Original Article

Assessment of the prophylactic and therapeutic effects of curcumin nanoemulsion in comparison with Spiramycin in mice simulating acute infection with *T. gondii* (RH strain)

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ABSTRACT

Background: Currently, there is no effective adjunct to therapeutic drugs against acute toxoplasmosis. Curcumin (CUR) is one of the most promising naturally occurring agents with anti-inflammatory, anti-oxidant, and anti-carcinogenic activity. It is hypothesized that improvement of CUR properties utilizing nanotechnology may be beneficial in enhancing its therapeutic effects.
Objective: To evaluate the prophylactic immunostimulatory and therapeutic effects of CUR nanoemulsion in acute toxoplasmosis in experimentally infected mice.
Material and Methods: A case-control experimental study was conducted including 45 Albino mice. Mice were divided into a control negative uninfected group I (5 mice) and experimental group II (40 mice) infected with the virulent RH strain to simulate acute toxoplasmosis. Group II was subdivided into four subgroups (10 mice each); IIa (control positive, infected non-treated), IIb (infected and prophylactically pre-treated with CUR nanoemulsion), IIc (post-infection treated with CUR nanoemulsion), and IId (post-infection treated with Spiramycin). The assessment parameters included estimation of the mortality rate, and parasite burden in impression smears from peritoneal fluids, livers, and spleens. Alterations in the tachyzoites morphological features among study groups were recorded using scanning electron microscopy (SEM).
Results: The mortality rate was relatively high (40%) by the 6th day in the infected non-treated subgroup (IIa); with no mortality recorded in all treated experimental subgroups. The prophylactic CUR nanoemulsion pre-treated subgroup (IIb) had the highest percentage of tachyzoites reduction in the peritoneal fluids (78.13%), and in the livers and spleens impression smears (both 88.89%). In the two treated subgroups (IIc and IId), the recorded reduction percentages were 71.88%, 75%, respectively for peritoneal fluids; 81.48% for livers in both subgroups; and 85.42%, 84.73%, respectively for spleen impression smears. Examination of the peritoneal exudates using SEM showed deformed tachyzoites in all the treated subgroups.
Conclusion: Our results indicate that CUR nanoemulsion is as effective as Spiramycin and has a promising medicinal effect on acute toxoplasmosis. Therefore, it may be used as an adjuvant to specific treatment with Spiramycin.

Keywords: curcumin; experimental study; nanoemulsion; prophylaxis; tachyzoites; toxoplasmosis.

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INTRODUCTION

Toxoplasmosis is a world-wild disease, with significant morbid complications in congenitally infected newborns and immunocompromised patients. It is caused by the protozoan parasite *T. gondii*, an obligate intracellular protozoan belonging to the phylum Apicomplexa[1]. In 2019, data from Germany indicated a high rate of toxoplasmosis ranging from 20-77% depending on age. Postnatally-acquired (food-related) infection proved predominant, but maternal-to-fetal transmission still played an important role. While most infections are asymptomatic, congenital toxoplasmosis and reactivated encephalitis in immunosuppressed patients were found to be sources of considerable morbidity[2]. American reviewers[3] claimed that although prevalence of acute toxoplasmosis apparently declined over the past two decades, it is still associated with high morbidity and mortality in the USA. Since they reviewed several previous reports, two issues were observed; low awareness of the disease burden amongst healthcare professionals, and high relapse rates after treatment. Accordingly, the reviewers hypothesized that low awareness of the disease may lead to delayed prescribed treatment or incomplete treatment with subsequent disease relapse[3]. A study conducted in Saudi Arabia showed that approximately one-third of the population had IgG seropositivity, and only 6.4% were IgM seropositive[4]. Among the Egyptian population, the seroprevalence of *Toxoplasma* IgG

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ranged in the different Egyptian Governorates from 3 (El-Wadi-El-Gadded) to 42.5% (Cairo)[5].

In a national perinatal survey to assess age standardized toxoplasmosis serological status among French women from 1995-2016, a decreased seroprevalence of 31.3% was recorded, in association with increase in age, residence in Paris or south-western regions, and in those with higher professional status[6].

