Universidade Federal do Pará

Núcleo de Teoria e Pesquisa do Comportamento

Programa de Pós-Graduação em Neurociências e Comportamento

HIDROXICLOROQUINA EM DOSES ELEVADAS PROVOCA ALTERAÇÕES NAS OSCILAÇÕES CEREBRAIS DE BAIXA FREQUÊNCIA EM REGISTROS ELETROCORTICOGRÁFICOS DE FORMA NÃO CONCOMITANTE AS ALTERAÇÕES NA FUNÇÃO CARDÍACA, HEPÁTICA E RENAL EM RATOS WISTAR

CAMYLA EMANUELLE MELÉM DE SOUZA

Belém - PA

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Dedico esta dissertação a Deus, meu refúgioe fortaleza, socorro bem presente na tribulação; ao meu pai, Emanoel Jr., meu conselheiro e apoiador; e à minha avó, Lourdes Souza, minha maior incentivadora.

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Souza, C. E. M. de (2024). Hidroxicloroquina em doses elevadas provoca alterações nas oscilações cerebrais de baixa frequência em registros eletrocorticográficos de forma não

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Resumo

Introdução: A pandemia de COVID-19 levou ao uso da hidroxicloroquina como possível tratamento, apesar de resultados inconclusivos. A droga é conhecida por seu uso em outras condições e teve ampla divulgação na mídia. No entanto, estudos mostraram falta de eficácia e efeitos colaterais. **Objetivo:** Este estudo questiona se a hidroxicloroquina pode causar toxicidade dependendo da dose e duração do uso. **Métodos:** O estudo utilizou 54 ratos Wistar machos adultos, alojados em condições controladas. Foram administradas drogas, incluindo anestésicos e hidroxicloroquina. Os ratos foram submetidos à cirurgia para implantação de eletrodos cerebrais e cardíacos. Foram divididos em grupos com diferentes doses de hidroxicloroquina por vários períodos. Registros eletrocorticográficos e eletrocardiográficos foram obtidos, e amostras de sangue foram coletadas para análises bioquímicas. A análise de dados foi realizada usando software estatístico. Após os procedimentos, os animais foram sacrificados. **Resultados:** Alteração dos traços eletrocorticográficos no córtex motor dos ratos, com mudanças comportamentais e variações na potência espectral das ondas cerebrais e diminuição da atividade cardíaca e aumento dos níveis das enzimas hepáticas AST e ALT quando administradas altas doses de HCQ. **Conclusão:** Elevadas dosagens de HCQ alteraram significativamente os traçados eletrocorticográficos no córtex motor, aumentaram componentes bioquímicos e diminuíram a atividade cardíaca em ratos.

Palavras-chave: Hidroxicloroquina; COVID-19; Toxicidade; Estudo Experimental.

Souza, C. E. M. de (2024). High doses of hydroxychloroquine cause changes in low-frequency brain oscillations in electrocorticographic recordings, not concomitant with

changes in cardiac, hepatic, and renal function in Wistar rats. Master's Dissertation Proposal. Belém: Postgraduate Program in Neurosciences and Behavior, Federal University of Pará. 40 pages.

Abstract

Introduction: The COVID-19 pandemic led to the use of hydroxychloroquine as a possible treatment despite inconclusive results. The drug is known for its use in other conditions and received extensive media coverage. However, studies have shown a lack of efficacy and side effects. **Objective:** This study questions whether hydroxychloroquine can cause toxicity depending on the dose and duration of use. **Methods:** The study used 54 adult male Wistar rats housed under controlled conditions. Drugs, including anesthetics and hydroxychloroquine, were administered. The rats underwent surgery for the implantation of brain and cardiac electrodes. They were divided into groups with different doses of hydroxychloroquine over various periods. Electrocorticographic and electrocardiographic recordings were obtained, and blood samples were collected for biochemical analyses. Data analysis was performed using statistical software. After the procedures, the animals were sacrificed. **Results:** High doses of HCQ altered electrocorticographic traces in the motor cortex of the rats, with behavioral changes and variations in the spectral power of brain waves, decreased cardiac activity, and increased levels of liver enzymes AST and ALT. **Conclusion:** High doses of HCQ significantly altered electrocorticographic patterns in the motor cortex, increased biochemical components, and decreased cardiac activity in rats.

Keywords: Hydroxychloroquine; COVID-19; Toxicity; Experimental Study.

Sumário

Introdução

A pandemia desencadeada pela replicação desenfreada do vírus SARS-Cov-2, com disseminação inicial ocorrida em meados do ano de 2019 em Wuhan, na China e declaração de um surto mundial em março de 2021, ocasionou uma enorme sobrecarga nos sistemas de saúde da maioria dos países (HABAS et al., 2020; OCHANI et al., 2021; WHO, 2020).

A resposta global para contenção foi a utilização de medidas protetivas de rápida implementação, como o isolamento social, o uso de máscaras e higienização das mãos, com a finalidade de atenuar a sobrecarga nos sistemas de saúde. Foram buscadas também possíveis medidas terapêuticas e preventivas como o reaproveitamento e reposicionamento de vários medicamentos, dentre eles a hidroxicloroquina (HCQ), com o objetivo de reduzir a gravidade da doença que primariamente dissemina-se nas vias aéreas superiores, produzindo a síndrome respiratória aguda, afetando a função pulmonar, visto que desenvolver novos medicamentos, causaria uma demora extensiva, então, estes medicamentos foram incentivados para investigação (CORRÊA & BARROSO, 2020; REHMAN et al., 2021).

A hidroxicloroquina (HCQ) é um composto químico da classe dos agregados anfifílicos catiônicos pertencente à família dos 4-aminoquinolina, com fórmula conhecida por 2-[4-[(7-cloro-4-quinoil) amino] pentil] etilamino] etanol sulfato (1:1). Este medicamento que comumente é usado no tratamento da malária, devido ao seu mecanismo de ação atuar sobre algumas espécies de Plasmodium, assim também como em doenças autoimunes e em distúrbios reumáticos, por atuar em diversas vias celulares diminuindo, assim, o potencial inflamatório, através da redução das citocinas pró inflamatórias e do aumento dos receptores Toll-like, durante a pandemia de COVID-19, começou a ser utilizada para tratamento das pessoas infectadas pelo vírus, após a decorrência de estudo científico desenvolvido, um ensaio clínico não randomizado, que sugeria propriedades antivirais deste medicamento pela diminuição da colonização das vias aéreas e da carga viral dos pacientes

participantes desta pesquisa que foram acompanhados durante dez dias, recebendo 600mg por dia de HCQ, e tendo seu resultado potencializado quando em uso combinado com a azitromicina (ALFARO-MURILLO *et al*., 2020; DANZA *et al*., 2020; GAUTRET et al., 2020; NIRK *et al*., 2020; OLIVEIRA et al., 2022).

As discussões acerca dessa droga e de suas possíveis ações antivirais foram ampliadas durante as pesquisas contra o SARS-Cov-2, ao longo do ano de 2020, em meio à pandemia do Covid-19, sobretudo, por conta de previamente ter apresentado efetividade agindo em outras patologias virais, como: HIV-1, vírus da raiva, Polivírus e membros da família coronavírus. Igualmente, essa substância possui uma fácil disponibilidade e custos não elevados, fazendo com que se tornasse potencialmente interessante para pesquisas. (RIBEIRO et al., 2020)

Ainda que a amostra utilizada na pesquisa não tenha sido significativa e que não tenha tido grupo controle para acompanhamento em longo prazo, o que resultou em dados não claros e nem conclusivos, a propagação da mídia foi massiva ao apoiar o uso desse medicamento no tratamento da COVID, que por ser meio formador de opinião da massa, colaborou ao uso indiscriminado deste medicamento por muitas pessoas no tratamento e na prevenção da doença (CORRÊA & BARROSO, 2020; LUCCHETTA et al., 2023; PAUMGARTTEN *et al.,* 2020).

Contudo, diversos estudos demonstram que a sua administração contra o SARS-Cov-2 não possui eficácia, embora tenha sido largamente utilizado pela população de forma indevida. Com base nisso, as pesquisas acerca da hidroxicloroquina adquiriram um novo foco, buscando descobrir os possíveis efeitos nos sistemas orgânicos que essa droga pode ocasionar quando manuseada em doses elevadas. (FIGUEIREDO et al., 2022; MENEZES et al., 2020; PACHECO et al., 2020).

