Immunomodulatory impact of natural oils loaded metal-organic frameworks on chronic toxoplasmosis in mice

Original Article

Mai A Shehata[1], Heba M El Naggar[1], Hagar F Abdelmaksoud[2], Ayman M El-Ashkar[1,3], Ashraf M Barakat[4], Reda M Abdelhameed[5], Shimaa A Atta[6], Omnia S Mohammad[1]

Departments of Medical Parasitology, Faculty of Medicine, Ain Shams University, Cairo[1], Parasitology, Theodor Bilharz Research Institute, Giza[2], Microorganisms and Clinical Parasitology, College of Medicine, University of Bisha, Bisha, Saudi Arabia[3], Zoonotic Diseases[4] and Applied Organic Chemistry, Chemical Industries Research Institute[5], National Research Centre[4,5], Giza, and Immunology, Theodor Bilharz Research Institute, Giza[6], Egypt

ABSTRACT

Background: Although several drugs and natural products were investigated in the treatment of chronic toxoplasmosis, brain cysts are still resistant to eradication.
Objective: To evaluate the immunomodulatory effect of *Nigella sativa* oil (NSO) and wheat germ oil (WGO) loaded metal-organic frameworks (MOF) on chronic toxoplasmosis in mice.
Material and Methods: Eighty male Swiss albino mice were divided into 8 groups (10 mice each); GI: non-infected non treated, GII-GVIII: infected with ME-49 avirulent strain of *T. gondii*; GII: non-treated, GIII-GVIII were treated with Spiramycin (GIII), Spiramycin-MOF (GIV), MOF (GV), WGO-MOF (GVI), NSO-MOF (GVII), and WGO+NSO-MOF (GVIII). Immunological evaluation was performed through immunohistochemical (IHC) staining of liver sections, and serological detection of immunological markers, *i.e.*, interferon-gamma (INF–γ), and tumor necrosis factor-alpha (TNF–α).
Results: The IHC examination revealed a high predominance of $CD8^+$ cells elicited in GVII, and GVIII followed by GVI. A statistically significant difference was recorded between the treated test and control groups regarding the levels of INF–γ, and TNF–α in their sera with the highest level recorded in GVII followed by GVIII, GVI, GIII, GIV, and GV, respectively.
Conclusion: Our study showed that NSO and WGO combinations with MOF exhibited significant immunostimulant effects against chronic murine toxoplasmosis.
Keywords: essential oils; metal-organic framework; *Nigella sativa*; spiramycin; *Toxoplasma*; wheat germ.

Received: 25 October, 2024; **Accepted:** 14 December, 2024.

Corresponding Author: Mai A. Shehata; **Tel.:** +20 1222230979**; Email:** maishehata@med.asu.edu.eg

Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 17, No. 3, December, 2024.**

INTRODUCTION

Globally, the significant intracellular protozoan parasite *T. gondii* is astonishingly capable of infecting, surviving, and replicating in almost all mammalian cells. Although toxoplasmosis in humans is typically mild or asymptomatic, it can induce life-threatening illnesses in immunosuppressed people and congenitally infected fetuses[1]. In toxoplasmosis, both innate and adaptive immunity are elicited. While innate immunity comprises natural killer (NK) cells and macrophages, adaptive immunity is predominated by cell-mediated immune response[2]. Through pattern recognition receptors, antigen-presenting cells recognize *T. gondii* and produce pro-inflammatory cytokines (IL-12 and TNF-α)[3]. When stimulated by IL-12, Th1 cells release IL-2, and IFN-γ. Both cytokines strongly activate $CD8^+$ cytotoxic T cells increasing IFN-γ production, and accordingly, macrophages become effective microbicides[4]. Therefore, adaptive immunity targets the quickly dividing tachyzoites in the acute infection transforming them into slowly developing bradyzoites in dormant cysts in various tissues in the chronic toxoplasmosis. Besides, a protective B-cell response is initiated by IL-6 influence in early chronic toxoplasmosis[5]. A critical equilibrium between pro-inflammatory (IL-12, IFN-γ, and TNF), and anti-inflammatory cytokines (IL-10, IL-27, TGF-1) determines the course of toxoplasmosis[6].

