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Original article

Effect of Ciprofloxacin loaded on Silver Nanoparticles on Acute Toxoplasmosis

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Abstract

Background: Toxoplasmosis is an infectious disease caused by *Toxoplasma gondii*. The *Toxoplasma gondii* strain has an impact on the severity of toxoplasmosis. The severity of infection is mostly determined by the host's immune response, so, infections in immunocompetent people typically asymptomatic. Infection can induce neurological, ocular, and systemic disorders in immunocompromised patients. Nanomedicine developments appear promising, allowing toxoplasmosis to be treated with nanotechnology. **Aim**:The aim of the present work was to assess the effect of ciprofloxacin and ciprofloxacin loaded on silver nanoparticles on murine acute toxoplasmosis. **Materials and methods**: Sixty Mice were used in this study divided into 7 groups. Group I:Non Infected non-treated mice (5mice), group II: Infected with acute strain (RH) non-treated mice (10 mice), group III: Infected mice treated with Spiramycin 100mg/kg (10 mice), group IV: Infected mice treated with ciprofloxacin 100mg/kg.(10 mice), group V: Infected mice treated with ciprofloxacin loaded silver nanoparticles 100mg/kg.(10 mice), group VI: Infected mice treated with silver nanoparticles (10 mice) and group VII: Non infected treated with silver nanoparticles(5mice). Effect of treatment was assessed by counting the number of tachyzoites in peritoneal fluid, survival rate and histopathological assessment of liver. **Results**:Ciprofloxacin treated group showed significant reduction in tachyzoite count in peritoneal fluid, induced morphological alteration in shape of tachyzoites and improved histopathological picture in liver tissue but didn't significantly improve survival rate in infected mice. The effect of Ciprofloxacin was augmented after loading it

on silver nanoparticles. **Conclusion**:Ciprofloxacin proved its effectiveness against experimental acute toxoplasmosis and its effect was augmented after loading it on silver nanoparticles.

Keywords: Toxoplasmosis; silver nanoparticles; Ciprofloxacin; mice.

Introduction

The most effective treatment for toxoplasmosis is a combination of pyrimethamine and sulfadiazine, which work together to inhibit *toxoplasma gondii* folic acid production [1**].** This medication, however, has a number of negative side effects, including hypersensitivity, bone marrow suppression, and teratogenic consequences [2**].**

Quinolones, offer a wide range of antimicrobial activity against bacteria, mycoplasma, and protozoan parasites (*Toxoplasma gondii and plasmodium falciparum*) [3**].** Fluoroquinolones are DNA replication inhibitors that target type II topoisomerases in prokaryotes (DNA gyrase and topoisomerase IV) [4**].**

Nanomedicine advancements appear promising, allowing nanotechnology to be used in the treatment of toxoplasmosis [5**].**

The present study aimed to evaluate the efficacy of ciprofloxacin and ciprofloxacin loaded on silver nanoparticles against acute toxoplasmosis.

Materials and methods

This study was conducted at the National Research Center (NRC). The research protocol was approved by Research Ethics Committee, Faculty of Medicine, Benha University, Egypt. The study started from July 2019 to September 2020. A prospective experimental study was conducted.

Parasite and animals

Inclusion criteria: sixty Laboratory-bred male Swiss albino mice, 10 weeks old and approximately 20-25gram were used in this study.

Exclusion criteria: Mice were free from infection and didn't receive any drugs.

RH strain of *T. gondii* was obtained from the National Research Center. Tachyzoites of this strain were collected by serial intraperitoneal passages in BALB/c mice. Parasites (1×10^5) were inoculated in the mice, and after 72 hours, tachyzoites were provided by repeated flushing of the peritoneal cavity by phosphate

buffered saline (PBS). Tachyzoites were then harvested and centrifuged at 200 g for 5 min at room temperature to remove peritoneal cells and cellular debris. The supernatants were collected and centrifuged at 800 g for 10 min [6**]** .A total of 60 laboratory-bred male Swiss albino mice 8-10 weeks-old, approximately 20-25gram were used in this study.

