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Spiramycin-chitosan Nanoparticles Decline Parasite Burden and Renovate Patent Histopathological Changes in Liver and Lung in Mice Experimentally Infected with Acute Toxoplasmosis

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THE present study was designed to evaluate the use of nanoparticles in improving the antiparasitic effect of Spiramycin, Spiramycin loaded on Carboxymethyl chitosan nanoparticles (CMC Np),) on experimental toxoplasmosis. Different doses of the drugs under study were assessed using parasitological and histopathological investigations. For this purpose, a total of 38 female Swiss albino mice were divided as follows; Group 1 (5 mice) as a negative control group (non-infected, non-treated group). Group 2 (5 mice) as a positive control group (infected, non-treated group); Group 3: In this group, 28 mice were infected, and treatment started after 10-15 days post-infection for 1 week on a daily basis. The third group was subdivided into four subgroups (7 mice for each), which were treated as follows; subgroup (3a); the infected mice treated with Spiramycin alone in a dose of 100 mg/ kg /day orally, sub group (3b); the infected mice treated with CMC Np orally. Sub group (3c); the infected mice treated with Spiramycin loaded on CMC Np of concentration of 0.35 gm/100 ml H_2O (low dose-LD) orally. Subgroup (3d); the infected mice treated with Spiramycin loaded on CMC Np of concentration of 0.70 g/ 100 ml H_2O (High dose-HD) orally. At the end of the experiment, liver and lung were dissected for detection of the parasite burden and the histopathological examination of the tissues was carried out to allocate the histopathological findings in these organs and detection of tissue cysts. Remarkably, a noticeable decrease in parasitic load was stated pooled with renovation of histopathological alterations were prominent with treated groups in association with infected non-treated group.

Keywords: Acute toxoplasmosis**,** Spiramycin, Carboxymethyl chitosan nanoparticles

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite scattered all over the world. It infects warm-blooded animal, counting mammals and birds, as well as humans [1]. Taxonomically, Toxoplasma belong to phylum Apicomplexa, Class: Conoidasida, Order: Eucoccidiorida, Sub order: Eimeriorina, Family: Sarcocystidae, Subfamily: Toxoplasmatinae [2].

T. gondii has three infective stages, namely tachyzoites, bradyzoites (in tissue cysts), and sporulated oocyst [3]. Infection with *T. gondii* occurs through many routes, including horizontally via oral consumption of sporulated oocysts within contaminated food and water, and digestion of bradyzoite cysts in undercooked meat as well as tachyzoites transmitted vertically from mother to offspring transplacentally or congenitally [4].

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Additionally, organ transplantation and blood transfusion are other routes of transmission but less common [5].

T. gondii tachyzoites have the ability to invade all body-nucleated cells, therefore, cysts can be distinguished in most organs. However, some organs are more susceptible than others are to containing cysts [3].

Infection with *T. gondii* is asymptomatic in 80% of *immunocompetent* hosts [6]. Self-limited mutual non-tender cervical lymphadenopathy is the distinctive sign of the infection, which may be associated with fevers and myalgia. *T. gondii* develops a latent infection due to the formation of dormant bradyzoite cysts inside tissue, but may reactivate again causing destructive effects in the invaded organs [7]. The impact of latent brain infection on behavior and psychological health in immunocompetent people is ongoing [8], while there is no relation between latent infection and psychiatric illness or behavioral abnormalities in infected people [9]. In contrast, infection is frequently fatal in immunocompromised persons, leading to encephalitis with distinctive brain, pulmonary and ocular lesions [10].

Congenital infection can also result in severe toxoplasmosis, which can affect up to 0.5% of newborns and ranges from asymptomatic to severe, with the majority of symptoms occurring in the brain and eyes [11].

The gold standard for diagnosing *T. gondii* infection typically assumed the sequestration of *T. gondii* by bioassay using lab animals. Potential specimens include excretions, body fluids, lymph nodes, muscle, and brain tissues [12]. In addition, many serological tests are used for diagnosis such as dye test (DT), modified agglutination test (MAT), enzyme-linked immunosorbent assays (ELISA), immunosorbent agglutination assay (ISAGA), indirect fluorescent antibody test (IFAT), and indirect haemagglutination assays (IHA) [13].

Real-time PCR used to estimate the course of toxoplasmosis and the efficiency of treatment as it could define the severity of the infection [14]. Matched to conventional PCR and nested-PCR, the real-time PCR assay is thought to be the most actual technique for detecting congenital toxoplasmosis [15].

The drugs (like a combination of pyrimethamine and sulfadiazine, azithromycin, Clindamycin, and dapsone) are used to treat toxoplasmosis, but occasionally these drugs have harmful side effects and lengthy courses last from a few weeks to over a year, from this standpoint, there has become an urgent need to find alternative methods of treatment [16].

Spiramycin is another substantial drug used for the treatment toxoplasmosis. It is also used to treat infections of digestive, respiratory, urinary, and reproductive systems [17]. Spiramycin is a safe antibiotic, it also, it hinders the parasite from entering the fetus over the placenta and has little fetal toxicity so it is applied to inhibit the transmission of *T.gondii* from the mother to the fetus [18]. It did not, however, reach useful concentrations in the brain and showed inadequate penetration across the blood-brain barrier (BBB) [19, 20]. Moreover, if such antibiotics are administered during the first trimester of pregnancy, they may have teratogenic consequences [21]. Furthermore, tissue cysts can remain latent in immunocompetent persons and reawaken in immunocompromised patients because existing medications like pyrimethamine and sulfadiazine are only active against tachyzoites and not bradyzoites. [21, 22].