Regrettably, available therapeutic drugs only have an impact on tachyzoites in acute infections, and are unable to eradicate tissue cysts in chronic infections completely[7]. The gold standard for treating toxoplasmosis is still the combination of pyrimethamine and sulphadiazine, which inhibits folic acid synthesis despite substantial side effects being identified[8]. Notably, Spiramycin was reported to prevent congenital toxoplasmosis due to its high efficiency against tachyzoites in acute infection, low toxicity, and capability of reaching elevated placental titers. However, it showed poor blood-brain barrier crossing, and failed to achieve adequate cerebral concentrations[9]. When used in combination with

 DOI: 10.21608/puj.2024.331310.1275

metronidazole, its effectiveness significantly increased against chronic cerebral toxoplasmosis[10]. It showed moderate anti-*Toxoplasma* activity in chronically infected mice with minimal to moderate alleviation of tissue damage[10,11]. It is noteworthy that bone marrow mesenchymal stem cells were also investigated against chronic murine toxoplasmosis but unfortunately, they were less effective than when combined with pyrimethamine, Spiramycin, and folinic acid or Spiramycin-metronidazole combination[10,12].

On the other hand, the usefulness of natural products in the treatment of parasitic diseases attracted much attention. Seeds of *N. sativa* provided *in vitro* schistosomicidal effects against *S. mansoni*[13,14], whereas its extracts exhibited effective lethal activity against *L. tropica*[15]. Moreover, several studies recorded its therapeutic efficacy against toxoplasmosis[16,17]. Regarding WGO, it also recorded potential efficiency against cryptosporidiosis[18,19], and toxoplasmosis[20]. Combined with propolis, it significantly reduced *Toxoplasma* cysts and exhibited promising efficacy against chronic toxoplasmosis[21].

Nanotechnology is anticipated to provide novel possibilities to treat and control many illnesses because it allows for atomic-scale material modification. Nanoparticles (NPs) are tiny molecules ranging from 1 to 100 nm in diameter. They can be classified into a variety of groups depending on their traits, forms, or sizes[20]. The MOFs are well known to have an enormous surface area, adaptable contour, and huge pore volume with good molecular stability. By altering the metal and/or organic linker, it is simple to change the chemical characteristics of MOFs, which increases their drug-loading capacity and prevents their quick breakdown[22,23]. One kind of frequently utilized MOFs is copper-benzene tricarboxylic acid (Cu-BTC)[24]. It is claimed that Cu^{2+} has a lethal effect on a variety of microorganisms[25,26]. As a result, additional studies examined Cu^{2+} biological activity in conjunction with BTC[24,27,28]. Notably, chronic toxoplasmosis was treated with aluminum-benzene dicarboxylate MOF-NPs[11], Cu-BTC MOF[17], nano-curcumin@MOFs[29], and chitosan NPs[30]. Guanabenz-loaded polyethylene glycol poly lactic-co-glycolic acid NPs showed excellent effects against chronic murine toxoplasmosis. It reduced the brain tissue cyst count with elevation of INF-γ[31]. In this research, we attempted the evaluation of the immunomodulatory effect of NSO and WGO-loaded MOF for the treatment of chronic murine toxoplasmosis.

MATERIAL AND METHODS

This experimental study was conducted at the Departments of Parasitology, Applied Organic Chemistry, and Immunology, National Research Centre (NRC), Giza, Egypt during the period from October 2023 to April 2024.

Study design: Male Swiss albino mice were divided into 8 groups. The test groups were infected with *T. gondii* ME-49 avirulent strain, treated with either spiramycin, NSO, or WGO either alone or loaded on NPs at 8 weeks post-infection (wpi), and sacrificed at 10 wpi. The efficacy of drugs was tested through IHC staining of liver specimens with serological detection of TNF-α and INF–γ (Fig. 1).

Animals: Eighty male laboratory-bred Swiss albino mice were obtained from the NRC's animal house. They were 6 weeks old and weighed 20-25 grams. Plastic cages containing white wood chips were used to keep 8 mice in every cage. Mice were maintained on a complete commercial food mixture for feeding and tap water for drinking. The animals were kept for 12 h in light and 12 h in dark successively at an average temperature of (25±2^{0}C).

Drugs and regimens: Spiramycin (Medical Union Pharmaceuticals, Cairo, Egypt) was administered at a dose of 200 mg/kg/d[32]. While Cu-BTC MOFs and WGO were obtained from Sigma-Aldrich Co., USA, NSO was obtained from Pharco-Pharmaceuticals, Alexandria, Egypt. The doses for Cu-BTC MOFs[33], WGO[34], and

