Original Article

Efficacy of *Cuminum cyminum* (L.) seed oil on acute toxoplasmosis: An experimental study on albino mice

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ABSTRACT

Background: The currently available therapeutics for treatment of toxoplasmosis are associated with various adverse effects, highlighting the urgent need for development of safer, more tolerable and cost-effective medications for treatment of such a disease. These properties can be properly afforded by natural compounds.

Objective: The purpose of the current work was to assess the efficacy of cumin seed oil (CSO) in the treatment of acute toxoplasmosis in mice models.

Material and Methods: The total phenol and flavonoid contents of CSO were estimated using Folin_ Ciocalteu assay and aluminum trichloride reaction, respectively. The CSO was orally administered at a dose of 5 ml/kg/d, starting 24 h post infection (PI) and continued for five consecutive days. In comparison to Septrin™, the therapeutic effectiveness was assessed using the following parameters: parasitological (animal survival, parasite burden, viability, and infectivity), ultrastructural (scanning electron microscopy, SEM), immunological (serum tumor necrosis factor alpha, TNF-α), and histopathological (liver, spleen, and brain).

Results: The total phenol and flavonoid contents of CSO were 84.65±0.22 mg gallic acid equivalents /g (GAE/g) and 52.08±0.18 mg quercetin equivalent/g (QE/g), respectively. Both Septrin™ and CSO markedly improved the animal survival and reduced the parasite burdens in different specimens. They significantly deteriorated tachyzoite viability and infectivity. In CSO-treated mice, distorted crescent-shaped tachyzoites, deep depressions, membrane pores and huge vesicular swellings were detected by SEM. The immunological study showed that treatment with either Septrin™ or CSO obviously decreased TNF-α, substantially reduced the necro-inflammatory reaction, and subsequently ameliorated the histopathologic changes in different organs.

Conclusion: The evidences gathered herein support that CSO may be useful as a safe, natural therapy for acute toxoplasmosis considering both the anti-inflammatory and the anti-*Toxoplasma* properties. However, the mode of antiprotozoal action of the oil components should be further investigated.

Keywords: acute toxoplasmosis, albino mice, cumin seed oil, hepatic necro-inflammation, RH strain, SEM, TNF-α.

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INTRODUCTION

Toxoplasmosis is caused by an obligate intracellular coccidian protozoan; *T. gondii*. It has a wide diversity of intermediate hosts and so a worldwide distribution. It is one of the most common parasitic infections affecting human and warm-blooded animals. Besides, *T. gondii* is a vastly significant economic and veterinary medical concern in livestock industry as an essential zoonosis[1]. The global *T. gondii* seroprevalence was estimated to be 32.9% in pregnant women[2]. Moreover, its worldwide prevalence in domestic and wild cats, the key definitive hosts, was reported to be 35% and 59%, respectively[3]. In Egypt, up to 95% of domestic cats are infected with *T. gondii*[4]. Man acquires the infection via ingestion of raw or undercooked meat, containing tissue cysts or by accidental consumption of food and drinks, polluted with mature oocysts. Trans-placental transmission is another principal route of infection that may lead to serious fetal consequences[5]. This protozoan has three infectious forms: bradyzoites in tissue cysts, sporozoites in oocysts and tachyzoites in pseudocysts. Tachyzoites, as rapidly proliferating forms, are the chief cause of the acute phase of the disease and are accountable for deleterious pathologic effects[6]. The infection is usually guarded by the host immune reaction and remains dormant in neural and muscle tissues for life of immunocompetent hosts. Most cases are asymptomatic however, individuals with compromised immune system could undergo reactivation and suffer life-threatening sequelae[7].

Protection against intracellular pathogens such as *T. gondii*, mainly depends on the host cell-mediated immune response. A crucial step in eliminating this parasite is the activation of macrophages and T lymphocytes essentially $CD8^+$ cells for production

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of T helper 1 (Th1) cytokines. In particular, tumor necrosis factor alpha (TNF-α) was reported to clearly elicit a protective role in toxoplasmic retinochoroiditis, by inhibiting the parasite proliferation in retinal pigment epithelial cells[8]. However, in early pregnancy increased levels of TNF-α due to *T. gondii* infection induced excessive inflammatory reaction, leading to abortion[9]. Moreover, AIDS patients with active ocular or cerebral toxoplasmosis were found to have high levels of TNF-α that contributed to the inflammatory-mediated tissue damage[10].

Currently, the recommended treatment regimens of toxoplasmosis include combined therapeutics such as pyrimethamine-sulfadiazine, pyrimethamine-clindamycin, and trimethoprim-sulfamethoxazole. They are commonly used in the treatment of toxoplasmic encephalitis and ocular toxoplasmosis[11,12]. Although the available drugs possess adequate efficiencies, they are often associated with adverse effects as allergy, bone marrow suppression, folic acid deficiency, and hematologic toxicity, osteoporosis, teratogenic effects, and renal problems[13]. These harmful outcomes highlight the urgent need for development of new, safe, and cost-effective medications for treatment of toxoplasmosis.

In this respect, natural stuffs and their composites are the most productive resources for the therapy of a wide scope of diseases. For instance, spices are beneficial bionutrients when inserted in food ingredients or in nutritional supplements. From ancient ages, spices were consumed as additives to enhance food taste and essence. They also comprise plentiful medicinal assets and are manipulated in treatment of several disorders. Cumin is one of the popular spices habitually utilized as a flavoring aromatic in the Mediterranean kitchen[14]. It possesses a tiny hairy, brownish, boat-shaped seed, retaining a spicy sweet aroma with faintly bitter and pungent flavor. It is a chief component of curry and chili powder used for flavoring lots of commercial food products. In conventional medicine, cumin was safely employed as antispasmodic and diuretic agent and also to relieve toothaches, epilepsy, jaundice, dyspepsia, flatulence, maldigestion, and diarrhea[14]. Its scientific name is *Cuminum cyminum* (L.) that belongs to the Apiaceae (Umbelliferae) family[15]. Cumin leaves, flowers, fruits, and seeds are the valuable portions of this plant. The pharmacological examinations revealed that *C. cyminum* possesses insecticidal, anti-inflammatory, analgesic, antioxidant, anticancer, antidiabetic, antiplatelet aggregation, hypotensive, bronchodilator and antimicrobial properties[16,17]. The cumin seed oil (CSO) exhibited an antibacterial influence on various Gram-positive and Gram-negative strains[18]. It showed a wide-spectrum antifungal competence against distinctive pathogenic *Candida* species[19]. Moreover, its antiviral influence on herpes simplex virus-1, was reported[20].

The reported antimicrobial and anti-inflammatory characteristics of *C. cyminum* (L.) and its influence on the pro-inflammatory pursuit of a Th1 cytokine "TNF-α"challenged us to assess CSO efficacy in the treatment of acute toxoplasmosis in mice, experimentally infected with the virulent *T. gondii* RH strain.

MATERIAL AND METHODS

This case control experimental study was conducted during the period from June 2021 to December 2021 at the Medical Parasitology Department, Faculty of Medicine, Alexandria University, Egypt.

Study design: Laboratory mice were infected with *T. gondii* RH strain. The total contents of phenol and flavonoid in CSO were estimated. The efficacy of CSO was evaluated in comparison to Septrin™, through parasitological, ultrastructural, immunological, and histopathological studies.

Experimental animals: Ninety-four laboratory-bred male Swiss albino mice, four to six weeks old, weighing 20-25 g were used in the current study. Animals were housed in a colony room in the animal house of Medical Parasitology Department, Alexandria Faculty of Medicine, with a 12:12 hours light/dark cycle at 20-25°C and 70% humidity. They were maintained in metallic, well-ventilated, clean cages. Food pellets and water were continuously supplied and bedding was changed daily.