Treating Toxoplasma encephalitis with spiramycin is not recommended since the medication does not pass the blood-brain barrier. [23]. A number of variables, including membrane permeability and solubility, influence Spiramycin's bioavailability. Studies were directed to improve the medications' water solubility and degree of dissolution, which are both poor [24, 25].

Employing nanoparticles to conveyance medications and regulating their discharge after binding to targets permits for local drug concentrations to be adujsted [26]. Chitosan nanoparticles (CS NPs) are a hopeful drug transport technology that can be used for vaccine transfer, oral drug administration, and drug distribution to the eye and brain [27]. Therefore, the current study was planned to estimate the use of Spiramycin loaded on Carboxy Methyl Chitosan nanoparticles *(CMC nanoparticles* experimentally on mice infected with acute toxoplasmosis using parasitological, and histopathological molecular parameters.

Material and Methods

Ethical Considerations

This study approved by the research ethics committee of the National Research Centre**,** Egypt, under approval number 6122012023.

Parasites

ME49 strain of *T. gondii* were provided by Zoonotic Department of National Research Center (NRC), Egypt. The strain was regularly reserved alive by recurrently oral suckling of Swiss albino mice with 0.1 ml of earlier infected mice's brain homogenate, which enclosed around 1×10^2 tissue cysts/ml, every eight weeks in order to progress acute toxoplasmosis [28]. Infected mice were anesthetized and sacrificed by cervical vertebral dislocation [29].

Experimental animals

A total of 38 laboratory-bred female Swiss albino mice, 6 weeks old, weighing roughly 20-25 grams, the mice were kept in white wood chips-filled plastic cages with five mice each, with unlimited access to food and water [30].

Preparation of Carboxymethyl chitosan nanoparticles (CSNPs)

CSNPs were prepared based on the modified ionotropic gelation process. Briefly, carboxymethyl Chitosan (0.5 g) was dissolved in 100 ml distilled water and left under stirring for 24 h. Calcium chloride (2 g) was liquefied distinctly in 100 ml of deionized water. Then, the calcium chloride solution was added to the carboxymethyl Chitosan solution dropwise at different concentrations under vigorous magnetic stirring at room temperature. The resulting suspension was then left under ultra-sonication for 45 min.

Spiramycin loaded on Carboxy Methyl Chitosan nanoparticles (CMC nanoparticles)

To get a final Spiramycin-CMC NP poly-load, 0.35 and 0.7 g of spiramycin were liquefied in 100 milliliters of distilled water. The mixture was then added to a solution of carboxymethyl Chitosan nanoparticles at the same molar ratio while being stirred for 30 minutes. After that, the suspension was subjected to ultrasonication for 45 minutes, and after that, it was agitated for an additional 30 minutes [31].

Experimental Procedure

a. Animal inoculation for histopathological investigation

Uninfected mice were inoculated orally with 0.1 ml of the brain cyst suspension (10 cysts per mouse), then all of the infected mice were slaughtered at 8 weeks post infection, and their brains were collected, split into two halves. One-half was utilized to count the number of cysts, and the other half was fixed in 10% formalin for histological analysis.

b. Grouping and sampling

The 38 female Swiss albino mice used in this study were divided as follows: Group 1 (5 mice) as a negative control group (non-infected non-treated group), Group 2 (5 mice) as a positive control group (infected non-treated group), Group 3: It includes animals with the acute phase of the infection. In this group, 28 mice were infected, and treatment started after 10-15 days post-infection for 1 week on daily basis. The third group was subdivided into four subgroups (7 mice for each), which were treated as follows: subgroup (3a), the infected mice treated with Spiramycin alone in a dose of 100 mg/ kg /day orally. Sub group (3b), the infected mice treated with CMC Np (carboxymethyl chitosan nanoparticles) orally, sub group (3c), the infected mice treated with Spiramycin loaded on CMC Np of conc of 0.35 gm/100 ml H_2O (low dose-LD) orally. Subgroup (3d), the infected mice treated with Spiramycin loaded on CMC Np of conc of 0.70 gm/ 100 ml H_2O (High dose-HD) orally (Figure 1).

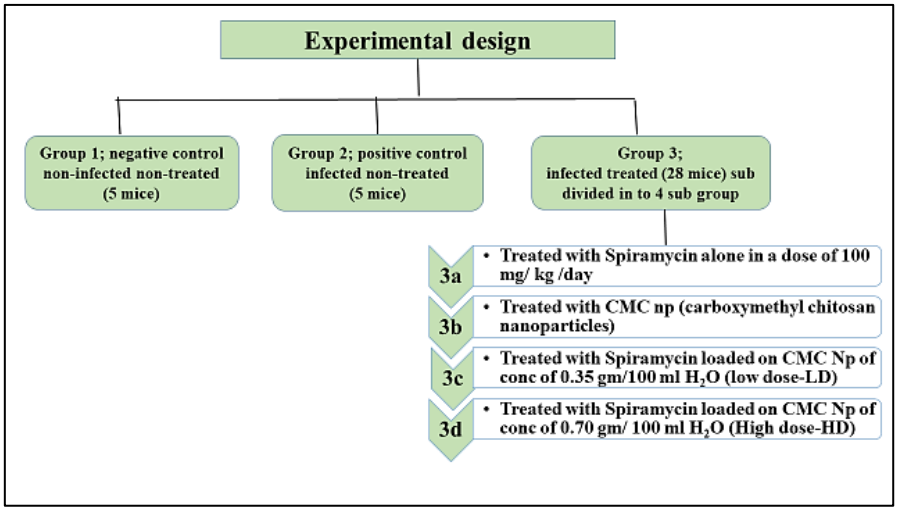

Fig. 1. Experimental design of the studied groups

Histopathological evaluation

All mice were scarified and their organs (liver, and lungs) were removed, and then fixed in 10% formalin, dehydrated in various alcohol concentrations, cleaned with xylol, and embedded in paraffin blocks, then stained with hematoxylin and eosin (H&E) and examined under a light microscope [(32].

