

Neuro-Histo-Morphological Effects of Hydroxychloroquine, Ivermectin and their Co-Administration on the Brain of Adult Wistar Rats

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ABSTRACT

Background: During the COVID-19 pandemic, hydroxychloroquine (HCQ) and ivermectin were widely used, sometimes in combination, despite limited regulatory approval. Concerns remain regarding their safety, particularly potential neurotoxic effects following prolonged or high-dose exposure.

Objective: This study evaluated the effects of hydroxychloroquine, ivermectin, and their co-administration on brain weight and histomorphology of Wistar rats.

Methods: Eighty-four adult Wistar rats were randomly assigned into seven groups ($n = 12/\text{group}$): control, hydroxychloroquine (two dose levels), ivermectin (two dose levels), and combined hydroxychloroquine–ivermectin (two dose levels). Drugs were administered orally for up to 28 days. Animals were sacrificed on treatment days 5, 14, and 28. Brain weights were recorded and histological assessment was performed using haematoxylin and eosin staining.

Results: No statistically significant differences were observed in brain weights across all treatment groups compared with controls. Histological examination revealed neuronal atrophy in rats administered low-dose hydroxychloroquine after 14 and 28 days. Acute neuronal necrosis and gliosis were observed in rats administered ivermectin at 0.2 mg/kg on day 5 only, with no observable lesions at later time points. Co-administration of hydroxychloroquine and ivermectin showed no observable histological alterations at any treatment duration.

Conclusion: Hydroxychloroquine administration beyond five days was associated with neuronal atrophy, while ivermectin within therapeutic doses showed minimal and transient histological effects. Co-administration of hydroxychloroquine and ivermectin was not associated with adverse brain histomorphology under the conditions of this study.

Keywords

Brain histology, Hydroxychloroquine, Ivermectin, Neurotoxicity, Wistar rats.

Introduction

The brain is a highly specialized organ responsible for sensory integration, cognition, motor coordination, and behavioural regulation, functions that are tightly dependent on the structural and functional integrity of neuronal and glial networks [1,2]. Preservation of brain integrity is essential for neurological health, and exposure to xenobiotics capable of crossing the blood–brain barrier (BBB) may compromise neuronal structure and function by disrupting cellular homeostasis and neural signaling pathways [3,4]. Experimental animal models, particularly Wistar rats, are widely employed for evaluating potential neurotoxic effects of pharmaceutical agents through histomorphological analysis [5,6].

Hydroxychloroquine (HCQ) and ivermectin are drugs with established clinical roles in the management of malaria, autoimmune disorders, and parasitic infections [7-9]. During the COVID-19 pandemic, both agents were widely used off-label, sometimes concurrently, following reports of potential antiviral activity from in vitro studies and observational clinical data [10,11]. This widespread use, including unsupervised self-medication, raised significant safety concerns, particularly regarding prolonged administration and high dosages [11,12]. Hydroxychloroquine is known to accumulate in lysosomes, alter intracellular pH, and modulate immune and inflammatory pathways [9,13].

Importantly, HCQ has been reported to cross the BBB and has been associated with adverse central nervous system (CNS) effects, including irritability, emotional disturbances, hallucinations, and psychosis [14,15]. Ivermectin, in contrast, is generally excluded from the CNS by P-glycoprotein efflux transporters at the BBB, although neurotoxicity has been reported at supratherapeutic doses or when BBB integrity is compromised [16,17].

Despite the extensive use of these agents during the pandemic, limited experimental data exist on their combined effects on brain morphology. This study therefore investigated the effects of HCQ, Ivermectin, and their co-administration on brain weight and histomorphology in Wistar rats.

Materials and Methods

Study Design and Experimental Animals

This experimental study used eighty-four adult Wistar rats (mean weight $\approx$ 230 g). Animals were housed under standard laboratory conditions with free access to feed and water and allowed to acclimatize for 14 days prior to treatment. Rats were randomly allocated into seven groups (n = 12 per group).

Drug Administration

Drug administration was done by direct feeding using a nasogastric tube. The rats in the treatment groups B and C were given

only HCQ, those in treatment groups D and E were given only Ivermectin while rats in treatment groups F and G were given a combination of HCQ and Ivermectin. The specific dosage of this drug administration is presented in Table 1. The day of treatment commencement (day 15 of rat arrival) was taken as treatment day 1 (TD₁).