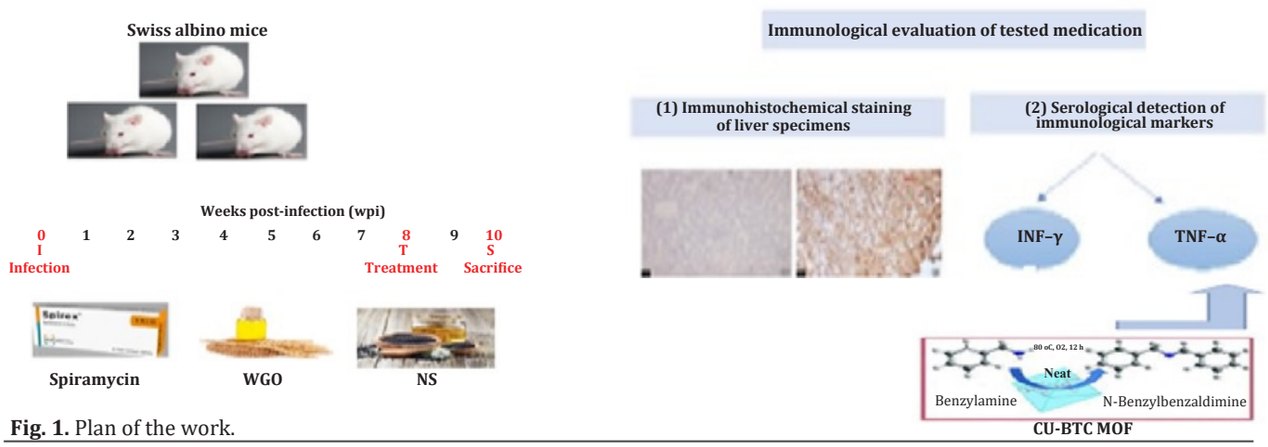

Fig. 1. Plan of the work.

NSO[35] were 100, 1400, and 10 mg/kg/day, respectively. In all treated groups, treatment started at 8 wpi for 2 w[36].

Mice infections and sacrifice: Mice were infected by 0.1 ml of brain suspension containing 10 cysts/ mouse[17] administered by gavage using an esophageal tube. They were sacrificed at 10 wpi by beheading[37].

Animal groups: The animals were divided into eight groups, ten mice each, designated as in table (1).

Table 1. The study groups.

Groups (n=10)	Characteristics
GI	Not infected, not treated.
GII	Infected, not treated.
GIII	Infected and treated with Spiramycin.
GIV	Infected and treated with Spiramycin + Cu-BTC MOF.
GV	Infected and treated with Cu-BTC MOF.
GVI	Infected and treated with WGO + Cu-BTC MOF.
GVII	Infected and treated with NSO + Cu-BTC MOF.
GVIII	Infected and treated with WGO + NSO + Cu-BTC MOF.

Cu-BTC: Copper-benzene tricarboxylic acid; **MOF:** Metal-organic frameworks; **WGO:** Wheat germ oil; **NSO:** *Nigella sativa* oil.

Preparation of *T. gondii* strain and experimental infection: Thirty Swiss albino mice were regularly used to maintain the avirulent ME-49 strain of *T. gondii* at the NRC's animal house facility. A 0.1 ml of the brain homogenate containing 10 cysts/mouse of a previously infected mouse was fed to the infected groups through a gastric tube. The cervical dislocation was used to kill the infected animals 8 wpi. The brains of the killed mice were extracted. Sterile pestle and mortar were used to homogenize the brain tissues. The homogenized brains were diluted in 1 ml of phosphate-buffered saline (PBS, pH 7.4) per each one[38]. To prepare the homogenate for the following infection by gastric gavage, the homogenate was adjusted to an average concentration of 1×10^2 cysts/ ml using a hemocytometer (Boeckel Co., Germany)[39].

Preparation of Cu-BTC MOF: The method described by Abdelhameed *et al*.[40] was adopted.

Characterization of MOF: Using a scanning electron microscope (SEM: Hitachi SU-70, Japan), the nanostructure morphology of MOF was studied[20].

Preparation of NSO and WGO@Cu-BTC MOF: In 100 ml of ethanol, NSO and WGO were dissolved in concentrations of 100-1000 ppm. A magnetic stirrer was used to mix the medication solutions with 1 g of Cu-BTC MOFs for ninety minutes at room temperature, followed by overnight incubation at room temperature, and then centrifugation of the suspension at 5,000 rpm for 5 min. To calculate the amount of loaded oil in the supernatant, the concentrations of NSO and WGO in the solution before and after loading were compared using the equation: A−B/A×100 [A= initial concentration of the drug and B = final concentration of the drug][29,40].

