RESEARCH ARTICLE

The superior efficacy of chloroquine over buparvaquone in reducing the chronic cerebral *Toxoplasma gondii* cysts load and improving the ultrastructural pathology in an immunocompromised murine model

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ABSTRACT

Toxoplasma gondii, the etiologic agent of toxoplasmosis, infects about 30 – 50% of the world population. The currently available anti-*Toxoplasma* agents have serious limitations. The present study aimed to investigate the effects of two antimalarials; buparvaquone (BPQ) and chloroquine (CQ), on immunocompromised mice with chronic cerebral toxoplasmosis, using spiramycin as a reference drug. The assessed parameters included the estimation of mortality rates (MR) among mice of the different study groups, in addition to the examination of the ultrastructural changes in the brain tissues by transmission electron microscopy. The results showed that only CQ treatment could decrease the MR significantly with zero deaths, while both spiramycin and BPQ caused an insignificant reduction of MR compared to the infected non-treated group. All the used drugs decreased the number of mature ruptured cysts significantly compared to the infected non-treated group, while only CQ increased the number of atrophic and necrotic cysts significantly. Furthermore, both spiramycin and BPQ improved the microvasculopathy and neurodegeneration accompanying the infection with different degrees of reactive astrocytosis and neuronal damage with the best results regarding the repair of the microvascular damage with less active glial cells, and normal neurons in the CQ-treated group. In conclusion, this study sheds light on CQ and its excellent impact on treating chronic cerebral toxoplasmosis in an immunocompromised mouse model.

Keywords: Cerebral toxoplasmosis; immunocompromised mice; antimalarials; buparvaquone; chloroquine.

INTRODUCTION

Toxoplasmosis is a widespread parasitic disease caused by *Toxoplasma gondii* (*T. gondii*) that inhabit the small intestine of felines which act as the definitive hosts. The infection is transmitted to almost all mammals and birds. Humans acquire the infection through the consumption of food and drinks contaminated with cat feces containing oocysts, or improperly cooked infected meat of intermediate hosts having tissue cysts, in addition to the transplacental transmission from mother to fetus. Besides the possible hazards of the congenital infection, toxoplasmosis is mostly asymptomatic in healthy individuals; it does not cause danger except to those with defective immunity. Hence, there is much less research interested in treating the *Toxoplasma* parasite compared to other parasitic diseases such as malaria (Secrieru *et al*., 2020).

In general, the critical factor in the pathogenesis of *T. gondii* is the ability of the immune system to convert the acute infection to an immunologically silent chronic one. This is achieved by transforming the rapidly multiplying tachyzoites to less permeable, slowly growing bradyzoites within true tissue cysts that may persist for life without causing any symptoms (Jeffers *et al*., 2018). The great problem lies in those with vulnerable immunity, where infection can continue in an active status, threatening the infected hosts with the possibility of fatal disseminated encephalomyelitis, given the neurotropic nature of the parasite (Aksoy *et al*., 2014; Schl ter & Barragan, 2019).

Chronic toxoplasmosis faces many difficulties regarding treatment, specifically in immunosuppressed subjects due to poor drug penetration into the brain (Chew *et al*., 2012b). Combination therapy has been used as sulfadiazine combined with pyrimethamine and atovaquone conjoined with clindamycin. Both combined medications were efficient to treat acute *T. gondii* infection and minimizing the severity of encephalitis relapses (Dunay *et al*., 2018). However, the prolonged usage of the former combination may lead to hematologic and renal toxicity (Crespo *et al*., 2000). While low

bioavailability of the latter combination, defective brain penetration, and emerging resistance stand as obstacles to its use (Hughes *et al*., 2011).

In general, currently available therapies are defective to eradicate *T. gondii* infection in vulnerable groups, exposing them to the risk of irreversible damage to vital organs, ending in the death of many affected cases. Repurposing the existing drugs as antimalarials in the treatment of *T. gondii* infection presents remarkable candidates. Antimalarial drugs are reported to provide more effectual therapeutic solutions for safer eradication and control of toxoplasmosis (Secrieru *et al*., 2020).

Chloroquine (CQ), a quinoline derivative drug that is commonly used in the treatment of malaria and other parasitic infections, displays direct and indirect anti-*Toxoplasma* effects. CQ can affect *T. gondii* directly through diminishing apicoplast and inhibiting the endocytic pathways via increasing the endosomal pH required for cell fusion, and indirectly through pro-apoptotic and anti-inflammatory actions along with the immunomodulation exerted by induction of IFN-γ, the key factor in combating toxoplasmosis (Kadri *et al*., 2014; Fantini *et al*., 2020; Gamea *et al*., 2022).

Buparvaquone (BPQ), a hydroxynaphthoquinone originally developed as an antimalarial compound and is now commercially available for use against theileriosis in cattle. It is also active against *Babesia, Eimeria, Leishmania donovani, and Pneumocystis carinii*. Buparvaquone inhibits the ubiquinone reductase cytochrome bc and blocks the parasite respiratory chain. Buparvaquone exerts excellent activity against transplacental transmission of toxoplasmosis in pregnant mice (Müller *et al*., 2016; Müller *et al*., 2017).

In the present study, we investigated the anti-*Toxoplasma* effect of two different antimalarials; BPQ and CQ on the ultrastructure of the brain cortical tissues of immunocompromised mice with chronic cerebral toxoplasmosis.

