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CIPROFLOXACIN-LOADED SILVER NANOPARTICLES EFFICACY ON CHRONIC TOXOPLASMOSIS INFECTED MICE

By

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Abstract

Toxoplasmosis is one of the commonest health problems especially in immunocompromised patients. The study evaluated the effect of ciprofloxacin loaded on silver nanoparticles in treating mice experimentally infected with toxoplasmosis. This study was conducted on mice infected with (ME 49 *Toxoplasma gondii* strain). Sixty mice were divided into: GI: 5 non infected non-treated mice (normal control). GII: 10 infected non-treated mice (infected control). GIII: 10 infected mice treated with Spiramycin 100mg/kg (drug controls). GIV: 10 infected mice treated with ciprofloxacin 100mg/kg. GV: 10 infected mice treated with ciprofloxacin loaded silver nano-particles (100mg/kg ciprofloxacin+2.5µg/kg silver nano). GVI: 10 infected mice treated with silver nanoparticles (2.5µg/kg). GVII: 5 non-infected mice treated with silver nanoparticles (2.5µg/kg). Mice were sacrificed 8 weeks (experimental end) and brain and liver from each group were processed for histopathological studies of the cysts numbers, and sizes.

Key words: Albino mice, *Toxoplasma gondii,* Ciprofloxacin, Silver nanoparticles.

Introduction

Toxoplasma gondii is an obligate intracellular zoonotic protozoan of phylum Apicomplexa, causes toxoplasmosis of worldwide (Abbas *et al,* 2020) among man, animals and birds (Hagras *et al*, 2019). Cats serve as the definitive host (Al-Kappany *et al,* 2010). In Egypt toxoplasmosis and anti-*Toxoplasma* antibodies were reported mainly among the childbearing age females (Saleh *et al,* 2014) and immunocompromised patients (Taman and Alhusseiny, 2020). Human toxoplasmosis is asymptomatic but, postnatal infection causes fatal encephalitis in immunodeficient patients such as those with HIV/AIDS or organ transplant recipients (El-Sayed and Morsy, 2021). Besides, congenital toxoplasmosis may cause spontaneous abortion, premature birth, neonatal defects and stillbirth in pregnant women depending on the gestational period (Ibrahim *et al,* 2017).

A combination of pyrimethamine and sulfadiazine that block formation of folic acid in the parasite is the most efficient toxoplasmosis treatment (Mahmoud, 1999). But, there were side effects of this treatment, as hyper sensitivity, bone marrow suppression, and teratogenic effects (Petersen, 2007).

Quinolones offer a wide range of antimicrobial activity against bacteria, mycoplasma, as well as *Toxoplasma gondii and Plasmodium falciparum* (Gouvea *et al,* 2013). They are frequently utilized in clinics and well tolerated by the majority of patients (Johannes *et al,* 2007). Fluoroquinolones are DNA replication inhibitors target type II topoisomerases in prokaryotes (Collin *et al,* 2011).

Nanotechnology is utilized to treat many zoonotic parasitosis including toxoplasmosis (Gutiérrez *et al,* 2016). Silver nanoparticles are the most prevalent with antibacterial and anti-inflammatory properties (Edmundson *et al,* 2014).

This study aimed to evaluate efficacy of Ciprofloxacin® with or without loaded on silver nanoparticles on *Toxoplasma gondii* ME49 strain experimentally infected mice.

Materials and Methods

Ethical approval: The approval was kindly obtained from the Ethical Committee of Faculty of Medicine Benha University, which agreed with the Helsinki (2000) guidelines dealing with experimental animals. Study was con- ducted between July 2019 and September 2020 at the Medical Parasitology Depart-

ment, Faculty of Medicine, Benha University, and National Research Center (NRC).

Non-virulent ME49 strain of *T. gondii* was kindly provided from The Zoonotic Department of NRC. The strain was maintained by sub-passage in Swiss albino mice with 0.1 ml of brain homogenate contained about $1x10^2$ tissue cysts/ml every 8 weeks (Nasr *et al,* 2020).

Animals: Sixty clean laboratories bred male Swiss albino mice 10 weeks old and weights about 25gm were provided from the NRC's animal house. Mice were kept in insect proof cages (10mice/cage) in experimental conditions and were provided with normal food and tap water, 12hrs cycle of light/dark (Grujić *et al,* 2005).