Table 1: Dosage of HCQ and Ivermectin.

Group	Agent	Dosage	
		Day 1 and 2	Day 3 to 28
A (Control)	-----	-----	-----
B	HCQ	6.66mg/kg body weight bd	3.33mg/kg body weight bd
C	HCQ	13.33mg/kg body weight bd	6.66mg/kg body weight bd
D	Ivermectin	0.2mg/kg body weight od	
E	Ivermectin	0.4mg/kg body weight od	
F	HCQ + Ivermectin	6.66mg/kg body weight bd	3.33mg/kg body weight bd
		0.2mg/kg body weight od	
G	HCQ + Ivermectin	13.33mg/kg body weight bd	6.66mg/kg body weight bd
		0.4mg/kg body weight od	

Sample Collection

Animals were sacrificed in batches on treatment days 5, 14, and 28. Brains were carefully dissected, blotted dry, weighed using a digital balance, and immediately fixed in 10% buffered formalin.

Histological Analysis

Fixed brain tissues were processed using standard paraffin-embedding techniques. Sections of 4 μ m thickness were stained with haematoxylin and eosin (H&E) and examined under light microscopy. Representative photomicrographs were obtained at appropriate magnifications.

Ethical Considerations

Ethical approval for this study was obtained from the Ambrose Alli University Research Ethics Committee. All experimental procedures complied with institutional guidelines for the care and use of laboratory animals.

Results

This section presents the data collected from the brain of Wistar rats using descriptive statistics on tables (showing the Mean $\pm$ Standard deviation) and histology pictographs in a logical order.

Effect of Hydroxychloroquine on Brain Weight and Histology:

The effects of HCQ administration on the weight and histology of the brain of Wistar rats are shown in Table 2 and Figures 1, 2 and 3 respectively. No statistically significant differences were observed in brain weights of HCQ-treated rats compared with control animals across all treatment durations as shown on Table 2. Histological examination revealed normal brain architecture at day 5. However, neuronal atrophy was observed in rats administered the lower HCQ dose after 14 and 28 days, while no observable

lesions were noted in rats receiving the higher dose.

Table 2: Weight of wistar rats’ brain (g).

Treatment Group	Treatment Days		
	TD5	TD14	TD28
A (Control)	1.50 ± 0.20	1.60 ± 0.10	1.50± 0.17
B	1.23± 0.12	1.40 ± 0.30	1.50± 0.10
C	1.43 ± 0.29	1.47 ± 0.15	1.37 ± 0.29

Effect of Ivermectin on Brain Weight and Histology

The effects of Ivermectin administration on the weight and histology of the brain of Wistar rats are shown in Table 3 and Figures 4, 5 and 6 respectively. Brain weights of ivermectin-treated rats did not differ significantly from controls at any treatment duration as shown on Table 3. Histological assessment revealed acute neuronal necrosis and gliosis in rats administered 0.2 mg/kg ivermectin at day 5. No observable lesions were detected on days 14 and 28 in either ivermectin dose group.

Effect of Co-administration of Hydroxychloroquine and Ivermectin on Brain Weight and Histology

The effects of co-administration of HCQ and ivermectin administration on the weight and histology of the brain of Wistar

rats are shown in Table 4 and Figures 7, 8 and 9 respectively. Co-administration of HCQ and ivermectin did not result in statistically significant changes in brain weight compared with controls as shown in Table 4. Histological examination showed no observable lesions at any treatment duration as shown in Figures 7, 8 and 9.

Table 3: Weight of wistar rats’ brain (g).

Treatment Group	Treatment Days		
	TD5	TD14	TD28
A (Control)	1.50 ± 0.20	1.60 ± 0.10	1.50 ± 0.17
D	1.43 ± 0.32	1.30 ± 0.17	1.47 ± 0.21
E	1.50 ± 0.60	1.47 ± 0.23	1.43 ± 0.21

Table 4: Weight of wistar rat brain (g).