Mesmo hoje as questões que envolvem a HCQ fomentam debates e investigação extensiva, porém, já não com a mesma perspectiva inicial. Agora, tendo como referencial os estudos clínicos e revisões sistemáticas realizadas até o momento, os resultados demonstrados têm sugerido inconsistência quanto aos benefícios do uso deste medicamento para tratamento da COVID. Questionam-se também os possíveis efeitos adversos que a utilização deste medicamento poderia ocasionar, buscando descobrir os possíveis efeitos nos sistemas orgânicos que essa droga pode ocasionar quando manuseada em doses elevadas (CORTEGIANI, A. et al., 2020)

É sabido que os fármacos, em geral, podem exercer influência na atividade cerebral, podendo afetá-la de diversas maneiras, dependendo de sua classe farmacológica e do sistema neural que visam, podendo modificar os neurotransmissores, estabilizar ou mesmo suprimir a atividade cerebral, entre outras atribuições (MOURA & REYES, 2002).

Logo, essa temática acerca da hidroxicloroquina ainda carece de estudos científicos mais específicos, o que acaba tornando as evidências já existentes sobre este assunto pouco divulgadas, apesar de efeitos colaterais neurológicos, cardíacos, renais e hepáticos terem sido relatados por pacientes que fizeram o uso desse medicamento, em doses elevadas. (DIAZ-GAGO *et al*., 2020 ; MELO, et al., 2021)

Nesse contexto, o presente estudo questiona se o consumo de hidroxicloroquina poderia provocar toxicidade dependendo da dosagem e tempo de administração, em seus usuários.

2 OBJETIVO GERAL

Avaliar a toxicidade da hidroxicloroquina em ratos wistar.

2. 1 Objetivos específicos

- Avaliar a toxicidade da hidroxicloroquina em relação à função cerebral através da avaliação eletrocorticográfica;

- Estimar a toxicidade da hidroxicloroquina em relação à função cardíaca através da avaliação eletrocardiográfica;

- Observar a toxicidade da hidroxicloroquina em relação à função hepática por meio de parâmetros bioquímicos do sangue.

Artigo- Pharmacological Research: é uma revista científica focada na farmacologia e suas aplicações clínicas na medicina, revisada por pares, publicada mensalmente, que abrange todos os aspectos da farmacologia experimental. (ISSN: 1043-6618).

O artigo titulado: "**High-Dose Hydroxychloroquine Induces Changes in Low-Frequency Brain Oscillations in Electrocorticographic Records Not Concurrent with Alterations in Cardiac, Hepatic, and Renal Function in Wistar Rats**" está estruturado em formato de artigo científico obedecendo às regras de formatação e estruturação descritas pela Revista "Pharmacological Research", em conformidade com as instruções para preparação em manuscrito.

Artigo

High-Dose Hydroxychloroquine Induces Changes in Low-Frequency Brain Oscillations in Electrocorticographic Records Not Concurrent with Alterations in Cardiac, Hepatic, and Renal Function in Wistar Rats.

Camyla Emanuelle Melém de Souza [1,2], Daniella Bastos de Araújo[1*], Rafaela Negrão Olivia Santos[1], Laís Helena Baptista Amoras[1], Ana Luiza Cordeiro de Campos[1], Clarissa Araújo da Paz[1], Thaysa de Sousa Reis[1], Luciana Eiró-Quirino[1], Lara Gabriele Silva da Silva[1], Maria Helane Noronha[1], Maria Klara Otake Hamoy[1], Daniela Lopes Gomes[2] & Moisés Hamoy[1*].

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Abstract

A toxicity of hydroxychloroquine (HCQ) can affect the functioning of vital organs, in addition to causing ocular and cardiovascular damage. This study aims to assess the toxicity of hydroxychloroquine through electrocorticographic evaluation and blood biochemical parameters in Wistar rats. The animals were subjected to an HCQ dose of 350mg/kg via intraperitoneal (i.p) every 12 hours for periods of 24 hours, 48 hours, 72 hours, and 96 hours, with each group containing n=9. After the treatments, the animals underwent electrocorticogram in the motor cortex, electrocardiogram, and blood samples were subjected to biochemical tests for hepatic and renal function. At high doses, HCQ altered the electrocorticographic trace of the animals, decreased cardiac activity throughout the treatment, and significantly increased the values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Evaluation of renal function after administration of high doses of HCQ, as determined by serum creatinine levels, did not show significant changes. The results indicate that exposure to high doses of HCQ in rats can alter structures and functions of vital organs.

Keywords: Hydroxychloroquine, electrocorticographic, toxicity, biochemical, Electrocardiogram.

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the causative agent of COVID-19, was first reported in December 2019 in the city of Wuhan, China. It was only in March 2021 that it was declared a global outbreak, with confirmed death cases increasing significantly to 227,368 deaths by April 29, 2020, causing a tremendous burden on the healthcare systems of most countries (WHO, 2020; Habas et al., 2020; Corrêa & Barroso, 2020; Ochani et al., 2021).

With the urgency to alleviate the burden on healthcare systems, scientists gathered in the search for possible therapeutic and preventive measures that could be quickly implemented, such as social isolation, the use of masks, and hand hygiene. However, for the treatment of established infections, the repurposing and repositioning of various drugs, including hydroxychloroquine, were undertaken. These were encouraged for investigation since developing new medications would entail extensive delays (Maciorowski et al., 2020; Rehman et al., 2021; Sanghai et al., 2021; Choudhury et al., 2022).

Hydroxychloroquine (HCQ) is a chemical compound belonging to the class of cationic amphiphilic aggregates in the 4-aminoquinoline family, with a known formula of 2-[4-[(7-chloro-4-quinolinyl) amino] pentyl] ethylamino] ethanol sulfate (1:1). This drug has historically been used for the treatment of malaria, owing to its mechanism of action against certain Plasmodium species. Additionally, its effects may also be beneficial in the treatment of autoimmune and rheumatological diseases, such as Systemic Lupus Erythematosus, by acting on various cellular pathways, thereby reducing inflammatory potential through the suppression of pro-inflammatory cytokines and the increase in Toll-like receptors (Danza et al., 2016; Nirk et al., 2020; Alfaro-Murillo et al., 2020).

Discussions about this drug and its potential antiviral actions were expanded during research against SARS-CoV-2 throughout the year 2020, amid the COVID-19 pandemic. This was primarily due to its previously demonstrated effectiveness against other viral pathologies, such as HIV-1, rabies virus, Poliovirus, and members of the coronavirus family. It began to be used for the treatment of individuals infected with the virus following the findings of a scientific study suggesting the antiviral properties of this medication, including the reduction of airway colonization and viral load in patients receiving 600mg of HCQ per day. The results were further enhanced when used in combination with azithromycin. Moreover, this substance is readily available and costeffective, making it potentially interesting for research (Gautret et al., 2020; Uzunova et al., 2020).

However, several studies demonstrate that its administration against SARSCoV- 2 is not effective, although it has been widely used by the population improperly (Paumgartten et al., 2020). Based on this, research on hydroxychloroquine has acquired a new focus, aiming to discover the possible effects on organic systems that this drug may cause when handled in high doses (Menezes et al., 2020; Pacheco et al., 2020; Figueiredo et al., 2022).

Nevertheless, this topic still lacks more specific scientific studies, making the evidence on this matter less widely disseminated, despite neurological, cardiac, renal, and hepatic side effects having been reported by patients who used this medication in high doses (Diaz-Gago et al., 2020). Additionally, retinopathy, neuromyotoxicity, and cardiomyotoxicity have been reported when used in the long term (Richter et al., 2003; Siddiqui et al., 2007; Parmar et al., 2000; Yusuf et al., 2017; Iselin & Marti & Pless, 2016; Chatre et al., 2018).

In this context, the present study aims to evaluate the effects of high doses of hydroxychloroquine on the homeostasis of cerebral oscillations (ECoG), cardiac activity (ECG) and blood biochemical parameters in Wistar rats.

2. Materials and methods

2.1 Experimental Animals

54 adults male Wistar rats (200 ± 20g, aged 8-10 weeks) from the Central Animal Facility of the Federal University of Pará were used. The animals were housed in acrylic boxes (48cm x 38cm x 21cm) under controlled conditions (22 ± 2°C; 12/12-hour light/dark cycle) with ad libitum access to food and water.
All experimental procedures were conducted from 8:00 to 11:00 AM following the principles of laboratory animal care (National Research Council, 2011), and all necessary precautions were taken to prevent the animals' suffering and distress. All procedures were only carried out after the animals had been completely anesthetizedand after approval by the ethics committee not only of the institution where this stud was carried out but also of all the arrive checklists. Project approval number: CEUA nº 9484220321 (ID 001669).