Immunological evaluation of the tested medications

- **Immunohistochemical (IHC) staining[41]:** Scarification of mice was performed at 10 wpi and the animals' livers were removed for IHC examinations. Micro-sections of the liver fixed with formalin and embedded in paraffin (FFPE) were applied on glass slides. The IHC process was performed following the instructions of a commercial avidin-biotin-peroxidase complex (ABC) kit (Vectastain® ABC-HRP kit, Vector labs, USA), which used Meyer's Hematoxylin as a counter stain and diaminobenzidine (DAB) as a chromogen. Primary mouse anti-CD4 monoclonal antibody (Elabscience®, USA, Cat Number E-AB-22098, Diluted 1:200), and secondary mouse anti-$CD8^+$ monoclonal antibody (Elabscience®, USA, Cat Number PA5036, diluted 1:100) were added to the liver slices from each group. To identify antigen-antibody complexes, marker $CD8^+$ T-cell expression was dyed with DAB (Sigma-Aldrich, USA) and peroxidase-labeled. The 1^{ry} or 2^{ry} antibodies were substituted with non-immune serum for the negative controls. IHC-stained sections were viewed under an (Olympus BX-53, Germany) light microscope at magnifications of 40x and 100x. In short, FFPE liver sections were exposed to 2 successive steps: deparaffinization by xylene and rehydration with ethanol in distilled water at decreasing concentrations. Hydrogen peroxide 3% solution in a 10 ml citrate buffer (pH 6.0) solution was used to inhibit the endogenous peroxidase activity. The liver sections were then blocked using an additional solution (1% bovine serum albumin, BSA, in 0.1 M PBS), then incubation was done for one hour at room temperature with mouse polyclonal anti-*T. gondii* ME49 antibodies (1: 2000 diluted with 1% BSA in tris-buffered saline with tween 20, TBST). To view the antigen-antibody reaction, immunoglobulin IgG HRP-conjugated and 0.2% DAB were added. Hematoxylin was used to color the background of all DAB-treated tissue sections before they were inspected under a light microscope.

Serological detection of immunological markers

- **Serum samples preparation:** Blood samples (30 to 50 µL blood) were collected from the jugular vein at 10 wpi before scarification[42], clotted at room

temperature, and then centrifuged at 2000 rpm for 10 min. The aliquoted serum samples were stored at -20^{0}C until needed.

- **Immune markers:** Cytokines (INF–γ, and TNF–α) levels were measured by commercially available ELISA kits (Quantikine, USA and Cusabio, China, respectively). In brief, serum samples obtained after completion of the treatment regimen were incubated with the immobilized specific antibodies to visualize the HRP-TMB reaction[43].

Statistical analysis: Data analysis was done by SPSS version 20 computer software. Data were presented as mean±SD. The ANOVA (F) with subsequent post-hoc Tukey test was applied for multiple comparisons between the different tested groups. The *P* value was considered significant at less than 0.05.

Ethical consideration: The study received approval from the Research Ethics Committee at the Faculty of Medicine, Ain Shams University with approval number (FMASU R25/2024).

RESULTS

Characterization of MOF and efficiency of drug loading on Cu-BTC: Five diffractions were produced for the powder X-ray diffraction (PXRD) of Cu-BTC at 2θ=6.7^o, 9.6^o, 11.6^o, 13.4^o, and 19.1^o. CuB-TC MOF was shown to have a homogenous 3D porous structure with an octahedral form using SEM investigation. The successful drug loading of Cu-BTC depends on the concentration of the drug in addition to the surface-to-volume ratio of Cu-BTC, therefore as these parameters rise, so does the drug loading percentage. Once it reached a certain level, it remained constant. A loading dose of 254.2 mg/g was used. With a drug concentration of 100 ppm and a longer stirring period, the drug loading increased. At 90 min, the maximum loading was achieved.

Immunohistochemical staining (IHC) of liver tissues: Liver tissues of all *Toxoplasma*-infected groups reacted with antibodies against the CD8$^+$ marker expressed as different densities. The result was interpreted as positive CD8$^+$ expression (dark brown color with different intensities), or negative CD8$^+$ expression (blue color). The IHC reaction was negative in the healthy control group (GI) (Fig. 2A), while it was strongly positive in the *Toxoplasma*-infected untreated group (GII) indicating predominance of CD8$^+$ cells (Fig. 2B).

Highly predominant CD8$^+$ cells were also noticed in GVII and GVIII (Fig. 3 A and B), and moderate expression was observed in GVI (WGO@Cu-BTC MOF-treated group) (Fig. 3C). Low or minimal expression of CD8$^+$ cells was apparent in GIII, GIV, and GV (Fig. 3 D, E and F).