Treatment Group	Treatment Days		
	TD5	TD14	TD28
A (Control)	1.50 ± 0.20	1.60 ± 0.10	1.50 ± 0.17
F	1.33 ± 0.12	1.17± 0.23	1.57 ± 0.32
G	1.37±0.38	1.23± 0.23	1.20± 0.50

Discussion

This study evaluated the effects of HCQ, Ivermectin, and their combined administration on brain morphology in Wistar rats. In clinical practice, when medications are administered, there is

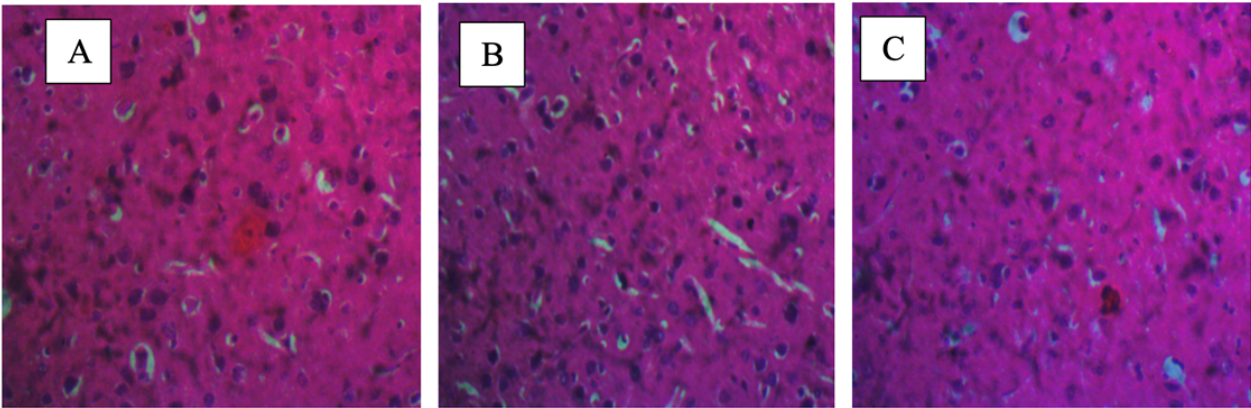


Figure 1: Photomicrographs of H & E-stained sections from the brain of Wistar rats administered HCQ for five days showing no observable lesion in groups A, B and C (HE x400).

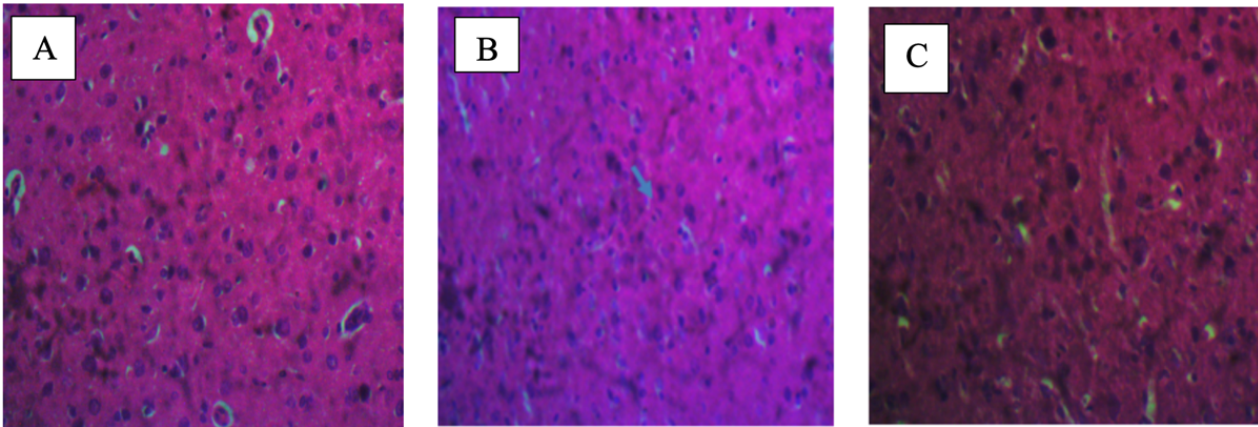


Figure 2: Photomicrographs of H & E-stained sections from the brain of Wistar rats administered HCQ for fourteen days showing no observable lesion in groups A and C but an atrophy of neurons (arrows) in group B (HE x400).

