Journal of Research in Pharmacy

Research Article

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Long-term administration of high doses of Hydroxychloroquine poses a risk of seizures

Yousef PANAHI [1]*, Tannaz HAGHGOUIE [2]

[1,2] Division of Pharmacology and Toxicology, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

* Corresponding Author. E-mail: y.panahi@tabrizu.ac.ir (Y.P.); Tel. +98-4136378743

Received: 05 August 2022 / Accepted: 07 October 2022

ABSTRACT: The coronavirus spreading has led to the development of many drugs to prevent and treat it. One of these drugs is hydroxychloroquine, so this study was designed to investigate the potential side effects of the drug when used long-term and at high doses in male rats. Physiologically, 30 male Wistar rats were randomly divided into two groups: control and hydroxychloroquine (10, 50, 100, and 200 mg/kg) (n=6 per group). Hydroxychloroquine was administered orally for seven consecutive days. On the seventh day, 30 minutes after receiving hydroxychloroquine, the animals were anesthetized with a combination of ketamine (70 mg/kg) and xylazine (7 mg/kg), the animal's head was fixed using a stereotaxic device, and an incision 1.5-2 cm long was made in the scalp. Drill the desired point with a perforated dental drill using the Paxinos coordinates (AP = -0.27, ML = -0.14, DV = -0.3). Pentylenetetrazol (80 mg/kg i.p.) was used to induce epileptiform activities. Finally, diazepam (10 mg/kg, i.p.) was given to reduce epileptiform activity. The number of seizure activities was reduced significantly ($p<0.001$) at dose of 10 mg/kg and significantly ($p<0.001$) increased at doses of 100 and 200 mg/kg. In contrast, the dose of 50 mg/kg had no discernible effect. Giving male rats high doses of oral hydroxychloroquine for one week has biphasic effects on the number of pentylenetetrazol-induced seizure activities. As a result, it has protective effects in low and stimulant effects in high doses.

KEYWORDS: CA1, in vivo; epileptiform activity, rat.

1. INTRODUCTION

A significant number of patients have been hospitalized with signs and symptoms suggestive of Coronavirus disease (COVID-19), and some have been referred to an emergency intensive care management program, which is straining the healthcare system. The two antimalarial drugs, hydroxychloroquine (HCQ) and chloroquine (CQ), have garnered significant attention due to positive results from a small number of studies and press [1]. Nowadays, hydroxychloroquine is preferred over chloroquine due to its superior safety profile [2]. Hydroxychloroquine, a hydroxylated analogue of chloroquine, has antimalarial and anti-inflammatory properties [3]. Immediately following oral administration, cardiac and neurotoxic effects are evident due to rapid and almost complete absorption. It is widely distributed, metabolized in the liver, and excreted by the kidneys [4]. Sodium channels in the heart and brain are blocked by hydroxychloroquine, causing its toxicity. Hydroxychloroquine also binds to sodium channels in the brain, which results in epileptiform seizures. It also weakens skeletal muscles when taken in high doses [5]. Liposomal inflammation is treated with hydroxychloroquine. The availability of these medications to physicians, academic institutions, and medical centers during this time of crisis despite their lack of substantial evidence of efficacy is testament to the current crisis [1]. Patients with epilepsy should be told about the risk of seizures caused by chloroquine or hydroxychloroquine, according to the package insert for chloroquine and hydroxychloroquine. People with epilepsy have been understandably concerned about the safety of chloroquine and hydroxychloroquine since this statement was issued. Most centers are offering the medication in clinical trials or as an off-label therapy. An observational study conducted recently found that hydroxychloroquine was not effective at combating COVID-19 [6]. Chloroquine or hydroxychloroquine is not associated with seizures in sufficient numbers to support any statistical relationship. Case series and case reports are the only sources of data on this particular topic. The risk of seizures caused by these medications cannot be proved conclusively. The purpose of this

How to cite this article: Panahi Y, Haghgouie T. Long-term administration of high doses of Hydroxychloroquine poses a risk of seizures. J Res Pharm. 2023; 27(1): 414-419.