Besides, reported drug resistance development of current drugs for acute toxoplasmosis have several adverse effects, which necessitates the search for new anti-*T. gondii* agents[7]. Curcumin (CUR), a hydrophobic polyphenol extracted from the rhizomes of *Curcumina longa*, has anti-inflammatory, anti-oxidant, anti-infectious and anti-carcinogenic activities[8]. The efficiency of CUR extracts was investigated against other infections by several protozoans such as *G. lamblia*[9], *T. cruzi*[10], *C. parvum*[11], *Leishmania* spp.[12], and *P. falciparum*[13], as well as against *S. mansoni* and *S. japonicum*[14]. However, the major disadvantages of CUR are its low bioavailability due to its poor water solubility, and poor absorption associated with its high rate of metabolism and rapid elimination[15]. Hence, CUR nanoemulsion is one of various formulations that were developed to enhance the bioavailability of CUR by improving its solubility rate[16].

Therefore, the objective of the present study is to evaluate the immunostimulatory prophylactic and therapeutic effects of CUR nanoemulsion on acute toxoplasmosis in experimentally infected mice as compared to specific treatment with Spiramycin.

MATERIAL AND METHODS

This case-control study was conducted at Medical Parasitology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt during the period from August, 2018 to January, 2019.

Study design: The study included two main groups of mice representing control negative, and experimental acute toxoplasmosis groups. The latter was divided into four subgroups, infected non-treated; prophylactically pretreated (CUR nanoemulsion); and two therapeutic subgroups treated by either CUR nanoemulsion or Spiramycin. To avoid loss of mice in the experimental subgroups, all mice were sacrificed on the 6th day post infection (dpi) for assessment of tachyzoites burden in the peritoneal fluids as well as in livers and spleens impression smears. Tachyzoites morphological alterations were recorded using SEM.

Experimental animals: Forty-five female Swiss albino pathogen-free mice CD1 strain (*Mus musculus domesticus*), 6-8 weeks age and weighing 20-25 g, were purchased from animal house, Faculty of Medicine, Suez Canal University. Animals were housed in Medical Parasitology Department, Faculty of Medicine, Suez Canal University, under controlled temperature and light conditions. Mice were provided with water and commercial chow *ad libitum*.

Parasites: The virulent RH strain was kindly obtained from Medical Parasitology Department, Faculty of Medicine, Alexandria University. It was maintained by serial intra-peritoneal inoculation of tachyzoites in Swiss albino mice every 3-4 days[17].

Drugs: Spiramycin powder was purchased from Sigma-Aldrich, Germany. Powder containing 0.5 million IU (equivalent to 468.75 mg) was dissolved in 18.75 ml distilled water to reach a concentration of 25 mg/ml. Each mouse was orally given 0.1 ml Spiramycin suspension, i.e. a dose of 100 mg/kg. On the other hand, CUR was delivered as emulsion with a concentration of 25 mg/ml, and each mouse received 0.02 ml; i.e., a dose of 20 mg/kg.

Preparation of CUR nanoemulsion: It was manufactured by Nanotech, Cairo, Egypt using the method previously described[18]. Briefly, hydrophilic, and biocompatible polymer polyethylene glycol with a molecular mass of 400 Da was used for the synthesis of CUR-polyethylene glycol. Preparation of CUR nanoemulsion was by dissolving CUR in polyethylene glycol using ultra sonication[18].

Characterization of CUR nanoemulsion: Using transmission electron microscope (TEM), CUR nanoemulsion appeared as a yellowish brown emulsion. Its mean particle size was 10±5 nm with a spherical like-shape in TEM (Figure 1). It is freely water soluble, and was measured by UV–visible spectroscopy which is one of the most widely used techniques to assess the optical properties. The absorption spectrum showed absorption band with a maximum wave length 425 nm[18].