Parasite maintenance and animal infection: *Toxoplasma gondii* RH HXGPRT (-) virulent strain was obtained from the laboratory of Medical Parasitology Department, Alexandria Faculty of Medicine, Alexandria, Egypt. It was maintained by serial intraperitoneal (IP) inoculation of tachyzoites in albino mice every five days. One milliliter of sterile isotonic saline (0.9%) was injected into the peritoneal cavity of infected mice on the fifth day post-infection (PI). The peritoneal fluid was withdrawn and examined for tachyzoites under light microscope (x400). This process was repeated three times to wash thoroughly the peritoneal cavity. The obtained tachyzoites were washed three times with phosphate buffered saline (PBS) pH 7.4 and then diluted to be used for experimental animal infection at a dose of 3.5 x 10^3 tachyzoites/mouse through IP route[21].

Tested compounds: Septrin™ oral suspension (40 mg trimethoprim, 200 mg sulphamethoxazole) (GlaxoSmithKline, S.A.E) was implemented as a reference therapy at a dose of 0.5 mg/kg/d[22]. A volume of 5 ml of the Septrin™ oral suspension was added to 995 ml PBS, pH 7.4, to obtain a final drug concentration of 240 mg/L for administration of 50 µl/mouse/d. Cumin seed oil (Sigma Aldrich, Germany) was orally

administered at a volume of 125 µl that was equivalent to the dose of 5 ml/kg/d[21].

Assessment of the bioactive compounds (phenol and flavonoid) in CSO: The total phenol content in CSO was estimated using Folin-Ciocalteu assay[23]. This estimation depends on the reduction of Folin-Ciocalteu reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds present in CSO. The absorbance of the reduced reagent was measured with a spectrophotometer at 760nm wavelength. The total concentration of phenol compounds was calculated and expressed as gallic acid equivalent per gram (GAE/g). The total flavonoid content was estimated by applying the aluminum trichloride reaction method[24]. Absorbance of the reaction mixture was read spectrophotometrically at 510 nm wavelength. The total flavonoid concertation was calculated and expressed as quercetin equivalent per gram (QE/g). Both analytic tests were carried out in triplicates.

Animal categorizing and treatment schedule: Of the total 94 animals included in the study, sixty-four were allocated in four groups (16 mice each) as follows: group I exemplified the normal, uninfected mice (non-infected control), they were injected with one ml PBS, pH 7.4 through IP route. Group II represented the infected, untreated animals (infected control). Group III included the infected, Septrin™-treated mice (therapeutic control). Group IV comprised the infected, CSO-treated animals. The employed treatment regimens began 24 h PI and were maintained for five consecutive days[21]. Six mice from each group were euthanized on the 7th d PI with an over-dose of thiopental sodium. The remaining ten mice in each group, were observed to estimate the survival rate. The other 30 naive mice were allocated to judge parasite infectivity.

Parasitological study

Survival rate: The number of living mice was recorded daily until all experimentally infected mice died. The survival rate was calculated in each group according to the following equation: Number of survived mice/ number of mice at the beginning of the experiment ×100[25].

Parasite burden: Livers, spleens and brains were isolated from euthanized experimentally infected mice. Impression smears were separately performed for each mouse from each organ and stained with Giemsa. *Toxoplasma gondii* tachyzoites were counted in different tissue smears under oil immersion objective (×100) lens. The mean values of ten different fields for each mouse and for each group were calculated[25]. The peritoneal fluid containing tachyzoites was collected separately from experimentally infected mice and centrifuged at 2000 rpm for ten minutes[21]. The sediment was suspended in one ml PBS (pH 7.4) and extracellular tachyzoites were counted three times under light microscope (x400) using Neubeuer haemocytometer. The mean parasite count was calculated for each mouse and for each studied group.

Parasite mean viability rate (VR): Tachyzoites from peritoneal fluid of experimentally infected mice, were separately stained with 0.4% trypan blue to test their viability[26]. Parasite viability rate was determined for each mouse by evaluating the number of viable tachyzoites/100 parasites, under light microscope using high power lens (X40). This step was repeated three times and the mean viability rate was calculated for each group[26].

Infectivity rate (IR): To assess parasite infectivity, ten naïve mice for each infected group (II, III, IV) were intraperitoneally inoculated with tachyzoites previously collected from infected animals, at a dose of $1x10^3$ tachyzoites/mouse. They were named groups II`, III` and IV`, respectively. Seven days later, mice were euthanized, peritoneal cavities were washed, and the IR was determined for each group by calculating the percentage of mice positive for tachyzoites infection (infected mice) following this equation: IR = (Number of infected mice at the sacrifice time/Number of naïve mice inoculated with *T. gondii* tachyzoites) X 100[27].

Ultrastructural study: Tachyzoites obtained from the peritoneal wash of the infected untreated group II and CSO-treated group IV were fixed in 2.5% glutaraldehyde and processed for scanning electron microscopy (SEM).

Immunological study: Two ml of blood was collected separately from mice, in the non-infected control group I and the experimentally infected groups II, III and IV, euthanized seven days PI. Sera were separated and stored at -20°C to be used for quantification of TNF-α using ELISA MAX™ Deluxe Set Mouse TNF-α (Biolegend.com) according to the manufacturer instructions[28].

Histopathological study: Equal parts from the liver, spleen and brain tissues were fixed in 10% buffered formalin, dehydrated in ascending series of ethyl alcohol, cleared in xylol, and embedded in paraffin. Serial sections, 4-micron thick, were cut and processed for staining with hematoxylin and eosin (H&E). They were dehydrated and then mounted in Canada balsam. Tissue sections were examined for pathological changes using standard light microscopy and the severity of inflammation was assessed in a blinded fashion. Both number of necro-inflammatory foci and degree of portal inflammation were assessed in liver sections. The necro-inflammatory foci were scored as (0= <1 focus per lobule, 1= at least one focus per lobule, 2= multiple foci per lobule). Meanwhile, portal inflammation was scored as (0= absent, 1= focal in some portal tracts, 2= focal in all or diffuse in some tracts, 3= diffuse inflammation in all portal tracts). Both scores were grouped in final activity grade using METAVIR

activity grading system[29]. A four-point scale was done, in which A0 indicates no activity, A1: minimal or mild, A2: moderate, and A3: severe activity.

Statistical analysis: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov was used to verify the normality of distribution of variables. Comparisons between groups for categorical variables were assessed using Chi-square test (Fisher or Monte Carlo), ANOVA was used for comparing the four studied groups and Post Hoc test (Tukey) for pairwise comparison. Significance of the obtained results was considered at the 5% level (P<0.05).

Ethical consideration: All animals were handled in strict accordance with the Egyptian National Animal Welfare Standards. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Alexandria University (Approval number: 0305203).

RESULTS

Assessment of the bioactive compounds (phenol and flavonoid) in CSO: The total phenol content of CSO was determined as 84.65±0.22 mg GAE/g, meanwhile the total flavonoid content was calculated as 52.08±0.18 mg QE/g.

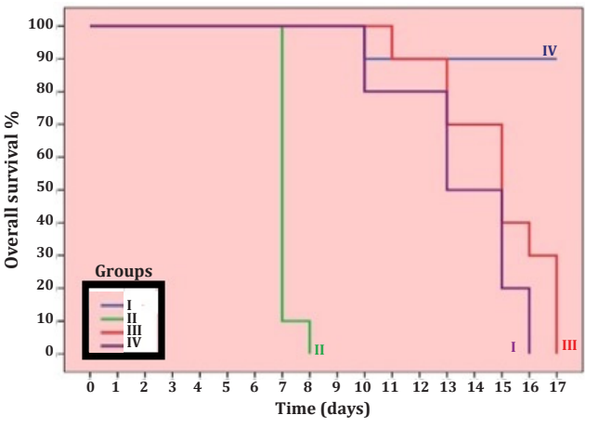

Fig. 1. Kaplan-Meier survival curve for overall survival rate in different studied groups. **Group I:** Non-infected control; **Group II:** Infected untreated control; **Group III:** Infected Septrin™-treated; **Group IV:** Infected CSO-treated.

Parasitological study

Survival rate: Figure (1) shows that 90% of the infected untreated mice (group II) died by the 7th day PI. However, all mice treated with either Septrin™ or CSO (group III and IV) were alive till the 9th d PI. Furthermore, by the 15th d PI, the survival rates of Septrin™-treated and CSO-treated groups were 70% and 50%, respectively. On statistical basis, oral treatment with Septrin™ and CSO markedly elevated (P<0.001) the mean survival duration (14.9±2.0 d, 13.6±2.2 d, respectively), when compared to the infected untreated control group II (7.1±0.3 d) (Table 1). Interestingly, CSO showed an equivalent effect on the animal survival as compared to Septrin™ (P=0.418) (Table 1).