ISSN: 2630-6344

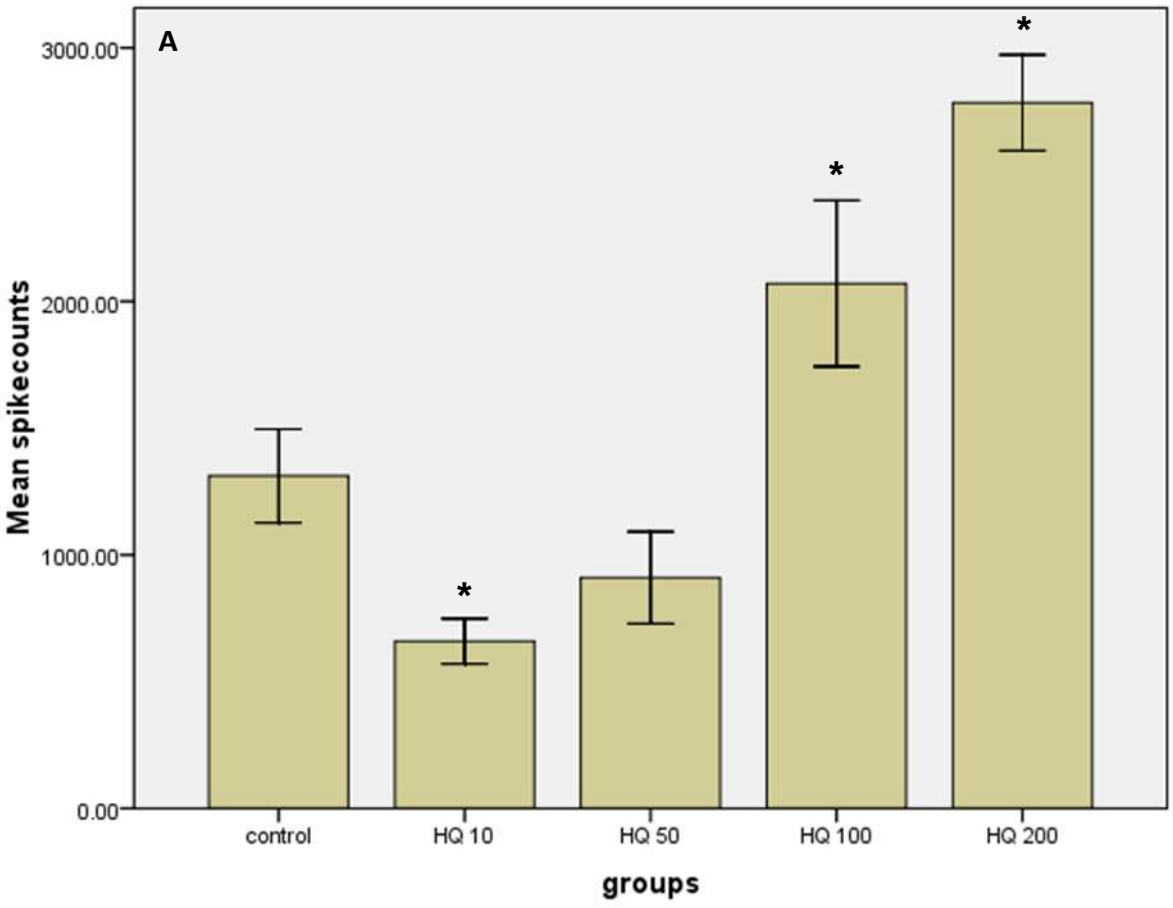
http://dx.doi.org/10.29228/jrp.323

Journal of Research in Pharmacy
Research Article

study is to determine whether long-term administration of high doses of hydrioxychloroquine will cause seizures in PTZ-induced model rats.