Drugs:

Spiramycin: 3 M.I.U (704 mg) tablet manufactured by Medical Union Pharmaceuticals Egypt (Pharaonia Pharmaceuticals analytical standard code: J01FA02). The tablets were crushed and dissolved in distilled water to make oral suspension. The dose used was adjusted at 100 mg/kg day according to [7**].**

Ciprofloxacin: was obtained as tablets **(**500mg) manufactured by European Egyptian Pharm. IND. The tablets were crushed and dissolved in distilled water to make oral suspension. The dose used was adjusted at100mg/k.g [8**].**

Ciprofolxacin loaded on silver nanoparticles: Administrated at a dose of 100mg/k.g.

Synthesis of the silver metal nanoparticles (Ag MNPs):

Silver metal nanoparticles were prepared by non-hydrolytic solution gel methods [9**].**

Preparation of Ciprofloxacin loaded silver nanoparticles (Ag NPs) *:*

Ciprofloxacin loaded on AgNPs were prepared by adding 0.001 ml aqueous solution of Ciprofloxacin to 100 mL of the synthesized AgNPs, with continuous stirring under ultra-sonication to enhance the interaction between the antibiotic and the AgNPs [10**].**

Silver nanoparticles characterization

The transmission electron micrograph of silver nanoparticles showed that, the average particle size is 20 nm. The particles are well separated from the neighbouring nanoparticles. The particles are spherical in shape **Fig. (1).**

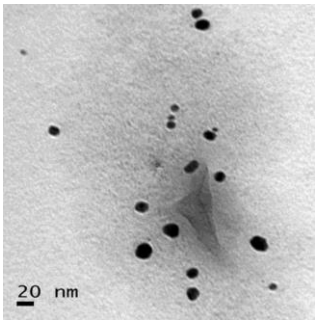


Fig.(1):Trasmission Electron Micrograph of crystalline silver nanoparticle

Mice infection and experimental Design:

Mice were injected intraperitoneally with 0.2ml of peritoneal exudates of previously infected mice containing 1×10^3 tachyzoites of *T. gondii* RH strain/ mouse [11**].**

Mice were divided into 7 groups each group contains 10 mice, except normal control contained 5 mice. Group I:Non Infected non-treated mice (normal control) (5mice), group II: Infected non-treated mice (infected control) (10 mice), group III: Infected mice treated with Spiramycin 100mg/kg (drug control)(10 mice), group IV: Infected mice treated with ciprofloxacin 100mg/kg.(10 mice), group V: Infected mice treated with ciprofloxacin loaded silver nanoparticles 100mg/kg.(10 mice), group VI: Infected mice treated with silver nanoparticles(10 mice) and group VII: Non-infected mice treated with silver nanoparticles(5mice).Treatment was administered orally to mice for 7 days via esophageal tube feeding starting from first day of infection. Five mice were sacrificed at 5^{th} day post treatment and the other mice were left for survival rate estimation.

Evaluation of drug efficacy

Tachyzoites counting in peritoneal fluid:

The peritoneal cavity of every mouse was washed by 1ml normal saline .A drop (25ul) of peritoneal fluid was placed on glass slide and examined by bright-field microscope at (×400) magnification **.**The mean tachyzoites count of each group was calculated.

Survival rate in mice after one week:

Daily observation of mice was done for one week to determine the survival rate [11**].** Results were compared to the infected untreated control group.

Histopathological assessment of liver tissue .

Liver specimens were collected from all mice/ group and then fixed in 10% neutral buffered formalin, dehydrated in different concentrations of alcohol, cleared with xylol, and embedded in paraffin blocks. Paraffin sections of 5μm thickness were prepared and stained with hematoxylin and eosin (H&E) and then examined by a light microscope [12**].**The slides were examined under the light microscope at x10, x40, x100 magnifications for the presence of any pathological changes resulting from *T. go*ndii as necrosis, congestion, hemorrhage.

Statistical analysis

- Collected data were coded and introduced to a PC using the Statistical

Package for Social Science (SPSS) for windows version 11.0. Data were represented as the mean ± standard deviation (SD) (n = 10). The analysis of variance (ANOVA) procedure was used to clarify statistically significant differences between the studied groups. Post hoc test *(Bonferroni)* for pairwise group comparison was used to assess inter group difference each 2 groups.

Values were considered statistically significant when $P < 0.05$. The infection reduction rate was assessed using the formula: (Mean value of the infected untreated group - mean value of infected treated group) X 100 / mean value of infected untreated group .