Mice infection: One ml of the peritoneal fluid was withdrawn after injection of sterile isotonic saline (0.9%) into the peritoneal cavity of infected mice on the 5th dpi. This process was repeated three times to wash thoroughly the peritoneal cavity, and the obtained tachyzoites were washed three times with phosphate

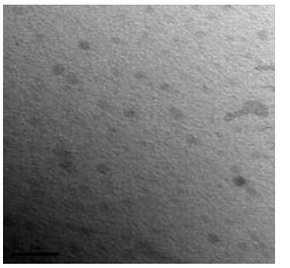

Fig. 1. CUR nanoemulsion shown by TEM

buffered saline (PBS) pH 7.4. Tachyzoites were counted and one ml of the processed peritoneal fluid containing 1.4×10^5 tachyzoites was diluted with 4 ml PBS. Mice were infected by intraperitoneal inoculation of 0.1 ml/mouse, the infective dose was adjusted as 3.5×10^3 tachyzoites[19].

Pilot study: A pilot study was conducted to determine the least effective dose of CUR nanoemulsion. The effect of different doses of CUR nanoemulsion (10, 20, 40 mg/kg/d) for treatment of acute toxoplasmosis was compared. The investigated doses of CUR nanoemulsion used in our pilot study were determined as described by Ghosh *et al*.[20], who assessed the antimalarial efficacy of nanotized CUR. Estimation of the parasite burden recorded in the pilot study confirmed that doses of 20 and 40 mg/kg/d gave the same effect up to the 6th dpi. Therefore, the dose of 20 mg/kg/d was concluded as the lowest effective dose.

Mice grouping: Table (1) describes the study groups.

Mortality rate (MR): It was calculated according to the following equation; MR= (number of dead mice at the sacrifice time)/(number of mice at start of experiment) X 100[24].

Estimation of parasite burden: For each mouse, a peritoneal lavage was performed by injection of one ml PBS using insulin syringe. Injection and extraction was repeated several times (~5-7) with gentle peritoneal massage in-between. One ml peritoneal fluid was carefully collected, and the tachyzoites were counted in 10 µ using Neubauer hemocytometer. The number was multiplied by 100 to obtain number of tachyzoites/ml[25]. The mean±SD was calculated for mice in each group. Three impression smears from different areas of each liver and spleen were stained with Giemsa stain and examined under oil immersion lens[26]. For each mouse, hepatic and splenic burden was estimated by counting the number of tachyzoites in 10 different high-power fields, and the mean number was calculated. Accordingly, the mean number of tachyzoites in each experimental subgroup was determined[27]. The percentages of reductions in the parasite count were calculated according to the following equation: Reduction% (R%) = [(C-E)/C] X 100, where C and E refer to control group and experimental groups, respectively[28].

Tachyzoites morphological features: The SEM study was performed in SEM unit in NRC, Dokki, Giza. The collected suspension of peritoneal fluid was washed twice with PBS, the sediment was fixed in glutaraldehyde, and re-washed 3 times by flooding with large volumes of sterile distilled water. The specimens were fixed for 1 hour in 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) at room temperature, dehydrated through graded ethyl alcohol, and then passed through 100% Freon 113. They were then dried, mounted on aluminum stubs, coated with gold-palladium in a Hitachi sputtering system, and examined using a QUANTA FEG250 scanning electron microscope, FEI Company, USA[29].

Statistical analysis: Results were collected, tabulated, statistically analyzed by statistical package SPSS. Two types of statistics were performed. Descriptive statistics including mean (X), percentage and standard deviation and analytical statistics using ANOVA (*F* test) and probability test (*P* value). Statistical significance was considered at $P<0.05$.

Ethical consideration: The study was approved by the ethical committee of Suez Canal University. Animal experiments were carried out according to The National Research Council's Guide for the Care and Use of Laboratory Animals.

RESULTS

Mortality rate: The highest MR (40%) was observed in the infected non-treated subgroup (IIa). One and three mice died on the 4th and 5th dpi, respectively. All the other subgroups had zero MR till the 6th dpi (with statistical significance ($P<0.01$) and were therefore sacrificed.

Estimation of parasite burden in peritoneal fluids: In the control positive infected non-treated subgroup (IIa), the mean tachyzoites count was $533.33\pm76.38 \times 10^3$/ml. The highest percentage of tachyzoites reduction (78.13%) was detected in CUR

Table 1. Study groups and subgroups.