2.2 Drugs used
For the execution of the work, the following chemical substances were used: Ketamine Hydrochloride (Köing Laboratory, Santana de Parnaíba, SP, Brazil); Xylazine Hydrochloride (Vallée Laboratory, Montes Claros, MG, Brazil); Lidocaine Hydrochloride (Hipolabor Laboratory, Sabará, MG, Brazil); Hydroxychloroquine (Plaquinol 400mg) from Sonafi-Synthelabo laboratory (São Paulo, SP, Brazil).

2.3 Experimental Design
Surgical implantation of electrodes was performed at stereotaxic coordinates of - 0.96 mm from Bregma, targeting the motor cortex region, following the technique employed by Hamoy et al. 2018. After the surgical procedure, the animals were divided into three groups: a) Control Group; b) Sham Procedure Group, receiving physiological saline in the same volume as the hydroxychloroquine (HCQ) group; c) Group receiving 350 mg/kg oral doses of HCQ every 12 hours for a period of 24 hours; d) Group receiving 350 mg/kg oral doses of HCQ every 12 hours for a period of 48 hours; e) Group receiving 350 mg/kg oral doses of HCQ every 12 hours for a period of 72 hours; f) Group receiving 350 mg/kg oral doses of HCQ every 12 hours for a period of 96 hours. Each group contains 9 individuals. After EEG recordings, electrodes were placed in the thoracic region for Electrocardiographic (ECG) recording, using lead D2. Following the recording period, blood samples were collected for biochemical analyses of liver function (AST, ALT) and assessment of renal function through creatinine levels.

2.4 Implantation of electrodes for electrocorticographic recordings
The ECoG (Electrocorticographic) recordings were obtained following the procedures described by Estumano et al. (2019). For this, the animals were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.), and after the abolition of the interdigital reflex, they were placed in a stereotaxic apparatus. The skull was exposed, and activated silver electrodes (tip exposure, 0.5 mm in diameter) were placed on the dura mater above the motor cortex at the coordinates of bregma – 0.96 with ± 1.0 mm lateral. On the third day after surgery, the HCQ treatment was initiated, with each animal receiving 350 mg/kg every 12 hours. Twelve hours after the last application, ECoG recordings were performed, which were recorded in a digital data acquisition system, and the traces were registered in mV (millivolts). Offline analysis was conducted as described by Hamoy et al. (2018).
The analyses were performed at a frequency of up to 30 Hz, divided into delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), and beta (12-28 Hz) bands for the interpretation of the dynamics of brain activity as described by Hamoy et al. (2018).

2.5 Amplication of electrodes for electrocorticographic recordings
The electrocardiographic activity was obtained in lead DII, with electrodes crafted in a non-conjugated manner. The reference electrode was positioned under the right armpit (0.5

cm), and the recording electrode was fixed in the tenth intercostal space, 3.5cm below the left armpit, following the recording vector. Each recording had a duration of 3 minutes, and the analyzed data included: Heart rate (BPM), Amplitude (mV), R-R interval (ms), P-Q interval (ms), QRS duration (ms), and QT interval (ms).

2.6 Blood's Biochemical Analysis

After the ECoG recordings, blood samples were collected for tests to assess the hepatic enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). These enzymes were measured by obtaining blood samples from the studied animals, which were analyzed at the Clinical Analysis Laboratory of the Institute of Biological Sciences, Federal University of Pará. The analysis was conducted using a biochemical analysis device for liver function from Wiener Lab GROUP, model CM 200. Blood samples were also used to assess serum creatinine levels (LABTEST).

2.7 Data analysis

The offline analysis was conducted using a tool created in the Python programming language (version 2.7). The "Numpy" and "Scipy" libraries were employed for mathematical processing, and the "Matplotlib" library was used for generating graphs and plots. A graphical interface was developed using the PyQt4 library. Spectrograms were calculated using the Hamming window with 256 points (256/1000 s). For Power Spectral Density (PSD), each frame was generated with na overlap of 128 points per window. For each frame, the PSD was computed using the Welch method for averaging periodograms. Frequency histograms were obtained by calculating the PSD of the signal using the Hamming window with 256 points without overlap, resulting in a resolution of 1Hz per bin. Each wave displayed in the PSD represents an average of a set of experiments. The PSDs were calculated for each group, and the means were displayed in individual boxes. Analyses were conducted in a frequency range up to 30 Hz, divided into frequency bands: Delta (1-4 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), and Beta (12-28 Hz), for the interpretation of cerebral dynamics during the development of intoxication.

2.8 Euthanasia of the animals

After the recording and blood collection period, the animals were euthanized using high doses of ketamine hydrochloride (300 mg/kg i.p.) and xylazine hydrochloride (30 mg/kg i.p.) intraperitoneally to prevent future issues, following institutional requirements for the euthanasia of these animals.

2.9 Statistical analysis

The normality and homogeneity of variances were assessed using the Kolmogorov-Smirnov and Levene tests, respectively. Since the residuals were normally distributed and the variances were equal, comparisons between the mean amplitudes of the traces and control values were conducted using ANOVA followed by Tukey's post hoc test. Mean values were presented with their respective standard deviation values (mean ± SD). The significance level was set at * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. GraphPad® Prism 8 software was used for statistical analyses.

3. Results

3.1 High doses of hydroxychloroquine altered the electrocorticographic trace in the motor cortex of the rats

Throughout the study, animals treated with hydroxychloroquine exhibited behavioral changes, decreased food intake, and softened feces compared to the control and SHAM groups, although they maintained the same water intake (data not shown).

The ECoG records for the control and sham groups showed amplitudes below 0.1 mV

(typically low amplitude, Figure 1 A and B), as demonstrated in the 1-second amplification (Figure 1 A and B, center) with a spectrogram showing the highest energy intensity below 10 Hz (Figure 1 A and B, right). The ECoG records for the hydroxychloroquine-treated group D1 and D2 had a higher distribution of power in frequencies above 10 Hz compared to the control group (Figure 1 C and D). In the group that received hydroxychloroquine D3 (72 hours), changes in the ECoG trace were observed, increasing power intensity in frequencies below 15 Hz with greater irregularity in the trace (Figure 1 E), as seen in the spectrogram (Figure 1 E). For the group that received treatment for 96 hours, the trace amplitude remained below 0.1 mV but exhibited changes in power intensity in frequencies below 20 Hz (Figure 1 F), as demonstrated in the right-sided spectrogram.

Insert Figure 1

Figure 1. Examples of electrocorticographic (ECoG) recordings lasting 3 minutes. ECoG trace for the control group (left), 1-second trace amplification (center), and energy distribution spectrum (right) (A); ECoG trace for the SHAM vehicle group (left), 1-second amplification of the recording (center), and corresponding spectrogram with power distribution in frequencies up to 40 Hz (right) (B); ECoG trace for group (D1) treated with 350mg/kg every 12 hours orally for a period of 24 hours (left), 1-second amplification of the recording (center), and spectrogram (right) (C); ECoG recording for group (D2) treated with 350mg/kg orally every 12 hours for 48 hours (left), 1-second amplification of the recording (center), and corresponding spectrogram (right) (D); ECoG trace for group (D3) treated with 350mg/kg every 12 hours for 72 hours (left), 1-second amplification (center), and spectrogram (right) (E); and ECoG recording for group (D4) treated with 350mg/kg orally every 12 hours for a period of 96 hours. The decomposition of the total spectral power distribution revealed changes in the power tracking of oscillations up to 30 Hz in animals treated with 350 mg/kg oral hydroxychloroquine every 12 hours for 24, 48, 72, and 96 hours (Figure 2 A).