2. RESULTS

The findings of this study show that oral hydroxychloroquine has biphasic effects on the number of pentylenetetrazol-induced seizure activities in male rats. As a result, it has protective effects in low doses and stimulant effects in high doses. This means that at low doses, hydroxychloroquine reduces the number of seizure activities while increasing the number of seizure activities at high doses. That is, after using low doses of this drug, the number of spikes per unit time decreases, and after using high doses of this drug, the number of spikes increases. This study showed that hydroxychloroquine at a dose of 10 mg/kg significantly reduced the number of seizure events compared with the control group ($p<0.001$). In contrast, the number of these activities increased significantly ($p<0.001$) after oral administration of hydroxychloroquine at doses of 100 and 200 mg/kg compared to the control group. It should be noted that when compared with controls, 50 mg/kg of this drug has no significant effect on epileptic seizures activities ($p>0.05$).



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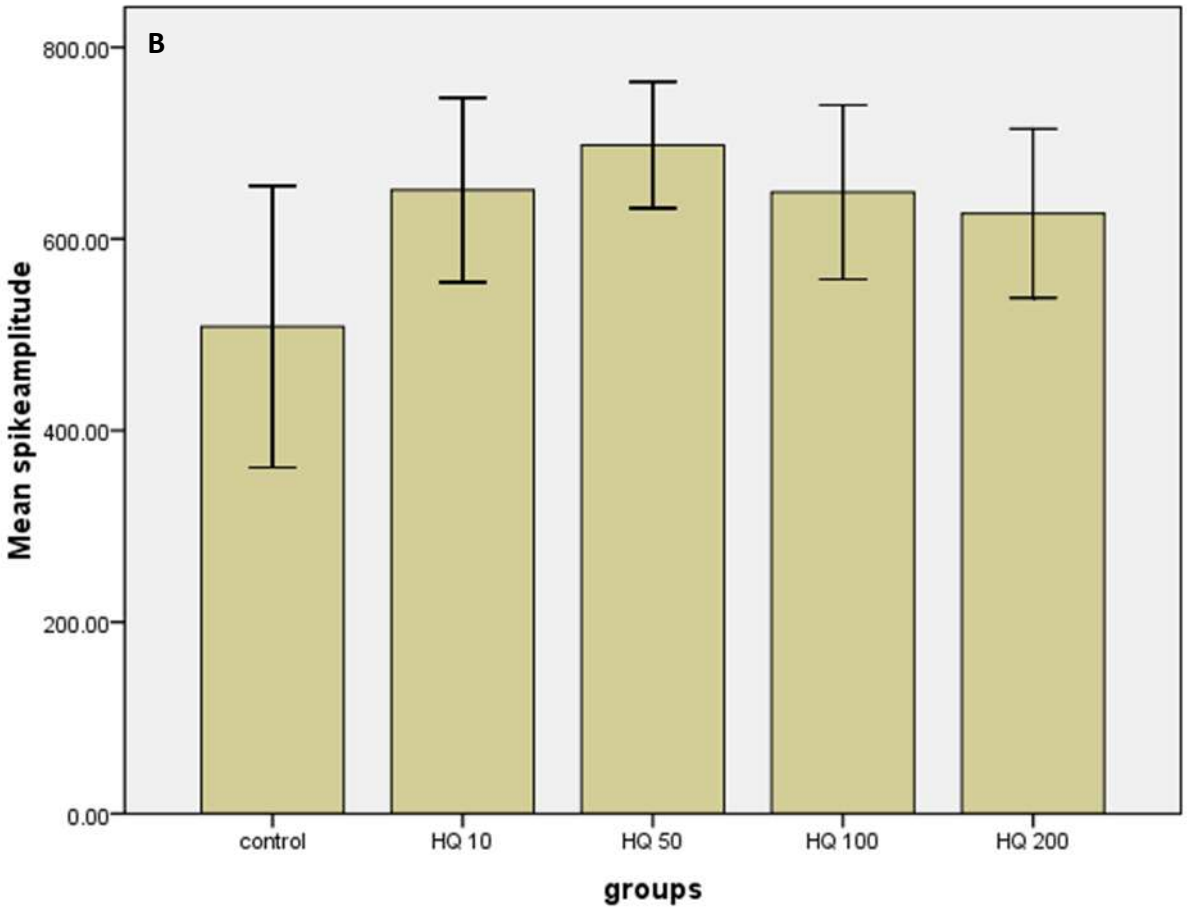