Results:

In ciprofloxacin treated group, there was significant reduction in the percentage of the mean tachyzoite count (75.9%,**Table 1)** and non-significant improvement of survival rate of infected mice (**Table, 2)** ,also there was alteration in the morphology of tachyzoites (**Fig., 2).**

As regard histopathological assessment of liver tissue: there was focal hepatocytic vacuolar degeneration, moderate mononuclear cellular infiltration and dilated sinusoids ,free tachyzoites (**Fig.,3).** The efficacy of ciprofloxacin was enhanced after loading it on silver nanoparticles as the percentage of reduction in tachyzoites count was 85.8% (**Table, 1) .**There was morphological alteration in shape of tachyzoites (**Fig.,2)** and non-significant improvement of survival rate of infected mice (**Table,2).** There was more improvement in histopathological picture of liver in this group than that of ciprofloxacin treated group (**Fig.,3).**

Table (1): Effect of ciprofloxacin and ciprofloxacin loaded on silver nanoparticles on mean tachyzoites count in comparison with spiramycin**:**

Experimental groups	Total number of mice	Mean tachyzoite counting Mean±SD	% of Reduction	Pairwise Group Significance				
				GII (P1)	GIII (p2)	GIV (p3)	GV (p4)	GVI (P5)
Normal control(GI)	5	-	-	-	-	-	-	-
Infected control(GII)	10	35.80±2.85	-	-	<0.001**	<0.001**	<0.001**	<0.001**
Spiramycin(GIII)	10	2.80±.44	92.2%	<0.001**	-	<0.001**	<0.001**	<0.001**
Cipro (GIV)	10	8.64±1.17	75.9%	<0.001**	<0.05*	-	<0.05*	<0.05*
Cipro with nano(GV)	10	5.08±1.14	85.8%	<0.001**	<0.001*	<0.001**	-	<0.001**
Mice infected and treated with silver nano(GVI)	10	13.44±2.82	62.5%	<0.001**	<0.05*	<0.05*	<0.05*	-
Non infected mice treated with silver nanoparticles(GVII)	5	-	-	-				

P < 0.05*: Significant; P <0.001** highly significant; P1:Comparison between GII(control) and other groups
P2: Comparison between GIII(spiramycin treated group) and other groups; P3: Comparison between GIV(Ciprofloxacin treated group)and other groups; P4: Comparison between GV(Ciprofloxacin loaded on silver nanoparticles)and other groups; P5: Comparison between GVI (silver nanoparticles treated group)and other groups.

ANOVA test:-Used to compare mean of more than two groups of quantitative data. *P* value <0.05 was considered statistically significant (*) while >0.05 statistically insignificant P value <0.01 was considered highly significant (**) in all analyses.

Table (2): Survival rate among different groups experimental groups over one week:

Survival	Number of mice/group	Survived No	%
Normal control(GI)	5	5	100%
infected control(GII)	5	0	0.0
Spiramycin(III)	5	2	40.0
Cipro (GIV)	5	1	20.0
Cipro with nano(V)	5	1	20.0
Mice infected and treatd with nano(VI)	5	0	0.0
Non infected mice treated with silver nanoparticles(GVII)	5	5	100%
P value	0.23		

ANOVA test:-Used to compare mean of more than two groups of quantitative data. *P* value <0.05 was considered statistically significant (*) while >0.05 statistically insignificant P value <0.01 was considered highly significant (**) in all analyses.

