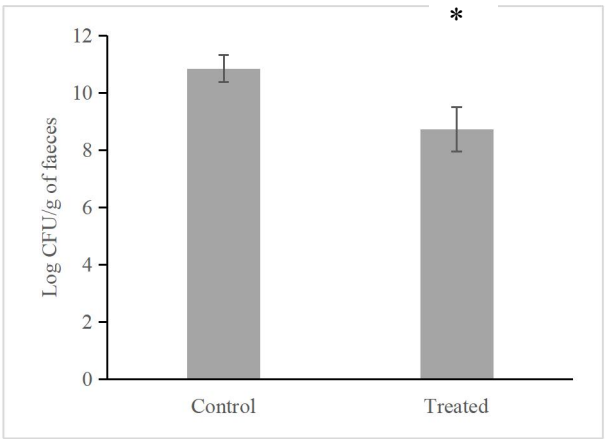

Figure 1. Bar diagram represents the significant difference in total anaerobic bacterial count in fecal sample in between Control and HCQ-treated rats at *$p<0.05$

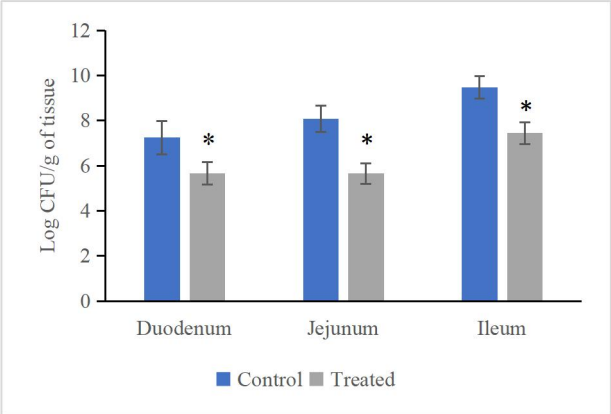

Figure 2. Bar diagram represents the difference in total anaerobic bacterial count in small intestinal tissues in between Control and HCQ-treated rats *p<0.05

Total aerobic bacterial count and analysis

After the isolation of bacteria from fecal sample of both control and treated rats, we found significant decrease in total bacterial colony count in the treated rats than the control as shown in Figure 3. In the three segments of small intestines duodenum, jejunum, ileum we found significant decrease in total colony count of bacteria in the treated rats as shown in Figure 4. Researchers found that due to exposure of different antibiotics viz. azithromycin, levofloxacin, cefpodoxime, the mean log CFU value is decreased in fecal samples of adult healthy volunteers (Anthony, W, et al 2022).

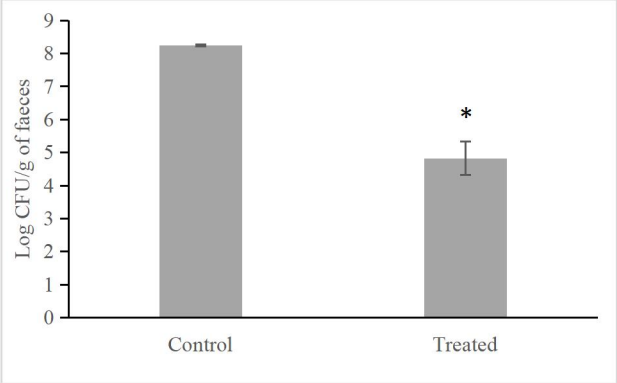

Figure 3. Bar diagram represents the difference in total aerobic or facultative anaerobic bacterial count between Control and HCQ-treated rats *p<0.05

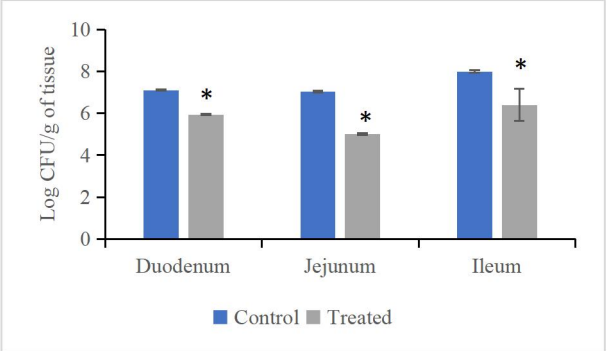

Figure 4. Bar diagram represents the difference in total aerobic or facultative anaerobic bacterial count between Control and HCQ-treated rats *p<0.05

After the evaluation of total colony count in both aerobic and anaerobic condition, significantly decreased colony count was found in treated group than that of control. Gut microbiota plays an important role in the maintenance of gut health. Altered or diminished gut microbiota might hamper the normal physiological system and might raise some diseases like inflammatory bowel diseases, colon cancer etc. (Guarner F et al., 2003).