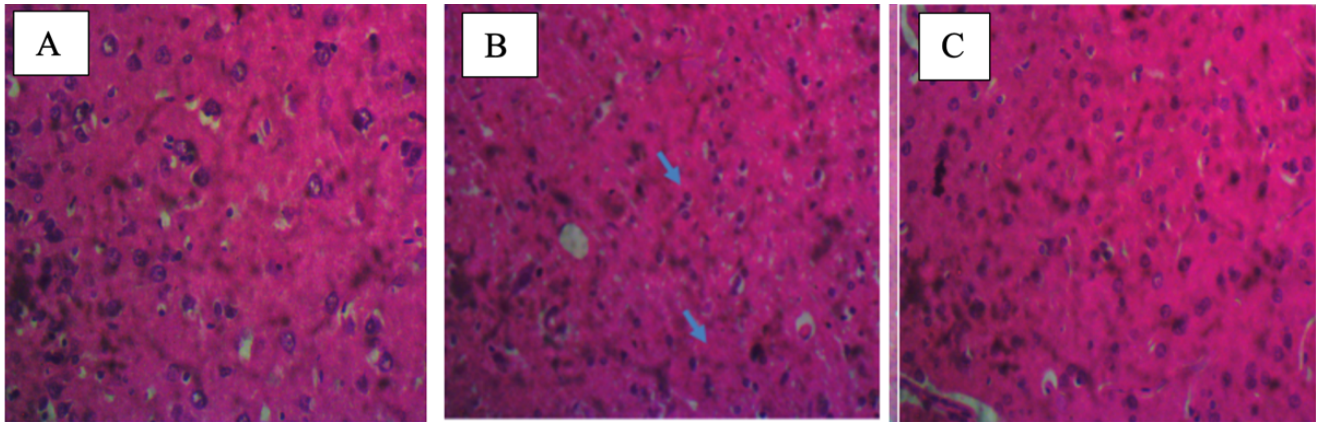


Figure 3: Photomicrographs of H & E-stained sections from the brain of Wistar rats administered HCQ for twenty-eight days showing no observable lesion in groups A and C but an atrophy of neurons (arrows) in group B (HE x400).

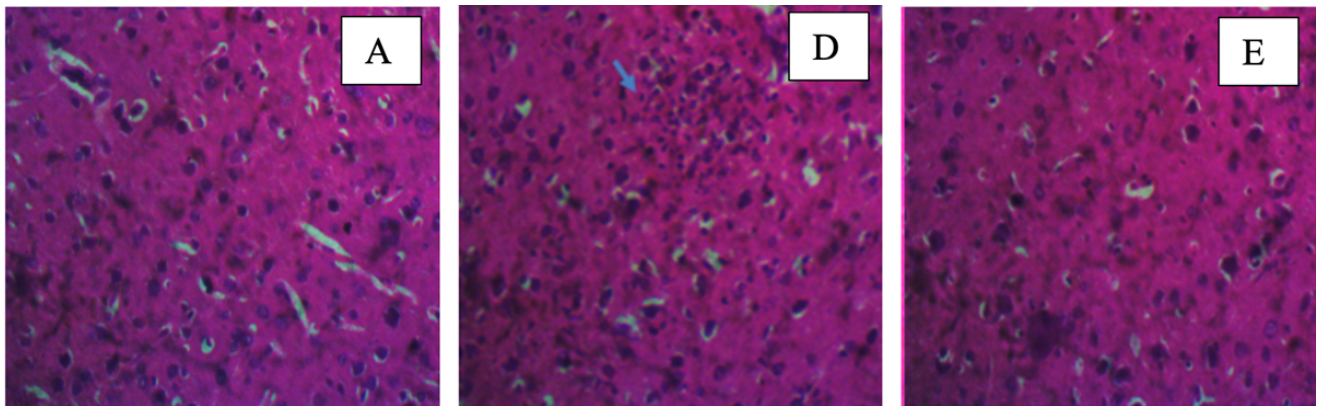


Figure 4: Photomicrographs of H & E-stained sections from the brain of Wistar rats administered Ivermectin for five days showing no observable lesion in groups A and E while an acute neuronal necrosis and gliosis (arrows) was observed in group D (HE x400).