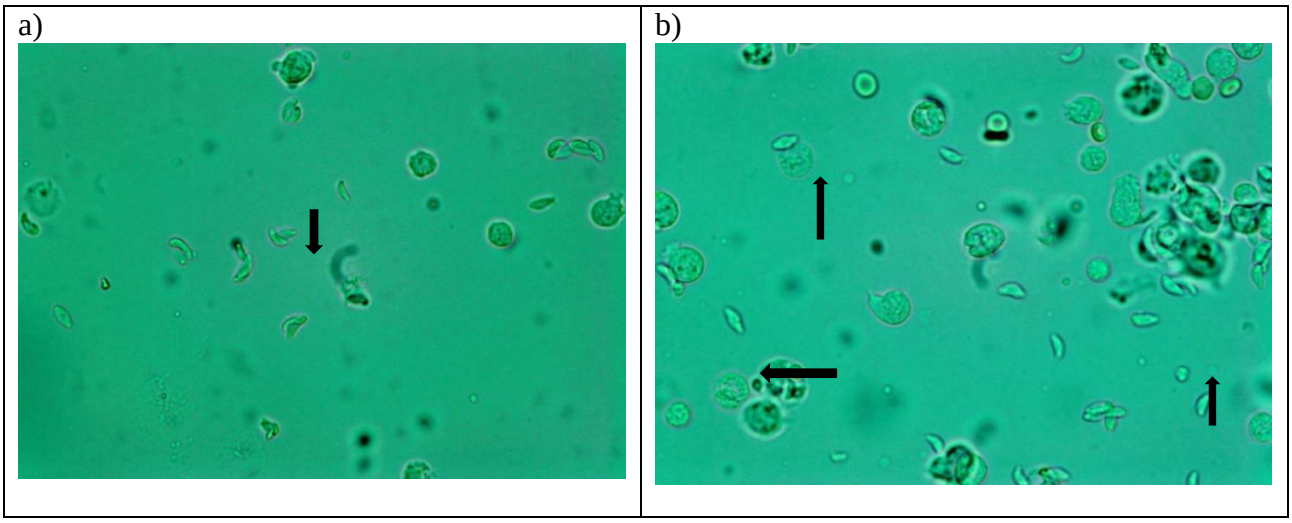
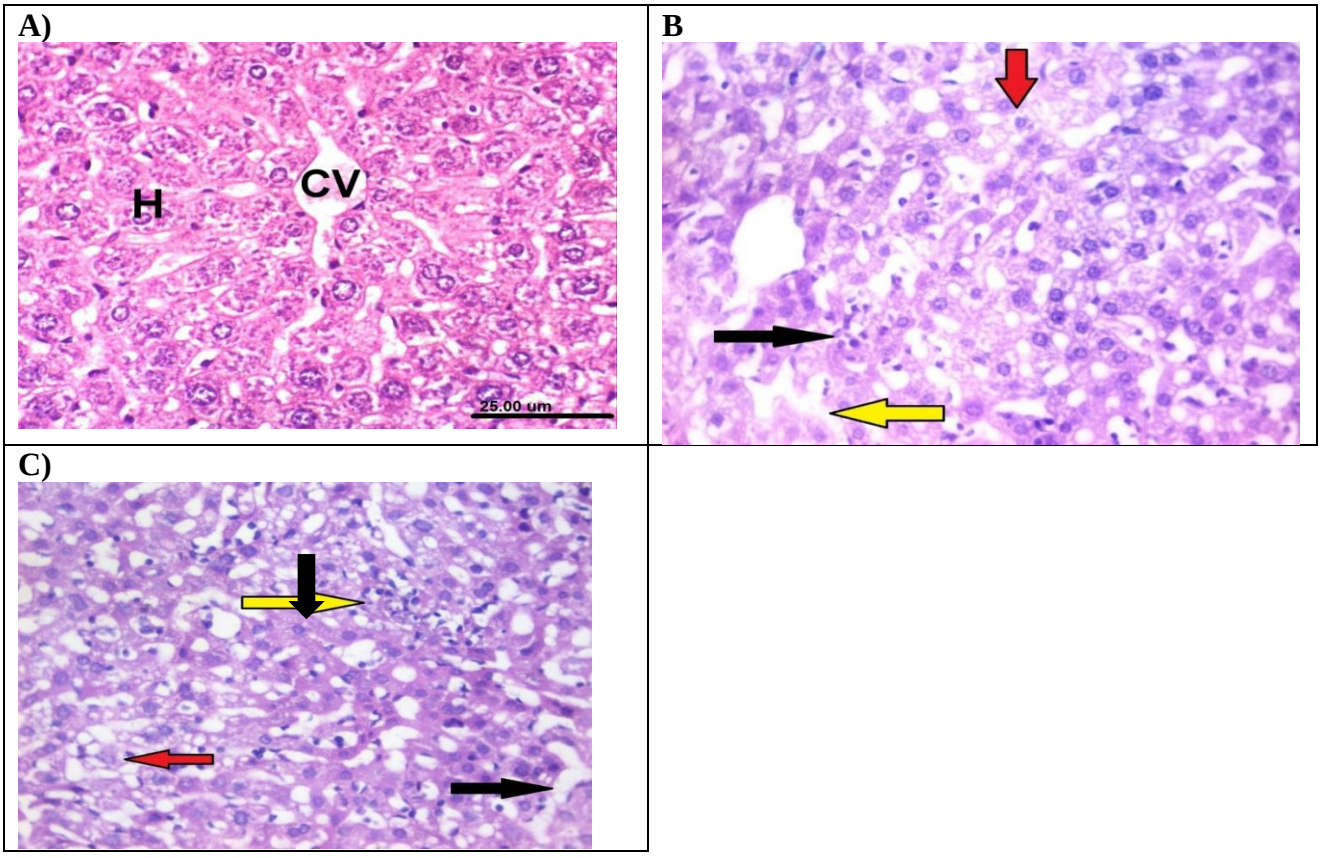