Estimation of parasite load

The obtained through determination of the cycle threshold (CT) which marked the cycle when the fluorescence of a given sample significantly exceeded the baseline signal. A lower CT value means higher *Toxoplasma* load (DNA), while a higher CT value means lower *Toxoplasma* load. Finally, negative CT means complete elimination (absence) of the parasite.

Statistical Analysis

In the statistical assessment among the different collections, the significance of difference was tested using: student's t-test, to equate the mean of two groups of numerical information. Paired t-test: to compare the mean of variables in different periods of quantitative data. ANOVA test: Used to compare the mean of more than two groups of quantitative data. P-value <0.05 was considered statistically significant (*), while *P* value <0.01 was considered highly significant (**).

Results

Histopathological findings

Group I: Non-infected -non-treated (Negative control)

The examination of the histological structures of the tissue samples from the liver, and lungs of female mice exposed normal construction and tissue of these organs.

Group 2: Infected non-treated mice (Positive control)

a-The Liver of female mice infected with *T. gondii* showed severe vascular degeneration and necrosis of hepatocytes with congestion hepatic blood vessels **(**Figure 2).

b-The lungs, histopathological examination showed focal areas and peribronchial inflammatory cell infiltration associated with rupture of some alveoli, dilatation of some alveoli, and rupture of some alveolar walls associated with congestion of some pulmonary blood vessels (Figure 3).

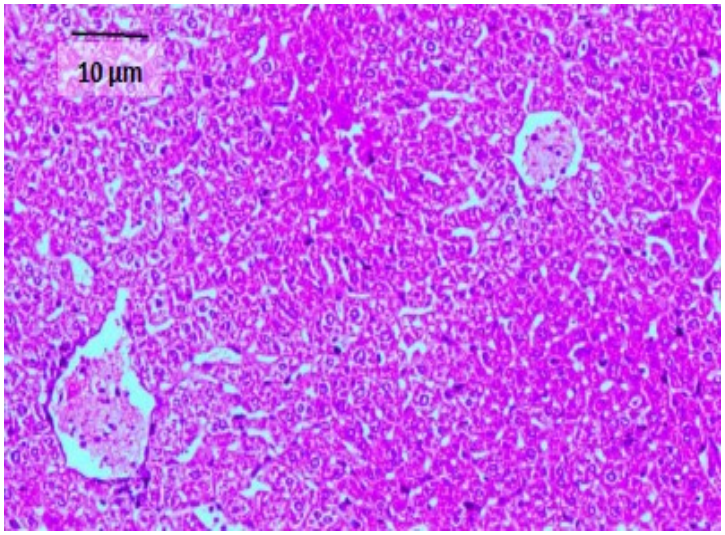

Fig. 2. Liver of female mouse infected with *T. gondii* (Positive control) showing severe vacuolar degenerative and necrosis of hepatocytes with congestion hepatic blood vessels.

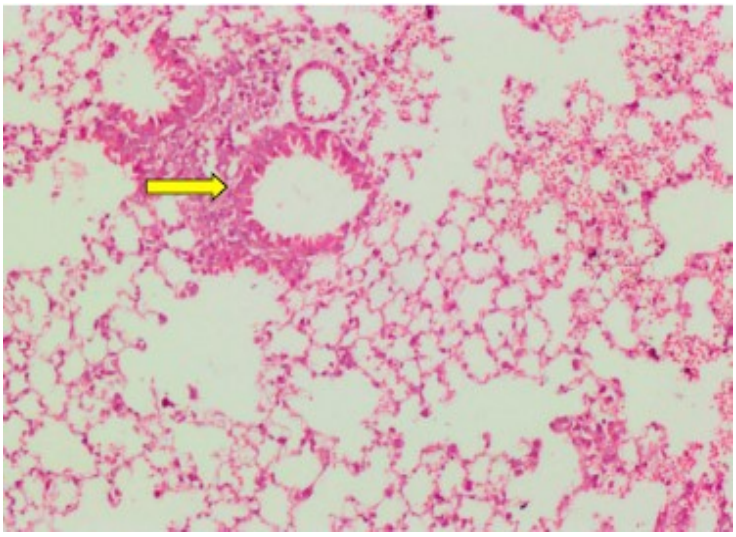

Fig. 3. Lung of female mouse infected with *T. gondii* (positive control) showing focal area and peribroncheal inflammatory cell infiltration (arrow) associated with rupture of some alveoli.

Group3-a. The infected mice treated with Spiramycin 100mg/kg/d alone (after 10-15 days post infection).

a-The liver of female mice infected with *T. gondii* and treated with spiramycin showed vascular degeneration of hepatic cells and congestion of some hepatic central veins with focal aggregation of inflammatory cells infiltration with necrosis of some hepatic cells (Figure 4).

b-The Lungs showed interalveolar inflammatory cell infiltration associated with mild to moderate thickening of alveolar wall and rupture of some alveolar walls forming giant alveoli associated with congestion of some pulmonary blood vessels (Figure 5**)**.