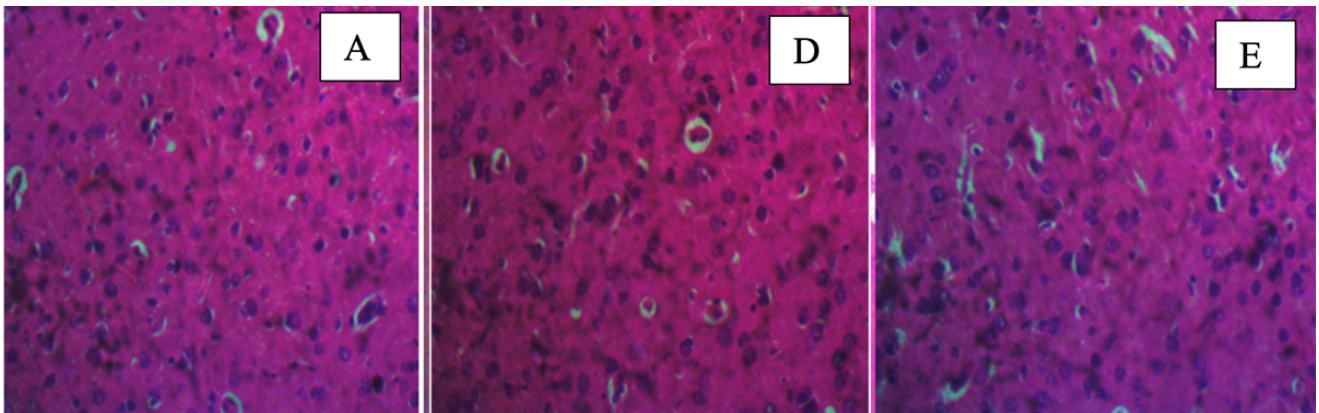


Figure 5: Photomicrographs of H & E-stained sections from the brain of Wistar rats administered Ivermectin for fourteen days showing no observable lesion in groups A, D and E (HE x400).

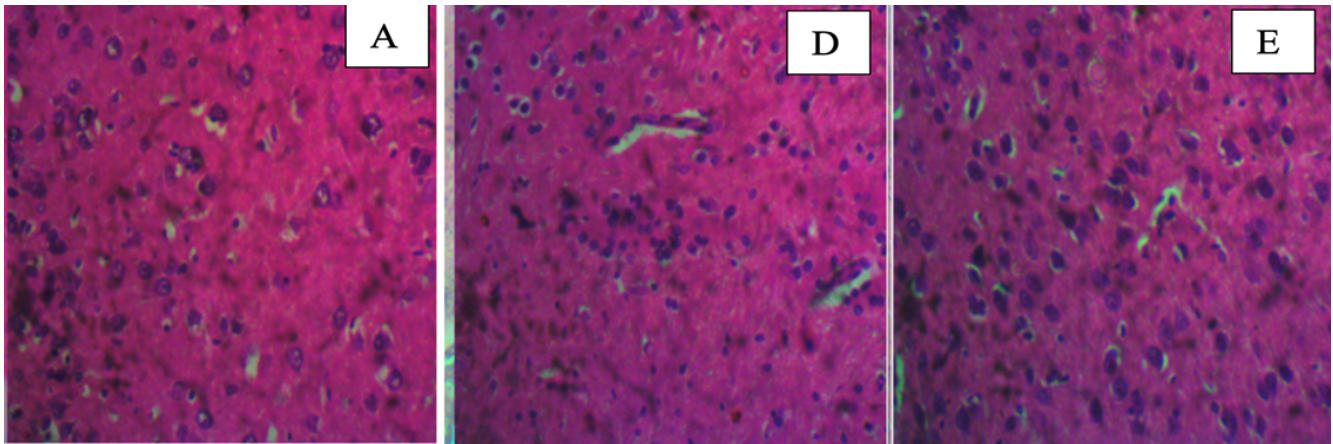


Figure 6: Photomicrographs of H & E-stained sections from the brain of Wistar rats administered Ivermectin for twenty-eight days showing no observable lesion in groups A, B and C (HE x400).