	No.	Name	Characteristics
Group I	5	Control negative group	Non-infected, non-treated.
Group II	40	Experimental group	Infected by RH strain in a dose of $3.5X10^3$ tachyzoites/mouse[19].
Subgroup IIa	10	Control positive subgroup	Infected, non-treated.
Subgroup IIb	10	CUR prophylactic subgroup	Infected and prophylactically pretreated with CUR nanoemulsion 20 mg\kg orally once daily for 4 days before infection[19,21].
Subgroup IIc	10	CUR therapeutic subgroup	Infected and treated with CUR nanoemulsion 20 mg\kg orally once daily for 5 dpi[22]. Treatment began 4 hours after infection[23].
Subgroup IId	10	Spiramycin therapeutic subgroup	Infected and treated with Spiramycin; 100 mg/kg orally once daily for 5 dpi. Treatment began 4 hours after infection[23].

nanoemulsion prophylactic pretreated subgroup (IIb) with a mean count of 116.67±28.87 X 10^3/ml. This was followed by 75% in the Spiramycin treated subgroup (IId) with a mean count of 133.33±28.87 X 10^3/ml; and lastly 71.88% in the CUR nanoemulsion treated subgroup (IIc) with a mean count of 150.22±50.12 X 10^3/ml (Table 2). The recorded difference between the experimental subgroups was statistically significant (P < 0.01) (Table 2).

Estimation of tachyzoites counts in livers impression smears: In the infected, non-treated control positive subgroup (IIa), the mean tachyzoites burden/10 HPFs was 9.2±1.17 X 10^3/ml. It was obvious that CUR nanoemulsion prophylactic pretreated subgroup (IIb) had the highest percentage of reduction (88.89%) with a drop in mean tachyzoites burden of 1.45±1.33 X 10^3/ml. The mean tachyzoites burden of CUR nanoemulsion treated subgroup (IIc) and Spiramycin treated subgroup (IId) were 1.67±1.53 and 1.67±1.16 respectively, with a similar percentage of reduction of (81.48%) (Table 2). There was a statistical significance (P<0.01) between subgroups (IIb, IIc, IId) and the non-treated control subgroup (IIa) (Table 2). Additionally, livers' smears showed normal impression consisting of multiple blood cells, decreased number of tachyzoites in CUR prophylactic pretreated subgroup (IIb) and CUR treated subgroup (IIc), versus the increased number of tachyzoites in infected non-treated (IIa) control subgroup (Figure 2).

Estimation of tachyzoites counts in spleens impression smears: The mean tachyzoites count/10 HPF in infected, non-treated control positive subgroup (IIa) was 12.04±1.33 X 10^3/ml. The highest reduction percentage (88.89%) occurred in CUR nanoemulsion prophylactic pretreated subgroup (IIb), with a mean count of 1.33±1.53 X 10^3/ml. Percentages of reduction in CUR nanoemulsion treated subgroup (IIc) and Spiramycin treated subgroup (IId) were 85.42% and 84.73% respectively with a mean count 1.75±1.39 X 10^3/ml and 1.83±0.76 X 10^3/ml, respectively.