MATERIALS AND METHODS

Animals and Ethical aspects
A total of 36 female Swiss albino CD1 mice 6-7 weeks old weighing 25-30 g were obtained from Theodor Bilharz Research Institute. Mice were housed in a constant temperature (22–24°C) and light-controlled room on an alternating 12:12 h light-dark cycle with free access to food and water. The experiment was conducted following the approval of the Animal Care and Use Committee of Theodor Bilharz Research Institute, Giza, Egypt (PT: 694/2022).

Dexamethasone-induced immune suppression
Induction of immune suppression in all mice was performed with oral administration of dexamethasone (DEX) (Dexazone, Al Kahira pharmaceutical & chemical industries company, Egypt). Mice were gavaged once daily with 0.25mg/g/day for 14 successive days before inoculation of *T. gondii* cysts and continued to receive DEX at the same dose throughout the experiment (Rehg *et al*., 1988).

Experimental infection
Mice were orally infected by gavage with 100 ME49 cysts in 0.2 ml of phosphate-buffered saline (pH 7.2) (PBS). Cysts of the ME49 strain of *T. gondii* were obtained from the brains of chronically infected mice after euthanasia. Their brains were removed and triturated in PBS with a mortar and pestle. An aliquot of 50 μl of the brain suspension was examined for the number of cysts, and the dose of infection was adjusted after appropriate dilution in PBS that each mouse was infected with 100 *T. gondii* cysts (El Fadaly *et al*., 2015).

Experimental design and drug administration

Study groups
After 14 days of DEX administration, the mice were randomly divided into 4 groups.

Group 1 (n=12): infected non-treated (control group); Group 2 (n=8): infected and treated with spiramycin; Group 3 (n=8): infected and treated with BPQ; Group 4 (n=8): infected and treated with CQ.

Administrated drug
Spiramycin® (Pharaonia Pharmaceuticals, Egypt), mice received 200 mg/kg/day once daily for ten successive days (Al-Zanbagi, 2007; Etewa *et al*., 2018); Buparvaquone (Sigma-Aldrich, Inc.), mice received 50 mg/kg/day once daily for five successive days (Müller *et al*., 2017); Chloroquine (Alexoquine®) (Alexandria Co. for Pharmaceuticals & Chemical industries, Egypt), mice received 20 mg/kg/day once daily for four successive days (Ishih *et al*., 2006).

The treatment regimens started on day 45 post-inoculation of infection. Spiramycin and CQ tablets were crushed into a powder form, weighed and their active ingredients were calculated per mouse per dose, then suspended in 200 μl of PBS and orally administered by gavage. While BPQ powder was emulsified in corn oil and heated to 37°C to enhance the solubility of the drug before oral gavage.

Mortality rate assessment (MR) and euthanasia of mice
The mortality rate of mice in each group in the study was determined according to the formula (FarahatAllam *et al*., 2020):

$$MR\% = \frac{\textit{Number of dead mice at the end of the experiment}}{\textit{Number of mice at the beginning of the study}} \times 100$$

The surviving mice in each group at the end of the study (70 days) were euthanized by intraperitoneal injection of anesthetic–anticoagulant solution (500 mg/kg thiopental and 100 units/ml heparin).

Preparation of brain tissues for transmission electron microscopy (TEM)
For TEM analysis, sections from the cerebral cortices of mice from all study groups were removed and embedded in a mixture of 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) as a fixative. Specimens were then washed, post-fixed, dehydrated, and embedded in epoxy resin. Sections were selected (2 samples/each mouse) and cut into semithin (1.5 μm) and then into ultrathin sections (60–80 nm) using an Ultracut UC7 ultramicrotome (Leica) following standard procedures. The ultrathin sections were collected on Formvar-coated copper grids. Sections were then double stained in uranyl acetate followed by lead citrate. Finally, the stained sections were analyzed by a transmission electron microscope equipped with a digital camera (JEOL-Ex; Japan 1010 transmission electron microscope at 70 kV at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University (Ulloa-Navas *et al*., 2021; Yosri *et al*., 2022).

Ultrastructural estimation of *T. gondii* cortical brain cysts burden
Semiquantitative scoring of *Toxoplasma gondii* cysts was performed in 5 fields (2500') magnification of electron microscopic cerebral cortical Sections. The score was represented as arbitrary units: 0–2, mild; 2–4, moderate; 4–6, severe; and above 6, very severe according to Atmaca *et al*. (2019) with modification.

Statistical analysis of data
Statistical analysis was performed by using one-way analysis of variance (ANOVA), the chi-square test, and post hoc tests for multiple comparative analyses between the study groups. *P*-values <0.05 were considered statistically significant, and <0.001 were considered highly significant.

RESULTS

Mortality rate

By the end of the experiment on the 70th day post-inoculation, the MR of the infected non-treated group was 41.6% which decreased significantly among the group of mice infected and treated with CQ. However, there was no significant difference between the other treated groups and the infected non-treated group (Table 1).