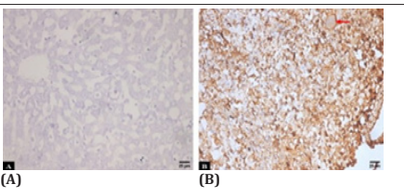

Fig. 2. IHC staining of normal healthy versus infected liver tissue. **A:** GI (uninfected, untreated negative control group) showing blue color (negative CD8$^+$ expression). **B:** GII (*Toxoplasma*-infected untreated positive control group) showing intense brown color (high predominant CD8$^+$ expression) and *Toxoplasma* cyst (red arrow in the right upper corner) (x400).

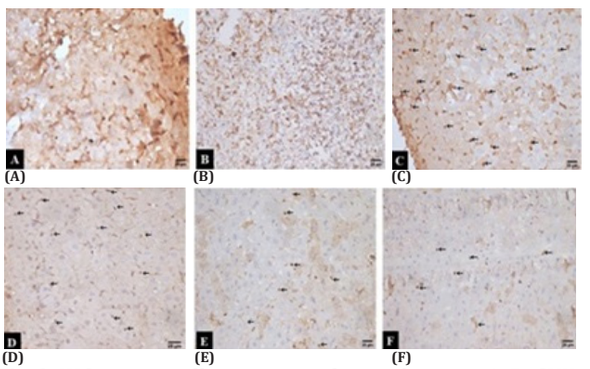

Fig. 3. IHC staining of liver tissues of treated groups. **A, B:** GVII (NSO@MOF-treated), and GVIII (WGO+NSO@MOF-treated) show diffuse brown color indicating high predominant CD8$^+$ expression. **C:** GVI (WGO@MOF-treated) showing moderate expression of CD8$^+$ (arrows). **D, E and F:** GIII (Spiramycin-treated), GIV (Spiramycin@MOF-treated) and GV (MOF-treated) showing low expression of CD8$^+$ (arrows) (x400), respectively.

Serological detection of immunological markers (INF-γ and TNF-α): Comparison between different test and control groups regarding the levels of serum INF-γ and TNF-α showed a statistically significant difference (*P*<0.0001) between the treated test and the control groups. Mice of GVII showed the highest level of serum INF–γ and TNF–α followed by GVIII and GVI respectively (Table 2).

Table 2. Comparison between different test and control groups regarding the levels of INF-γ and TNF-α.

Study groups	INF–γ (pg/ml) Mean ± SD	*P* value	TNF–α (pg/ml) Mean ± SD	*P* value
GI	57±1.5		45.02±2.64	
GII	97±1.8^{a}		71.77±2.09^{a}	
GIII	187±2.1^{ab}		102.1±2.05^{ab}	
GIV	150±3.8^{abc}	<0.0001*	97.19±2.38^{abc}	<0.0001*
GV	147±2^{abc}		90.54±3.59^{abcd}	
GVI	254±2.4^{abcde}		125.80±2.45^{abcde}	
GVII	283±3.6^{abcdef}		141.90±2.42^{abcdef}	
GVIII	265±3.1^{abcdefg}		133.8±2.21^{abcdefg}	

GI: Non-infected non-treated (negative control), GII-GVIII: Infected with ME-49 avirulent strain of *T. gondii*; **GII:** Non-treated (positive control), GIII-GVIII: Treated with: **GIII:** Spiramycin, **GIV:** Spiramycin@Cu-BTC MOF, **GV:** Cu-BTC MOF, GVI: WGO@Cu-BTC MOF, **GVII:** NSO@Cu-BTC MOF, **GVIII:** WGO+NSO@Cu-BTC MOF. ANOVA test was used for group comparison. The significance between groups was determined through a post hoc test (Tukey). **a:** Significant versus GI; **b:** Significant versus GII; **c:** Significant versus GIII; **d:** Significant versus GIV; **e:** Significant versus GV; **f:** Significant versus GVI; **g:** Significant versus GVII; *: Significant (*P*<0.05).