Figure 1: effects of hydroxychloroquine 10, 50, 100, and 200 mg/kg on PTZ-induced epileptiform activity spike counts and spike amplitudes in anesthetized male rats. Note- HQ10, Hydroxychloroquine 10 mg/kg; HQ50, Hydroxychloroquine 50 mg/kg; HQ100, Hydroxychloroquine 100 mg/kg; HQ200, Hydroxychloroquine 200 mg/kg. **A**: Applying hydroxychloroquine 10 mg/kg decreased spike count numbers and, Hydroxychloroquine 50, 100 and 200 mg/kg increased spike count numbers but, the increasing effect of hydroxychloroquine 50 mg/kg on the number of spike counts was not significant. **B**: Gavaging hydroxychloroquine 10, 50, 100 and 200 mg/kg had no effects on spike amplitudes. All the values are expressed as mean ± SEM (n = 6); means of various groups were statistically compared by one-way ANOVA followed by tukey's multiple comparison test using spss version 27. * Indicates significant differences ($p<0.001$) vs the control.

http://dx.doi.org/10.29228/jrp.323
J Res Pharm 2023; 27(1): 414-419

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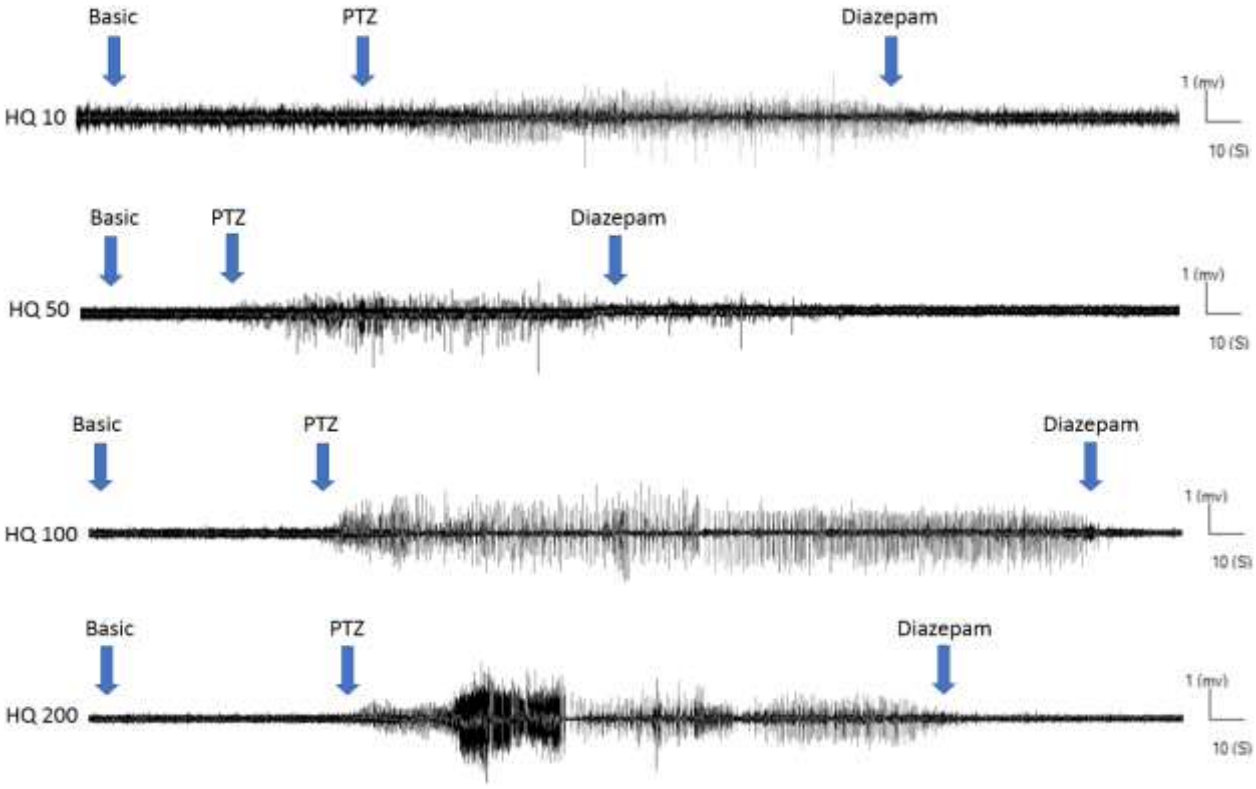