Number of cortical brain *T. gondii* cysts

Three different forms of parasitic cystic lesions were identified and counted; mature cysts, atrophic cysts, and necrotic cysts (Figure 1). The mature *T. gondii* cysts appeared as tachyzoite escaping cystic lesions with the irregular wall enclosing bradyzoites (Figures 1A and B), while the atrophic cysts were smaller in size with fewer bradyzoites without surrounding tachyzoites (Figure 1C). The necrotic cysts had disturbed morphology with large areas of debris and translucency with no apparent tachyzoites or bradyzoites (Figure 1D).

The presence of mature *T. gondii* cysts was most pronounced and frequently found in the cortical brain tissues of the infected non-treated group (G1) (42.9% of mice recorded severe number/5 fields and 57.1% of mice recorded very severe number/5 fields). There was a significant difference between G1 and all the infected treated groups with the highest significance between G1 and the group infected and treated with CQ (G4) (p-value <0.001). Concerning the atrophic cysts, G4 showed significantly higher numbers compared to all the study groups, The percentage of brain tissues of mice in G4 harboring necrotic cysts was significantly different from that of mice in G1 (Table 2).

Table 1. Comparison between study groups regarding the MR

Groups	Number of mice at the beginning of the study	Number of mice at the end of the study	Mortality rate
Group (1): infected non treated	12	7	41.6%[a]*
Group (2): infected and treated with spiramycin	8	5	37.5%[a, b]
Group (3): infected and treated with BPQ	8	7	12.5%[a, b]
Group (4): infected and treated with CQ	8	8	0[b]*

One-way ANOVA test and LSD posthoc test were used for pairwise comparisons a, and b: groups with the same subscript letter have no significant difference. *: statistical significance between groups (P<0.05).

Table 2. Comparison between the percentage of the brains of mice harboring different types of cysts among study groups

			Mild	Moderate	Severe	Very severe	χ^2	P-value	Post-hoc test
			Semiquantitative score						
Mature cysts	Group 1	N	0	0	3	4	36.52	0.000**	P1=0.045* P2=0.003* P3=0.001* P4=0.038* P5=0.002* P6=0.013*
		%	0.0%	0.0%	42.9%	57.1%			
	Group 2	N	0	2	3	0			
		%	0.0%	40.0%	60.0%	0.0%			
	Group 3	N	3	4	0	0			
		%	42.9%	57.1%	0.0%	0.0%			
	Group 4	N	8	0	0	0			
		%	100.0%	0.0%	0.0%	0.0%			
Atrophic cysts	Group 1	N	1	6	0	0	24.56	0.003*	P1=0.305 P2=1.00 P3=0.009* P4=0.310 P5=0.020* P6=0.009*
		%	14.3%	85.7%	0.0%	0.0%			
	Group 2	N	2	3	0	0			
		%	40.0%	60.0%	0.0%	0.0%			
	Group 3	N	1	6	0	0			
		%	14.3%	85.7%	0.0%	0.0%			
	Group 4	N	0	1	5	2			
		%	0.0%	12.5%	62.5%	25.0%			
Necrotic cysts	Group 1	N	7	0	0	0	8.61	0.035**	P1=0.217 P2=0.299 P3=0.010* P4=0.793 P5=0.135 P6=0.057
		%	100.0%	0.0%	0.0%	0.0%			
	Group 2	N	4	1	0	0			
		%	80.0%	20.0%	0.0%	0.0%			
	Group 3	N	6	1	0	0			
		%	85.7%	14.3%	0.0%	0.0%			
	Group 4	N	3	5	0	0			
		%	37.5%	62.5%	0.0%	0.0%			