Parasite burden: Both Septrin™ and CSO (groups III and IV) significantly reduced the tachyzoite burdens, when compared to the infected untreated group II (P<0.05) (Table 2). This was evidenced in all studied hepatic, splenic and cerebral impression smears and also in peritoneal fluid of groups III and IV. However, the efficacy of CSO was not as potent as Septrin™, when compared to each other regarding this valuable parameter (P<0.05) (Table 2).

Parasite mean viability rate (VR): By staining with trypan blue, viable tachyzoites appeared crescent in shape with faint blue cytoplasm and dark blue nucleus however, dead ones had dark blue cytoplasm (Fig. 2). Statistically, both Septrin™ and CSO significantly reduced the viability rates of *T. gondii* tachyzoites in comparison to the infected untreated control group II (P<0.001) (Table 3). Meanwhile, considering this parameter as the reference therapy in group III, Septrin™ elicited a significantly (P<0.001) higher efficacy (lower reduction) than CSO (greater reduction) (Table 3).

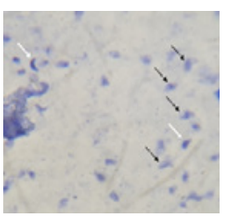

Fig. 2. Parasite viability test with trypan blue stain. Viable tachyzoites are crescent in shape with faint blue cytoplasm (black arrows) and dark blue nucleus however, dead tachyzoites have dark blue cytoplasm (white arrows) (x400).

Table 1. A comparison between the different studied groups according to survival duration.

Days PI	Study groups				Statistical analysis	
	I (n=10)	II (n=10)	III (n=10)	IV (n=10)	*F* test	*P* value
Mean ± SD.	16.3 ± 2.2	7.1 ± 0.3	14.9 ± 2	13.6 ± 2.2		
Median (Min.-Max)	17 (10–17)	7 (7 – 8)	15 (11–17)	14 (10–16)	**47.190**	**<0.001***
***P*0**		**<0.001***	0.353	0.014*		
Significance			***P*1<0.001*, *P*2<0.001*, *P*3=0.418**			

I: Non-infected control. **II:** Infected untreated control. **III:** Infected Septrin™-treated. **IV:** Infected CSO-treated. ***:** Statistically significant (P≤0.05). ***F:*** For ANOVA test, a pairwise comparison between each two groups using Post Hoc Test (Tukey). ***P:*** *P* value for comparing between the studied groups. ***P*0:** *P* value for comparing between group I and each other group. ***P*1:** *P* value for comparing between groups II and III. ***P*2:** *P* value for comparing between groups II and IV. ***P*3:** *P* value for comparing between groups III and IV.

Table 2. A comparison between the experimentally infected groups according to parasite burdens in different organs' impression smears (tachyzoites/10 oil fields) and peritoneal wash (tachyzoites/ml x 10^4)

Parasite burden	Study infected groups			Statistical analysis	
	II (n=6)	III (n=6)	IV (n=6)	*F* test	*P* value
Liver					
Median (Min. - Max)	13.5 (10.6 - 15.8)	2.7 (1.2 – 3.2)	7.5 (6.1 – 8.2)	**100.348**	**<0.001***
Mean ± SD	13.3 ± 2	2.4 ± 0.8	7.3 ± 0.8		
Significance	***P*1<0.001*, *P*2<0.001*, *P*3<0.001***				
Spleen					
Median (Min. - Max)	7.3 (6.8 – 8.6)	1.9 (1.6 – 2.4)	3.7 (3.2 – 4.5)	**153.888**	**<0.001***
Mean ± SD	7.5 ± 0.7	1.9 ± 0.3	3.8 ± 0.5		
Significance	***P*1<0.001*, *P*2<0.001*, *P*3<0.001***				
Brain					
Median (Min. - Max)	2.7 (2.4 – 3.6)	0.9 (0.4 – 1.1)	1.5 (1.2 – 2)	**54.016**	**<0.001***
Mean ± SD	2.9 ± 0.5	0.8 ± 0.3	1.5 ± 0.3		
Significance	***P*1<0.001*, *P*2<0.001*, *P*3<0.007***				
Peritoneal wash					
Median (Min. - Max)	49.5 (42 – 52)	4 (3 – 5)	16.8 (13 – 21.5)	**317.172**	**<0.001***
Mean ± SD	48 ± 4	4 ± 0.7	17 ± 3.5		
Significance	***P*1<0.001*, *P*2<0.001*, *P*3<0.001***				

II: Infected untreated control. **III:** Infected Septrin™-treated. **IV:** Infected CSO-treated. ***:** Statistically significant ($P \leq 0.05$). ***F:*** For ANOVA test, a pairwise comparison between each two groups using Post Hoc Test (Tukey). ***P:*** *P* value for comparing between the three studied groups. ***P*1:** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV. ***P3:*** *P* value for comparing between groups III and IV.

Table 3. Comparison between the experimentally infected groups according to parasite mean viability rate.

Viability rate	Study infected groups			Statistical analysis	
	II (n=6)	III (n=6)	IV (n=6)	*F* test	*P* value
Median (Min. - Max)	100 ± 0.0	15.0 ± 1.79	46.0 ± 7.59	**547.796**	**<0.001***
Mean ± SD	100.0 (100.0 -100.0)	14.5 (13.0 -18.0)	45.0 (38.0 -56.0)		
Significance	***P*1<0.001*, *P*2<0.001*, *P*3<0.001***				

II: Infected untreated control. **III:** Infected Septrin™-treated. **IV:** Infected CSO-treated. ***:** Statistically significant ($P \leq 0.05$). ***F:*** For ANOVA test, a pairwise comparison between each two groups using Post Hoc Test (Tukey). ***P:*** *P* value for comparing between the three studied groups. ***P*1:** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV. ***P3:*** *P* value for comparing between groups III and IV.

Infectivity rate: Both treated groups, III and IV showed a statistically significant deterioration in parasite infectivity (*P*=0.001 and *P*=0.033, respectively), when compared to the infected untreated control group II (Table 4). Interestingly, the comparison between these two groups, III and IV revealed that CSO apparently affected the tachyzoite infectivity close to the reference treatment; Septrin™ (*P*=0.350) (Table 4).

Ultrastructural study: By SEM, *T. gondii* tachyzoites collected from the peritoneal exudate of infected untreated group II showed the normal crescent shape with a completely regular smooth surface (Fig. 3A). However, those obtained from the infected CSO-treated group IV revealed obvious parasite enlargement with irregular surface, longitudinal furrows and small thin projections from the anterior end (Fig. 3B). Additionally, tachyzoites possessing an abnormally elongated anterior portion with multiple deep depressions and an anterior membrane-pore were also detected (Fig. 3C). Furthermore, there was a swollen tachyzoite with an obviously distorted crescent shape, surface dimples and a huge vesicular swelling (Fig. 3D).

Table 4. A comparison between the infected studied groups according to parasite infectivity rate.

	Study infected groups			Statistical analysis	
	II` (n=10)	III` (n=10)	IV` (n=10)	X^2	^{MC}P
	No. (%)	No. (%)	No. (%)		
Infectivity rate	10 (100%)	2 (20%)	5 (50%)	**547.796**	**<0.001***
Significance	^{FE}***P*1<0.001*, ^{FE}*P*2<0.033*, ^{FE}*P*3<0.350**				

II`:** Corresponding to infected untreated control group. **III`: Corresponding to infected Septrin™-treated group. **IV`:** Corresponding to infected CSO-treated group. ***:** Statistically significant ($P \leq 0.05$). ***X^2:*** Chi square test. **FE:** Fisher Exact test. **MC:** Monte Carlo test. **No:** Number. ***P:*** *P* value for comparing between the three studied groups. ***P*1:** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV. ***P*3:** *P* value for comparing between groups III and IV.