DISCUSSION

In the present study, liver sections were selected to perform the IHC examination for the detection of $CD8^+$ T-cells to throw light on the activation of $CD8^+$ cells during chronic toxoplasmosis and with subsequent administration of therapy. In fact, IHC is a diagnostic tool that utilizes an immunologic reaction to demonstrate the quantity of a particular substance in tissues while preserving the histologic architecture[44]. Moreover, since the liver is well-equipped with innate immunological phagocytic Kupffer cells to fight off invasive pathogens, it serves as the 2nd most effective barrier against pathogens after the intestinal epithelium, the body gatekeeper[45]. However, in chronic toxoplasmosis, tachyzoite invasion inside normal hepatocytes leads to the eventual induction of apoptosis, interrupting this innate immunity[46]. Instead, cytotoxic $CD8^+$ T-cells are essential contributors to the developed resistance and the cornerstone in fighting intracellular infection. Functional T-cell subsets are crucial for inducing protection for a long time and maintaining the chronic state of infection thus, preventing the development of a disease due to parasite reactivation[47]. Based on the fact that the liver is a highly vascularized organ and a frequent target for pathogens, it was chosen for the IHC procedure in the current research. Our study showed that IHC reactivity to $CD8^+$ molecule attached to the membrane of cytotoxic $CD8^+$ T-cells was evident in the infected liver tissue of mice (GII) sacrificed 10 wpi compared to a negative reaction in GI, indicating a prominent T-cell reaction against *T. gondii* throughout the dormant stage of the infection. This result agrees with Yahia *et al*.[41] who reported significant upregulation of cytotoxic T-lymphocytes in the hepatic sections of mice with toxoplasmosis sacrificed 9 wpi. Tiwari *et al*.[48] also performed an IHC examination and illustrated the role of cytotoxic T-lymphocytes in killing target cells. Not only do they reduce the number of *Toxoplasma* cysts by inhibiting the proliferation of tachyzoites *via* direct cell-cell contact, but they can also invade and damage the cyst wall.

A note of interest is the remarkable upregulation of $CD8^+$ T-cells revealed by a strongly positive IHC reactivity in GVII and GVIII followed by GVI compared to the lower expression in GIII, GIV, and GV. Mahmoud *et al*.[49] addressed the immunostimulatory function of *N. sativa*, which was evident by enhancing the total antioxidant capacity, stimulation of cell-mediated cytokine production (IL-12, IFN-γ, TNF-α), and promotion of $CD8^+$ expression. This also concurs with studies that revealed the anti-*Toxoplasma* activity of NSO@Cu-BTC MOF and refer it to the antioxidant, anti-inflammatory, and cytoprotective activity of thymoquinone (a compound found in NSO), in collaboration with the enhanced bioavailability of the nano-porous Cu-BTC[50, 51]. This agrees with Mady *et al*.[16] who demonstrated NSO as a potent activator of cell-mediated immunity cells, preventing *T. gondii* from using the immunological evasion mechanisms. These results may also be supported by a study that reported WGO as having powerful anti-inflammatory activity in addition to a considerable antagonism to oxidative stress[52]. Besides, these results are consistent with a study that used MOF-NPs, spiramycin, and spiramycin loaded on MOFs-NPs against chronic murine toxoplasmosis in which low-density $CD8^+$ infiltration was noted in the livers from infected treated mice emphasizing the fact that spiramycin and the MOF NPs are weak enhancers of the cell-mediated immune response against toxoplasmosis[11].

Previously, we reported 100% survival, 64.3% and 51.4% decrease in brain cysts in mice with chronic toxoplasmosis treated with the combination of NSO with Cu-BTC and combination of WGO, and NSO with Cu-BTC, respectively with an obvious amelioration of pathological lesions. When the WGO and Cu-BTC combination was applied in the infected mice, the survival rate was 90%, the reduction of tissue cysts was 49.5% and obvious improvement of the affected tissues was found. Moreover, when spiramycin was used alone or in combination with Cu-BTC, the survival rate was 80%, and the reduction of brain cysts was 42.4% and 41.8% consecutively with mild to moderate reduction of tissue damage noted. The lowest effect was obtained with the infected group treated with Cu-BTC in which the survival rate was only 70% and the reduction of brain cysts was 24.4%[17].

In the current work, a statistically significant difference (P<0.0001) was recorded between the treated test and the control groups regarding the levels of INF–γ and TNF–α. Our results were consistent with a study that revealed the highest level of INF–γ (300.50±22.22 pg/ml) in the infected mice group treated with (NSO+pyrimethamine) followed by (295.50 ±41.22 pg/ml) in the group treated with NSO only, and lastly (88.90±14.61pg/ml) in the group treated with (clindamycin+pyrimethamine)[16].

In conclusion, NSO and WGO could be coupled successfully with Cu-BTC inducing a desirable cell-mediated immunity in mice with chronic toxoplasmosis. This was evidenced by increased $CD8^+$ expression in the liver with enhanced INF–γ, and TNF–α secretion in the sera of the treated infected mice.

Author contribution: Shehata MA, El Naggar HM, Abdelmaksoud HF, and Mohammad OS contributed in the study design. Barakat AM infected and handled the experimental mice throughout the experiment. Abdelhameed RM performed the immunohistochemical study. Atta SA performed the serological detection of immunological markers (INF-γ and TNF-α). El-Ashkar AM performed the formal analysis and wrote the manuscript. All authors approved the authorship and critically revised the manuscript before publication.