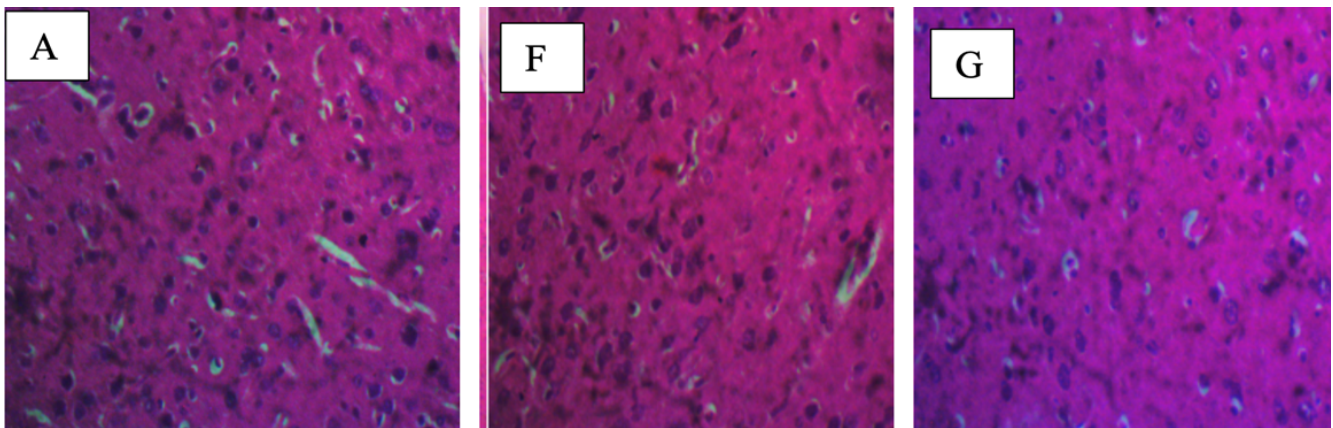


Figure 7: Photomicrographs of H & E-stained sections from the brain of Wistar rats co-administered HCQ and Ivermectin for five days showing no observable lesion in groups A, F and G (HE x400).

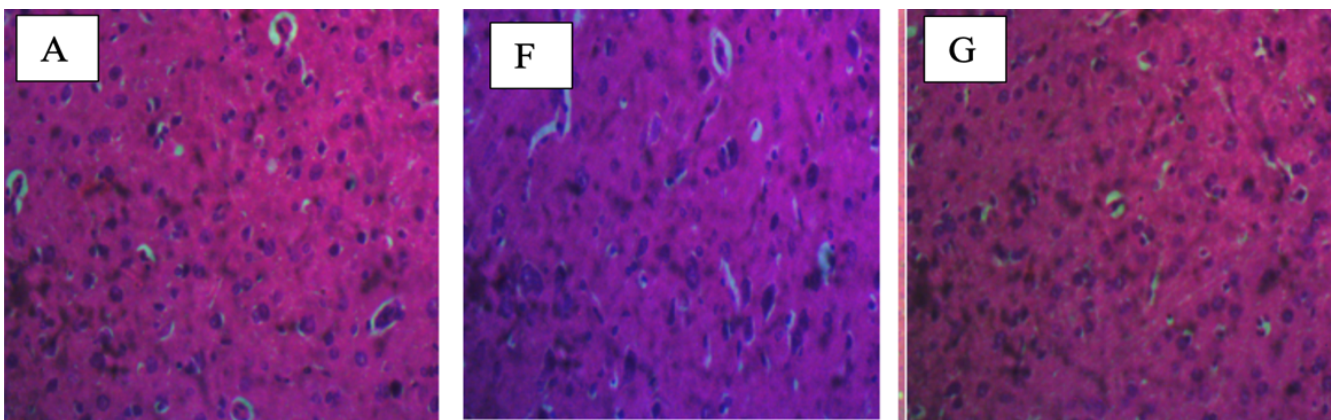


Figure 8: Photomicrographs of H & E-stained sections from the brain of Wistar rats co-administered HCQ and Ivermectin for fourteen days showing no observable lesion in groups A, F and G (HE x400).