Figure 2: Graphic representation of the effects of hydroxychloroquine 10, 50, 100, and 200 mg/kg on PTZ-induced epileptiform activities recorded from the CA1 of the rat hippocampus. Basic: CA1 activities were recorded without the use of any drugs. PTZ: epileptiform activity was measured after an intraperitoneal injection of pentylenetetrazol (80 mg/kg). Diazepam: Epileptiform activity was suppressed by an intraperitoneal injection of diazepam (10 mg/kg). Note- HQ10, Hydroxychloroquine 10 mg/kg; HQ50, Hydroxychloroquine 50 mg/kg; HQ100, Hydroxychloroquine 100 mg/kg; HQ200, Hydroxychloroquine 200 mg/kg.

3. DISCUSSION

The findings of this study show that taking hydroxychloroquine orally for one week in various doses has a biphasic effect on pentylenetetrazol-induced epileptiform activity. As a result, it has protective effects in low doses and pro-convulsant effects in high doses on epileptiform activities. It means that at low doses, it reduced the number of PTZ-induced epileptiform activities, but at high doses, it increased the number of epileptiform activities. However, according to the findings of this study, the pro-convulsant effects of hydroxychloroquine in higher doses are dose-dependent, i.e., as the drug dose is increased, the resulting stimulatory effects become stronger. As a result, the increase in the number of spikes compared to the control group is not significant ($p>0.05$) after using the dose of 50 mg/kg, but after using the doses of 100 and 200 mg/kg, the difference in the number of spikes compared to the control group is significant ($p<0.001$). Because many people use hydroxychloquine for the prevention and even treatment of Covid 19 disease, it can be overdosed and cause a variety of side effects. In animal studies, hydroxychloroquine is approximately 40% less toxic than chloroquine when taken orally. It is a 4-aminoquinone that was initially synthesized by adding a hydroxyl group to chloroquine [5]. Due to its low toxicity and increased accessibility, hydroxychloroquine is the preferred medication for treating malaria and rheumatoid arthritis. Although their structures, therapeutic effects, pharmacokinetics, and toxicological differences continue, the two drugs share a wide range of similarities [8]. Therefore, both drug's clinical features of poisoning are nearly identical and the recommended treatment is nearly the same [9]. Fish and Espir [10] reported in 1988 that, despite the fact that 30 percent of Systemic Lupus Erythematosus (SLE) patients receive hydroxychloroquine, epilepsy or any other serious complaint has never been experienced by any of them, However, chloroquine has been linked to several neuropsychiatric events [11]. When taking antimalarial drugs prophylactically, one of four previously healthy