Conflict of interest: None.
Funding statement: No funds were received for this research article.

REFERENCES

1. Lima T, Lodoen M. Mechanisms of human innate immune evasion by *Toxoplasma gondii*. Front Cell Infect Microbiol 2019; 9:103.
2. Sasai M, Yamamoto M. Innate, adaptive, and cell-autonomous immunity against *Toxoplasma gondii* infection. Exp Mol Med 2019; 51(12):1-10.
3. Yarovinsky F. Innate immunity to *Toxoplasma gondii* infection. Nat Rev Immunol 2014; 14:109–121.
4. Tosh KW, Mittereder L, Bonne-Annee S, Hieny S, Nutman T, Singer S, *et al*. The IL-12 response of primary human dendritic cells and monocytes to *Toxoplasma gondii* is stimulated by phagocytosis of live parasites rather than host cell invasion. J Immunol 2016; 196:345–356.
5. Zhao XY, Ewald SE. The molecular biology and immune control of chronic *Toxoplasma gondii* infection. J Clin Invest 2020; 130:3370–3380.
6. Moghaddami R, Mahdipour M, Ahmadpour E. Inflammatory pathways of *Toxoplasma gondii* infection in pregnancy. Travel Med Infect Dis 2024; 62:102760.
7. Montazeri M, Rezaei K, Ebrahimzadeh M, Sharif M, Sarvi S, Ahmadpour E, *et al*. Survey on synergism effect of ketotifen in combination with pyrimethamine in treatment of acute murine toxoplasmosis. Trop Med Health 2017; 45:1-5.
8. Shammaa A, Powell T, Benmerzouga I. Adverse outcomes associated with the treatment of *Toxoplasma* infections. Sci Rep 2021; 11(1):1035.
9. Hagras N, Mogahed N, Sheta E, Darwish A, El-Hawary M, Hamed M, *et al*. The powerful synergistic effect of spiramycin/propolis loaded chitosan/alginate nanoparticles on acute murine toxoplasmosis. PLoS Negl Trop Dis 2022; 16:e0010268.
10. EL-Ashkar A, El-Hosseiny L, Abu Zahra F, Abd El-Samee N, Barakat A, Elgohary S, *et al*. Potential therapeutic effect of allogenic mesenchymal stem cells on chronic cerebral murine toxoplasmosis. AEJI 2020; 10(2):129–140.
11. El Naggar H, Anwar M, Khayyal A, Abdelhameed R, Barakat A, Sadek S, *et al*. Application of honeybee venom loaded nanoparticles for the treatment of chronic toxoplasmosis: Parasitological, histopathological, and immunohistochemical studies. J Parasit Dis 2023; 47(3):591-607.
12. Etewa S, Al-Hoot A, Abdelmoaty S, Mohammad S, Moawad H, Sarhan M, *et al*. The outcomes of mesenchymal stem cells therapy for experimental toxoplasmosis. PUJ 2019; 12(1):34-44.
13. Assi MA, Noor MH, Bachek NF, Ahmad H, Haron AW, Yusoff MS, *et al*. The various effects of *Nigella sativa* on multiple body systems in human and animals. PJSRR 2016; 2(3): Corpus ID: 73570780.
14. Abd El-Hack ME, Alagawany M, Farag MR, Tiwari R, Karthik K, Dhama K. Nutritional, healthical and therapeutic efficacy of black cumin (*Nigella sativa*) in animals, poultry and humans. Int J Pharmacol 2016;12(3):232-248.
15. Mahmoudvand H, Sharififar F, Rahmat MS, Tavakoli R, Dezaki ES, Jahanbakhsh S, *et al*. Evaluation of antileishmanial activity and cytotoxicity of the extracts of *Berberis vulgaris* and *Nigella sativa* against *Leishmania tropica*. J Vector Borne Dis 2014; 51(4):294-299.
16. Mady RF, El-Hadidy W, Elachy S. Effect of *Nigella sativa* oil on experimental toxoplasmosis. Parasitol Res 2016; 115:379–390.
17. Mohammad O, El Naggar H, Abdelmaksoud H, Barakat A, Abdelhameed R, Shehata M. The effect of *Nigella sativa* oil and wheat germ oil-loaded metal organic frameworks on chronic murine toxoplasmosis. Acta Trop 2023; 239, 106823.
18. Abdelmaksoud H, Aboushousha T, El-Ashkar A. Deep glance on the antiparasitic anticancer activities of wheat germ oil in chronically infected immunosuppressed mice with cryptosporidiosis. J Parasit Dis 2022; 46(3):785-794.
19. Abdelmaksoud H, Osman E, Abdel-Hameed S, Aboushousha T, Naggar H. *In vivo* evaluation of anti-cryptosporidial effects of wheat germ extracts in immunocompromised mice. J Parasit Dis 2022; 46(3):833-844.
20. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arab J Chem 2019; 12:908–931.
21. Elmahallawy EK, El Fadaly HAM, Soror AH, Ali FAZ, Abd El-Razik KA, Soliman YA, *et al*. Novel insights on the potential activity of propolis and wheat germ oil against chronic toxoplasmosis in experimentally infected mice. Biomed Pharmacother 2022; 156:113811.
22. Karimi Alavijeh R, Akhbari K. Biocompatible MIL-101(Fe) as a smart carrier with high loading potential and sustained release of curcumin. Inorg Chem 2020; 59:3570–3578.
23. Trotta F. Metal organic frameworks in medicine. Acta Sci Pharma Sci 2019; 3:107–109.
24. Soltani S, Akhbari K. Cu-BTC metal-organic framework as a biocompatible nanoporous carrier for chlorhexidine antibacterial agent. J Biol Inorg Chem 2022; 27:81–87.
25. Emam HE, Darwesh OM, Abdelhameed RM. In-growth metal organic framework/synthetic hybrids as antimicrobial fabrics and its toxicity. Colloids Surf B Biointerfaces 2018; 165:219–228.
26. Soltani S, Akhbari K, White J. Sonochemical synthesis, crystal structure and antimicrobial property of one-dimensional dinuclear coordination polymer. Z Anorg Allg Chem 2021; 647:442–447.
27. Soltani S, Akhbari K, Phuruangrat A. Incorporation of silver nanoparticles on Cu-BTC metal–organic framework under the influence of reaction conditions and investigation of their antibacterial activity. Appl Organomet Chem 2022; 36(6):e6634.
28. Soltani S, Akhbari K, Phuruangrat A. Investigation of effective factors on antibacterial activity of Pillared-Layered MOFs. J Mol Struct 2021; 1225:129261.
29. El-Shafey A, Hegab M, Seliem M, Barakat A, Mostafa N, Abdel-Maksoud H, *et al*. Curcumin@metal organic frameworks nano-composite for treatment of chronic toxoplasmosis. J Mater Sci Mater Med 2020; 31:1–13.
30. Etewa S, El-Maaty D, Hamza R, Metwaly A, Sarhan M, Abdel-Rahman S, *et al*. Assessment of spiramycin-loaded