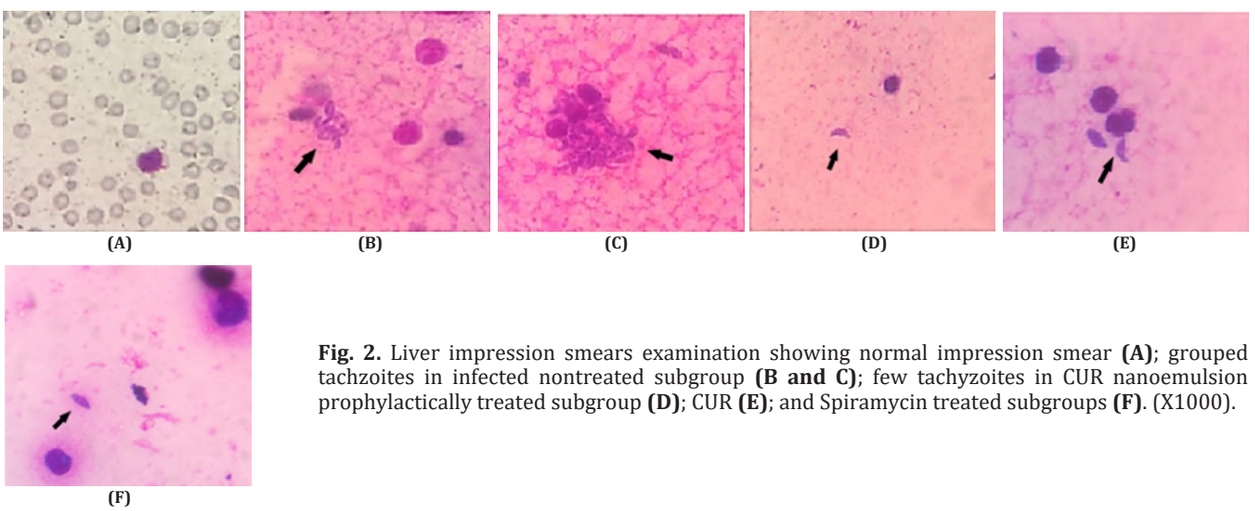

Fig. 2. Liver impression smears examination showing normal impression smear **(A)**; grouped tachzoites in infected nontreated subgroup **(B and C)**; few tachyzoites in CUR nanoemulsion prophylactically treated subgroup **(D)**; CUR **(E)**; and Spiramycin treated subgroups **(F)**. (X1000).

Table 2. The effect of CUR nanoemulsion and Spiramycin treatments on mean tachyzoites count and reduction% in peritoneal fluids, livers, and spleens impression smears of *T. gondii* RH strain infected subgroups.

Subgroups	Mean ± SD X 10^3/ml	Reduction%	Statistical analysis	
			F-test	*P* value
Peritoneal fluids (X 10^3)				
Infected non-treated	533.33 ± 76.38	--		
CUR prophylactic	116.67 ± 28.87	78.13	48.22	**<0.01***
CUR treated	150.22 ± 50.12	71.88		
Spiramycin treated	133.33 ± 28.87	75.00		
Livers impression smears (/10 HPFs)				
Infected non-treated	9.20 ± 1.17	--		
CUR prophylactic	1.45 ± 1.33	88.89	30.43	**<0.01***
CUR treated	1.67 ± 1.53	81.48		
Spiramycin treated	1.67 ± 1.16	81.48		
Spleens impression smears (/10 HPFs)				
Infected non-treated	12.04 ± 1.33	--		
CUR prophylactic	1.33 ± 1.53	88.89	55.11	**<0.01***
CUR treated	1.75 ± 1.39	85.42		
Spiramycin treated	1.83 ± 0.76	84.73		

*: Significant (P < 0.05).

There was a statistical significance (P<0.01) among experimental groups (Table 2). The spleen impression smear of normal mice consisted of marked number of hemic cells (Figure 3A), as compared to a large number of tachyzoites in infected non-treated subgroup (IIa) (Figure 3B). Spleen impression smear examination of both CUR treated (IIc) and CUR nanoemulsion prophylactically pretreated (IIb) subgroups showed few numbers of tachyzoites (Figures 3C and 3D).

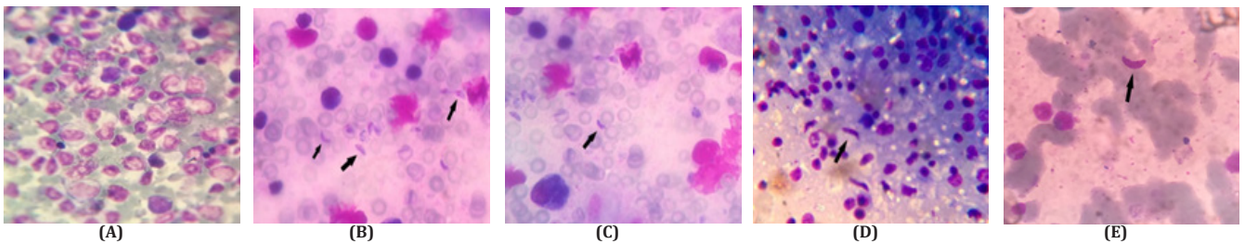

Fig. 3. Spleen impression smears showing normal architecture **(A)**; many tachyzoites in infected non-treated subgroup **(B)**; few tachyzoites in CUR prophylactic pretreated subgroup **(C)**; CUR treated, and Spiramycin treated subgroups **(D and E)**, respectively.