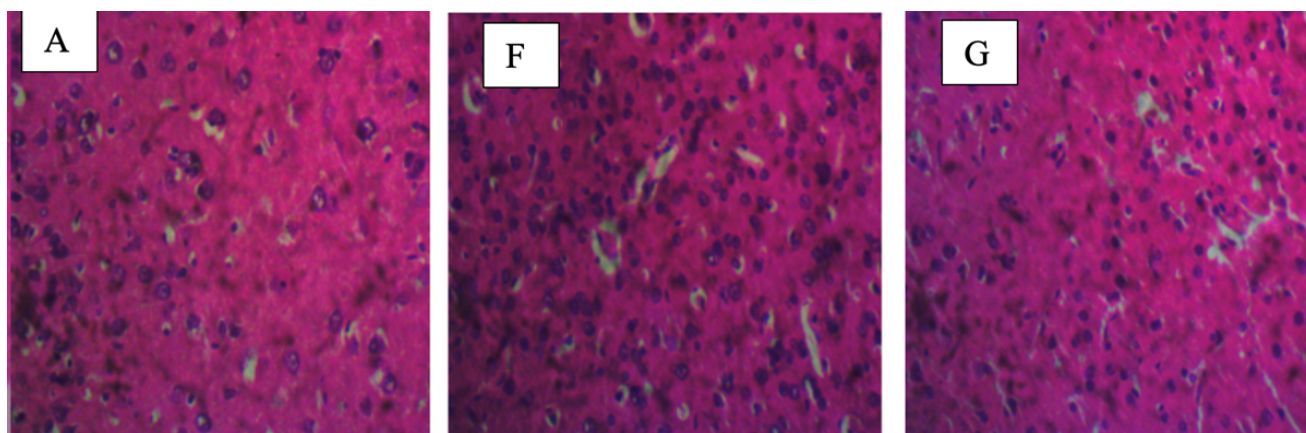


Figure 9: Photomicrographs of H & E-stained sections from the brain of Wistar rats co-administered HCQ and Ivermectin for twenty-eight days showing no observable lesion in groups A, F and G (HE x400).

always the consideration of their untoward effects [18,19]. More often than none, these considerations are related to the drugs' pharmacokinetics and pharmacodynamics, medication errors and in some low and middle income (LMICs), the concern of counterfeit medications [20]. Authors have advocated that a patient-centered approach will better guarantee medication safety [21]. Commonly, little attention is paid to the central nervous system effects except when the medication potentially becomes concerning for altered mental status as to cause falls in the elderly [22,23]. Meanwhile, there are more serious adverse effects of medications on the brain, some of which are not immediately evident, while some cause irreversible damage [24,25].

This study focused on the possible neurotoxic effect of coadministration of HCQ and ivermectin considering their widespread use during the COVID-19 outbreak in the management of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [26,27]. This is because adaptive cellular responses and variations in drug metabolism and transport alter pharmacokinetics and pharmacodynamics, both genetic and physiological factors directly influence how organisms respond to xenobiotics and experience adverse drug reactions [28,29].

In this study, HCQ administration over five days was associated with neuronal atrophy, suggesting a potential time-dependent neurotoxic effect [30]. The absence of lesions at the higher hydroxychloroquine dose may reflect a non-linear dose–response relationship, adaptive cellular responses, or inter-animal variability, phenomena that have been previously described for certain adverse drug reactions [31–33]. The observed HCQ-induced neuronal changes are consistent with reports indicating that HCQ can cross the blood brain barrier (BBB) and exert central nervous system (CNS) effects, possibly through lysosomal dysfunction, altered neurotransmitter balance, or immune-mediated mechanisms [14,15,34].

Ivermectin administration done within therapeutic dose ranges produced minimal neuro-histological alterations, with transient neuronal changes observed only at early exposure. These findings align with previous studies demonstrating limited CNS penetration

of ivermectin due to P-glycoprotein–mediated efflux at the BBB [17,35]. Neurotoxicity associated with Ivermectin has largely been reported in cases of overdose or impaired BBB function [16,36].

Interestingly, co-administration of hydroxychloroquine and ivermectin was not associated with observable histological abnormalities. This suggests that combined administration, under the conditions studied, did not exacerbate neurotoxicity. Potential explanations include altered BBB transport dynamics, modulation of drug distribution, or pharmacodynamic interactions, although these mechanisms remain speculative and require further investigation [3,26,37].

Conclusion

HCQ administration for periods exceeding five days was associated with neuronal atrophy in Wistar rats, whereas Ivermectin within therapeutic doses showed minimal and transient histological effects. Co-administration of HCQ and Ivermectin did not result in observable adverse brain histomorphology. These findings highlight the need for caution in prolonged HCQ use and underscore the importance of further studies on combined drug exposure and neurotoxicity.

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