Preparation of silver nanoparticles: A- Silver acetate and benzylamine were purchased from the American company Aldrich, and 99% of ethanol from Egyptian Chemical and Pharmaceutical Co. B- Silver metal nanoparticles were prepared using non-hydrolytic solution gel method (Küünal *et al,* 2016). Silver acetate (0.5g) was dissolved in 10ml benzylamine; mixture was put into an autoclave for 24hrs and then placed in an oven at 200°C for 48 hrs. Mixture was centrifuged and the precipitates were washed five times with ethanol and dried in air at 70°C.

Preparation of Ciprofloxacin loaded silver nanoparticles (Ag NPs)*:* A total of 0.001ml ciprofloxacin aqueous solution was added to 100ml of the produced Ag NPs with continuous stirred under ultra-sonication (Mohsen *et al,* 2020).

Characterization of Ag NPs: Using carbon-coated copper grids as substrates, TEM pictures were captured using Philips Morgagni 268 Electron Microscopy operating at 200 KV. A drop of silver-particles mixed with water was applied to a copper grid coated with carbon for natural dried. Transmission electron micrograph showed the Ag NPs as spherical particles with an average size of 20 nm, which were well separated from the neighboring nanoparticles.

Experimental infection: Mice were orally inoculated with ME49 strain 0.1ml of brain cysts suspension contained 10 cysts/mouse (El-Sayed and Aly, 2014).

Drugs: 1- Spiramycin® or Spirex 3 M.I.U tablet of 704mg (Medical Union Pharmaceuticals Egypt). Tablets were crushed and dissolved in distilled water for oral administration daily dose of 100mg/kg (Etewa *et al,* 2018). 2- Tablets of (Ciprofloxacin® 500mg) European Egyptian Pharm (IND stands for industries). Tablets were crushed and dissolved in distilled water for oral administration in a dose of 100mg/kg (Duarte *et al,* 2015). 3- Ciprofolxacin loaded on silver nanoparticles (Ag NPs) was given in a dose of 100mg /kg as ciprofloxacin & 2.5µg/kg Ag NPs in a daily dose of 2.5µg/kg in 200µl PBS (Mulfinger *et al,* 2007).

Experimental design: Treatment started at 6^{th} week post infection (P.I.) for a week and scarification was two weeks later. GI: Neither infected, nor treated mice (normal control). GII: Infected non-treated mice (positive control). GIII: Infected mice spiramycin 100mg/kg treated (drug control). GIV: Infected mice ciprofloxacin 100mg/kg treated. GV: Infected mice ciprofloxacin loaded Ag NPs treated (100mg-/kg ciprofloxacin & 2.5µg/kg Ag NPs). GVI: Infected mice Ag NPs treated (2.5-µg/kg). GVII: Non-infected mice Ag NPs treated (2.5µg/kg) to assess toxicity.

All mice were sacrificed and brains were collected at the experimental end (8 weeks). Each mouse brain was split into two halves, one to count brain cysts, and the second was fixed in 10% buffered formalin for histopathological examinations.

Brain cysts count: One half of each brain was minced individually in a sonicator for cell lysis and 1ml of formalin 10% was added to the minced brain tissue of each mouse from emulsified homogenate. A drop (25µl) from brain suspension was put on glass slide and examined by light microscope at (x40) magnification for mean cysts number in mice individually.

Measurement of tissue cyst: A drop of the

brain suspension was put on a glass slide and measured with micrometer in eyepiece at x400 magnification.

Histopathological examination: Brain and liver specimens were individually collected fixed in 10% buffered formalin, dehydrated in different ascending series of ethanol, cleared in xylol, and embedded in paraffin blocks. Paraffin sections of 5µm thickness were prepared stained in H&E and examined by a light microscope at different magnifications for the histopathological changes (Abdel-Bary *et al*, 2012).

Statistical analysis: Data were computerized and analyzed by using Statistical Package for Social Science (SPSS) for windows version 11.0. Data were represented as mean ± standard deviation (SD) (n = 10). ANOVA was used to clarify significant differences between groups. Post hoc test *(Bonferroni)* for pairwise group assessed difference among each 2 groups.

Results

There was highly significant difference between infected control and treated groups (P-<0.001), significant difference between spiramycin treated and ciprofloxacin loaded Ag NPs treated mice (P <0.001), significant difference between spiramycin treated and ciprofloxacin treated mice (P <0.05), and a significant difference between spiramycin treated and Ag NPs treated groups (P <0.05).