Scanning electron microscopy study: SEM of tachyzoites of *T. gondii* in control positive infected non-treated subgroup (IIa) appeared elongated and were often crescent-shaped with a rounded pole at one end and a pointed pole at the other (Figure 4A). In subgroups treated with CUR nanoemulsion (prophylaxis IIb, or treatment IIc), some tachyzoites were elongated with dimpled surfaces and disorganized conoid (Figure 4B). Other deformed tachyzoites had deep ridges on their surface (Figure 4C). Few were broadened and homogenous, with irregular surface; but the conoid was preserved (Figure 6D). Other tachyzoites were clumped together with some of the previous abnormal characters noticed on their surfaces (Figures 4E and 4F).

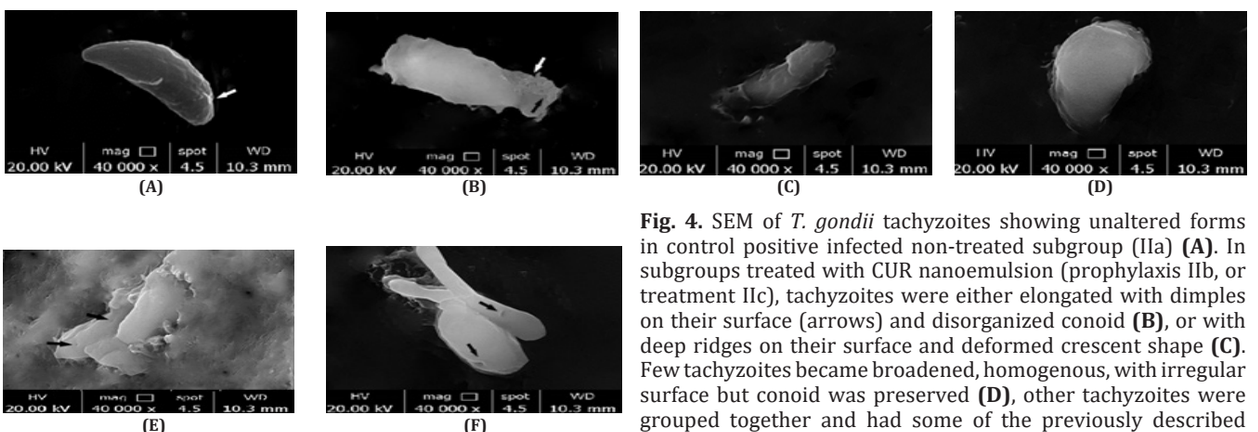

Fig. 4. SEM of *T. gondii* tachyzoites showing unaltered forms in control positive infected non-treated subgroup (IIa) **(A)**. In subgroups treated with CUR nanoemulsion (prophylaxis IIb, or treatment IIc), tachyzoites were either elongated with dimples on their surface (arrows) and disorganized conoid **(B)**, or with deep ridges on their surface and deformed crescent shape **(C)**. Few tachyzoites became broadened, homogenous, with irregular surface but conoid was preserved **(D)**, other tachyzoites were grouped together and had some of the previously described abnormal characters on their surfaces **(E and F)**.