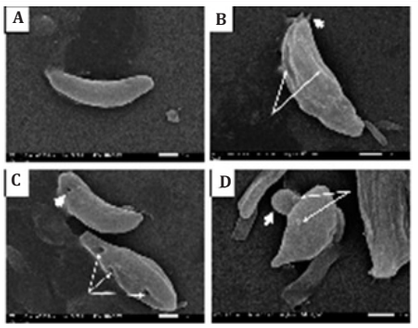

Fig. 3. SEM of *T. gondii* tachyzoites collected from the peritoneal exudate of infected untreated group II **(A)** and infected CSO-treated group IV **(B-D)**. **A:** A normal tachyzoite showing the crescent shape with a completely regular smooth surface. **B:** A treated parasite demonstrating a markedly enlarged tachyzoite with irregular surface, longitudinal furrows (arrows) and small thin projections from the anterior end (arrowhead). **C:** Two treated tachyzoites, one possessing an abnormally-elongated anterior portion and irregular surface with multiple deep depressions (arrows), the other one revealing a pore at the anterior part (arrowhead) with preservation of its crescent shape. **D:** A treated swollen tachyzoite showing an obviously-distorted crescent shape, surface dimples (arrows) and a huge vesicular swelling (arrowhead).

Immunological study: There was an obvious elevation in the serum level of TNF-α in the infected untreated control group II as compared to the non-infected group I (P<0.001) (Table 5). Both treatment regimens, Septrin™ and CSO markedly decreased TNF-α serum levels, when compared to the untreated group II (P<0.001) (Table 5). Moreover, there was no statistically significant difference in TNF-α serum levels between Septrin™-treated mice (group III) and the non-infected control ones (P=0.097) (Table 5). Notably, treatment with CSO showed a reducing effect on TNF-α-serum-level, when compared to Septrin™ (P=0.053) (Table 5).

Histopathological study: On histopathologic examination of H&E-stained hepatic sections, the infected untreated control group II showed a thick edematous hepatic capsule covered by inflammatory exudate that is rich in tachyzoites and lymphocytes. The hepatic architecture showed mild disorganization with multiple parenchymal necro-inflammatory foci (Fig. 4A). Evident congestion and vascular dilatation of portal vessels, sinusoids and central veins were observed (Fig. 4A). Hepatocytes showed diffuse feathery degenerative changes. Lobular inflammatory infiltrates composed of neutrophils admixed with lymphocytes, plasma cells and rare giant cells were detected. Multiple foci were seen per lobule (score 2). Focal interface hepatitis and moderate portal mononuclear infiltrate composed of lymphocytes and plasma cells were observed in most of the portal tracts (score 2) (Fig. 4B). Necrotic hepatocytes and intra/extra cellular tachyzoites were also detected (Fig. 4C). The necro-inflammatory grade was A3 (sever activity) according to METAVIR scoring system.

In infected Septerin™-treated group III, there was an evident improvement of liver histopathology with restoration of the hepatic architecture. The capsular edema was minimal, the parenchymal necro-inflammatory foci were few, and the portal inflammation was minimal with mild congestion of portal vessels, sinusoids, and central veins (Fig. 4D). Hepatocytes showed no degenerative changes and there was a mild, focal mononuclear infiltration of the portal tract with no interface hepatitis (score 0), and with improved portal vascular dilatation (Fig. 4E). Lobular inflammatory foci declined to one per liver lobule (score 1) showing necrotic hepatocytes, while tachyzoites were rarely detected (Fig. 4F). The necro-inflammatory grade dropped to A1 (minimal activity). Of note, CSO-treated group IV showed a marked amelioration of hepatic pathology and an obvious improvement of the necro-inflammatory scoring similar as Septerin™-treated group but with focal interface hepatitis (score 1) (Fig. 4G-I).

The H&E-stained splenic sections of the infected control group II showed splenic capsule infiltrated by lymphocytes, severely congested red pulp, possessing numerous megakaryocytes and epithelioid histiocytes with intracellular parasites (Fig. 5A, B). The lymphoid pulp appeared compressed with rare germinal centers. Both Septrin™ and CSO had obviously improved the splenic architecture. Lymphoid hyperplasia was noted with mild red pulp congestion and rarely observed tachyzoites (Fig. 5C-F).

Table 5. Comparison between the different studied groups according to the levels of TNF-α in serum samples.

TNF-α (pg/ml)	Study groups				Statistical analysis	
	I (n=6)	**II (n=6)**	**III (n=6)**	**IV (n=6)**	***F* test**	***P* value**
Mean ± SD.	35.3 ± 0.7	402.5 ± 70.3	85.4 ±4.5	141.8 ± 1.9		
Median (Min.-Max)	35.3(34.4–36.1)	404.3(330.8–472.4)	85(80.2–91.1)	141 (140.2–144.3)	**129.074**	**<0.001***
***P*0**		**<0.001***	0.097	**0.014***		
Significance	***P*1<0.001*, *P*2<0.001*, *P*3=0.053**					

I: Non-infected control. **II:** Infected untreated control. **III:** Infected Septrin™-treated. **IV:** Infected CSO-treated. ***:** Statistically significant (P≤0.05). ***F:*** For ANOVA test, a pairwise comparison between each two groups using Post Hoc Test (Tukey). ***P:*** *P* value for comparing between the studied groups. ***P*0:** *P* value for comparing between group I and each other group. ***P*1:** *P* value for comparing between groups II and III. ***P*2:** *P* value for comparing between groups II and IV. ***P*3:** *P* value for comparing between groups III and IV.

Histopathologic brain sections of the infected untreated group II revealed sever meningitis with focal parenchymal mononuclear infiltrates. Neuronal degenerative changes were widely seen in different cerebral areas as well as perivascular edema however, tachyzoites were not detected by H&E stain (Fig. 6 A, B). An obvious improvement was observed in both Septrin™ and CSO-treated groups, in terms of mild meningitis, focal neuronal degeneration, focal perivascular edema and non-detectable mononuclear infiltrates (Fig. 6 C, D).

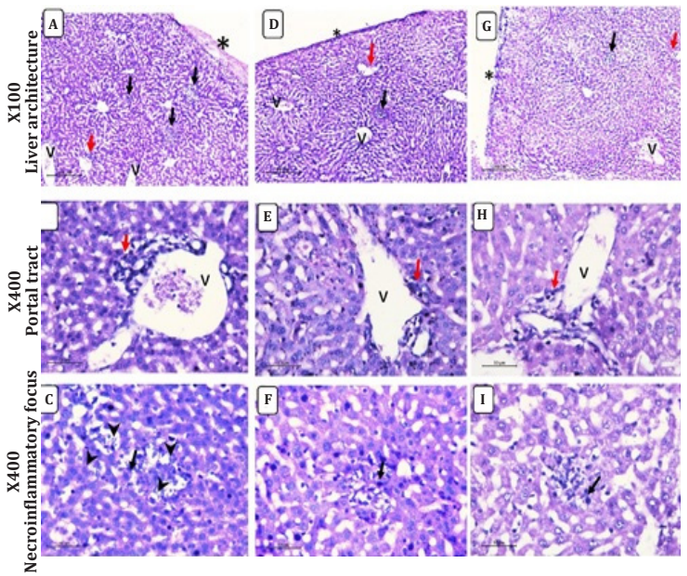

Fig. 4. Histopathologic examination of H&E-stained hepatic tissues, obtained from *T. gondii* RH strain infected mice (infected untreated control, infected Septrin™-treated and infected CSO-treated groups; II, III and IV, respectively). **Group II (A-C), III (D-F) and IV (G-I). A)** The capsule (asterisk) is edematous and covered by inflammatory exudate, multiple necro-inflammatory foci (black arrows) and moderate portal inflammation (red arrow) were observed with marked congestion of portal vessels and central veins **(v)**. **B)** High power of portal tract with moderate mononuclear infiltration (red arrow), interface hepatitis and dilatation of portal vessels **(v)**. **C)** High power of one necro-inflammatory focus showing necrotic hepatocytes (black arrow) and intra/extra cellular tachyzoites (arrow heads). **D)** Minimal capsular (asterisk) edema, few necro-inflammatory foci (black arrow) and minimal portal inflammation (red arrow) were demonstrated with moderate congestion of portal vessels and central veins **(v)**. **E)** High power of portal tract with minimal mononuclear inflammatory infiltrate (red arrow) and no interface hepatitis. **F)** High power of one necro-inflammatory focus showing necrotic hepatocytes (black arrow) while tachyzoites were not detected. **G)** The liver capsule (asterisk) was slightly edematous with residual inflammatory exudate, few necro-inflammatory foci (black arrow) minimal portal inflammation (red arrow) and dilatation of portal vessels and central veins **(v)**. **H)** high power of portal tract with minimal mononuclear infiltration (red arrow) and no interface hepatitis. **I)** High power of one necro-inflammatory focus showing necrotic hepatocytes (black arrow) with no detectable tachyzoites.