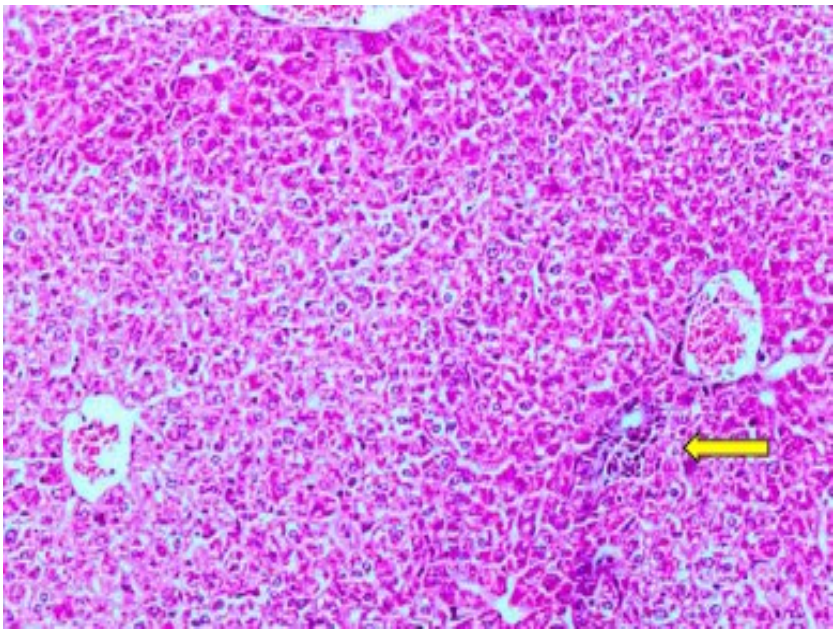

Fig. 4. Liver of female mouse infected with *T. gondii* treated with Spiramycin 10 days post infection showing congestion of some hepatic central veins with focal aggregation of inflammatory cell infiltration (arrow) with necrosis of some hepatic cells

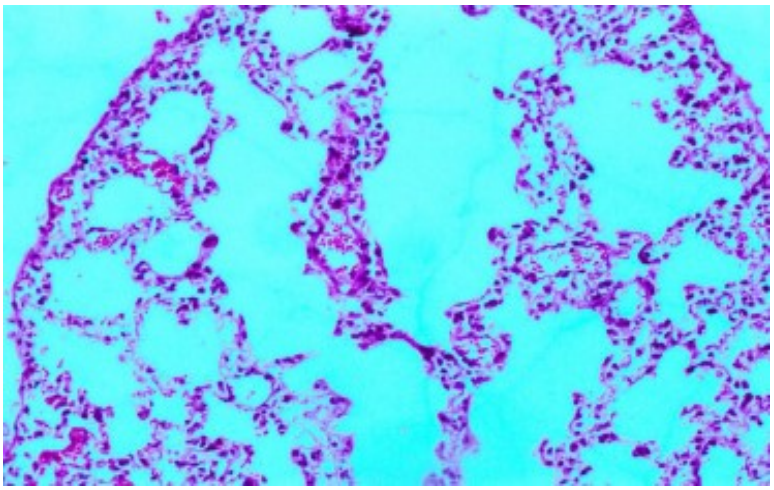

Fig. 5. Lung of female mouse infected with *T. gondii* treated with Spiramycin 10 days post infection showing mild to moderate thickening of alveolar wall with rupture of some alveolar wall forming giant alveoli associated with congestion of some pulmonary blood vessels.

Group 3-b. The infected mice treated CMC nanoparticles alone:

a-The Liver of the infected female mouse with *T. gondii* and treated with CMC NP showed vascular and granular degeneration of hepatic cells associated with a focal area of inflammatory cell infiltration in the portal areas (Figure 6**).**

b-The Lungs showed rupture of some alveolar walls with blood extravasation, rupture of some alveolar walls and focal areas of inflammatory cell infiltration (Figure 7).

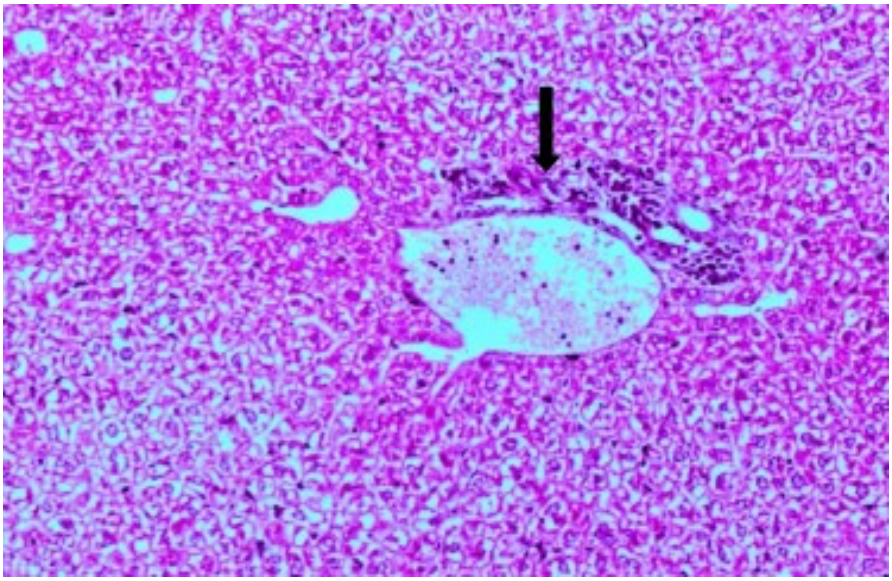

Fig. 6. Liver of a female mouse infected with *T. gondii* treated with CMC NP 10 days post infection showing vacuolar and granular degeneration of hepatic cells associated with a focal area of inflammatory cell infiltration in the portal area (arrow).