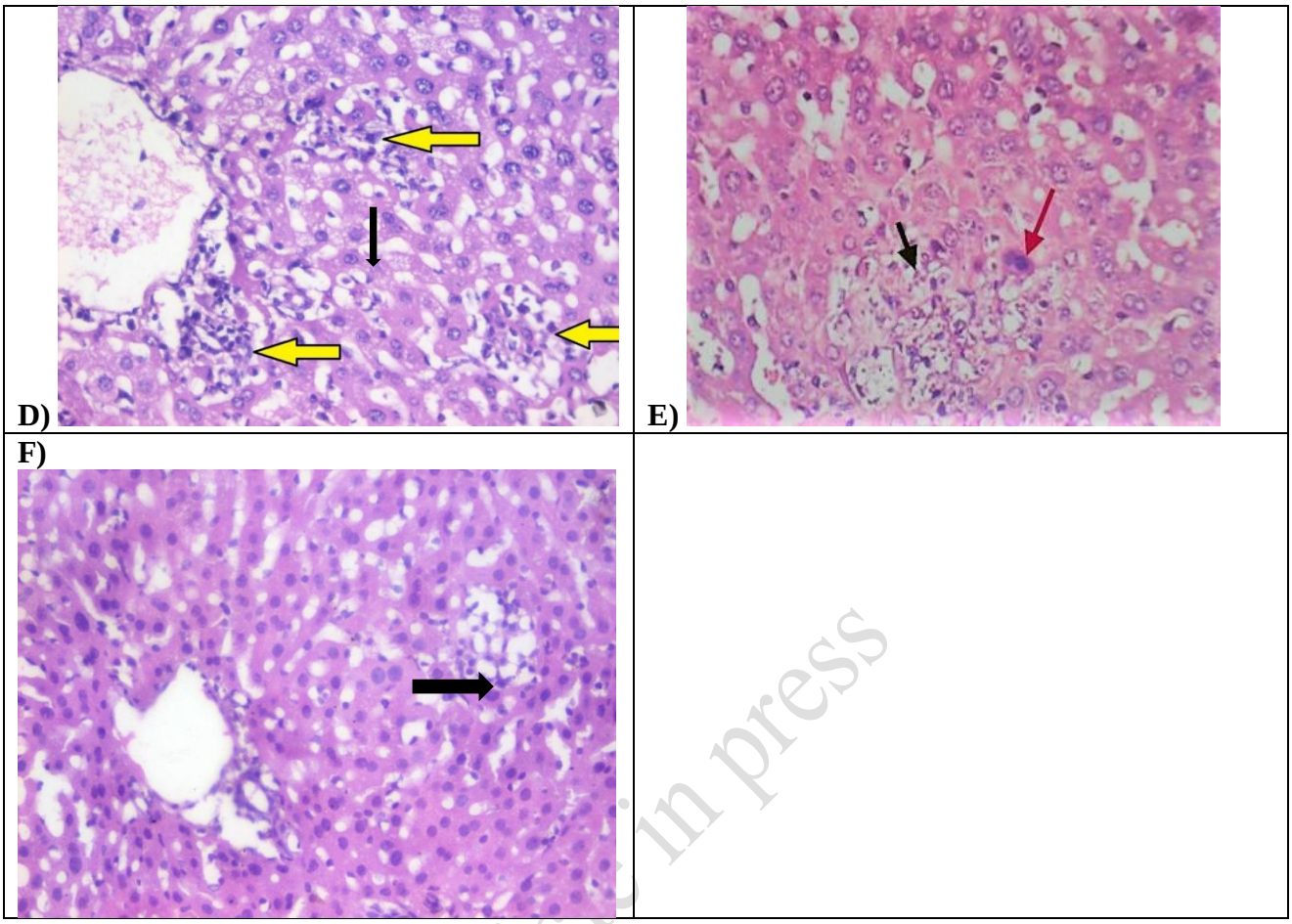