DISCUSSION

It was reported that CUR is stable in acids and at high temperatures, but unstable under light conditions and in alkaline media[15]. Being hydrophobic in nature, it has poor oral bioavailability and instability, besides its rapid metabolism results in low plasma and tissue CUR levels. Efforts were conducted to avoid these restrictive pharmacokinetic characteristics in order to maximize its potential clinical applications. Previous studies proved that nanotechnology improved drug efficacy and decreased its toxicity by increasing bioavailability and preventing its degradation[30,31]. In this respect, the survival time of experimental mice was reported to extend 8-10 dpi with CUR nanoemulsion treatment, versus 5-8 dpi with CUR suspension[32]. Besides, the same investigators conducted another study to evaluate Atovaquone nanoemulsion in treatment of acute toxoplasmosis in comparison to Atovaquone suspension. Results revealed that untreated control mice showed infection after four days of tachyzoite inoculation and died within six days, whereas no signs of infection were reported in mice treated with Atovaquone nanoemulsion until the end of the experiment (14th dpi). Notably, mice treated by Atovaquone suspension died 7-8 dpi[33]. Additionally,

the investigators reported significant differences between the mean counts of peritoneal tachyzoites in both groups. Increased tachyzoites number was significantly high at 4-7 dpi in Atovaquone suspension treatment, while no tachyzoites were detected until the 7th d (end of the experiment) in mice treated by Atovaquone nanoemulsion[33].

In the current study, 40% MR was observed in the infected non-treated group (IIa), while no mice died in the other subgroups [CUR nanoemulsion prophylactic and treated subgroups (IIb, and IIc, respectively) and Spiramycin treated subgroup (IId)] till termination of the study (6th dpi). This recorded MR agrees with a number of other studies that evaluated the use of nanotechnology or therapeutic drugs or natural products[32,34,35]. Azami *et al*.[32] evaluated CUR nanoemulsion treatment of acute toxoplasmosis in mice, and observed that the mean survival time in all treated groups was longer than that in the non-treated group. The survival time extended 8-10 dpi in CUR nanoemulsion treatment, while mice treated with Soybean oil nanoemulsion died 5-7 dpi[32]. In the same year, Etewa *et al*.[34] evaluated Spiramycin loaded chitosan nanoparticle (SLCN) in treatment of acute toxoplasmosis. The investigators observed that the lowest MR among RH infected mice was recorded in the SLCN-treated group, where 75% of mice survived up to the 7th dpi. In 2020, Allam *et al*.[35] designed a study to evaluate the efficacy of Spiramycin alone or combined with metronidazole (MTZ), nitazoxanide (NTZ), and trimethoprim/sulfamethoxazole (TMP-SMZ), against virulent RH *T. gondii* in acute experimental toxoplasmosis. The investigators observed that 90% of the infected untreated group died by the 7th dpi (the sacrifice time). Meanwhile, none of the mice died after TMP-SMZ or Spiramycin-metronidazole (SP-MTZ) combined therapy, whereas one mouse and two mice died after Spiramycin and NTZ alone, respectively. Two points were concluded from the study conducted by Allam *et al*.[35]. First, it was a good decision to sacrifice all mice on the 6th dpi because 9 mice died by the 7th dpi in the infected non-treated group. Second, only one mouse died by the 6th dpi in Spiramycin-treated group (IId), while none had died by 6th dpi (end of the experiment) in our study. We were cautious to avoid losing mice if we did not sacrifice them by the 6th dpi. In their report, the investigators claimed that SP-MTZ gave the best effect on both survival time and parasite load in liver, spleen and brain. The efficacy of this combination was attributed to parasite protein synthesis inhibition by Spiramycin together with high tissue penetration capacity of MTZ. The second successful combination was SMZ-TMP, and Spiramycin came third, while NTZ was the least effective in parasite load reduction in the examined tissues that may have been due to the low dose used[35].

More recently, Gomaa and Sheta[36] investigated cumin oil seed (CSO) in treatment of *T. gondii* (RH strain)-experimentally infected mice. Results revealed 90% MR in the infected untreated mice at the 7th dpi, while all mice treated with either Septrin™ or CSO were alive till the 9th dpi[36]. Furthermore, in our investigation, survival of mice in CUR nanoemulsion prophylaxis treated subgroup (IIb) up to the 6th dpi correlates with the report of Gomaa *et al*.[37] who investigated the course of infection with *T. gondii* RH strain in mice pre-immunized with gamma-irradiated tachyzoites. In their report, none of the mice in the infected control group stayed alive beyond the 8th dpi, whereas all mice in the immunized group lasted with 100% survival rate. Differences regarding mice survival and mortality rates may be explained by variation in infection routes, the number of inoculated tachyzoites, the different doses of used drugs, mode of drug administration and duration of treatment.