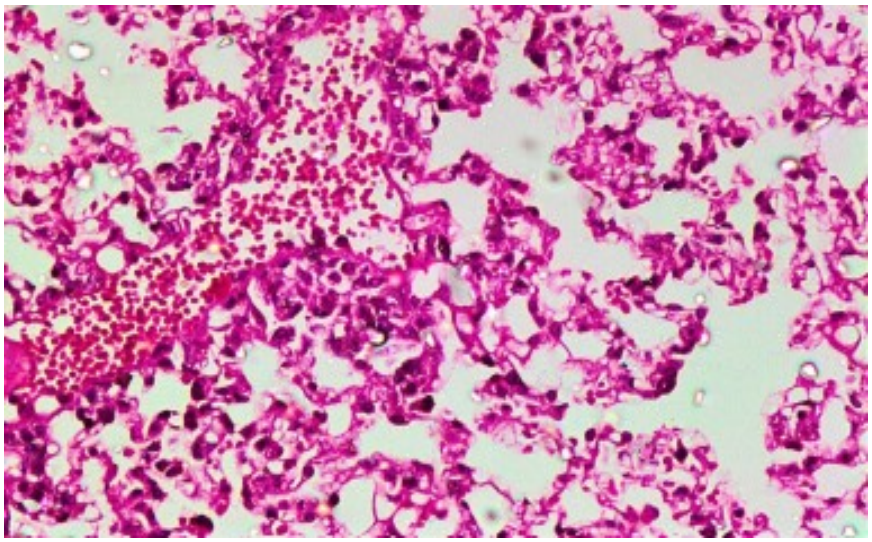

Fig. 7. Lung of female mouse infected with *T. gondii* treated with CMC NP 10 days post infection showing rupture of some alveolar wall with blood extravasation.

Group 3-c: The infected mice treated with low dose of Spiramycin-CMC NPs (0.35gm/100ml water)

a-The liver of mice infected with *T.gondii* and treated with Spiramycin-CMC NPs (0.35gm/100ml water) showed focal aggregation of inflammatory cell infiltration and vascular degeneration of hepatic cells (Figure 8).

b-The Lungs showed rupture of some alveoli forming large ones associated with congestion of pulmonary blood vessels, edema and rupture of some alveoli (Figure 9).

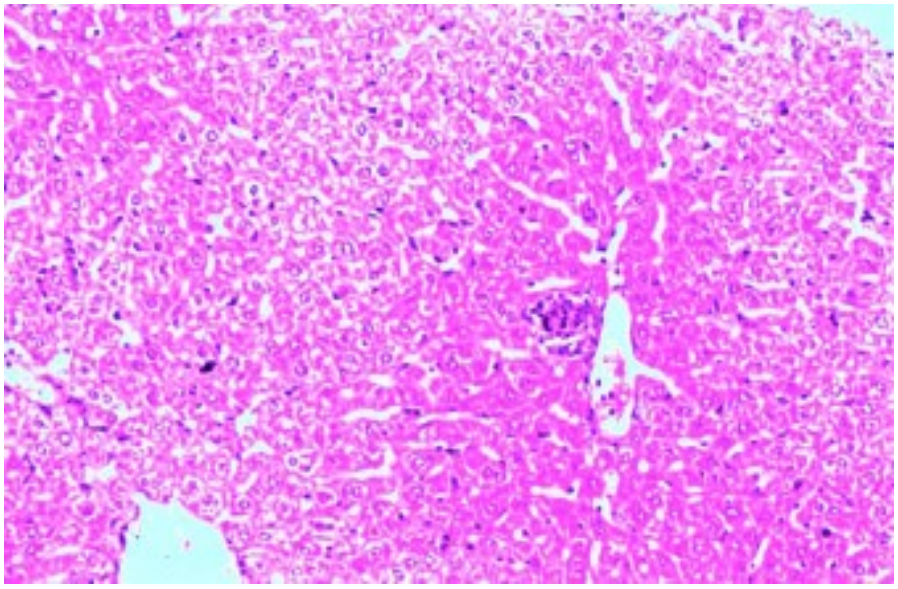

Fig. 8. Liver of female mouse infected with *T. gondii* treated with Spiramycin-CMC NPs (0.35 g/100ml water) 10 days post infection showing focal aggregation of inflammatory cell infiltration and vacuolar degeneration of hepatic cells.

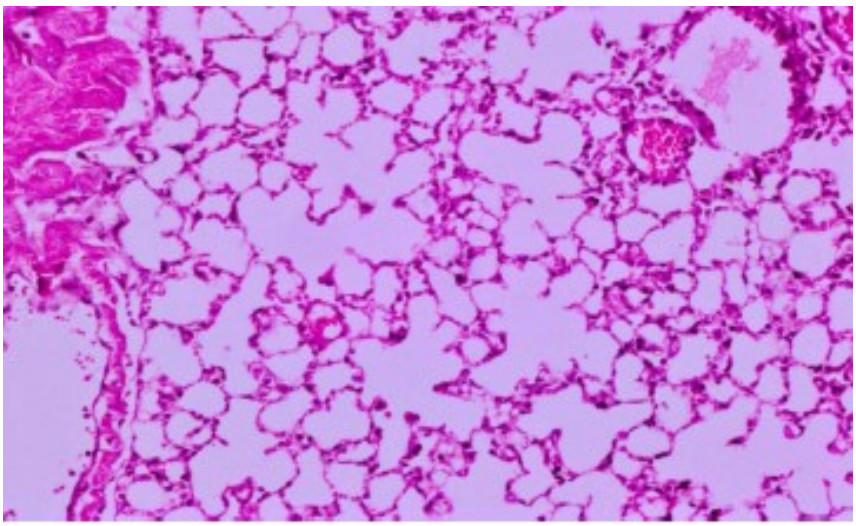

Fig. 9. Lung of female mouse infected with *T. gondii* treated with Spiramycin-CMC NPs (0.35 g/100ml water) 10 days post infection showing rupture of some alveoli forming large one associated with congestion of pulmonary blood vessels and edema.