Fig.(2):a)Image from infected not treated group showing normal crescent shape of tachyzoites.(x400).b)Image from treated groups showing distortion of the crescent shape of tachyzoites, swelling of some tachyzoites and deformity of other.(x400).

Histopathological assessment of liver:





Fg.(3):A) Section in mouse liver from non infected not treated group showing the normal histological architecture of hepatic lobule, normal central vein (CV) and hepatocytes (H).(H&E X400). B)Section in mouse liver from infected not treated group showing, focal hepatocytic degeneration (red arrow), lobular mononuclear cellular infiltration around tachyzoites (black arrow) and dilated sinusoids (yellow arrow) (H&E, X400). C)Section in mouse liver treated with spiramycin showing focal hepatocytic vacuolar degeneration (red arrow), focal mononuclear cellular infiltration around tachyzoites (yellow arrow) and hyperplasia of von kupffer cells(small black arrow)(H&E, X400). D)Section in mouse liver treated with ciprofloxacin showing focal hepatocytic vacuolar degeneration, moderate mononuclear cellular infiltration (yellow arrows) and dilated sinusoids ,free tachyzoites (black arrow)(H&E, X400). E) Section in mouse liver treated ciprofloxacin loaded on silver nanoparticles with showing free tackyzoites (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (red arrow) (H&E, X400).F)Section in mouse liver treated with silver nanoparticles showing more or less preserved hepatocytes and occasional portal and lobular mononuclear cellular infiltration(black arrow) (H&E, X400).

Discussion

T. gondii infection in humans is still difficult to treat [13]. Spiramycin (SP) and azithromycin are the most often utilized medications for treating asymptomatic T. gondii infection in the general population or pregnant women. However, the lack of brain penetration and poor bioavailability, may limit the full therapeutic potential of these drugs. The idea of combined therapy is currently the treatment of choice for toxoplasmosis. In the treatment of toxoplasmic encephalitis in humans, combination treatments such as pyrimethamine-sulfadiazine, pyrimethamine-clindamycin, and atovaquone-clindamycin were widely applied. However, long-term usage of these combinations may result in serious negative effects [14**].**

The present work studied the therapeutic effects of ciprofloxacin and ciprofloxacin loaded on silver nanoparticles against the virulent *T. gondii* strain.

This study showed that there was significant reduction in mean tachyzoite count in all treated groups in comparison to infected untreated group**.** The highest percentage of reduction was shown in Spiramycin treated group (92.2%) ,followed by ciprofloxacin loaded on silver nanoparticles treated group (85.8%). Our result coincide with another study [7**],** who reported that examining impression smears stained with Giemsa from several tissues (brain, liver, and spleen) of virulent RH infected mice subgroups revealed a considerable reduction in parasite count in the infected group treated with Spiramycin .The efficacy of Nitazoxanide (NTZ), Spiramycin (SP) and SP-metronidazole was evaluated against the virulent RH *T. gondii* strain in acute experimental toxoplasmosis by[14**]**. Spiramycin and SP-metronidazole were effective against acute murine toxoplasmosis and induced abnormalities in the tachyzoites ultrastructure, according to the results, SP-metronidazole gave the best results on both mice survival rate and parasite load in the brain and liver.