Also, there was significant difference between treated and control groups (P <0.001). Mice treated with ciprofloxacin loaded Ag NPs showed reduction of 60.82% followed by mice treated with Ag NPs, which showed reduction of 50.6%.

Details were given in tables (1 & 2) and figures (1 & 2)

Table 1: Effect of ciprofloxacin & ciprofloxacin loaded on silver nanoparticles on tissue cyst count compared to spiramycin:

Groups	Mice	Cyst count	Reduction %	Pairwise group significance against other groups				
				GII (P1)	GIII (P2)	GIV (P3)	GV (P4)	GVI (P5)
GI	10	-	-	-	-	-	-	-
GII	10	21.36±1.32	-	-	<0.001**	<0.001**	<0.001**	<0.001**
GIII	10	8.88±0.73	58.5%	<0.001**		<0.001**	<0.001**	<0.001**
GIV	10	15.05±0.61	29.5%	<0.001**	<0.05*	-	<0.05*	<0.05*
GV	10	10.84±0.88	49.3%	<0.001**	<0.001**	<0.001**	-	<0.001**
GVI	10	16.08±1.09	24.7%	<0.001**	<0.05*	<0.05*	<0.0.05*	-
GVII	5	-	-	-	-	-	-	-

P< 0.05: Significant. p<0.001** highly significant p<0.05: non-Significant.

Table 2: Effect of ciprofloxacin and ciprofloxacin loaded on silver nanoparticles on tissue cyst size:

Groups	Mean tissue cyst size	%of reduction of cyst size
GI	-	-
GII	34.2±5.12	-
GIII	22±4.74	35.7%
GIV	22.2±5.93	35.1%
GV	13.4±2.07	60.82%
GVI	17.0±2.24	50.6%
GVII		-

Discussion

In the present study, with *T. gondii* ME49 strain, the best result in reduction of tissue cyst count was in spiramycin treated mice with a rate of 58.5% (P < 0.001) followed by ciprofloxacin loaded on silver nanoparticles treated mice with a reduction rate of 49.3%. This agreed with Etewa *et al.* (2018) in Egypt, who treated *T. gondii* infected mice with spiramycin and/or with spiramycin loaded chitosan reported that both showed a significant decrease in number of cysts in brain. Also, Farag *et al.* (2019) in Egypt denoted that spiramycin decreased cyst burden in chronic murine toxoplasmosis. Moreover, Omar *et al.* (2021) who reported that spiramycin significantly caused reduction in mice brain cysts' number as compared to positive control.

In the present study, there was significant reduction in tissue cyst count in ciprofloxacin treated group with a rate of 29.5% **(**P

<0.05). The in-vivo fluoroquinolone enrofloxacin caused efficacy in a murine model infected with *T. gondii* ME49 strain, which reduced brain cysts by 68% with *Calomys callosus* compared to 79% with sulfadiazine (Gottstein *et al,* 1999).

In the present study, loading of ciprofloxacin on silver nanoparticles led to significant reduction of *T. gondii* tissue cyst when compared ciprofloxacin alone (P <0.05), which may be attributed to the nanoparticles ability to deliver drug to specific tissues and proving controlled release therapy. This agreed with targeted and sustained drug delivery decreasing its related toxicity (Rizvi and Saleh, 2018).

In the present study, the least reduction rate was reached with silver nanoparticles treated mice with significant rate of 24.7% (P <0.001). This agreed with Johnston *et al.* (2010) who reported highly toxic to bacteria and fungi associated with ion release and induction of oxidative stress Also, this agreed with Cameron *et al.* (2016), they found that silver nanoparticles caused strongly decreased in live cryptospodiosis *parvum* oocysts of infected patients.

In the present study, the best reduction in cysts' size was with ciprofloxacin loaded on silver nanoparticles treated mice (60.82%) followed by silver nanoparticles treated ones with a reduction of 50.6%. No doubt, ciprofloxacin affected the cell membrane integrity when loaded on silver nanoparticles, which increased its efficacy. Choi and Hu (2008) found that the nanoparticles (NPs) have altered the structure of glycoprotein and lipophosphoglycan molecules on the parasites' surfaces. Abdel Hallim *et al.* (2015) reported that SEM images of *C. parvum* showed that oocysts became inactive and changes in structure of its wall were observed after treated with NPs.