(χ2): Chi-square Test   **p:** p-value for comparing the studied groups   *: p-value <0.05 is significant   **: p-value <0.001 is highly significant
P1: G1 vs. G2; **P2:** G1 vs. G3; **P3:** G1 vs. G4; **P4:** G2 vs. G3; **P5:** G2 vs. G4; **P6:** G3 vs. G4
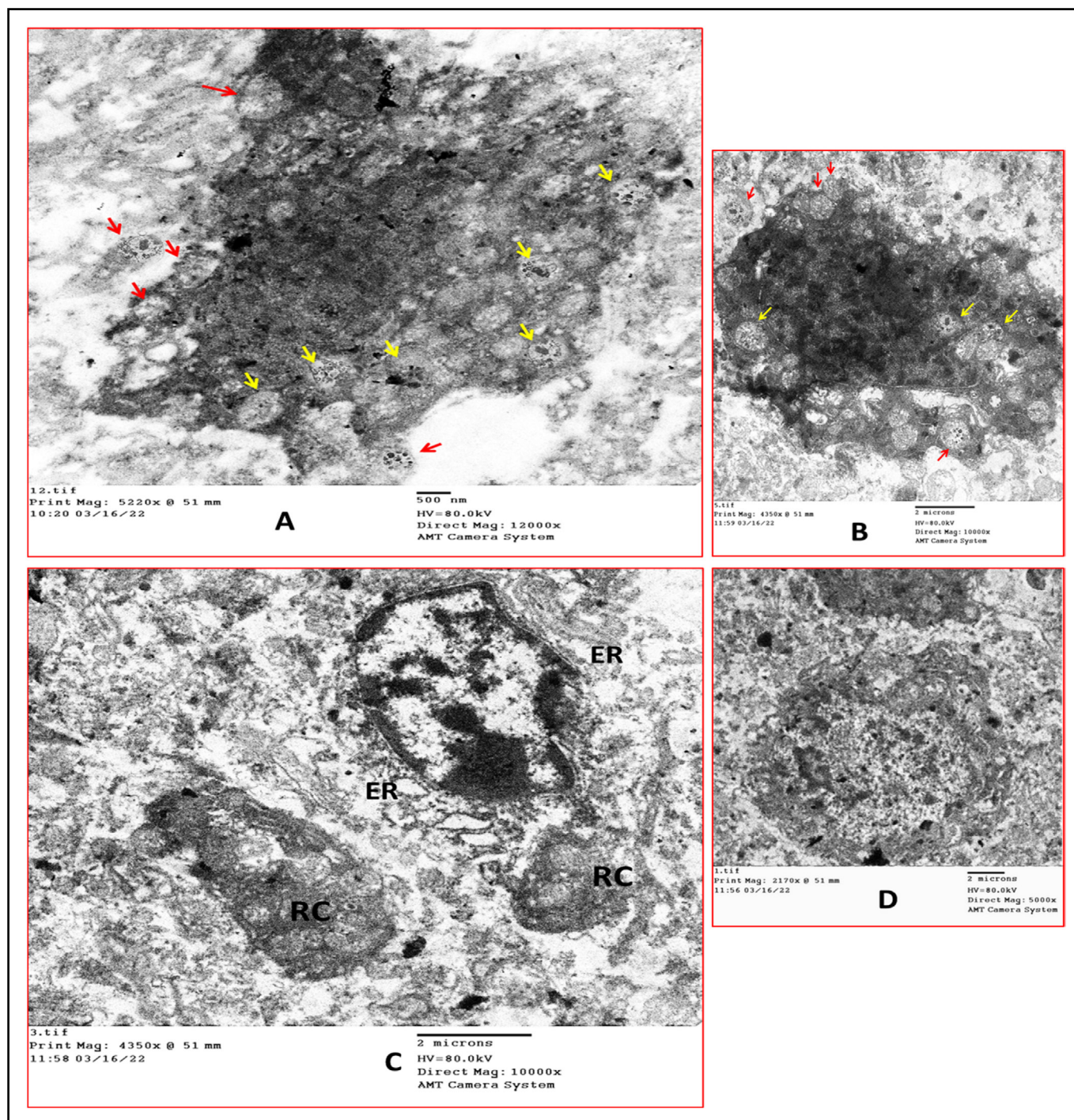
Figure 1. Microphotographs showing various cortical brain *T. gondii* cysts. A & B: Mature cysts with bradyzoites inside (yellow arrows) and tachyzoites near the outer border (red arrows) as seen in the infected non-treated group (extracellular cyst). **B:** infected microglial cell, carrying tachyzoites (Trojan horse) (intracellular cysts). **C:** active glial cell surrounded by endoplasmic reticulum (ER), trying to engulf an atrophic residual cyst (AC). **D:** completely necrotic cysts. (Magnification power; A, 12000×; B & C, 10000×; D, 5000×).

Morphological study of cortical brain tissues

Brain capillaries
Brain capillaries in the infected non-treated group were dilated with marked perivascular edema and interrupted endothelial junctional complexes. There was a separation of the endothelial cells from the basement membrane in some capillaries (Figure 2A). The treated groups showed reactive pericytes, swollen endothelial cells, and mitochondrial activity (Figure 2B and C). Marked improvements in the ultrastructural integrity of the tight junctions and peri

parenchymal edema were seen in the group treated with CQ (Figure 2D).