In our current study, the tachyzoite count in peritoneal fluids as well as livers and spleens impression smears recorded statistically significant reduction percentage in CUR nanoemulsion prophylactic subgroup (IIb). El Temsahy *et al*.[38] recorded statistically significant reduction in parasitic counts in the livers and spleens of chitosan immunized mice compared to their corresponding control. However, this reduction in the mean tachyzoites count was found to be more evident in mice receiving encapsulated chitosan in comparison to those receiving booster combination with Freund's incomplete adjuvant. Similarly, Gomaa *et al*.[37] recorded 100% reduction rate in the mean tachyzoite count in impression smears from organs of mice immunized with gamma irradiated tachyzoites.

On the other hand, we found that CUR nanoemulsion (IIc) and Spiramycin-treated (IId) subgroups showed more or less similar results presenting the highest reduction percentage in impression smears of both livers (81.48% in both groups), and spleens (85.42% and 84.73%, respectively). This finding is also in agreement with several studies using nanotechnology[19,32,34] or therapeutic drugs[35] or natural products[32,36]. Gaafar *et al*.[19] recorded that only silver nanoparticles (NPs) and combined (chitosan and silver) NPs produced statistically significant reduction in liver and spleen parasite counts compared to the corresponding control group. A higher growth inhibition rate of tachyzoites (90%) was previouy recorded in mice receiving CUR nanoemulsion with a statistically significant difference in comparison to Soybean oil nanoemulsion (11%)[32]. Etewa *et al*.[34] also reported a significant reduction in tachyzoites count in Spiramycin and SLCNs treated subgroups as compared to other subgroups. Additionally, SLCNs treated group recorded the highest reduction percentage (96.2% and 98.6% for liver and spleen, respectively). Allam *et al*.[35] observed that the percent reductions in counts in the livers of mice treated with TMP-SMZ and Spiramycin-metronidazole were significant as compared to untreated mice, while reductions in the counts in the spleens were

not significant. Recently, both CSO and Septrin treated groups significantly reduced tachyzoite burdens when compared to an infected untreated group. However, the efficacy of the former was comparatively not as potent as the latter[36].

Regarding morphological changes of *T. gondii* tachyzoites in peritoneal fluids, our SEM results showed deformed tachyzoites in pre-immunized and treated subgroups (IIb, and IIc respectively). Similar structural changes were reported in several studies[19,34,36]. The degree of deformity and tachyzoites immobilization were reportedly more pronounced in subgroups treated by silver NPs and combined chitosan and silver NPs. On the other hand, the same report attested that in subgroups pre-immunized with chitosan NPs then treated with pyrimethamine, the tachyzoites were shown to move normally without any signs of deformity[19]. In that study, the investigators suggested that the changes in tachyzoites shape might be secondary to changes resulting from interference of the drugs with parasite DNA synthesis or interference with folic acid cycle[19]. Etewa *et al*.[34] observed that tachyzoites displayed sluggish movement with deformity of the crescent shape in Spiramycin, and SLCNs treated subgroups. In a study conducted recently in Alexandria, the investigators demonstrated several tachyzoites deformities in a CSO-treated group. These included 1) swelling with irregular surface and small thin projections from the anterior end; 2) abnormal elongated anterior portion with multiple deep depressions and an anterior membrane-pore in some tachyzoites; and 3) surface dimples and a huge vesicular swelling[36]. We hypothesized that these changes explain the loss of invasive power and reproduction capability of the treated tachyzoites that in turn lead to decreased parasite burden and consequent increase in the survival time of the treated mice.

In conclusion, CUR nanoemulsion proved to be as effective as Spiramycin and has a promising medicinal effect on acute toxoplasmosis. Therefore, it may be used as an adjuvant to specific treatment with Spiramycin.

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Author contribution: Rageh EA performed mice infection, all parasitological parameters and analyzed the results. El-Gayar EK and Alabbassy MM proposed the study topic, and the study design, interpreted the results, and wrote the draft. Abaza SM supervised and finalized the article for publication. All authors agreed over the authorship and final version of publication.

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