In the present study, there was significant reduction in tissue cyst size in spiramycin treated mice as compared to infected untreated ones with a rate of 35.7%. This agreed with Zhang *et al.* (2001) who reported that the spiramycin was able to inhibit the protein synthesis and subsequent alteration in glycoprotein synthesis, the major component of cyst wall

In the present study, all treated mice showed reduction in the inflammatory infiltrate with regeneration of hepatocytes together with minimal mononuclear inflammatory infiltrate. The present best result was achieved with spiramycin treated mice followed by ciprofloxacin loaded on silver nanoparticles treated ones**.**

In the present histopathogical assessment of brain tissue in ME49 strain control mice, showed normal tissue architecture. But, the infected untreated ones showed severe inflammatory infiltration around *T. gondii* cyst. This agreed with Atmaca *et al.* (2014), who showed that *T. gondii* infection caused an intense inflammatory reaction in brain tissues with infiltration of mononuclear cells in the perivascular and meningeal regions. Also, in the present study, the best result in treating infected mice was achieved with silver nanoparticles followed by spiramycin, and then ciprofloxacin loaded on silver nanoparticles.

In the present study, pathological changes improvement were in spiramycin treated mice, which brain sections showed moderate inflammatory reaction in form of shrunken, severe necrosis pyknosis of neurons and reduction in degree of brain gliosis in when compared to positive control ones. Etewa *et al.* (2018), reported that spiramycin loaded nanoparticles caused improvement of pathological pictures of brain tissues in both acute and chronic stages of *T. gondii* infection.

In the present study, ciprofloxacin treated mice showed more or less gliosis inhibition and degeneration of *Toxoplasma* cyst. This agreed with Morena *et al.* (2019) who reported that ciprofloxacin reduced the release of pro-inflammatory cytokines by lipopolysaccharides (LPS) activated microglia. They added that inhibited LPS-induced activation of transcription nuclear factor (NF-κB), as one of the major transcription factors implicated in toll-like receptors (TLR4) signaling. Gen-

erally, fluroquinolones (FQs) prevented the engagement of the LPS to toll like receptor 4 (TLR4), myeloid differentiation protein-2 (MD-2) complex and its dimerization, indicating that the binding between LPS and the receptor complex is the target for the anti-inflammatory properties of the ciprofloxacin (Zusso *et al*, 2019).

In the present study, silver nanoparticles treated mice showed preservation of neuronal tissue, less proliferation of glial cell. This agreed with Wong *et al*. (2009) reported evidence for the anti-inflammatory properties of AgNPs, in both in *vivo* and in *vitro* models and that AgNPs down regulated the inflammatory quantities proving that AgNPs suppressed inflammatory process. Besides, Ferdous and Nemmar (2020) reported that the AgNPs cytotoxic effects documented in in vitro studies in various cell lines were controlled by factors such as the size, shape, coating, and dose and cell type. They added that in vivo, following various routes of exposure, like inhalation, instillation, oral, dermal and intravenous, have established the Ag translocation, accumulation, and distributed toxicity to various organs.

Conclusion

The ciprofloxacin showed significant reduction in the histopathological count of *Toxoplasma gondii* (ME 49 strain) tachyzoites, cysts number & size in both brain and liver tissues of infected mice. This was mainly achieved when augmented by loaded silver nanoparticles.