Glial cells and neurons
The infected non-treated group showed reactive microglia and astrocytes. Microglial activation was evidenced by increased and enlarged lysosomes in the cytoplasm. While reactive astrocytosis was characterized by increased cytoplasmic Golgi body, endoplasmic reticulum, mitochondria, and pseudopodal expansions. Neuronal cells showed chromatin migration condensing into dense blocks
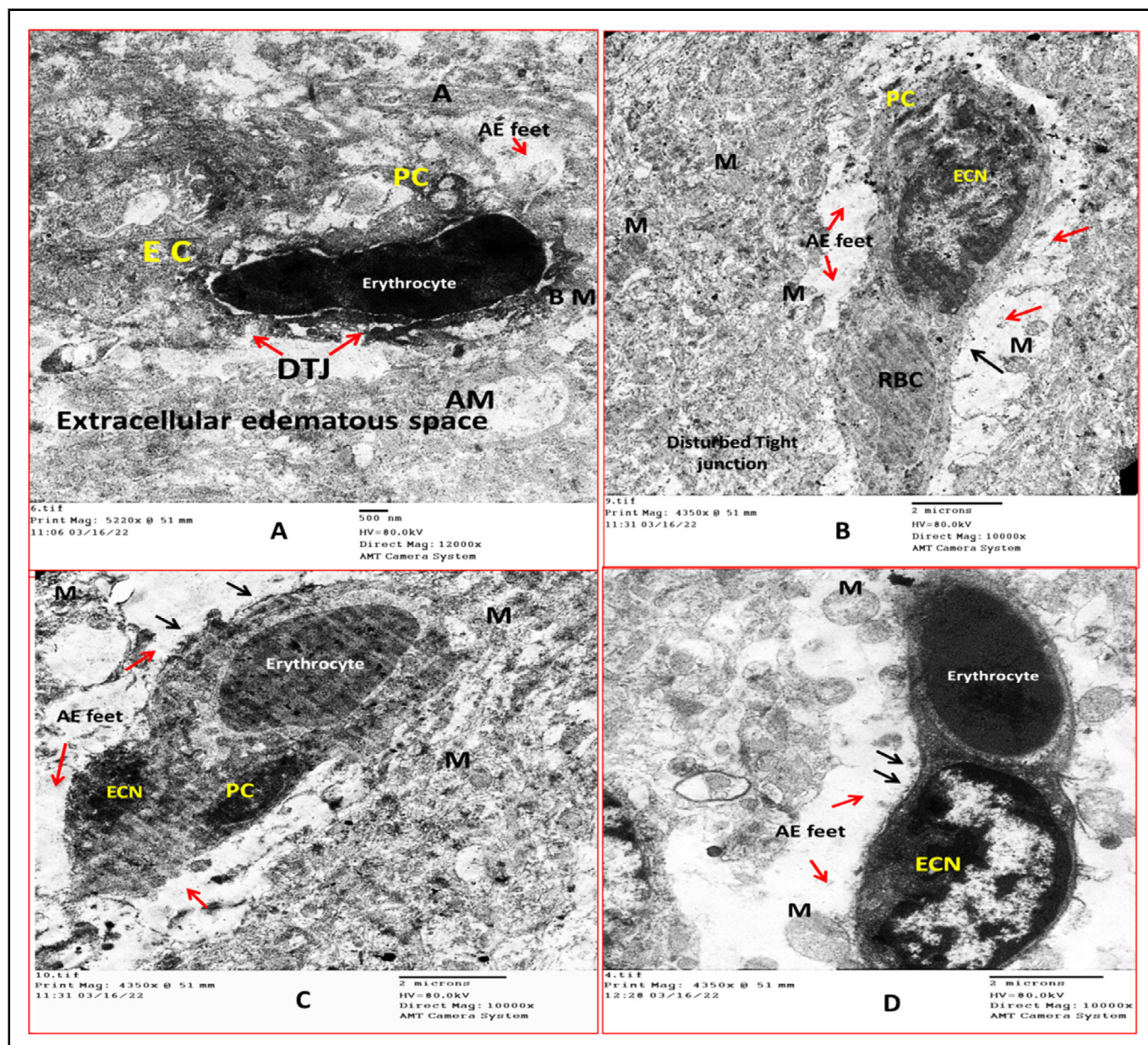
Figure 2. Microphotographs showing ultrastructural features of cerebral capillaries in different groups. **A:** infected non-treated group shows extensive damage to the endothelial tight junction (DTJ), abnormal nerve myelination (AM), with extracellular edematous spaces, and abnormal location of astrocyte end (AE) feet. **B, C & D:** Intact tight junction, normal location of AE feet, electron-dense tiny structures (black arrows), and mitochondrial activity are noticed in the infected group treated with spiramycin (B) with BPQ (C) and with CQ (D). (ECN: endothelial cell nucleus; PC: pericyte), (Magnification power; A, 12000×; B, C & D, 10000×).

and some of them have degenerated and shrunken others were harboring the *T. gondii* parasite. These findings were seen with different degrees in the group treated with spiramycin and improved in the group treated with BPQ while the group treated with CQ showed less active glial cells and normal neurons (Figure 3 and Figure 4).

Myelin sheaths
Disorganized myelin sheaths and electron-dense or swollen axoplasms with distorted myelin sheaths were noticed in the infected non-treated group (Figure 3B) which disappeared in the group treated with CQ completely (Figure 4B).

DISCUSSION

The aim of our work was to assess the efficacy of two different antimalarial drugs with different modes of action; buparvaquone and chloroquine in treating chronic cerebral toxoplasmosis in an immunocompromised mouse model on the ultrastructural level.