people experienced tonic-clonic seizures. One of these cases involves a patient who suffered from complex partial seizures for many years and only experienced tonic-clonic seizures while taking chloroquine [10]. Chloroquine-related side effects have been reported in 15 out of 100 patients with rheumatoid arthritis and SLE. They included nervous system side effects like headache, confusion, ototoxicity, and polyneuropathy but not epilepsy [12]. Despite the fact that the mechanisms of action of hydroxychloroquine and chloroquine are still being studied in modern molecular medicine [13]. Hydroxychloroquine and chloroquine are likely to have similar mechanisms of action and toxicities [14]. Consider our research, several studies have found that hydroxychloroquine has both pro- and anti-convulsant effects. Identifying predictors of seizure occurrence in newly diagnosed Lupus patients through a prospective observational study conducted at several centers, for example, discovered that using hydroxychloroquine resulted in delayed onset of seizures, implying that the medication can protect against seizure development. In a preclinical trial, a dose-dependent mechanism for causing seizures was observed with chloroquine by inhibiting GABAergic neurotransmission [15]. Preclinical studies have found a lower dose of chloroquine (1-5mg/kg) can prevent seizures, whereas a higher dose (10-50mg/kg) is convulsant [16]. Given the similarities in the pharmacokinetic properties of chloroquine and hydroxychloroquine, the GABAergic pathway may be involved in hydroxychloroquine's seizure effects [5]. In another case, following two weeks of therapeutic hydroxychloroquine 200 mg/day (5 mg/kg), a young woman with focal epilepsy and lupus had her first tonic-clonic seizure. She did not witness any further seizures during follow-up., despite the fact that focal seizures continued [17]. The drug binds to sodium channels in the brain, causing seizures, in high doses, and to skeletal muscle, affecting weakness in low doses, according to various reports. However, because diazepam reduces the seizure effects of hydroxychloroquine, it can be concluded that the GABAergic system is more involved in seizures than other systems [5]. However, other pathways and neurotransmitters may be involved in the neurotoxic effects of hydroxychloroquine, which needs to be investigated further.

4. CONCLUSION

Long-term administration of high doses of hydroxychloroquine has a biphasic effect on PTZ-induced seizure activity in rats, with low doses having a protective effect and high doses having a seizure aggravating effect. Therefore, hydroxychloroquine should be used with caution and under medical supervision in humans with underlying diseases such as epilepsy as well as in healthy individuals, and indiscriminate use should be avoided.

5. MATERIALS AND METHODS

In this study, 30 male Wistar rats weighing 200-230g were divided into two groups: control and hydroxychloroquine (doses of 10, 50, 100, and 200 mg/kg). In this experiment, a minimum number of animals was used. The mandates of the Helsinki Declaration have been adhered to in full. Hydroxychloroquine was given to the animals as an oral gavage for seven days in a row, while normal saline was used in the control group. On the seventh day, 30 minutes after receiving hydroxychloroquine, the animals were anesthetized with ketamine + xylazine (70 + 7 mg/kg), and after confirming the depth of anesthesia using pedal reflex, the animal's hair was shaved and their heads were fixed using a stereotaxic device. After cleaning the scalp with povidone iodine, an incision 1.5-2 cm long was made in the scalp to reveal the molded animal's skull. After cleaning the skin-to-skull connections and determining the Bergma point, use a perforated dental drill to achieve the CA1 region using the Paxinos coordinates (AP = -0.27, ML = -0.14, DV = -0.3). Basic activities were recorded from the CA1 area after a tungsten dipole electrode was placed for 10 minutes, and then intraperitoneal pentylenetetrazol (80 mg/kg) was used to induce epilepsy-like activities. Induced epileptiform-like activities were monitored for 10 minutes before being suppressed with diazepam (10 mg/kg) administered intraperitoneally [7]. All animal procedures in the present study conformed to mandates of the Helsinki Declaration. We tried to use the fewest number of animals possible in the experiment.

Acknowledgements: The authors acknowledge that this research has been done using the facilities of the Physiology and Pharmacology Laboratory of the Faculty of Veterinary Medicine of University of Tabriz, and here they thank Dr. Vafaei.

Author contributions: Concept – Y.P.; Design – Y.P., T.H.; Supervision – Y.P.; Resources – Y.P.; Materials – Y.P., T.H; Data Collection and/or Processing – Y.P.; Analysis and/or Interpretation – Y.P., N.; Literature Search – Y.P.; Writing – Y.P.; Critical Reviews – Y.P.

Conflict of interest statement: They guarantee that no more ethical conflicts will arise between the authors and experimental methodology as a result of conflicts of interest. Additionally, this project was not funded by any formal agency.

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