QUININE PROPHYLAXIS INDUCED HEPATOTOXIC AND HEPATOCELLULAR EFFECTS IN ANIMAL MODEL: TARGETING MISUSE AGAINST SARS-COV-2 IN COVID-19 ERA

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ABSTRACT

Background: Quinine prophylaxis against SARS-CoV-2 is on the rise particularly in the face of COVID-19.

Aim: this study examines hepatic tissues and enzymes following quinine prophylaxis in animal models representing quinine abuse in COVID-19 era.

Materials and Methods: Twenty male Albino wistar rats (180-200g) were randomly picked into four groups (n=5). Rats were maintained in a clean and aerated environment and fed with standard pellets and water provided *ad libitum*. Group I served as control. While group II, III and IV were treated with quinine at concentrations of 10, 20, and 30mg/kg b.w respectively on the left thigh using 5mL syringe. Rats were treated for 28 days at two days intervals and sacrificed by cervical dislocation. Blood was collected for the assessment of the liver function from the cardiac region while liver samples were excised, grossed and fixed in 10% formalin for histology. Serum alanine aminotransferease (ALT), aspartate aminotransferease (AST), alkaline phosphatase (ALP) and total bilirubin were determined.

Results: Serum activities of AST, ALT, and total bilirubin in group II to IV were elevated against the untreated group 1 ($p<0.05$). While, ALP in group II to IV reduced tremendously compared with the control ($p<0.05$), which is strongly suggestive of hepatotoxicity. Histopathology showed hepatocellular degeneration, vacoulation, periportal inflammation and necrosis.

Conclusion: Quinine prophylaxis in animal model induced hepatotoxic effects leading to increased hepatic enzymes particularly (AST, ALT), and bilirubin with hepatocellular damages in this study. Therefore, quinine prophylaxis against SARS-CoV-2 should be discontinued except COVID-19 has been diagnosed while hepatic enzymes of patients should be monitored along treatment.

Keywords: Quinine, prophylaxis, liver enzymes and hepatocellular damages

BACKGROUND

Quinine is naturally obtained from the bark of *C. officinalis* (Achan *et al.*, 2011), which is now synthesized in the laboratory as an antimalarial prescription drug (Jomsky and Nicholas, 2020). Due to emergence of SARS-CoV-2 infection responsible for COVID-19 pandemic (WHO, 2021), many people turned to treatment using herbal preparations (Odigie *et al.* 2020b) while some opted for quinine prophylaxis with the hope that it combats the virus. Grobe *et al.*, (2021) hypothesize that quinine may exert antiviral effects on SARS-CoV-2 and as such infected individuals may not go down with COVID-19. In Indonesia, health authorities are trialing quinine as a possible treatment for COVID-19 patients (Andriyanto, 2020). In Russia, quinine derivatives, mefloquinein is undergoing clinical trials for possible intervention (Sputnik, 2020) while, an American randomized placebo-controlled study assessed the effect of a quinine-containing nasal spray in healthcare professionals (US-NLM, 2021).

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Quinine has reportedly been used to treat viruses and has been demonstrated in-vitro against dengue (Marois *et al.,* 2014), herpes simplex, and influenza A. viruses (Malakar *et al.*, 2018). Effects of quinine on hepatic tissues and enzymes function is paramount and should not be overlooked. We should be mindful of the liver function while on prophylaxis against SARS-CoV-2, as it is the processing house for major metabolic activities in vertebrates (Grobe *et al.*, 2021). From the foregoing, there is limited information on quinine prophylaxis against SARS-CoV-2 particularly as new strains keep re-emerging by the day. SARS-CoV-2 which is the causative agent has not been experimented in this study rather, the use of quinine as prevention regimen is our primary concerns. Therefore, possible outcomes like hepatocellular and hepatic enzymes effects of quinine prophylaxis in animal models were investigated.

MATERIALS AND METHODS

Description and preparation of Quinine

Quinine was obtained from a government approved pharmaceutical stores with NAFDAC Registration Number: 06-0881 while distilled water was used as vehicle in preparing the stock solution in accordance to manufacturer's instructions.

Animal Housing and Ethical Clearance

Twenty inbred male Albino wistar rats weighing (180-200g) were obtained from the animal housing facility of Anatomy Department, University of Benin, Nigeria. Animals were housed in steel wire cages and acclimatized for 14days under ambient temperature (25 ± 3^{0C}), humidity (45-55%), and periodicity (12:12hourly) prior to experimentation. Clean and aerated environment was maintained regularly by the animal house keeper. Rats were fed with standard pellets (Standard feed Nigeria Plc.) and water provided *ad libitum*. Rats were handled according to international guidelines as reported by the Institute for Laboratory Animal Research (NRC, 2011). Ethical clearance (V.1034/36) was obtained from the Ministry of Agriculture and Natural Resources, Benin City; Edo State, Nigeria.