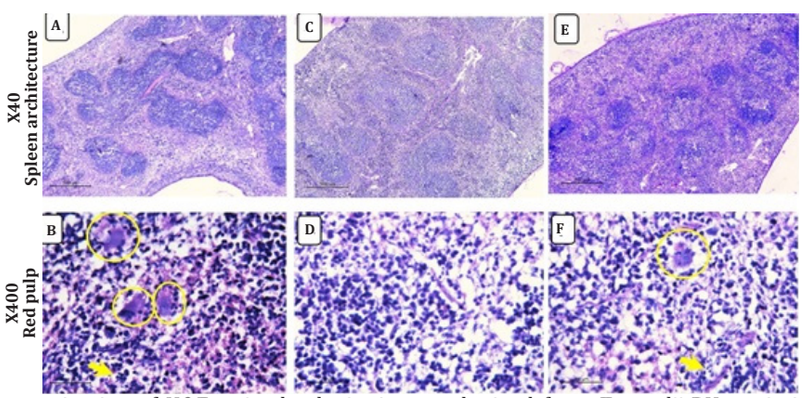

Fig. 5. Histopathologic examination of H&E-stained splenic tissues, obtained from *T. gondii* RH strain-infected mice (infected untreated control, infected Septrin™-treated and infected CSO-treated groups **II (A, B), III (C, D) and IV (E, F),** respectively). **A)** Hyperplastic severely congested red pulp with dilated sinusoids. **B)** High power of congested red pulp possessing numerous epithelioid histiocytes (yellow circles) with numerous extra cellular tachyzoites (yellow arrows). **C)** Mild congestion of red pulp and hyperplastic lymphoid follicles. **D)** High power of red pulp with no detectable epithelioid cells. **E)** Mild congestion of the red pulp and hyperplastic lymphoid follicles. **F)** High power of few epithelioid cells (yellow circles) with scanty tachyzoites (yellow arrows) in the red pulp.

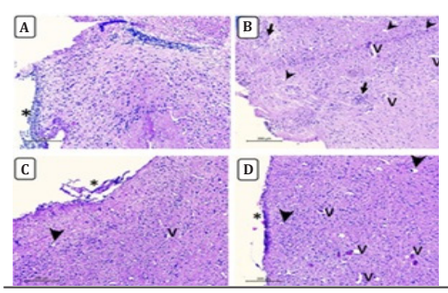

Fig. 6. Histopathologic examination of H&E-stained cerebral tissues, obtained from *T. gondii* RH strain-infected mice (infected untreated control, infected Septrin™-treated and infected CSO-treated groups; **II (A, B), III (C) and IV (D)**, respectively). **A)** Sever meningitis (asterisk). **B)** Multiple parenchymal nodules of inflammatory mononuclear cells (black arrows), perivascular edema **(v)** and neuronal degenerative changes (arrowheads). **C and D** represent mild meningitis (asterisk), focal perivascular edema **(v)** and focal neuronal degeneration (arrowheads) with no parenchymal mononuclear cell infiltrates.

DISCUSSION

Treatment of human toxoplasmosis represents a true challenge. The currently available therapeutics cannot clear latent infection with *T. gondii* and are often intolerable by patients. Considering these constraints, the search for alternative drugs with novel mechanisms of action needed upgrading[30]. Since many centuries, plants and their derivatives have been used as a valuable natural resource for traditional remedies. Recently, there is an increasing interest in the field of ethno-pharmacological research for the treatment of parasitic infections[31].

Cuminum cyminum (L.) was reported to possess ovicidal properties where the exposure to its essential oil-vapours resulted in 100% mortality of the eggs of stored-product insects such as flour beetle (*Tribolium confusum*) and Mediterranean flour moth (*Ephestia kuehniella*)[32]. Moreover, cuminaldehyde, a major constituent of CSO, exerted high irritant activity against female *A. gambiae* mosquitoes[17]. Notably, thymol and carvacrol were proved to possess a synergistic insecticidal effect on the larvae of *Amblyomma sculptum* and *Dermacentor nitens*[33,34]. Hence, the current experiment was designed to explore the anti-*Toxoplasma* activity of CSO in mice models. We chose CSO in our study because the essential oils of medicinal plants seemed to possess a better antimicrobial effect in comparison to powder, alcoholic and aqueous extracts, that are related to the main components of such oils[35].

In the present work, untreated mice, experimentally infected with *T. gondii* RH strain (group II) had rapidly died within seven days PI. This agrees with previous experiments conducted on similar parasite strains[21,36-38]. The low survival rate of animals after infection was attributed to rapid invasion of the host cells, uncontrolled parasite proliferation and aggressive tachyzoite dissemination in different organs[39]. Both Septrin™ (group III) and CSO (group IV) had significantly prolonged the mean survival duration to nearly 15 d. Comparable results were demonstrated when *Sophora flavescens* alkaloids (oxymatrine and matrine) were used to treat experimental toxoplasmosis in comparison to spiramycin as a reference drug[40]. Additionally, Azami *et al*.[37] recorded a marked increase in animal survival time when they used curcumin nanoemulsion alone in a similar experiment. The same findings were reported upon implementing trimethoprim-sulfamethoxazole, spiramycin-metronidazole and two different pyrimethamine combination therapies in treatment of acute toxoplasmosis[21,38].

The concept of using trimethoprim in a fixed combination with sulfamethoxazole (Septrin™) is attributed to their complementary and synergistic mechanisms of action. Sulfamethoxazole is recognized as a structural analogue of para-aminobenzoic acid, possessing a competitive inhibitory effect on dihydropetroate synthetase. This inhibits the synthesis of intermediary dihydrofolic acid from its precursors. Meanwhile, trimethoprim acts as a structural analogue of pteridine part of dihydrofolic acid which competitively inhibits dihydrofolate reductase. This subsequently downregulates tetrahydrofolic acid production; the physiologically active form of folic acid and a necessary cofactor in the synthesis of thymidine, purines, and DNA[41]. This sequential blockade of two enzymes in one pathway results in an effective microbicidal action as *T. gondii* was recognized as an obligate thymidine manufacturer and was suggested to lack alternative mechanisms for folate acquisition. These facts justify the more potent efficacy of Septrin™ than CSO on the parasite proliferation and burden in different tissues and on the tachyzoite viability in our study.

Herein, the oral five-day therapy with CSO succeeded to decrease the tachyzoite burdens significantly, in all studied tissue smears and also in peritoneal exudates, when compared to the infected untreated group II. Similar results were reported upon use of the essential oil of *Bunium persicum* (Boiss), another member of Apiaceae family, for the same duration to treat experimental acute toxoplasmosis with promising yields[42]. Counting tachyzoites in peritoneal exudate was reported to be valuable in the assessment of the anti-*Toxoplasma* activity of natural compounds[37]. Furthermore, the essential oil isolated from an Apiaceae member "*Daucus crinitus desf*" exerted a marked growth inhibitory effect on chloroquine-resistant *Plasmodium falciparum* culture[43].

Regarding the tachyzoite mean viability rate, it was significantly reduced by both Septrin™ and CSO therapies in comparison to the infected untreated controls. Similarly, the tachyzoite viability was extremely affected, when methanolic extracts of *Sambucus nigra*, Caprifoliaceae family were applied on *Toxoplasma* RH strain-infected macrophages[44]. Assessment of this parameter was crucial to evaluate the anti-*Toxoplasma* potentials of different compounds[36,38,40].