Insert Figure 2

Figure 2. Linear power distribution chart among groups with frequencies up to 40 Hz (A); Average power distribution chart in delta frequencies (1-4 Hz) (B); Mean power distribution in theta frequencies (4-8 Hz) (C); Linear power distribution chart for alpha oscillations (8-12 Hz) (D); Linear power distribution in beta (12-28 Hz) (E). (*). (After ANOVA followed by Tukey, * $P<0.01$ ** $P<0.001$ ***$P<0.0001$, n=9). Significant variation between hydroxychloroquine treatments at D3 and D4 and the other groups was found in the analysis of linear frequency distribution up to 40 Hz ($F(5, 48) = 101.1$, $p <0.0001$). In this case, the hydroxychloroquine groups D3 showed an average power (0.5812 ± 0.07263 mV² / Hz x 10^{-3}), and D4 (0.8067 ± 0.07926 mV² / Hz x 10^{-3}) had higher total spectral power than control, Sham, D1, and D2 groups (control: 0.3533 ± 0.03060 mV² / Hz x 10^{-3}; SHAM: 0.3415 ± 0.04745 mV² / Hz x 10^{-3}; D1: 0.3452 ± 0.04784 mV² / Hz x 10^{-3}; D2: 0.3995 ± 0.04304 mV² / Hz x 10^{-3}; $p <0.0001$ for all comparisons; Figure 2A). The decomposition of brain waves was also analyzed for the distribution of power levels in delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), and beta (12-28 Hz). Significant variation was observed in delta wave frequencies ($F(5, 48) = 107.2$; $p < 0.0001$), with a significant increase observed for animals treated with hydroxychloroquine at D3 (0.2207 ± 0.03211 mV² / Hz x 10^{-3}) and D4 (0.3079 ± 0.05072 mV² / Hz x 10^{-3}), compared to control (0.09893 ± 0.01394 mV² / Hz x 10^{-3}, $p < 0.0001$), SHAM group (0.09325 ± 0.01562 mV² / Hz x 10^{-3}), D1 (0.08917 ± 0.009373 mV² / Hz x 10^{-3}), and D2 (0.09233 ± 0.01357 mV² / Hz x 10^{-3}); $p <0.001$ (Figure 2 B). For theta waves, differences were also observed between groups ($F(5, 48) = 11.49$; $p <0.0001$). Animals in groups D2 (0.1557 ± 0.01970 mV² / Hz x 10^{-3}), D3 (0.1584 ± 0.01725 mV² / Hz x 10^{-3}), and D4 (0.1643 ± 0.02160 mV² / Hz x 10^{-3}) showed statistically different mean

power in theta compared to the Control group (0.1185 ± 0.01640 mV^2 / Hz x 10^{-3}), SHAM (0.1248 ± 0.01856 mV^2 / Hz x 10^{-3}), and D1 (0.1200 ± 0.01910 mV^2 / Hz x 10^{-3}) (Fig. 2 C). Differences were observed in alpha waves between groups ($F(5, 48) = 27.03$; $p < 0.0001$). Animals in groups D2 (0.05778 ± 0.007628 mV^2 / Hz x 10^{-3}), D3 (0.06312 ± 0.006231 mV^2 / Hz x 10^{-3}), and D4 (0.06235 ± 0.005248 mV^2 / Hz x 10^{-3}) showed statistically different mean power in alpha compared to the control group (0.04108 ± 0.007640 mV^2 / Hz x 10^{-3}), SHAM (0.04120 ± 0.005691 mV^2 / Hz x 10^{-3}), and D1 (0.03969 ± 0.006478 mV^2 / Hz x 10^{-3}) (Fig. 2 D). For beta oscillations, the D4 group (0.08390 ± 0.009952 mV^2 / Hz x 10^{-3}) showed statistical difference ($F(5, 48) = 6.970$, $p < 0.001$) compared to the control group (0.06225 ± 0.009218 mV^2 / Hz x 10^{-3}), SHAM (0.06453 ± 0.01369 mV^2 / Hz x 10^{-3}), D1 (0.06683 ± 0.009085 mV^2 / Hz x 10^{-3}). The D3 group (0.06497 ± 0.007952 mV^2 / Hz x 10^{-3}) showed statistical difference ($P<0.01$) compared to the control group (Fig. 2 E).

3.2 Hydroxychloroquine decreased cardiac activity throughout the treatment

Figure 3A shows the cardiac activity of the control group, presenting the amplitude of the tracings and sinus rhythm (Figure 3A). When amplified, all cardiac deflections could be observed, with atrial activity represented by the P wave, ventricular activity represented by the QRS complex, and ventricular repolarization represented by the T wave (Figure 3A). Animals in the SHAM group exhibited similar characteristics to the control group, demonstrating cardiac activity in sinus rhythm with all cardiac deflections (Figure 3B). For the groups treated with hydroxychloroquine at D1 and D2, patterns remained similar to those found in the control and SHAM groups, with sinus rhythm (Figures 3 C and D). For groups D3 and D4, the rhythm remained sinus, but there was a decrease in heart rate, which can be observed in the amplifications of Figures 3 E and F.

Insert Figure 3

Figure 3 – Control electrocardiogram in lead D-II in the mouse lasting 3 minutes (left), Amplification of the record over a 2-second period in red traces represents the intervals to be analyzed: Amplitude (mV), R-R Interval (ms), P-Q Interval (ms), Q-T Interval (ms), Duration of the QRS complex (ms) (Right) (A). Electrocardiographic recording of the SHAM group lasting 3 minutes (left); 2-second amplification of the record demonstrating components related to cardiac deflections (Right) (B). Electrocardiogram in mice treated with hydroxychloroquine D1 lasting 3 minutes (left); Amplification of the record in 2 s (record period 49 to 51 s) showing the presence of P deflections, QRS complex, and T for the treated group (C). ECG recording represented in the trace lasting 3 minutes for the Treated D2 group (left) and amplification of 2 s (49 to 51s) demonstrating sinus rhythm after treatment (D); Electrocardiographic trace of animals undergoing treatment D3 (left) with amplification of 2 s (49 to 51s) (right) (E), ECG recording of the D4 group (left) and 2- second amplification (right) (F). The cardiac activity of the groups was analyzed from the electrocardiogram, where the heart rate for the control group had an average of 233.3 ± 17.32 bpm, showing no statistical difference from the SHAM group with an average of 232.9 ± 12.93 bpm. However, after the first day of hydroxychloroquine application (D1), the average was 216.4 ± 17.85 bpm, and in D2, the average was 207.8 ± 27.28 bpm, with no statistical difference from the control and SHAM groups. Group D3 (176.7 ± 11.18 bpm) showed statistical differences from the control, SHAM, and D1 groups ($P<0.0001$) and from the D2 group ($P<0.001$). Group D4 showed a marked decrease in heart rate (156.2 ± 9.922 bpm) compared to the control, SHAM, D1, and D2 groups ($P<0.0001$) (Fig. 4A).

Insert Figure 4

Figure 4. Mean heart rate (bpm) recorded in the control, SHAM, D1, D2, D3, and D4 groups (A); Evaluation of average amplitudes (mV) of electrocardiograms for the groups (B); Assessment of average R-R intervals (ms) for the groups (C); Mean P-Q intervals (ms) recorded in the groups (D); Evaluation of average QRS complex duration (ms) (E); Assessment of average Q-T intervals (ms) (F). [ANOVA and Tukey's test ($p < 0.0001$, $n = 9$)] * $P<0.01$, ** $P<0.001$, and ***$P<0.0001$. There was no variation in amplitude between groups; the control group had an average of 0.3294 ± 0.03291 mV, the SHAM group 0.3232 ± 0.02945 mV, the D1 group had an average of 0.3133 ± 0.01978 mV, D2 group (0.3133 ± 0.01920 mV), D3 (0.3137 ± 0.0160 mV), and D4 (0.3103 ± 0.01369 mV) (P=0.343) (Fig. 4B). The mean P-Q intervals did not show a difference during hydroxychloroquine use; for the control group, the mean was 55.90 ± 2.117 ms, SHAM group (58.09 ± 2.580 ms), D1 group (61.33 ± 5.596 ms), D2 group (60.61 ± 3.593 ms), D3 group (56.34 ± 5.782 ms), and D4 group (57.87 ± 7.372 ms) (P=0.1235) (Fig. 4C). The mean R-R intervals between the control group (257.9 ± 18.76 ms), SHAM group (258.0 ± 14.92 ms), and D1 group (278.8 ± 23.12 ms) showed no statistical difference (P=0.0512). Group D2 (292.9 ± 37.62 ms) showed statistical diferences from the control and SHAM groups ($P<0.01$). Group D3 (340.4 ± 22.47 ms) showedstatistical differences from the control, SHAM, and D1 groups ($P<0.0001$) and from the D2 group ($P<0.001$). Group D4 (385.2 ± 22.44 ms) showed differences from the control, SHAM, D1, and D2 groups ($P<0.0001$) and from the D3 group ($P<0.001$) (Fig. 4D). In the analysis of QRS duration, no differences were observed between groups. The mean values obtained for the control group were 28.67 ± 1.414 ms, for the SHAM group 27.67 ± 1.414 ms, the D1 group (28.19 ± 3.179 ms), D2 (28.87 ± 1.601 ms), D3 (30.04 ± 1.713 ms), and D4 (29.98 ± 2.178 ms) (P=0.0925) (Fig. 4E). For the Q-T interval, the control group (70.01 ± 2.785 ms), SHAM group (69.62 ± 3,009 ms), and Group D3 (74.41 ± 5.070 ms) showed no statistical difference (P=0.022). Group D2 (82.82 ± 4,372 ms) showed statistical differences from the D1 group ($P<0.01$). Group D3 (92.17 ± 8.246 ms) showed statistical differences from the control, SHAM, and D1 groups ($P<0.0001$) and from the D2 group ($P<0.01$). For group D4 (94.03 ± 10,39 ms), differences were observed from the control, SHAM, and D1 groups ($P<0.0001$) and from the D2 group ($P<0.001$) (Fig. 4F). Activity of the liver enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) indicates damage to hepatocyte membranes due to increased permeability after the use of high doses of hydroxychloroquine. For the evaluation of AST activity, the mean for the control group was 89.16 ± 4.80 mg/dL, for the SHAM group (88.12 ± 4.700 mg/dL), group D1 (96.00 ± 18.30 mg/dL), and D2 (102.9 ± 29.87 mg/dL) showed no statistical difference (P=0.283). Group D3 (122.4 ± 17.51 mg/dL) showed statistical differences from the control and SHAM groups ($P<0.001$), and Group D4 (147.7 ± 30.15 mg/dL) showed statistical differences from the control, SHAM, D1, and D2 groups ($P<0.0001$) (Fig. 5 A).