In this study, we used laboratory mice as animal models. They were used in most *T. gondii* studies due to their relative ease of handling and management, in addition to being highly susceptible to toxoplasmosis (Zenner *et al*., 1998; Calero-Bernal *et al*., 2022). Concerning immunosuppression of mice, we chose oral dexamethasone administration as Rusináková *et al*. (2009)
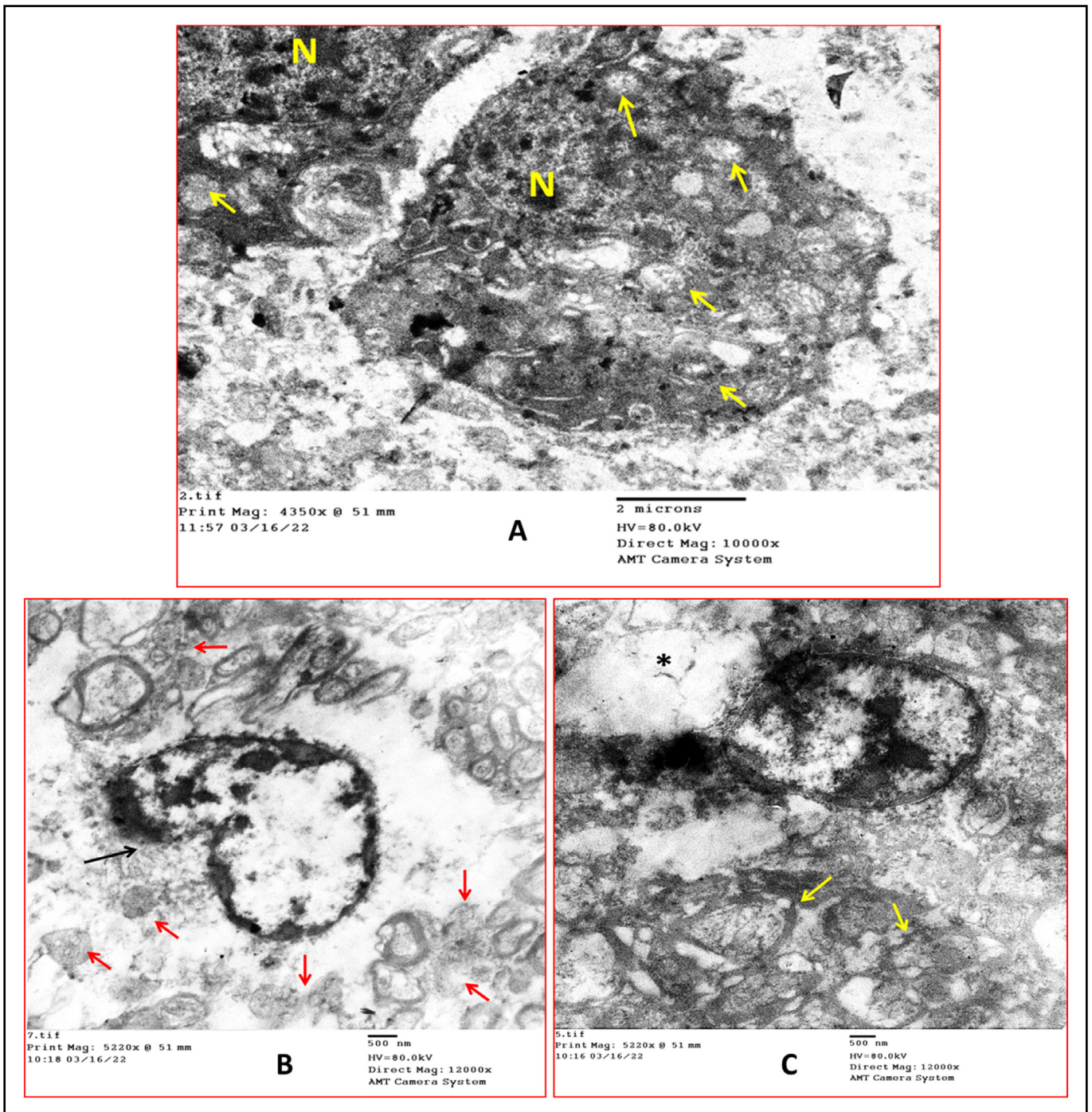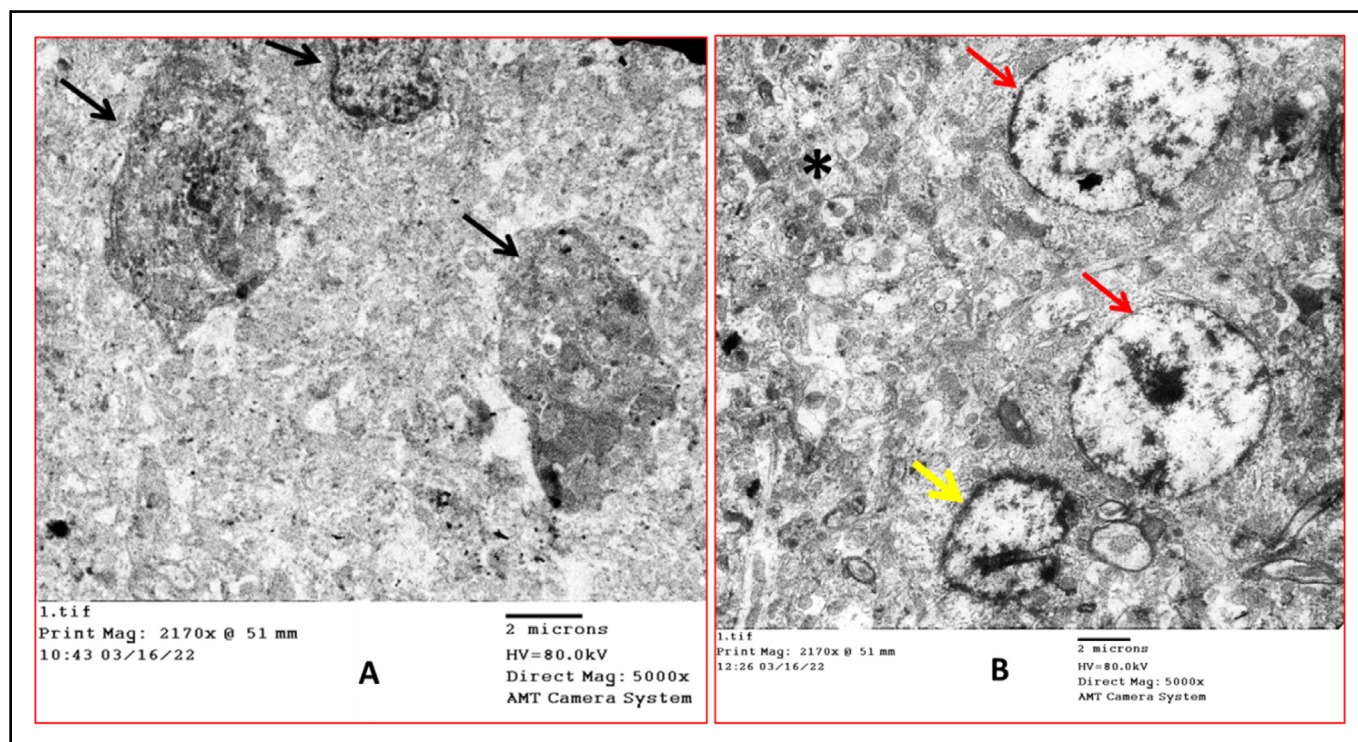
Figure 3. Microphotographs showing microglial cells within different conditions. A: microglial cells recently infected with tachyzoites, note that the nucleus is still visible (N). **B:** active microglial cell during engulfment of degenerated neurons and surrounded by neurons with deformed myelin (red arrows). **C:** microglial cell with widened perinuclear cistern with an enlarged lysosome (black star). (Magnification power; A,10000×; B & C, 12000×).