The mechanism of the growth inhibition and viability deterioration elicited by CSO has not been studied in a detailed manner, considering the great number of different groups of chemical compounds that exist in essential oils. It is worth noting that their antimicrobial activity is not attributed to one specific mechanism but they have many targets in the cell[45,46]. Not all the involved mechanisms are separate targets; some are affected as an outcome of another mechanism being targeted[35]. Regarding the mechanism by which CSO might exert its antiprotozoal effect, it was reported that phenolic compounds, such as thymol and carvacrol, may inactivate essential enzymes, react with the cell membrane, or disturb genetic material[46].

This assumption could also explain the drastic deterioration in parasite infectivity in our study since plasma membrane and parasitic enzymes are crucial in tachyzoite attachment and mammalian host cell invasion[48]. Parasite infectivity is a good indicator for anti-*Toxoplasma* pursuit of candidate agents[36].

The antimicrobial activity of essential oils is assigned to a number of terpene, terpenoid and phenolic constituents[49]. Phytochemical analysis revealed monoterpenoids (camphor, carvacrol, thymol, linalool… etc) and monoterpene hydrocarbons (α-pinene and β-pinene) as chemical constituents of CSO[20]. Such monoterpenoids were proved to have an antiprotozoal activity against *P. falciparum*[50], *G. lamblia*[51] and *T. brucei*[52]. Furthermore, α-pinene showed a considrable antiparasitic effect on *L. brasiliensis* and *T. cruzi*[53]. Cuminaldehyde was pointed out as the major compound associated with the pharmacological properties of *C. cyminum* seeds[54]. Although cuminaldehyde is a special monoterpenoid possessing an aromatic ring which makes it resemble phenyl propanoids with a proven antiplasmodial activity, it couldn't exert an antimalarial action[55]. Notably, studies using *C. cyminum* oil or the oil monoterpenic components, α-pinene and β-pinene proved their anthelmintic activities against the third stage larvae of *Anisakis simplex*[56,57].

The essential oil of *C. cyminum* (L.) potentiated the efficacy of ciprofloxacin on multiple bacteria[58,59]. It had a synergistic antibacterial activity with the food preservative "nisin" against food-borne pathogens and markedly inhibited the growth of such pathogens[18,60]. Similarly, it elicited a good antimicrobial effect on different bacteria including multidrug resistant strains of *Salmonella* spp. It was assumed that the bioactive compounds in this oil may have halted the proper assembly of cell membrane, which ultimately affected the cell shape and inhibited its growth[61].

Scanning electron microscopy revealed major ultrastructural changes in CSO-treated group. Comparable findings were recorded upon treatment of experimentally infected mice with *Nigella sativa* oil, aluvia, nitazoxanide, spiramycin-metronidazole[21,36,38]. Essential oils interact very closely with the cell membrane phospholipids by the aid of short carbon chains and high hydrophobicity of their constituents[62]. Like typical lipophiles, they can pass through the cytoplasmic membrane, disrupt the structure of different layers of polysaccharides, fatty acids, and phospholipids. Moreover, they could interact with proteins embedded in the cytoplasmic membrane, causing structural deformation, and increasing its permeability[62,63]. These facts might explain the ultrastructural changes revealed in our experiment in which, there was an obvious elevation in the serum level of TNF-α in the infected untreated control mice. This was supported by previous investigators[64,65]. Increased level of such a pro-inflammatory cytokine commences with *Toxoplasma* glycosylphosphatidylinositol recognition by host toll-like receptors (TLRs). Specifically, TLR2 and TLR4 are activated and subsequently trigger TNF-α production by macrophages[64]. Expression of indoleamine 2,3-dioxygenase, an enzyme involved in the catabolism of tryptophan and an amino acid important for tachyzoites replication, was modulated by TNF-α, contributing to parasite-growth inhibition[66]. Notably, neutrophils represent a valuable army in the early fight against *Toxoplasma* through induction of various cytokines involving TNF-α. This cytokine synergizes with IL-12 to produce IFN-γ, thereby promoting the T cell-dependent pathway of host resistance to the parasite. TNF-α seemed to play a vital role in host defense against *T. gondii*; mice exhibiting neutrophils depletion displayed uncontrolled tachyzoite replication and lethal systemic pathology[67]. Additionally, it was postulated that, in the early phase of infection, TNF-α can induce egress of tachyzoites from infected cells to help parasites invade other host cells for mass replication. Meanwhile, activated macrophages can eliminate TNF-α -induced egressed tachyzoites in the late infection phase[68].

In the present study, treatment with CSO showed a reducing effect, comparable to Septrin™ considering TNF-α-serum-level. This could be attributed to the anti-inflammatory property of CSO, since it markedly inhibited the expression of messenger RNA (mRNAs) of multiple pro-inflammatory mediators: inducible nitric oxide synthase, cyclooxygenase-2, IL-1 and IL-6[69]. Moreover, CSO efficiently blocks lipopolysaccharide-induced transcriptional activation of nuclear factor-kappa B (NF-κB) and inhibits the phosphorylation of extracellular signal-regulated kinase and c-Jun N-terminal kinase inflammatory pathways[69]. Subsequently, TLR2 and TLR4 become unable to induce macrophages to produce TNF-α. Our results also might be explained by the concomitant inhibitory effects on parasite proliferation and deteriorating pursuit of tachyzoite viability and infectivity, exerted by Septrin™ and CSO. Hence, the reduced host cell invasion and decreased inflammatory reaction.

On histopathologic basis, the infected untreated control group II revealed disorganized hepatic architecture, diffuse hepatocyte degeneration, lobular and portal inflammation score 2, and necro-inflammation grade A3. This group showed splenic-capsule lymphocytic infiltration, congested red pulp with megakaryocytes and epithelioid histiocytes laden with tachyzoites. Comparable histopathologic outcomes were previously reported[21,70]. The concomitant high level of TNF-α in such a group can cause the infiltration with the inflammatory cells and the necrotic cellular damages[71].

Administration of CSO (group IV) showed marked reduction in the necro-inflammatory reaction and

amelioration of the histopathologic changes, observed in hepatic, splenic and cerebral tissues. This finding could be attributed to the anti-*Toxoplasma* activity as well as its anti-inflammatory property. Our results are supported by previous studies in which the essential oil of *Nigella sativa* was employed for treatment of schistosomiasis and acute toxoplasmosis in experimentally infected mice[21,72]. The substantial improvement in the histopathologic sequels especially hepatitis and meningitis provide another explanation for the prolonged animal survival duration upon treatment with both Septrin™ and CSO. On the other hand, the lymphocytic tissue infiltration was still detected in CSO-treated mice. This inflammatory reaction may be due to the failure of CSO to eliminate tachyzoites completely. Furthermore, *C. cyminum* was reported to possess an immunomodulatory activity through stimulation of T lymphocytes and induction of Th 1 cytokines[73].

Overall, the evidence gathered herein, support that CSO can be useful as a safe, natural therapy for acute toxoplasmosis considering both the anti-inflammatory and the anti-*Toxoplasma* possessions. However, the mode of antiprotozoal action of the oil components should be further investigated.

Author contribution: Gomaa, MM proposed the study topic and design, and performed the parasitological experimentation. Sheta, E performed the histopathologic processing and examination. Both authors contributed in results analysis and interpretation and writing the manuscript.