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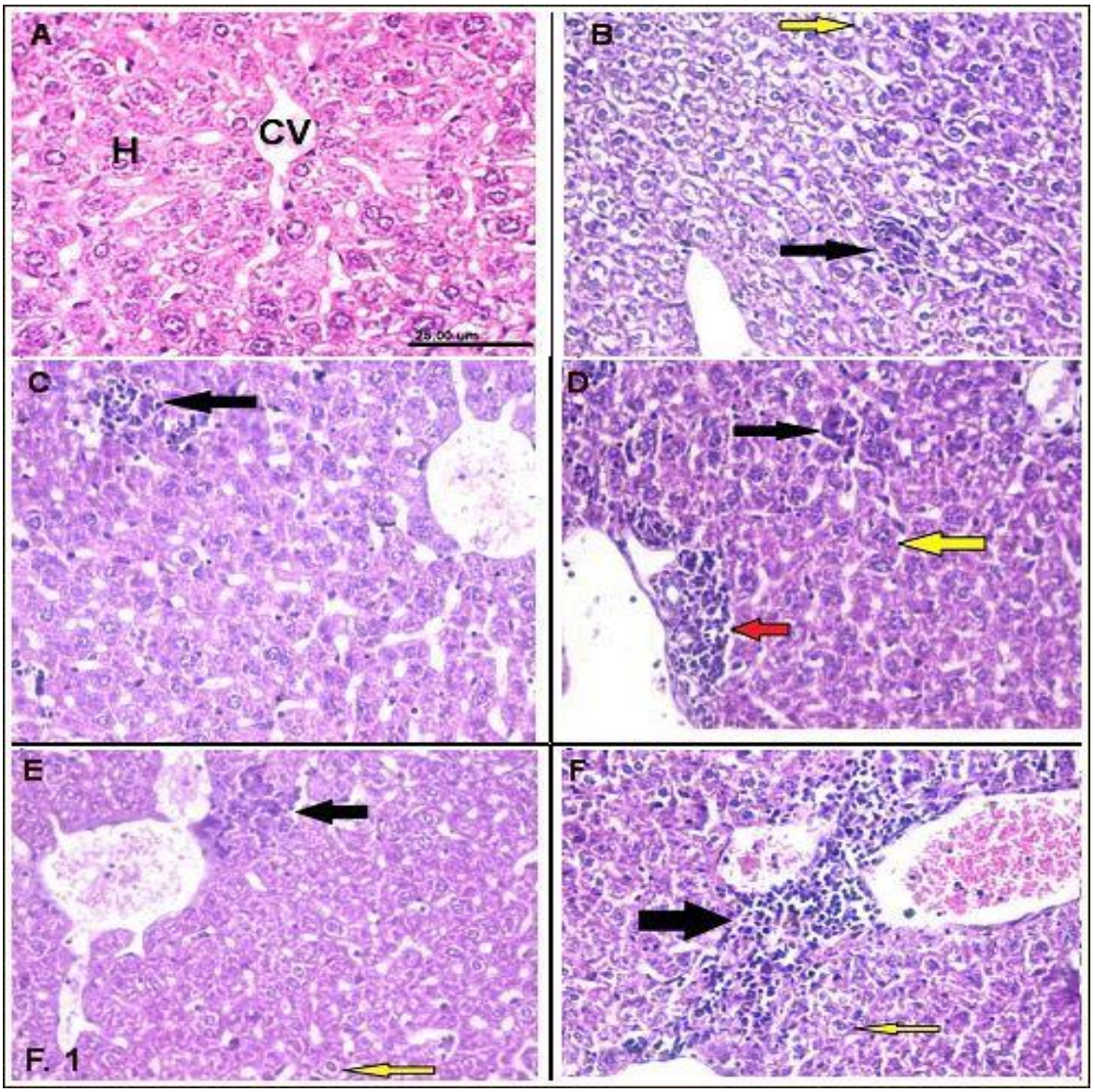
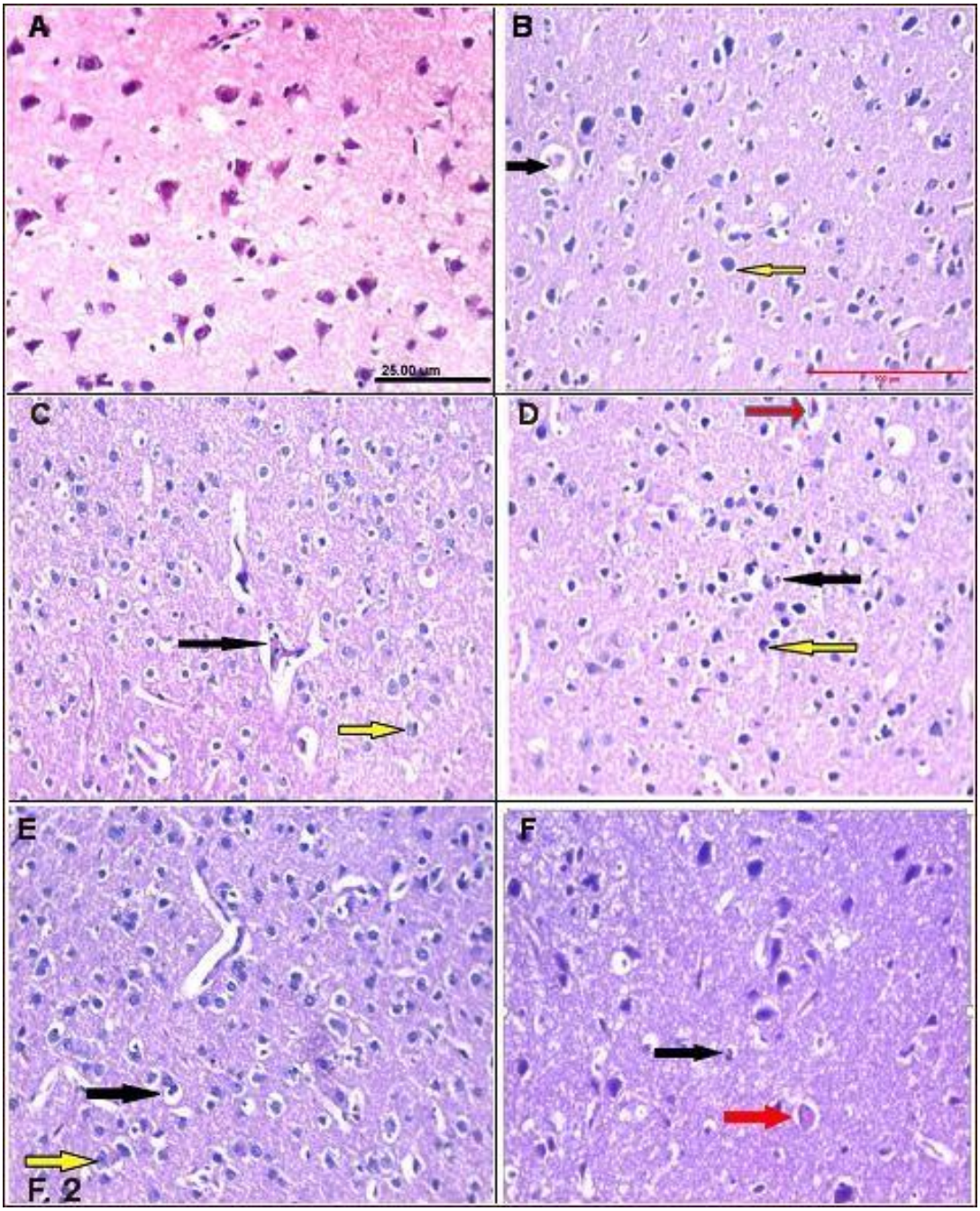
Explanation of figures