Insert Figure 5

Figure 5. Shows the mean AST (mg/dL) in the groups (A); Mean ALT (mg/dL) (B), and mean levels of creatinine (mg/dL) (C). [ANOVA and Tukey's test (**$P<0.001$, *** $P<0.0001$, $n = 9$)]. ALT activity for the control group had a mean of 55.14 ± 5.916 mg/dL, the SHAM group (54.82 ± 7.164 mg/dL), Group D1 (58.09 ± 7.70 mg/dL), and D2 (67.79 ± 13.50 mg/dL) showed no statistical difference (P=0.0152). Group D3 (75.38 ± 12.41 mg/dL) showed statistical differences from the control, SHAM, and D1 groups ($P<0.001$). For Group D4 (98.60 ± 18.84 mg/dL), statistical differences were observed from the Control, SHAM, D1, and D2 groups ($P<0.0001$) and D3 ($P<0.001$) (Fig. 5 B). For the evaluation of renal function after the administration of high doses of hydroxychloroquine, it was assessed by measuring serum creatinine, where the means for the control group were 0.6733 ± 0.08411 mg/dL, Group SHAM with an average of 0.6897 ± 0.08860 mg/dL, Group D1 (0.7544 ± 0.1305 mg/dL), Group D2 (0.8166 ± 0.1848 mg/dL), Group D3

(0.8212 ± 0.1881 mg/dL), and D4 (0.800 ± 0.2014 mg/dL) showed no statistical difference (P=0.1698) (Figure 5C).

4. Discussion

Recent efforts to repurpose HCQ for treating 2019 novel coronavirus disease (Covid-19) have raised concerns because this drug may prolong the QT segment on the electrocardiogram (Mazzanti et al., 2020; Ochani et al., 2021). The prolongation of the QT interval induced by medications is correlated with an elevated risk of a distinct type of ventricular arrhythmias known as Torsade de Pointes (TdP), which can result in cardiac arrest (Roden, 2004) The underlying mechanism of TdP is due to a delay in repolarization of the cardiac action potential at the end of its plateau phase, which prematurely reactivates the slow-inactivating CaV1.2 L-type calcium channels. The action potential is repolarized by outward potassium currents flowing through channels encoded by the human ether-a-go-go-related gene (hERG channels).

The inhibition of hERG function was the first molecular mechanism associated with TdP Rampe & Brown (2013) However, the drug's effects may also influence repolarization and induce arrhythmic events on voltage-gated K V 7.1 channels or inward rectifier K ir 2.1 channels. Furthermore, blocking Na+ and Ca2+ inward currents could counteract the QT prolonging effects of K+ outward current inhibition, a hypothesis underlying the Comprehensive in vitro Pro-Arrhythmia (CiPA) initiative aimed at refining the assessment of the electrocardiographic risk of new drugs. (Gintant et al., 2016).

Recent attempts to repurpose hydroxychloroquine (HCQ) for the treatment of Covid-19 have raised concerns due to its potential to prolong the QT segment on the electrocardiogram, which is associated with an increased risk of ventricular arrhythmias, including Tor. This risk is due to the drug's impact on cardiac action potential repolarization, primarily through the inhibition of hERG channels. Furthermore, the effects of HCQ on other potassium channels and the blockade of sodium and calcium inward currents could contribute to arrhythmic events. It is crucial to understand these mechanisms in order to evaluate the cardiac risks associated with HCQ and similar drugs. Initiatives like Comprehensive in vitro Pro-Arrhythmia (CiPA) are aimed at refining the assessment of such risks for new medications. (Ballet et al., 2022).

The present study demonstrated, for the first time, that high doses of HCQ induced changes in the electrocorticographic traces of low-frequency brain oscillations in the motor cortex of rats. These findings confirm what the drug label and studies already report, that HCQ can lower the seizure threshold, potentially increasing the risk of seizures, especially in epileptic patients (Jafri et al., 2017; Pati et al., 2020; Doyno et al., 2021; Solano et al., 2022).

The toxicity of hydroxychloroquine depends on the susceptibility of each patient, as these effects can be observed both at higher doses and at lower doses (Kushlaf 2011; Melles & Marmor, 2015; Browning, 2016; Basta et al., 2020; Emmanuel & Östlundh, 2020; Stokkermans et al., 2024).

In this study, it was proven that high doses (350 mg/kg PO) administered every 12 hours for 24, 48, 72 and 96 hours, where the toxicity of HCQ became more evident according to the podiatry and the time of contact with the damn it. For beta oscillations, associated with motor impairment and seizures, Group D4 showed statistically significant differences compared to most groups (control, SHAM and D1), and Group D3 showed significant differences only compared to the control group. These changes, indicating abnormal electrical activity, potential neuropharmacological effects and na influence on ECoG trace stability, emphasize the need for a controlled approach to the use of this substance. n this way, we observed that the effects of intoxication worsen, which corroborates some articles (Nicol et al., 2020; Gasmi et al., 2021; Bansal et al., 2021).

Another finding in our study was a significant decrease in cardiac activity during D3 (72h) and D4 (96h) treatments, with a decrease in the animals' heart rate, alterations in R-R and Q-T intervals indicating potential effects on the heart's electrical system.

These findings underscore the importance of careful evaluation of cardiac activity during HCQ treatment. The use of HCQ is associated with cardiac adverse reactions, which occur at therapeutic doses in the treatment of diseases such as lupus, more frequently in elderly or cardiac patients (Chatre et al., 2018). A specific case study associated with HCQ reports the development of heart failure in a patient diagnosed with SARS-CoV-2. This case highlights the need for a careful approach and continuous monitoring during treatment, especially considering the context of the COVID-19 pandemic (Diaz-Gago et al., 2020).

The mechanism of HCQ toxicity is not fully understood, although speculation suggests it may involve the accumulation of HCQ in cellular lysosomes, interfering with lysosomal digestion, leading to intracellular accumulation of glycogen and phospholipids in cardiomyocyte plasma membranes (Soong et al., 2007).

Compared to control and SHAM animals, high doses of HCQ significantly increased AST and ALT values. Elevated AST and ALT levels indicate hepatocyte membrane damage due to increased permeability after the use of high doses of hydroxychloroquine, providing additional information about the type of liver damage, emphasizing the complexity of this substance's interactions with different body systems (Komatsu et al., 2002; Ramesh et al., 2017).

Elevations in serum levels of AST and ALT are used as biomarkers for liver injury, detecting necrosis and hepatocyte damage (Singh & Sharma, 2011). Studies using HCQ for treatment have reported increased liver enzymes AST and ALT, supporting our results (El-Shishtawy et al., 2015; Srivats et al., 2016; Sayed & Soliman, 2021; Alruwaili et al., 2023).

The overall analysis of this information highlights the crucial importance of clinical monitoring during HCQ treatment, especially concerning the cardiac, cerebral, and hepatic systems. Early detection of changes in clinical parameters is essential for assessing treatment safety and making informed clinical decisions. This processincludes the possibility of adjusting or discontinuing medication, if necessary, to ensure the appropriate balance between benefits and potential risks.