documented the association between using immunosuppressants and the significant risk of toxoplasmosis. Moreover, Zhang *et al.* (2017) reported that the long-term use of DEX can easily lead to acute toxoplasmosis in patients and can promote the proliferation of *T. gondii in vitro*. ME-49 *Toxoplasma* strain was used in this study as it is proven to cause chronic toxoplasmosis with the associated formation of brain cysts (Ferguson *et al.*, 1994; Gigley *et al.*, 2009; Galván-Ramírez *et al.*, 2019). The reference drug used in our study was spiramycin, a macrolide antibiotic commonly prescribed to prevent congenital toxoplasmosis as an alternative drug to pyrimethamine which has many disadvantages such

as being myelotoxic, unavailable, and/or unaffordable in many countries (Konstantinovic *et al.*, 2019, Hagras *et al.*, 2022). The anti-*Toxoplasma* effect of spiramycin in murine models of infection has been approved by many authors (Araujo *et al.*, 1991; Grujić *et al.*, 2005; Chew *et al.*, 2012b; Etewa *et al.*, 2018; Omar *et al.*, 2021; Hagras *et al.*, 2022).

As regards the mortality rate on the day of euthanasia (the 70th day of the experiment) among mice of the studied groups, we found that only the CQ- treated group showed a significant and complete reduction in MR, without any deaths, compared to the infected non-treated group. While both spiramycin and BPQ treatments

Figure 4. Microphotographs showing the condition in the neurons. **A:** Infected neurons with abnormal surrounding neuropil in the infected non-treated group (black arrows). **B:** Normal neurons (red arrows) surrounded by normal neuropil (black star) and microglial cell (yellow arrow). (Magnification power; 5000×).

couldn't reduce the deaths significantly. In this study, the infected non-treated group showed a MR of 41.6% which is in agreement with Rageh *et al.* (2022) who reported a 40% mortality rate in the infected non-treated group. However, they reported no deaths in the group treated with spiramycin which may be explained by infecting the mice orally with only 20 cysts of the ME49 strain compared to 100 cysts of the same strain used in our study, and the euthanasia day of mice was on the 9th week compared to the 70th day of our experiment. In addition, Hagras *et al.* (2022) documented 100% MR among the mice of the infected non-treated group, and FarahatAllam *et al.* (2020) found 90% MR among the same group. Both studies used the RH virulent strain which could be the reason for higher MR.

Examination of brain tissues from the different study groups revealed three patterns of parasite cysts; the mature *T. gondii* cyst mostly surrounded by tachyzoites, the atrophic cyst, and the necrotic cyst. Regarding tissue cyst rupture and release of *Toxoplasma* parasites, Ferguson *et al.* (1989) found that the incidence of such rupture within the brain of immunocompetent infected mice was extremely rare with no evidence of new cyst formation as a result of the effective immune response. However, if the host acquired immunosuppression, brain cysts containing the bradyzoites may rupture, releasing the tachyzoites following their transformation, thus initiating acute on top of chronic infection or what is called recrudescence that may extend according to the condition causing life-threatening encephalitis (Chew *et al.*, 2012a). The condition would be much worse if the host had impaired immunity from the beginning; this may expose the host to fatal complications (Cabral *et al.*, 2016) which is the case in our animal models, hence the high mortality rate (41.6%) and the ultrastructural pathological features reported within the infected non-treated control group in this work.

All treatment regimens used in the present study reduced the number of *T. gondii* cysts in comparison to the infected non-treated group. Similarly, Grujić *et al.* (2005) and Rageh *et al.* (2022) reported a significant reduction of ME49 brain cyst burden after treatment with spiramycin compared to the control group. Likewise, Gamea

et al. (2022) reported a highly significant reduction of RH strain tachyzoites numbers in peritoneal fluid and liver smears after CQ treatment. Conversely, Müller *et al.* (2017) documented that BPQ couldn't reduce the cerebral parasitic cysts burden of ME49 strain significantly because of the inability to cross the blood-brain barrier (BBB) and the poor solubility of the drug, although the drug exerted excellent activity against transplacental transmission in the pregnant mice.