Evaluation of faecal parameters

After analyzing the fecal pellet count, HCQ-treated rats had significantly lower pellet count than the control rats. Fecal water content was found significantly higher in treated rats than the control rats as shown in Table 1. In a previous study, Ge, X., et al., found the similar result that due to antibiotics exposure fecal pellet count decreased and water content increased in mice.

Table 1. Showing the difference in Faecal pellet count /8 h and Faecal water content % between Control and HCQ-treated groups.

Parameters	Control group (mean±SD)	HCQ-treated group (mean±SD)	Statistical p value
Faecal pellet count /8 h	15.62 ± 1.62	8.6±1.95	p=0.000574
Faecal water content %	38.31± 1.03	69.11± 9.83	p= 0.000252

Evaluation of transit time (minutes)

After evaluating the transit time, significantly higher colonic and GI transit time were found in the HCQ-treated rats than the control, which means there is slower movement of the gut in treated rats than the control as shown in Figure 5 and this might be due to the abnormal contractility of the intestine.

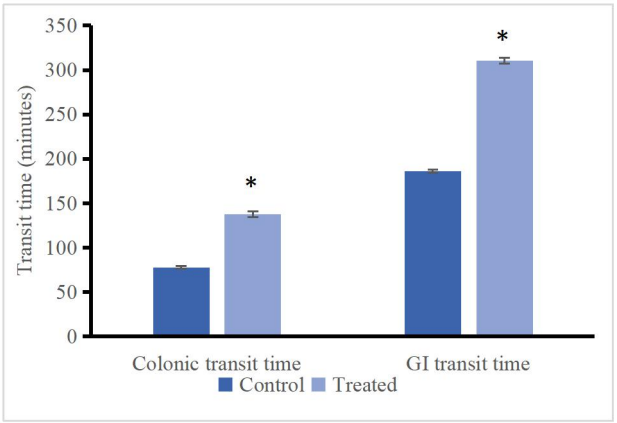

Figure 5 Bar diagram represents the difference in GI transit time (min) and Colonic transit time (min) between Control and HCQ-treated rats *p<0.05

Analysis of Smooth muscle contractility

The amplitude was significantly lower in the treated rats (p<0.05). Treated rats have abnormal duodenal movement and sometimes it stops which is shown by the straight line. In case of the jejunum of control group, amplitude was similar in the entire recorded curve but in treated rats, the curve pattern showed lower amplitude and in the mid-time, it touched the baseline, and then again, the lower amplitude continued. The curve

amplitude is lower in treated rats than in the jejunum of control rats. Thus, an abnormal Jejunum movement was observed in the case of HCQ-treated rats. In case of the treated ileum, the amplitudes were lower than in control rats and showed irregular movements as shown in Fig 6. Ge, X et al. (2017) found that due to the antibiotics viz. ampicillin, neomycin, and vancomycin exposure, gut motility decreased and as a result, the GI transit time and colonic transit time increased.

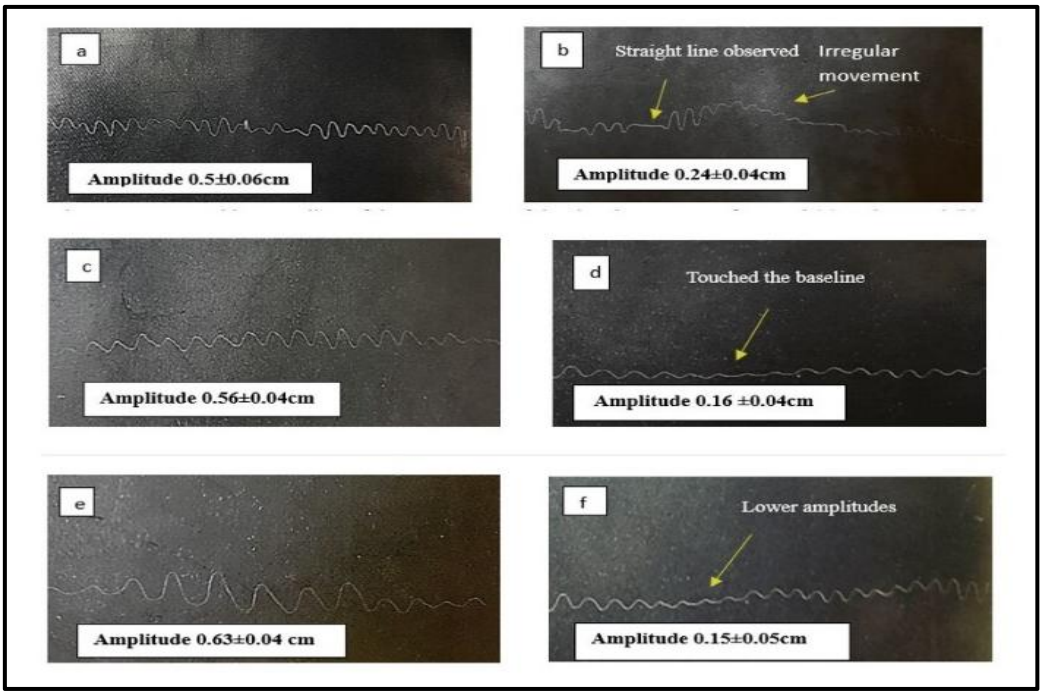

Figure 6. Kymographic recording of the movement of duodenal segments of control (a) and treated (b) rats, jejunal segments of control (c) and treated (d) rats and Ileal segments of control (d) and treated (e) rats. Drum speed 2.5 mm/sec.