In summary, the discussion on medications, exemplified by the HCQ case, underscores the ongoing need for research, monitoring, and careful evaluation to ensure that therapeutic benefits outweigh potential risks, ensuring safety and effectiveness in treating medical conditions.

High doses of HCQ significantly altered electrocorticographic patterns in the motor cortex, increased biochemical components significantly, and decreased cardiac activity in rats. The toxicity of HCQ is not fully elucidated; however, it may be associated with the formation of reactive oxygen species. Further studies are needed to better understand the potential risk of HCQ in humans.

Author contributions

CEMS, DLG and MH conceived and design the experiments. CEMS, RNOS, LHBA, ALCC, LGSS, MHN and MKOH performed the experiments. DBA, CAP, TSR and MH critically analyzed the data and adjusted, CEMS, DBA, RNOS, LHBA, LEQ and ALCC drafted the MS and all the authors critically revised and approved the final MS.

Competing interests

The authors declare no competing or financial interest.

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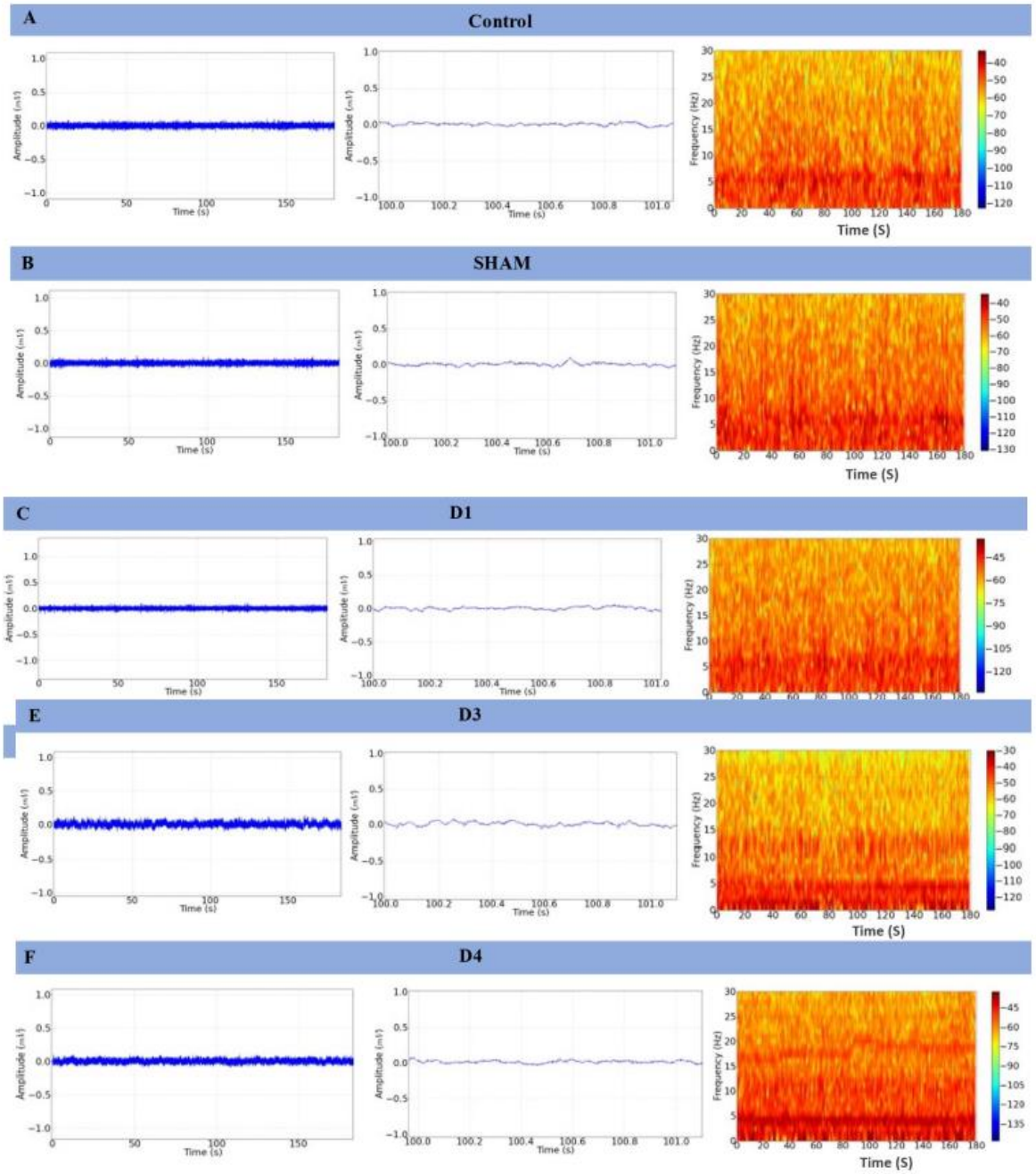


Figure 1. Examples of electrocorticographic (ECoG) recordings lasting 3 minutes. ECoG trace for the control group (left), 1-second trace amplification (center), and energy distribution spectrum (right) (A); ECoG trace for the SHAM vehicle group (left), 1-second amplification of the recording (center), and corresponding spectrogram with power distribution in frequencies up to 40 Hz (right) (B); ECoG trace for group (D1) treated with 350mg/kg every 12 hours orally for a period of 24 hours (left), 1-second amplification of the recording (center), and spectrogram (right) (C); ECoG recording for group (D2) treated with 350mg/kg orally every 12 hours for 48 hours (left), 1-second amplification of the recording (center), and corresponding spectrogram (right) (D); ECoG trace for group (D3) treated with 350mg/kg every 12 hours for 72 hours (left), 1-second amplification (center), and spectrogram (right) (E); and ECoG recording for group (D4) treated with 350mg/kg orally every 12 hours for a period of 96 hours.