Fig. 1: A- Section in control mouse liver showed normal histological of hepatic lobule, normal central vein (CV) and hepatocytes (H).(H & E, X400). B- Section in positive control mouse liver showed focal lymphoplasmacytic cellular infiltrate around *Toxoplasma* cyst (black arrow) and hydropic degeneration of hepatocytes (yellow arrow). C- Section in spiramycin treated mouse liver showed focal lymphoplasmacytic cellular infiltrate around *Toxoplasma* cyst (arrow) with preserved hepatocytes. D- Section in ciprofloxacin treated mouse liver showed moderate portal infiltration by mononuclear inflammatory cells (red arrow) and focal lymphoplasmacytic cellular infiltrate around *Toxoplasma* cyst (black arrow) and cloudy swelling of hepatocytes (yellow arrow). E- Section in ciprofloxacin treated loaded on silver nanoparticles mouse liver showed focal lymphoplasmacytic cellular (black arrow) and mild cloudy swelling of hepatocytes (yellow arrow) with preservation of hepatic architecture. F- Section in silver nanoparticles treated mouse liver showed dense portal infiltration by mononuclear inflammatory cells (black arrow) and mild cloudy swelling of hepatocytes (yellow arrow).

Fig. 2: A- Section in control mouse brain showed normal histological structure. (H & E, X400).B- Section in positive control mouse brain showed degenerated neuron (yellow arrow) and *Toxoplasma* cyst (black arrow). C- Section in spiramycin treated mouse brain showed degenerated glial cells (yellow arrow) with perivascular lymphocytes (perivascular cuffing) (black arrow). D- Section in mouse brain from infected not treated group showed neurophagia (yellow arrow) with scattered plasma cells (black arrow) and lymphocytes,vaculation and edema of neuron(red arrow). E- Section in mouse brain treated with spiramycin showed neurophagia (yellow arrow) and some plasma cells and lymphocytes (black arrow). F- Section in mouse brain treated with spiramycin showed neurophagia (black arrow) and *Toxoplasma* cyst (red arrow) with more or less brain archticture (H&E, X400).

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