Group 3-d: The infected mice treated mice with high dose of Spiramycin-CMC NPs (0.70 gm/100ml water):

a-The Liver of the female mice infected with *T. gondii* treated with Spiramycin-CMC NPs (0.70 gm/100ml water) showed mild inflammatory cells infiltration with congestion of blood vessels also mild focal areas and per vascular inflammatory cell infiltration in the portal areas (Figure 10).

b-The Lungs showed focal areas of inflammatory cell infiltration, moderate thickening of some alveolar walls, and rupture of some alveolar walls (Figure 11).

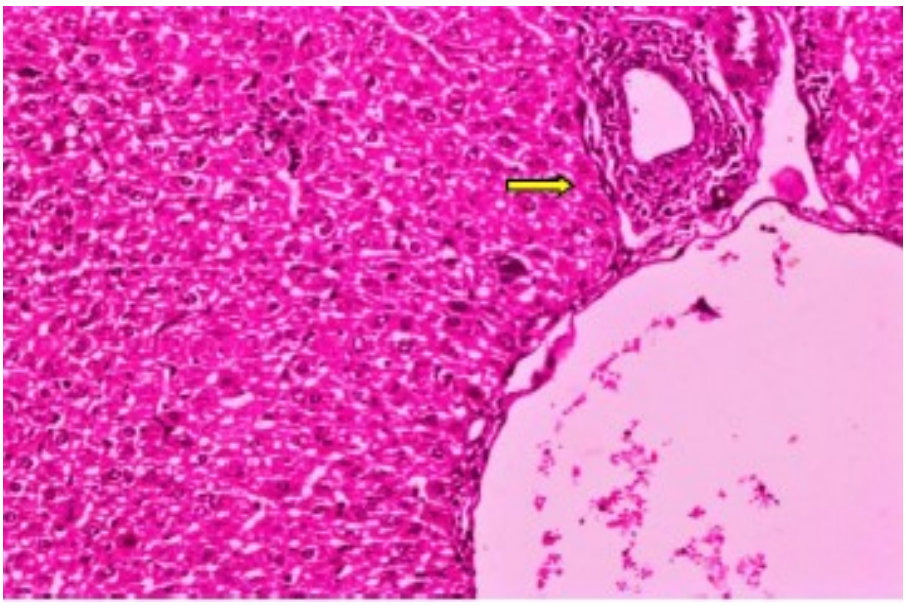

Fig. 10. Liver of female mouse infected with *T. gondii* treated with Spiramycin-CMC NPs (0.70 g/100ml water) 10 days post infection showing mild focal area and perivascular inflammatory cell infiltration in the portal area (arrow).

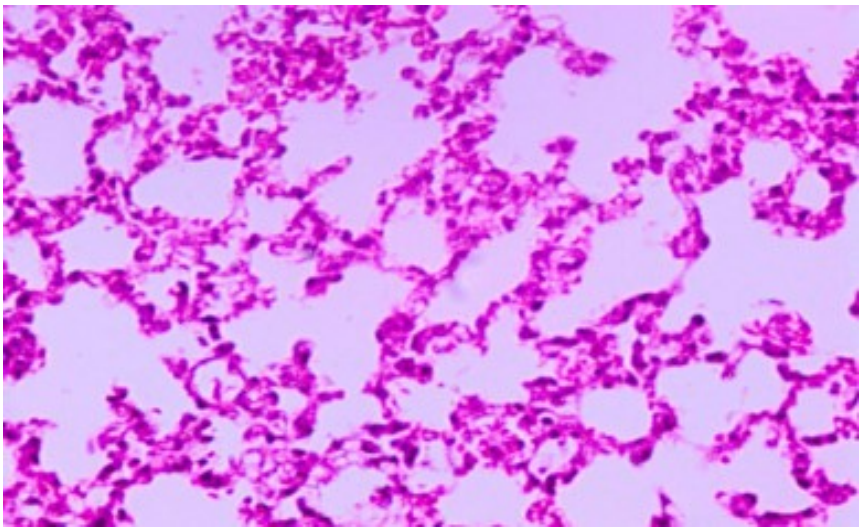

Fig. 11. Lung of female mouse infected with *T. gondii* treated with Spiramycin-CMC NPs (0.70 g/100ml water) 10 days post infection (acute phase) showing moderate thickening of some alveolar wall.

Parasitic load

The results of parasitological assessment represented by average liver parasitic load (ALPL) are shown in Table (1). As illustrated, a significant decrease in ALPL was noticed in the infected animals treated with Spiramycin (G3), CMC NP (G4), and Spiramycin loaded on CMC-NPs of different concentrations G5 and G6 (Table 1 & Figure 12)

From Table (2**),** all tested samples gave positive results except for samples A5 (negative control). The highest Ct samples that means it had the lowest parasite load were as follows: A4 (spiramycin given to the mice with acute infection), A3 (High dose of spiramycin –

CMC NP given to the mice with acute infection) and finally A2 (Low dose of spiramycin – CMC NP given to the mice with acute infection)**.**

TABLE 1. Average liver parasite load (ABPL) of treated mice at different treatment points as compared with untreated group.

Groups	Treatments	Day 14	P-value
2	Control positive	270.24±8.019^{c}	0.0010
3	Spiramycin	207.12±1.211^{b}	0.0080
4	CMC-NPs	170.21±1.261^{b}	0.0040
5	Spiramycin+ CMC-NPs (0.35gm)	116.31±14.212^{a}	0.0001
6	Spiramycin+ CMC-NPs (0.75gm)	97.45±12. 375^{a}	0.0001

Means with different superscripts (a, b, c, and d) within Colum are significantly different at P<0.05.

As shown in Fig. 1, there were significant ($p < 0.05$) statistical differences in the parasitic load and the number of the counted cysts among the treated groups. There was a significant decrease in parasite load in the groups treated with the CMC-NPs substances (G5 and G6) compared to the positive control group (0.0001).