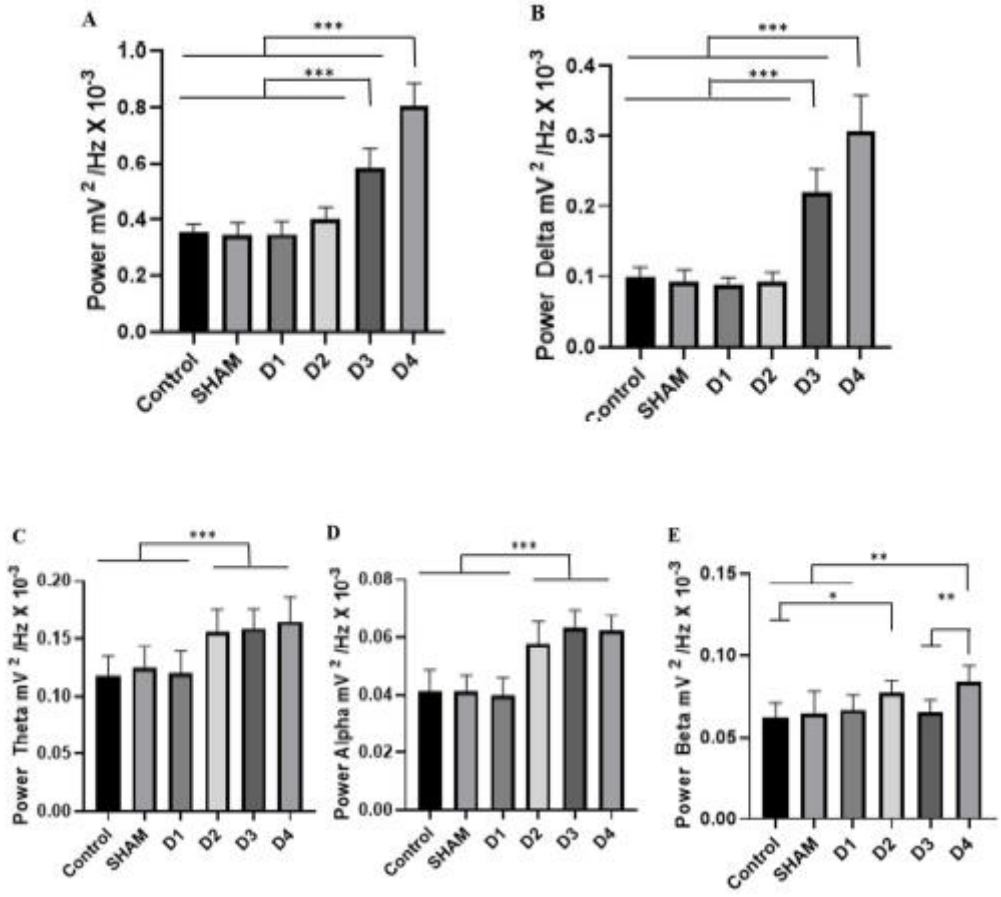
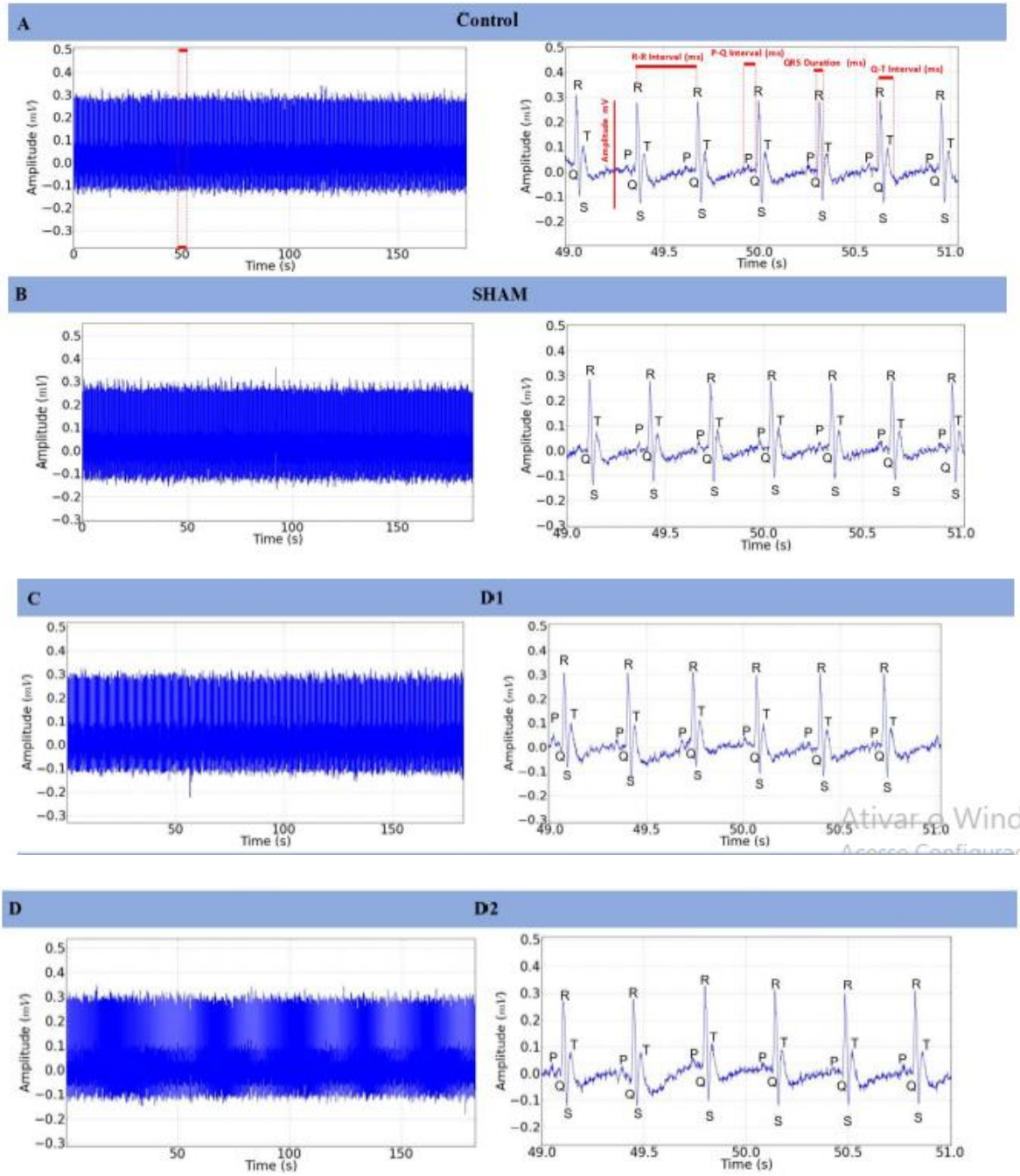


Figure 2. Linear power distribution chart among groups with frequencies up to 40 Hz (A); Average power distribution chart in delta frequencies (1-4 Hz) (B); Mean power distribution in theta frequencies (4-8 Hz) (C); Linear power distribution chart for alpha oscillations (8-12 Hz) (D); Linear power distribution in beta (12-28 Hz) (E). (*). (After ANOVA followed by Tukey, * P<0.01 ** P<0.001 ***P<0.0001, n=9).



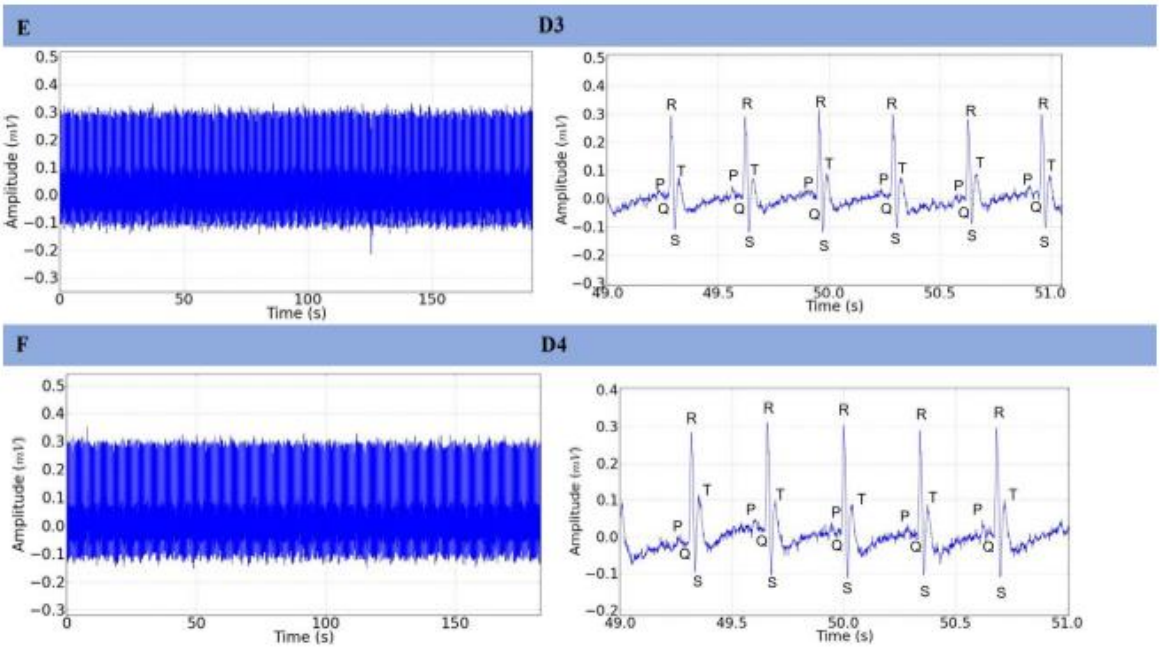
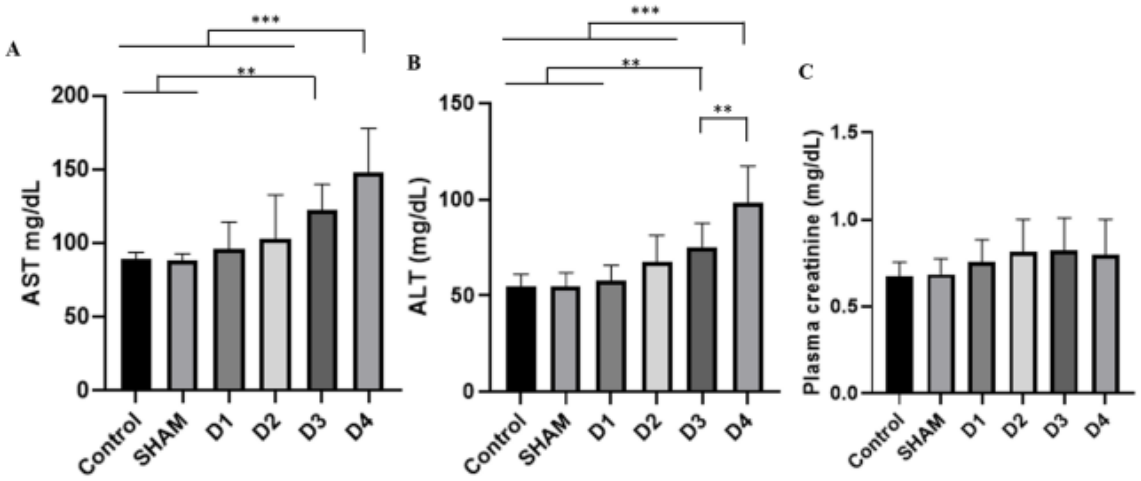