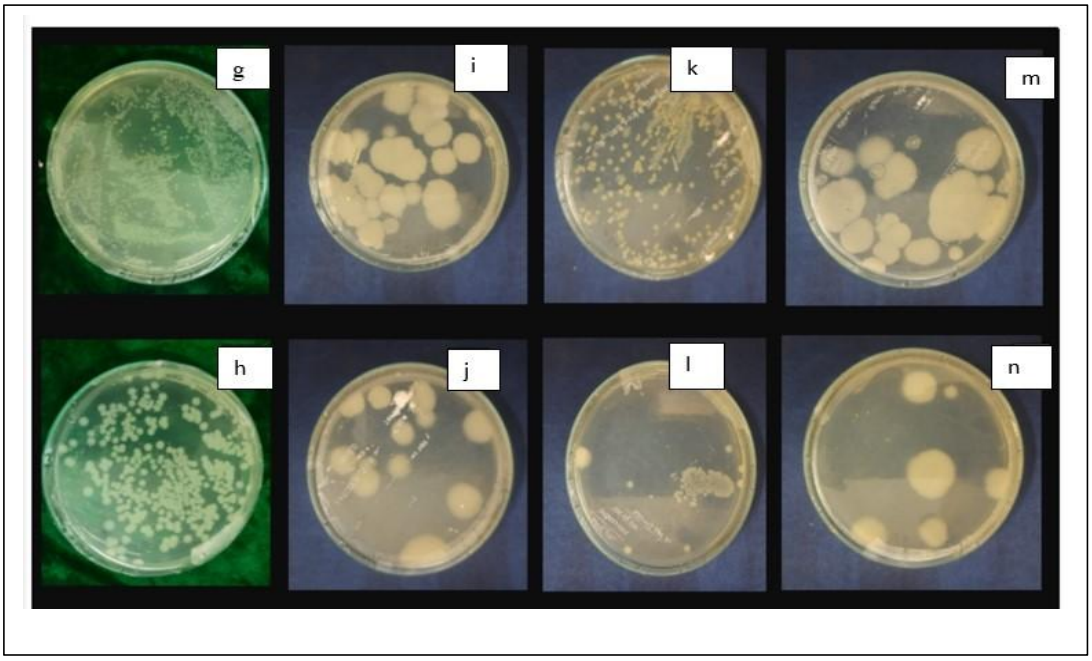

Figure 7. Photographs of the cultured bacterial plates of fecal and tissue samples at different dilution level (g) Control fecal bacteria at 10^{-4} dilution (h) Treated fecal bacteria at 10^{-1} dilution (i) Control Ileal bacteria in 10^{-4} dilution (j) Treated Ileal bacteria in 10^{-2} dilution. (k) Control jejunal bacteria in 10^{-3} dilutions (l) Treated jejunum bacteria in 10^{-1} dilutions (m) Control Duodenal bacteria in 10^{-3} dilution (n) Treated duodenal bacteria in 10^{-2} dilution

Limitation

From our study we can conclude that total bacterial count decreased but to confirm this 16SrRNA analysis is needed for the detailed study of microbes. To find out whether pathogenic bacteria grows or not further study is required.

Conclusion

From the above study we can conclude that due to the Hydroxychloroquine exposure faecal pellet frequency is decreased and faecal water content is significantly increased. Along with this the Gastrointestinal transit time and Colonic transit time both are significantly increased in the treated rats, this signifies that the contraction of smooth muscle might be decreased due to the Hydroxychloroquine exposure. The lower amplitude of the curve of the movements of three intestinal segments also showed the decreasing intestinal motility of the treated rats. This decreasing movement may occur due to the gut microbial dysbiosis. Lower bacterial colony in the treated rats in both aerobic and anaerobic cases, portended that the decreased gut microbiota might occurs due to Hydroxychloroquine exposure. Thus, this study can conclude that complete impairment of GI system might occur after consecutive HCQ exposure.

Acknowledgement

We would like to express our gratitude to Dr. Jayshree Laha, Principal, Raja N.L Khan women's college for providing us laboratory facilities and equipment to complete this work.

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