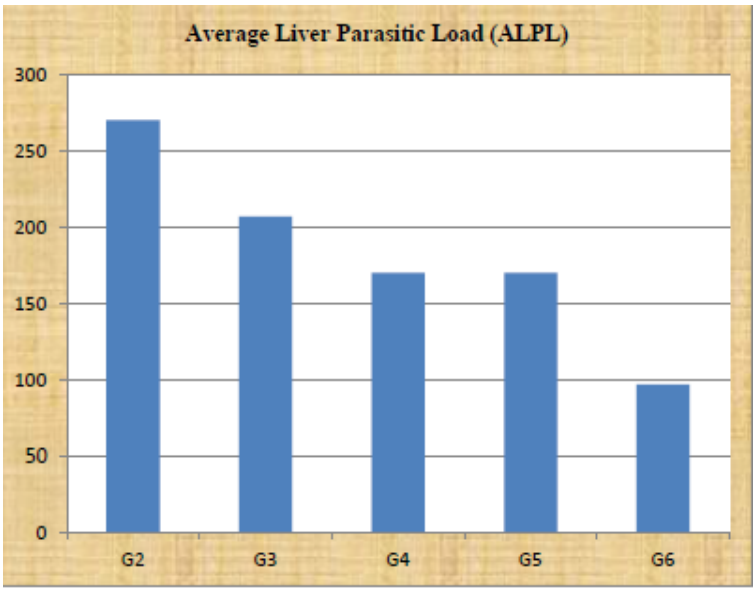

Fig. 12. Average Liver parasite load (ABPL) in 1 mL/liver homogenate of treated mice as compared with untreated mice during the acute phase. Significant differences (G2) vs. other groups are marked by asterisks), (G3 vs. G4 and G5), ((G4 vs. G5 were measured using a one-way ANOVA with Tukey's post hoc test: p _ 0.001).

TABLE 2. The CT, melting temperature of both the standard (A1 &A5) and the treated samples (A2-A4)

Well	Well Name	Assay	Ct	Final	Tm
A1: Infected non-treated group of mice	1	SYBR	24.55	+	82.44
A2: Infected mice and treated with spiramycin – CMC NP 0.35gm/100 ml water after 10-15 days post-infection	2	SYBR	10.20	+	82.94
A3: Infected mice and treated with spiramycin-CMC NP 0.70 gm/100 ml water after 10 days post-infection	4	SYBR	22.30	+	82.44
A4: Infected mice and treated with spiramycin after 10 days post-infection	6	SYBR	15.36	+	81.38
A5: Non infected, nontreated group of mice	ntc	NTC	No Ct	-	71.54

NTC: no template control (negative control).

SYPR green: Gel stain

Discussion

Treatment of toxoplasmosis is a worthy goal to track, especially given that the parasite is intracellular and can pass the blood-brain barrier [33**]**. In the present work, spiramycin, CMC Np, spiramycin loaded on CMC Np in the concentrations of 0.35 gm/100 ml H_2O, and spiramycin loaded on CMC Np of conc of 0.70 gm/100 ml H_2O were evaluated for treatment acute toxoplasmosis regarding parasite load, drug toxicity and histopathology of the liver, and lung of infected female Swiss albino mice.

Our study showed that when compared to the infected, untreated control group, the parasite burden in the liver and lungs of all treated mice decreased. After treatment with spiramycin loaded on CMC Np of conc. of 0.70 g/ 100 ml H_2O, the least amount of parasites was found compared to the other groups regarding the organs tested, and the results were analysed histopathologically. A similar considerable reduction was observed in mice treated with silver nanoparticles alone or in combination with chitosan nanoparticles in a different study **[**34**]**, as well as in mice treated with *Nigella sativa* oil [35]. Additionally, Spiramycin-loaded chitosan nanoparticles used at a concentration of 400 mg/mL to a group of mice that had been infected with acute toxoplasmosis leading to longest survival times, up to 18 days, with no mortality, and considerable drop in tachyzoites compared to the untreated group [19].

In the present work, the histopathological study demonstrated severe tissue damage in the liver, and lung of *T. gondii*-infected untreated mice, with marked

inflammation, congestion, areas of vacuolar degeneration. The free tachyzoites attracted inflammatory cells, which then sparked an inflammatory response and led to cell lysis within tissue sections compared to those of the healthy control and of the treated mice. These results are consistent with other reports from earlier investigations [36, 37, 38].

However, the Me49 parasite strain has a high rate of cyst rupture associated with the cellular immune response, which is why the decrease in the number of liver cysts observed with infection has been explained. [39].

The disrupted liver architecture revealed by the present study in GII was previously reported as a result *T. gondii* infection [40, 41].

The adverse effects on the liver, and lung of *T. gondii*-infected mice were all controlled by the treatments that were applied. The liver of infected untreated mice exhibited extensive vacuolar degeneration, necrosis of hepatocytes, isolated regions of inflammatory cell infiltration, congestion of hepatic blood vessel. These results verified with those provided by [35, 36]

Histopathological analysis of mice liver treated with Spiramycin loaded on CMC Np of conc of 0.35 gm/100 ml H_2O revealed focal aggregation of inflammatory cell infiltration and vacuolar degeneration of hepatic cells. However, mice receiving spiramycin loaded on CMC Np of conc. of 0.70 g/100 ml H_2O displayed vacuolar hepatocyte degeneration, mild inflammatory cell infiltration with blood vessel congestion, and mild focal areas with perivascular inflammatory cell infiltration in the portal area. These findings are consistent with a study by [42] who revealed a noticeable decrease in the number of tachyzoites, inflammatory cellular infiltration, and lobular, portal tract inflammatory reactivity in the infected group that received Spiramycin-Loaded Chitosan Nanoparticles (SLCN), Hagras *et al.,* [20] who demonstrated that the combined effect of spiramycin/Propolis loaded chitosan/alginate nanoparticles on acute murine toxoplasmosis had the best effect in the treatment of toxoplasmosis with no seen capsular edema. Portal inflammation and vascular dilatation were focal and minimal, with lobular mononuclear infiltration.