Figure 3 – Control electrocardiogram in lead D-II in the mouse lasting 3 minutes (left), Amplification of the record over a 2-second period in red traces represents the intervals to be analyzed: Amplitude (mV), R-R Interval (ms), P-Q Interval (ms), Q-T Interval (ms), Duration of the QRS complex (ms) (Right) (A). Electrocardiographic recording of the SHAM group lasting 3 minutes (left); 2-second amplification of the record demonstrating components related to cardiac deflections (Right) (B). Electrocardiogram in mice treated with hydroxychloroquine D1 lasting 3 minutes (left); Amplification of the record in 2 s (record period 49 to 51 s) showing the presence of P deflections, QRS complex, and T for the treated group (C). ECG recording represented in the trace lasting 3 minutes for the Treated D2 group (left) and amplification of 2 s (49 to 51s) demonstrating sinus rhythm after treatment (D); Electrocardiographic trace of animals undergoing treatment D3 (left) with amplification of 2 s (49 to 51s) (right) (E), ECG recording of the D4 group (left) and 2- second amplification (right) (F).



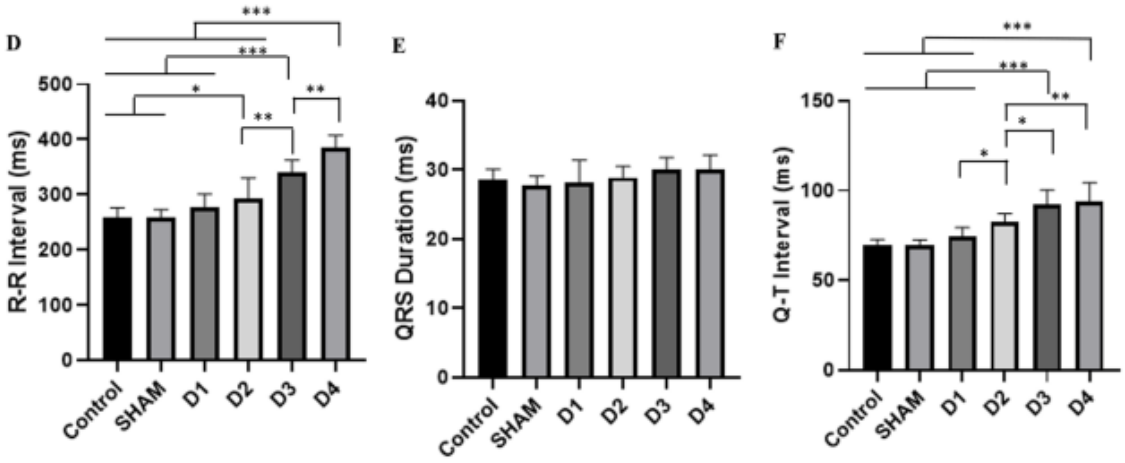


Figure 4. Mean heart rate (bpm) recorded in the control, SHAM, D1, D2, D3, and D4 groups (A); Evaluation of average amplitudes (mV) of electrocardiograms for the groups (B); Assessment of average R-R intervals (ms) for the groups (C); Mean P-Q intervals (ms) recorded in the groups (D); Evaluation of average QRS complex duration (ms) (E); Assessment of average Q-T intervals (ms) (F). [ANOVA and Tukey's test ($p < 0.0001$, $n = 9$)] * P<0.01, ** P<0.001, and ***P<0.0001.

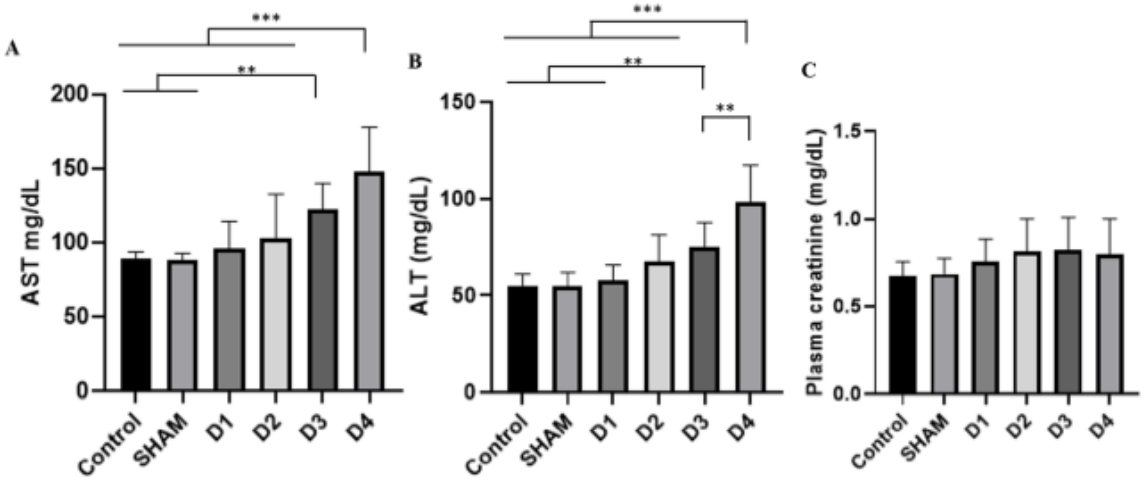


Figure 5. Shows the mean AST (mg/dL) in the groups (A); Mean ALT (mg/dL) (B), and mean levels of creatinine (mg/dL) (C). [ANOVA and Tukey's test (**P<0.001, *** P<0.0001, n = 9)].

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for *[Journal name]* and was not involved in the editorial review or the decision to publish this article.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CONSIDERAÇÕES FINAIS

Este estudo oferece uma contribuição importante ao conhecimento tido acerca da Hidroxicloriquina, pois demonstra alterações significativas nas oscilações cerebrais de baixa frequência no córtex motor de ratos, relacionadas à administração de medicamento em altas doses, e esses achados corroboram a informação de que a HCQ pode diminuir o limiar convulsivo e aumentar o risco de convulsões, especialmente em pacientes epilépticos, como descrit na bula deste medicamento.

Com o presente estudo, também foi possível estabelecer a dose pré-clínica de 350 mg/kg, que foi administrada em intervalos de 12 horas ao longo dos períodos de 24, 48, 72 e 96 horas. Os resultados mostraram que a toxicidade da HCQ aumenta com a duração do tratamento. Também se observou diferenças significativas nas oscilações em beta, associadas a distúrbios motores e convulsões nos grupos tratados, sugerindo atividade elétrica anormal e possíveis efeitos neurofarmacológicos.

Foi observada uma redução significativa na atividade cardíaca nos tratamentos de 72 e 96 horas, com mudanças nos intervalos R-R e Q-T, indicando possíveis efeitos no sistema elétrico do coração como achado relevante. A necessidade de monitorizar cuidadosamente a atividade cardíaca durante o tratamento com HCQ é reforçada por esses resultados, devido à sua associação com reações adversas cardíacas, especialmente em doses terapêuticas usadas para doenças como o lúpus.

O aumento significativo nos níveis de AST e ALT em resposta a altas doses de HCQ também aponta para danos hepáticos, destacando a complicação das interações da substância com diferentes sistemas corporais. Estes resultados estão de acordo com relatos anteriores sobre o aumento das enzimas hepáticas em tratamentos com HCQ.

É fundamental realizar uma monitorização clínica rigorosa ao longo do tratamento com HCQ para identificar precocemente quaisquer alterações nos parâmetros cardíacos,

cerebrais e hepáticos, possibilitando ajustes terapêuticos visando equilibrar benefícios e riscos. A importância contínua da pesquisa, vigilância e avaliação criteriosa para garantir a segurança e eficácia da HCQ no tratamento de condições médicas é enfatizada por este estudo. Resumindo, a conversa sobre a utilização de medicamentos como a HCQ enfatiza como é importante ter uma abordagem equilibrada e bem informada para maximizar os resultados do tratamento ao mesmo tempo em que se minimizam os riscos.

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