In this work, there was a significant reduction in the mean tachyzoite count in ciprofloxacin treated group in comparison to infected control group with 75.9% as a percentage of reduction**,** this result coincide with a previous study[8**],**who reported that Ciprofloxacin derivatives significantly reduced the number of parasitophorous vacuoles harboring tachyzoites compared with untreated control group. Fluoroquinolones recognize DNA replication inhibitors that target prokaryotic

type II topoisomerases (DNA gyrase and toposomerase IV), causing apicoplast genome replication suppression and thus affecting parasite vitality [4**].**

The efficacy of ciprofloxacin was enhanced after it was loaded onto silver nanoparticles, as nanoparticles improve medication delivery to tissues[16**].**

Enrofloxacin is a fluoroquinolone antibiotic used in veterinary medicine to treat bacterial infections. Treatment with enrofloxacin was more efficient than sulfadiazine in reducing T. gondii infection index in human foreskin fibroblast (HFF) cells. Enrofloxacin and sulfadiazine were similarly found to significantly reduce the percentage of infected cells by RH strain tachyzoites as compared to untreated cells. The antibiotic enrofloxacin inhibited parasite multiplication by 60%. [17**].**

In this study, silver nanoparticles treated group showed significant reduction (62.5%) in mean tachyzoites count, this is attributed to anti *toxoplasma* activity of silver nanoparticles. This result coincide with another study [18**],** who reported that the number of parasites in the liver and spleen of Swiss mice infected with tachyzoites of *T. gondii* virulent strain RH were reduced after treatment with silver nanoparticles (AgNP) and chitosan nanoparticles (CSNP + AgNP).

The increased concentration of interferon-gamma (IFN-) in response to Ag NPs explains the results of the parasite burden decrease in tissues. The effect of biogenic silver nanoparticles (AgNp-Bio on HeLa cells infected with *T. gondii* (RH strain) was evaluated by other researchers[19**]**; they noticed that infection, proliferation, and intracellular parasite load all significantly decreased. There was also an increase in reactive oxygen species (ROS) and interleukin-6 (IL-6) levels. Furthermore, the study of the parasite's action mechanisms revealed that AgNp-Bio acts directly on tachyzoites, causing depolarization of the mitochondrial membrane, an increase in ROS, the accumulation of lipid bodies, and the triggering of an autophagic process, as well as damage to the parasite membrane and phosphatidylserine exposure.

Concerning the survival rate ,there was non-significant difference in the survival rate between treated groups and infected untreated group**.** This, may be due to in adequate dose or inadequate duration of treatment and this coincide with Grujić et al.,[20**]** who reported that, despite some dose-dependent increase of survival, treatment with Spiramycin at doses of 100 and 200 mg kg/day had only a minimal effect on survival rate and was unable to protect mice from death.

In this work, there was no improvement in survival rate in ciprofloxacin treated group, our study coincide with another [8**],** who examined the activity of the Cipro derivatives *in vivo* in a model of acute murine toxoplasmosis. They compared Cipro treatment (7-day treatments) with Cipro derivatives and found that 13–25 percent of mice survived for up to 60 days post-infection in Cipro derivatives, whereas total death was reported 10 days post-infection in Ciprofloxacin treated animals.

In this study, morphological examination of tachyzoites by light microscopy revealed deformity of organisms in the peritoneal exudates from mice of different treated experimental groups, tachyzoites from the infected control mice appeared elongated, often crescent-shaped with a rounded pole at one end while in treated groups, there was swelling and deformity in shape of tachyzoites**.**

Gaafar et al., [18**],** suggested that the alterations in the morphology of the organisms could be secondary to changes caused by medication interference with parasite DNA synthesis or the folic acid cycle. Spiramycin, like other macrolides, works by attaching to the bacterial 50S ribosomal subunit, blocking bacterial protein synthesis [21]. Ciprofloxacin inhibits DNA gyrase and topoisomerase IV, which decreases parasite survival by inhibiting DNA replication [4]. The cytotoxicity of ciprofloxacin was evaluated in *Giardia lamblia* culture, with optical and transmission electronic microscopy used to examine morphological changes. Ciprofloxacin caused morphological changes in the cells as well as a loss of membrane integrity [22**].**

Our study showed morphological alteration in shape of tachyzoites in silver nanoparticles treated group this coincide with Sousa and Poiares-da-Silva study [23**],** who evaluated the effects of nano silver colloid on tachyzoites and bradyzoites of *T. gondii*. After 2 hours of incubation with nan osilver colloid, tachyzoites of *T. gondii* revealed unusual and irregular shape. Furthermore, treatment with silver nanoparticles resulted in motion paralysis and deformities in peritoneal exudate tachyzoites [18].

Conclusion

Ciprofloxacin has potential effect on toxoplasmosis and its efficacy was increased by loading it on silver nanoparticles that was shown by decrease tachyzoites count and improvement of pathological picture in liver.

Re

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