As regards infection of the lungs, in our study, the histopathological examination showed congested blood flow in some pulmonary blood vessels with dilatation of some pulmonary alveoli and rupture of some pulmonary alveolar walls as reported by [43]. According to the present study, the mice that treated with CMC NP showed discrete areas of peribronchial inflammatory cell infiltration together with the rupture of certain alveolar walls, whereas the mice that inoculated with spiramycin loaded on CMC Np at a conc. of 0.35 gm/100 ml in H_2O, some alveoli ruptured, forming big ones along with pulmonary blood vessel congestion and edema, and the mice treated with Spiramycin loaded on CMC Np at a conc. of 0.70 gm/100 ml in H_2O, some alveolar walls ruptured, while others fused to produce massive alveoli with thickened walls.

Conclusion

Toxoplasma gondii is a common protozoan parasite of warm-blooded animals, it is thought to infect one-third of the world's population of people, while toxoplasmosis in healthy adults is often asymptomatic or relatively mild, immunocompromised or pregnant people are more likely to experience terrible concerns. As a result, finding a safe and effective medication to replace the traditional course of treatment, which includes the use of Spiramycin loaded nanoparticles (NPs) as anti-parasitic medicines has grown significantly, also Chitosan nanoparticles (CS NPs) are a promising drug delivery technology that can be used for vaccine and drugs delivery, additionally increase the therapeutic efficacy of the drugs that combined with them.

Conflicts of interest

"There are no conflicts to declare".

Funding statement

"There is no funding statement to declare".

Author`s contributions

"Authors contribute equally in this work"

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تأثير جسيمات سبيراميسين-شيتوزان النانوية على تقليل عبء الطفيليات وتجديد التغيرات النسيجية المرضية في الكبد والرئة في الفئران المصابة تجريبيًا بداء المقوسات الحاد

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وجد طفيل التوكسوبلازما في جميع أنحاء العالم. وتقريبًا جميع الحيوانات عرضة للاصابة به، بما في ذلك الثدييات والطيور، وكذلك البشر. منذ أن اكتشف نيكول ومانكو الطفيلي في جوندي، وهو قوارض من شمال إفريقيا، في عام 1908، أصبح من المفهوم تدريجيًا أنه سبب انتشار داء الحيوان الحيواني. ومع ذلك، لم يتم فهم دورة حياتها الكاملة أخيرًا إلا في أواخر الستينيات، عندما أدرك أن القطط لعبت دورًا حاسمًا كمضيف نهائي، حيث تأوي دورة الطفيليات الجنسية وتخرج الطور المعدى من خلال برازها.

تم اجراء الدراسة على 38 فأر تجارب مقسمين الى مجموعات كالتالى:

صممت الدراسة الحالية لتقييم استخدام الجسيمات النانوية في تحسين التأثير المضاد للطفيليات للسبيراميسين، والسبيراميسين المحمل على جسيمات CMC النانوية، والجسيمات النانوية CMC على داء المقوسات التجريبي. تم تقييم جرعات مختلفة من الأدوية قيد الدراسة باستخدام التحقيقات الطفيلية والنسيجية. ولهذا الغرض، تم تقسيم إجمالي 38 فأرًا ألبينو سويسريًا على النحو التالي: المجموعة الأولى (5 فئران) كمجموعة مراقبة سلبية (مجموعة غير مصابة وغير معالجة)؛ المجموعة 2 (5 الفئران) كمجموعة مراقبة إيجابية (مجموعة مصابة غير المعالجة)؛ المجموعة 3: في هذه المجموعة أصيب 28 فأراً، وبدأ العلاج بعد 10-15 يوماً من الإصابة لمدة أسبوع يومياً. تم تقسيم المجموعة الثالثة إلى أربع مجموعات فرعية (7 فئران لكل منها)، والتي عولجت على النحو التالي: المجموعة الفرعية (3 أ)، الفئران المصابة المعالجة بالسبيراميسين وحده بجرعة 100 ملغم/كغم/يوم عن طريق الفم، المجموعة الفرعية (3ب)؛ الفئران المصابة التي عولجت بـ) CMC Np جسيمات كربوكسي ميثيل الشيتوزان النانوية) عن طريق الفم ، المجموعة الفرعية (3 ج) ؛ الفئران المصابة التي عولجت بسبيراميسين محملة على CMC Np بجرعة 0.35 جم/100 مل) H2O جرعة منخفضة (LD-عن طريق الفم، مجموعة فرعية (ثلاثية الأبعاد)؛ الفئران المصابة المعاملة بالسبيراميسين المحملة بـ CMC Np بتركيز 0.70 جم/ 100 مل) H2O جرعة عالية (HD-عن طريق الفم. في نهاية التجربة تم تشريح الكبد والرئة للكشف عن عبء الطفيلي وتم إجراء الفحص النسيجي للأنسجة لتخصيص النتائج النسيجية في هذه الأعضاء والكشف عن الأكياس النسيجية. ومن اللافت للنظر أن الانخفاض الملحوظ في الحمل الطفيلي تم ملاحظته مع تجديد التغيرات النسيجية المرضية التي كانت بارزة في المجموعات المعالجة بالاشتراك مع المجموعة المصابة غير المعالجة.

الكلمات الدالة: داء المقوسات الحاد، سبيراميسين، الجسيمات النانوية كربوكسي ميثيل الشيتوزان