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REFERENCES

1. Stelzer S, Basso W, Silván JB, Ortega-Mora LM, Maksimov P, Gethmann J, *et al*. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. Food Waterborne Parasitol 2019; 12:e00037.
2. Bigna JJ, Tochie JN, Tounouga DN, Bekolo AO, Ymele NS, Youda EL, *et al*. Global, regional, and country seroprevalence of *Toxoplasma gondii* in pregnant women: a systematic review, modelling and meta-analysis. Scientific Reports 2020; 10:12102.
3. Montazeri M, Galeh TM, Moosazadeh M, Sarvi S, Dodangeh S, Javidnia J, *et al*. The global serological prevalence of *Toxoplasma gondii* in felids during the last five decades (1967–2017): A systematic review and meta-analysis. Parasit Vectors 2020; 13:82-91.
4. Abbas IE, Villena I, Dubey JP. A review on toxoplasmosis in humans and animals from Egypt. Parasitology 2020; 147(2):135-159.
5. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2009; 39:1385–1394.
6. Mcauley JP, Boyer KM, Remington JS, Mcleod RL. Toxoplasmosis. in: Cherry JD, Harrison GJ, Kaplan SL, Hotez PJ, Steinbach WJ, ED. Feigin and Cherry's Textbook of Pediatric Infectious Diseases, 7th ed. Elsevier/Saunders, 2014; 2986–3001.
7. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. Semin Immunopathol 2012; 34:793–813.
8. Delair E, Creuzet C, Dupouy-Camet J, Roisin MP. *In vitro* effect of TNF-α and IFN-γ in retinal cell infection with *Toxoplasma gondii*. Invest Ophthalmol Vis Sci 2009; 50:1754–1760.
9. El-Sherbini MS, Abd El-Aal AA, El-Sherbiny WS, Attia SS, Abdel Aziz IZ, Nasr GM, *et al*. Toxoplasmosis and abortion: Pro- and anti-inflammatory cytokines gene expression of the host immune cells. Egypt J Med Human Genetics 2019; 20:1-10.
10. Meira CS, Pereira-Chioccola VL, Vidal JE, Mattos CCB, Motoie G, Costa-Silva TA, *et al*. Cerebral and ocular toxoplasmosis related with IFN-γ, TNF-α, and IL-10 levels. Frontiers Microbiol 2014; 5:492.
11. Francis P, Patel V, Bill P, Bhigjee A. Oral trimethoprim-sulfamethoxazole in the treatment of cerebral toxoplasmosis in AIDS patients-a prospective study. S Afr Med J 2004; 94(1):51–53.
12. Soheilian M, Sadoughi MM, Ghajarnia M, Dehghan MH, Yazdani S, Behboudi H, *et al*. Prospective randomized trial of trimethoprim/sulfamethoxazole versus pyrimethamine and sulfadiazine in the treatment of ocular toxoplasmosis. Ophthalmol 2005; 112 (11):1876–1882.
13. Wei HX, Wei SS, Lindsay DS, Peng HJ. A systematic review and meta-analysis of the efficacy of anti-*Toxoplasma gondii* medicines in humans. PloS One 2015; 10(9):e0138204.
14. Singh RP, Gangadharappa HV, Mruthunjaya K. *Cuminum cyminum*: A popular spice: An updated review. Pharmacogn J 2017; 9(3):292–301.
15. Zargari A. Medicinal Plants, 5th ed. Vol. 2. Tehran University Publications, Tehran, 2001.
16. Patil SB, Takalikar SS, Joglekar MM, Haldavnekar VS, Arvindekar AU. Insulinotropic and β-cell protective action of cuminaldehyde, cuminol and an inhibitor isolated from *Cuminum cyminum* in streptozotocin-induced diabetic rats. Br J Nutr 2013; 110(8):1434–1443.
17. Deletre E, Chandre F, Williams L, Duménil C, Menut C, Martin T. Electro-physiological and behavioral characterization of bioactive compounds of the *Thymus vulgaris*, *Cymbopogon winterianus*, *Cuminum cyminum* and *Cinnamomum zeylanicum* essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments. Parasit Vectors 2015; 8:316.
18. Tavakoli HR, Mashak Z, Moradi B, Sodagari HR. Antimicrobial activities of the combined use of *Cuminum*

cyminum (L.) essential oil, nisin and storage temperature against *Salmonella typhimurium* and *Staphylococcus aureus in vitro*. Jundishapur J Microbiol 2015; 8(4):e24838.
19. Naeini A, Naderi NJ, Shokri H. Analysis and *in vitro* anti-*Candida* antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic Candida strains. J Mycol Med 2014; 24(1):13–18.
20. Romeilah RM, Fayed SA, Mahmoud GI. Chemical compositions, antiviral and antioxidant activities of seven essential oils. J Applied Sci Res 2010; 6(1):50–62.
21. Mady RF, El-Hadidy W, Elachy S. Effect of *Nigella sativa* oil on experimental toxoplasmosis. Prasitol Res 2016; 115:379–390.
22. Bottari NB, Baldissera MD, Tonin AA, Rech VC, Nishihira VS, Thome, GR, *et al*. Sulfamethoxazole-trimethoprim associated with resveratrol for the treatment of toxoplasmosis in mice: Influence on the activity of enzymes involved in brain neurotransmission. Microb Pathog 2015; 79:17–23.
23. Gulcin I, Sat GI, Beydemir S, Elmastas M, Kufrevioglu OI. Comparison of antioxidant activity of clove (*Eugenia caryophylata Thunb*) buds and lavender (*Lavandula stoechas*). Food chem 2003; 87:393- 400.
24. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing antioxidant activity. J Agric Food Chem 2002; 50:3010-3014.
25. Eissa MM, El-Azzouni MZ, Mady RF, Fathy FM, Baddour NM. Initial characterization of an autoclaved *Toxoplasma* vaccine in mice. Exp Parasitol 2012; 131:310–316.
26. Crowley LC, Marfell BJ, Christensen ME, Waterhouse NJ. Measuring cell death by trypan blue uptake and light microscopy. Cold Spring Harb Protoc 2016; 7. DOI: 10.1101/pdb.prot087155.
27. Abou-El-Naga IF, Gomaa MM, ElAchy SN. Effect of HIV aspartyl protease inhibitors on experimental infection with a cystogenic Me49 strain of *Toxoplasma gondii*. Path Glob Health 2021; 116(2):107-118.
28. Berthold-Losleben M, Himmerich H. The TNF-alpha system: Functional aspects in depression, narcolepsy and psychopharmacology. Curr Neuropharmacol 2008; 6(3):193-202.
29. Bedossa P, Poynard T. METAVIR cooperative study group, an algorithm for the grading of activity in chronic hepatitis C. Hepatol 1996; 24: 289–293.
30. Ma CI, Diraviyam K, Maier ME, Sept D, Sibley LD. Synthetic chondramide A analogues stabilize filamentous actin and block invasion by *Toxoplasma gondii*. J Nat Prod 2013; 76:1565–1572.
31. Arab-Mazar Z, Kheirandish F, Rajaeian S. Anti-toxoplasmosis activity of herbal medicines: Narrative review. Herbal Med J 2017; 2(1):43–49.
32. Tunc I, Berger BM, Erler F, Dagli F. Ovicidal activity of essential oils from five plants against two stored product insects. J Stored Prod Res 2000; 36:161–168.
33. Novato TPL, Araujo LX, Monteiro CMO, Maturano R, Senra TOS, Matos RS, *et al*. Evaluation of the combined effect of thymol, carvacrol and cinnamaldehyde on *Amblyomma sculptum* (Acari: ixodidae) and *Dermacentor nitens* (Acari: ixodidae) larvae. Vet Parasitol 2015; 212:331–335.
34. Tak JH, Isman MB. Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in *Trichoplusia ni*. Sci Rep 2015; 5:12690.
35. Burt S. Essential oils: Their antibacterial properties and po¬tential applications in foods, a review. Int J Food Microbiol 2004; 94(3):223–253.
36. Abou-El-Nagaa IF, El Kerdanya ED, Mady RF, Shalaby ThI, Zaytoun EM. The effect of lopinavir/ritonavir and lopinavir/ritonavir loaded PLGA nanoparticles on experimental toxoplasmosis. Parasitol Int 2017; 66:735–747.
37. Azami SJ, Teimouri A, Keshavarz H, Amani A, Esmaeili F, Hasanpour H, *et al*. Curcumin nanoemulsion as a novel chemical for the treatment of acute and chronic toxoplasmosis in mice. Int J Nanomed 2018; 13:7363–7374.
38. Allam AF, Shehab AY, Mogahed NMFH, Farag HF, Elsayed Y, Abd El-Latif NF. Effect of nitazoxanide and spiramycin metronidazole combination in acute experimental toxoplasmosis. Heliyon 2020; 6:e03661.
39. Sibley LD, Mordue DG, Su C, Robben PM, Howe DK. Genetic approaches to studying virulence and pathogenesis in *Toxoplasma gondii*. Philos Trans R Soc Lond B Biol Sci 2002; 357:81–88.
40. Zhang X, Jin L, Cui Z, Zhang C, Wu X, Park H, *et al*. Antiparasitic effects of oxymatrine and matrine against *Toxoplasma gondii in vitro* and *in vivo*. Exp Parasitol 2016; 165:95-102.
41. Eyler RF, Shvets K. Clinical pharmacology of antibiotics. Clin J Am Soc Nephrol 2019; 14(7):1080-1090.
42. Kareshk AT, keyhani A, Mahmoudvand H, Oliaei RT, Asadi A, Andishmand M, *et al*. Efficacy of the *Bunium persicum* (Boiss) essential oil against acute toxoplasmosis in mice model. Iran J Parasitol 2015; 10(4):625–631.
43. Lanfranchi DA, Laouer H, El Kolli M, Prado S, Maulay-Bailly C, Baldovini N. Bioactive phenylpropanoids from *Daucus crinitus* desf. from Algeria. J Agric Food Chem 2010; 58:2174–2179.
44. Daryani A, Ebrahimzadeh MA, Sharif M, Ahmadpour E, Edalatian S, Esboei BR, *et al*. Anti-*Toxoplasma* activities of methanolic extract of *Sambucus nigra* (Caprifoliaceae) fruits and leaves. Int J Trop Biol 2015; 63(1):7–12.
45. Skandamis P, Koutsoumanis K, Fasseas K, Nychas GJE. Inhibition of oregano essential oil and EDTA on *Escherichia coli* O157:H7. Italian J Food Sci 2001; 13:65–75.
46. Carson CF, Hammer KA, Riley TV. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob Agents Chemother 2002; 46:1914–192.
47. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils: Present status and future perspectives. Medicines 2017; 4: 58-79.
48. Portes J, Barrias E, Travassos R, Attias M, de Souza W. *Toxoplasma gondii* mechanisms of entry into host cells. Front Cell Infect Microbiol 2020; 10:294.

49. Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, *et al*. Antibacterial activity of terpenes and terpenoids present in essential oils. Molecules 2019; 24:2471-2483.
50. Rodrigues GH, Kimura EA, Peres VJ, Couto AS, Aquino DFA, Katzin AM. Terpenes arrest parasitic development and inhibit biosynthesis of isoprenoids in *Plasmodium falciparum*. Antimicrob Agents Chemother 2004; 48:2502–2509.
51. de Almeida I, Alviano DS, Vieira DP, Alves PB, Blank AF, Lopes AH, *et al*. Antigiardial activity of *Ocimum basilicum* essential oil. Parasitol Res 2007; 101:443–452.
52. Nibret E, Wink M. Trypanocidal and antileukaemic effects of the essential oils of *Hagenia abyssinica*, *Leonotis ocymifolia, Moringa stenopetala*, and their main individual constituents. Phytomedicine 2010; 17:911–920.
53. Pereira PS, Oliveira CVB, Maia AJ, Tintino SR, Oliveira-Tintino CDM, Vega-Gomez MC, *et al*. Cytotoxicity of essential oil *Cordia verbenaceae* against *Leishmania brasiliensis* and *Trypanosoma cruzi*. Molecules 2021; 26:4485-4498.
54. Mnif S, Aifa S. Cumin (*Cuminum cyminum* L.) from traditional uses to potential biomedical applications. Chem Biodivers 2015; 12:733–742.
55. Mojarraba M, Rezaeic M, Miraghaeeb SS, Abdid G, Shokoohinia Y. Antimalarial evaluation of cuminaldehyde, an aromatic monoterpenoid, using cell free β-hematin formation assay. JRPS 2014; 3(1):36-41.
56. Navarro MC, Noguera MA, Romero MC, Montilla MP, González-de Selgas JM, Valero A. *Anisakis simplex* s. l.: larvicidal activity of various monoterpenic derivatives of natural origin against L3 larvae *in vitro* and *in vivo*. Exp Parasitol 2008; 120:295–299.
57. Pérez MGM, Moll CN, Espinosa GM, López AV. Evaluation of different Mediterranean essential oils as prophylactic agents in anisakidosis. Pharm Biol 2017; 55:456–461.
58. Derakhshan SSM, Bigdeli M. Effect of cumin (*Cuminum cyminum*) seed essential oil on biofilm formation and plasmid integrity of *Klebsiella pneumoniae*. Pharmacogn Mag 2010; 6:57-61.
59. Monteiro-Neto V, de Souza CD, Gonzaga LF, da Silveira BC, Sousa NCF, Pontes JP. Cuminaldehyde potentiates the antimicrobial actions of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. PLoS ONE 2020; 15(5):e0232987.
60. Wongkattiya N, Sanguansermsri P, Fraser IH, Sanguansermsri D. Antibacterial activity of cuminaldehyde on food-borne pathogens, the bioactive component of essential oil from *Cuminum cyminum* (L.) collected in Thailand. J Compl Integr Med 2019; 16(4):195-206.
61. Naveed R, Hussain I, Abdul-Tawab, Tariq M, Rahman M, Hameed S, *et al*. Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against *Salmonella* and other multi-drug resistant bacteria. BMC Complement. Alternat Med 2013; 13:265–275.
62. Maryam I, Huzaifa U, Hindatu H, Zubaida S. Nanoencapsulation of essential oils with enhanced antimicrobial activity: A new way of combating antimicrobial resistance. J Pharmacogn Phytochem 2015; 4:165–170.
63. Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, *et al*. Biological activities of essential oils from plant chemoecology to traditional healing systems. Molecules 2017; 22:70.
64. Guo YZ, He P, Feng AM. Effect of curcumin on expressions of NF-κBp65, TNF-α and IL-8 in placental tissue of premature birth of infected mice. Asian Pac J Trop Med 2017; 10:175–178.
65. Simanjuntak TP, Hatta M, Rauf S, Prabandari SA, Siagian C, Dwiyanti R. Tumor necrosis factor-alpha levels and histopathology finding after intervention with *Curcuma longa* extract. J Med Sci 2018; 18:56–62.
66. Chaves ACL, Cera´ volo IP, Gomes JAS, Zani CL, Romanha AJ, Gazzinelli RT. IL-4 and IL-13 regulate the induction of indoleamine 2,3-dioxygenase activity and the control of *Toxoplasma gondii* replication in human fibroblasts activated with IFN-γ. Eur J Immunol 2001; 31:333–344.
67. Bliss SK, Gavrilescu LC, Alcaraz A, Denkers EY. Neutrophil depletion during *Toxoplasma gondii* infection leads to impaired immunity and lethal systemic pathology. Infect Immunity 2001; 69(8): 4898–4905.
68. Yong Y, Liu M, Ren C, Shen J, Ji Y. Exogenous tumor necrosis factor-alpha could induce egress of *Toxoplasma gondii* from human foreskin fibroblast cells. Parasite 2017; 24:45-52.
69. Wei J, Zhang X, Bi Y, Miao R, Zhang Z, Su H. Anti-inflammatory effects of cumin essential oil by blocking JNK, ERK, and NF- κB signaling pathways in LPS-stimulated RAW 264.7 cells. Evid Based Complement Alternat Med 2015; 2015:474509.
70. Eissa MM, Barakat AMA, Amer EI, Younis LK. Could miltefosine be used as a therapy for toxoplasmosis? Exp Parasitol 2015; 157:12e22.
71. Haider S, Knofler M. Human tumor necrosis factor: Physiological and pathological roles in placenta and endometrium. Placenta 2009; 30:111-123.
72. Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. Ethnopharmacol 2002; 79:1–11.
73. Chauhan PS, Satti NK, Suri KA, Amina M, Bani S. Stimulatory effects of *Cuminum cyminum* and flavonoid glycoside on cyclosporine-A and restraint stress induced immune-suppression in Swiss albino mice. Chem Biol Interact 2010; 185(1):66-72.