Ferguson *et al.* (1994) described the ME49 strain infection in immunocompetent mice as an unstable chronic infection with a continuous turnover of cysts and documented the rupture of only 0.8% of the cysts surrounded by inflammatory cells. This is a very low percentage compared to the widespread ruptured cysts with emerging tachyzoites in the brains of the immunocompromised infected non-treated mice in our study. Additionally, tissue cysts were observed within the neurons and the active microglial cells, intracellularly, and others were seen extracellularly which indicates the extensive spread of *Toxoplasma* infection within the cerebral cortex of the immunocompromised mice. However, these findings contrast with previous studies of Pavesio *et al.* (1992) who reported that ME49 tissue cysts were only extracellularly located, and Ferguson *et al.* (1994) who found the cysts to be located only intracellularly within viable-appearing host cells. Both publications used only 10 cysts of the ME49 strain to infect each immunocompetent mouse intraperitoneally.

The degenerated cysts showed no surrounding active tachyzoites and fewer bradyzoites inside which indicates the efficacy of the treatment while the necrotic cysts showed neither tachyzoites outside nor bradyzoites inside, which means better elimination of infection by the drug. There was a significant difference between the percentage of mice brains with atrophic cysts in the group treated with CQ and the other groups. Also, the CQ treatment increased the number of necrotic cysts significantly compared to the infected non-treated group which is in agreement with Gamea *et al.* (2022) who stated that CQ triggers apoptosis of cells harboring *Toxoplasma*

tachyzoites. Estimation of brain cysts burden in the treated groups reflected the superior effect of CQ over BPQ and spiramycin in treating chronic cerebral toxoplasmosis.

Concerning the integrity of brain capillaries, the normal blood-brain barrier (BBB) is resistant to dexamethasone penetration (Meijer *et al.*, 1998); therefore, the immunocompromised non-infected group wouldn't show any ultrastructural changes. In this work, tight junctions between the microvascular endothelial cells were interrupted in the infected non-treated immunocompromised mice. In addition, signs of severe degenerative effects were recorded within the control non-treated group as deformed myelination of the axons, abnormality within the network of nerve fibers, their branches, and the glial filaments (neuropil) surrounding the infected neurons. As reported by Estato *et al.* (2018), their work documented the deleterious effect of acquired toxoplasmosis on cerebral microcirculation and the development of microvasculopathy, which promotes neuroinflammation with microglial activation and microcirculatory dysfunction in the brain, thus contributing to the neurodegenerative process accompanying toxoplasmosis. Also, *T. gondii* infection was capable of disrupting tight junction immunoreactivity and organization rate, as well as transendothelial electrical resistance *in vitro*. This imperfection within the BBB may clarify the relative entry of spiramycin and BPQ via these defective capillaries to affect the parasitic stages achieving a significant reduction of *T. gondii* cysts. These signs improved in both G2 and G3, with completely normal neurons surrounded by normal neuropil and areas showing autophagy in G4 treated with CQ, indicating its excellent impact on the process of neurogenesis. In addition, it was noticed that the outer surface of the cerebral capillaries is covered by electron-dense tiny structures and surrounded by astrocyte end feet in the treated groups only. In fact, astrocytes' end feet ensheath the blood vessels and are essential for the structural integrity of the cerebral vessels (El-Khoury *et al.*, 2006). Furthermore, the expression of vital vasculotrophic and neurotropic growth factors termed angioneurosis is believed to be through these astrocytes' feet (Saito *et al.*, 2011) which may clarify the presence of the electron-dense tiny structures as a helpful effect of treatment. This may reflect the ability of the effective treatment in stimulating B.V growth and improving their structure towards normal. In this work treatment significantly reduced parasite intensity and was successful to repair microvascular damages, caused by the infection with the best results in the CQ-treated group.

Regarding neurogenesis, neurons are subjected to serious harm, when exposed to an accumulation of toxins. To avoid neurodegeneration, neurons rely on autophagy which is generated in the axon tips to maintain their hemostasis as well as remove damaged cellular organelles. In this study, autophagy was reported only within the group treated with CQ indicating the successful return to normal vital processes after effectual treatment (Maday & Holzbaur, 2016). Moreover, active microglial cells were observed engulfing the degenerated axons. This process is vital in remodeling axons during neurogenesis (axon pruning) (Watts *et al.*, 2004).

On the other hand, mitochondria were observed in considerable quantity in groups that received the medication, especially around the capillaries and the infected sites. This is in agreement with Pernas *et al.* (2018) who reported that the host mitochondria play a vital role in restricting the growth of the established parasitic stages by limiting its uptake of fatty acids (FAs), liberated from lipophagy and are essential for *Toxoplasma* multiplication. Consequently, mitochondrial activity can be considered a host defense mechanism justifying the presence of various mitochondria near the capillaries and the site of infection in all treated groups. This finding indicates the positive effect of the used medications on constraining parasite multiplication.

CONCLUSION

The study results highlighted the superior efficacy of CQ over BPQ and spiramycin in reducing brain cysts significantly, as well as improving the ultrastructural pathological changes induced by chronic cerebral toxoplasmosis in immunocompromised mice, without any deaths. Novel drug formulations may be needed for spiramycin and BPQ to increase their efficacy in treating the infection.

Conflicts of Interest Statement
The authors declare that they have no conflict of interest.

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