Lethal Dose Determinations (LD_{50})

Acute toxicity study was conducted according to an existing report in the manufacturer's instruction manual for 7days. Lethal dose (LD_{50}) determination was an adaptation to Lorke's (1983) method. Three rats were used and extrapolated doses (20mg/kg b.w, 30mg/kg b.w, and 40mg/kg b.w respectively) from a previous study (Gorbes, 2021) were administered. They were monitored within the first 4hrs for mortality and other signs like restlessness, paw licking, salivation, and stretching.

Experimental Designs

The rats were caged per group (I to IV, n=5) while group I served as the control. Group II was administered 10 mg/kg b.w of quinine, group III 20mg/kg b.w and group IV 30mg/kg b.w. Each rat was picked with a hand towel while 5mL syringe / needle gauge was used to inject the dosages on the left thigh to ensure adequate deliveries. They were weighed before and after experimentation and treated for twenty-eight (28) days, at 2 days interval. The animals were decapitated using cervical dislocation after experimentation. They were incised from the chest wall to the stomach while 4mL of whole blood was collected by retro-orbital puncture from the medial canthus into plain tubes and centrifuged (BROADBENT, UK) at 3000 rpm for 5 minutes to obtain the serum for liver function tests (ALT, AST, ALP and bilirubin). Liver were excised, washed in normal saline and cut in slabs of about 5mm thickness before fixing in 10% formalin. They were processed according to the paraffin wax method using an automatic tissue processor. Sections were cut using rotary microtome to obtain 3µ thickness and stained with H&E.

Photomicrography and Statistics

We examined each section with the Olympus Binocular Microscope® and photographed with a digital microscope camera (Samsung model SS850).

Results were expressed as mean±standard error of mean (SEM), and differences between values determined using ANOVA and Tukey's post hoc test, and $p<0.05$ considered significant.

RESULTS

Quinine prophylaxis with 30mg/kg b.w showed that AST (46.13±1.15U/L), ALT (24.01±0.03U/L) and total bilirubin (0.20±0.16U/L) levels were elevated ($p<0.05$) compared with rats on 10mg/kg [AST (41.50±0.07U/L), ALT (16.12±1.15U/L), total bilirubin (0.12±0.12U/L)] and 20mg/kg [AST (43.05±0.03), ALT (19.12±1.01), total bilirubin (0.15±0.13)] against untreated group 1: AST- 38.02±1.03U/L; ALT-14.05±0.12U/L; total bilirubin-0.10±0.06U/L. However, ALP levels reduced ($p<0.05$) compared with control group 1 (21.36±1.21U/L). High dose treated rats (30mg/kg b.w) revealed a gross reduction in ALP (17.13±0.07U/L) compared with the 20mg/kg (19.11±0.13U/L) and 10mg/kg (20.42±1.01U/L) treatments respectively. Histopathology revealed periportal inflammation and diffused vacuolation, focal area of lymphoid aggregation, nuclei hepatocytes appearing hyperchromatic, multi-focal and diffused necrosis including hepatic granuloma (Plate 1A-D).

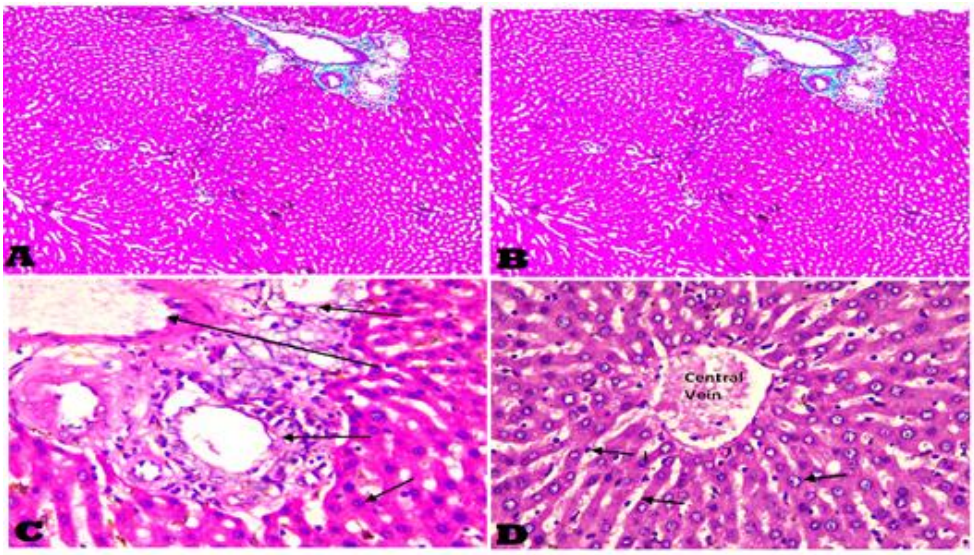

Plate 1: Showed control sections (A) and treated sections (B to D) with arrows pointed to pathological features earlier explained. H&E stains at x400magnification.

DISCUSSIONS

Since the advent of COVID-19 pandemic the world has been thrown into pandemonium on the way forward with new variant surfacing every other day. Health practitioners world-wide are increasingly getting overwhelmed while at a time there was a complete breakdown of major hospital work forces in most countries (Odigie *et al.*, 2020a). Despite the devastation globally, self-medication and personal intervention should never be encouraged because it often results to liver damages (Odigie *et al.*, 2020b). Recall that self-medication or personal intervention often leads to drug abuse and has been reported as one of the leading causes of liver damage globally (Atoigwe *et al.*, 2016). Also, the liver is prone to xenobiotics-induced injury because of its central role in xenobiotic metabolism and its portal location within the circulatory system (Adesanya *et al.*, 2012; Odigie and Odigie, 2015). Many drugs and chemicals often have adverse effects on the liver and lead to the distortion of liver architecture (Olurishe *et al.*, 2011).

Therefore, the liver must be protected being at the receiving end while trying to metabolize drugs in its abused state (Odigie and Obaseki, 2015).

Changes in liver architecture (histology) following prophylactic treatment of quinine and liver marker enzymes are indicative of hepatocellular damages and hepatotoxicity. This report is consistent with Ajiboso (2012) that reported the effects of chloroquine and sulfadoxine on biochemical and hematological indices in an animal experimentation, which led to the conclusion that chloroquine was more hepatotoxic than other antimalarial of sulfa derivatives. Our study thus corroborated the findings in which the toxicity observed in hepatic enzymes biochemistry exerted closely related effects as those reported by Ajiboso (2012). Our study is in agreement with Odigie (2013) that demonstrated prophylactic consumption of sulfa drugs on the liver of rats. Sulfa drugs are often sold across the counter as antimalarial but were reported to have hepatocellular effects in rats. Although this study centered on quinine as against the former that dwelled on pyrimethamine / sulfadoxine combined therapy. Elevated serum ALT, AST and total bilirubin indices indicated injuries in the hepatocyte owing to cell membrane alteration that often makes the enzymes to leak from tissues to serum. Umana *et al.,* (2013) suggested that ALT and AST are sensitive hepatocellular biomarkers that points to liver distress and sometimes evaluate the degree to which the liver is damaged while elevated AST parameter may be due in part to liver injury, cardiac infarction and occasionally muscular degeneration. Ajithkumar *et al.,* (2012) earlier informed that ALT is more specific to the liver and served as a profound parameter for detecting liver injury.

Elevated serum liver enzymes may also be a pointer to liver necrosis particularly in high dose (30mg/kg b.w.) quinine treated rats while serum ALP and bilirubin levels relate to hepatic cells functionality. Decreased serum ALP may be due to reduced bilirubin synthesis in the absence of biliary pressure but increased bilirubin observed in this study is largely from hepatic injury, which is evident via histopathology report. The exact mechanism by which quinine exerts its hepatotoxicity and hepatocellular effects has not been derived in this study. We suggest further research to unravel the why that has left a lacuna in the present research. However, Suh (2020); reported that drug induced liver injury occurs via multiple mechanisms involving various intracellular organelles with consequent disruption of intracellular calcium homeostasis, declined ATP levels and inflammation or bursting of the hepatocyte. High doses of prophylactic quinine treatment resulted to elevated activities of ALT and AST, and total bilirubin concentration. This is strongly related to the report by Sule *et al.,* (2012) stating that elevated liver enzymes may point to hepatic damages resulting from several mechanisms like toxic species induction or cellular membranes peroxidation. In this study, histology reports are in keeping with hepatocellular degeneration, which are not dissimilar to those revealed by Sule *et al.* (2012); Odigie (2013). Similar histological reports with evidences relating to cellular degenerations were observed by Buchweit *et al*. (2002) resulting from quinine exposure to rats. Authors however concluded that hepatocellular necrosis and other forms of hepatotoxic effects observed in their study were dose related (Buchweit *et al*. 2002), suggesting the reason for increased hepatocellular damages as dosage increases in this study. Recall that the more we increase the dosage, the more the damages caused by quinine treatment in rats.

CONCLUSION AND RECOMMENDATION

Prophylactic administration of quinine induces hepatotoxic effects in animal model in our study, leading to increased hepatic enzymes (AST, ALT and bilirubin), and hepatocellular damages.

This study agrees with existing reports on indiscriminate use of quinine. Therefore, quinine prophylaxis against SARS-CoV-2 should be discontinued. It should be administered following diagnosis while monitoring hepatic enzymes of patients.

Conflicting Interests

No conflicts of interest declared

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