chitosan nanoparticles treatment on acute and chronic toxoplasmosis in mice. J Parasit Dis 2018; 42:102–113.
31. Elgendy AWM, Haggag YA, El-Nouby KA, El-Kowrany SI, El Marhoumy SM. Evaluation of the effect of guanabenz-loaded nanoparticles on chronic toxoplasmosis in mice. Exp Parasitol 2023; 246:108460.
32. Grujić J, Djurković-Djaković O, Nikolić A, Klun I, Bobić B. Effectiveness of spiramycin in murine models of acute and chronic toxoplasmosis. Int J Antimicrob Agents 2005; 25:226–230.
33. Alajmi R, AL-Megrin W, Metwally D, AL-Subaie H, Altamrah N, Barakat A, *et al*. Anti-*Toxoplasma* activity of silver nanoparticles green synthesized with phoenix dactylifera and *Ziziphus spina-christi* extracts which inhibits inflammation through liver regulation of cytokines in Balb/c mice. Biosci Rep 2019; 39(5):BSR20190379.
34. Karabacak M, Kanbur M, Eraslan G, Soyer Sarica Z. The antioxidant effect of wheat germ oil on sub-chronic Coumaphos exposure in mice. Ecotoxicol Environ Saf 2011; 74:2119–125.
35. Mahmoudvand H, Dezaki ES, Kheirandish F, Ezatpour B, Jahanbakhsh S, Harandi MF. Scolicidal effects of black cumin seed (*Nigella sativa*) essential oil on hydatid cysts. Korean J Parasitol 2014; 52:653–659.
36. Rayan HZ, Wagih HM, Atwa MM. Efficacy of black seed oil from *Nigella sativa* against murine infection with cysts of Me49 strain of *Toxoplasma gondii*. PUJ 2011; 4:165–176.
37. Boivin G, Bottomley M, Grobe N. Responses of male C57BL/6N mice to observing the euthanasia of other mice. J Am Assoc Lab Anim Sci 2016; 55:406–411.
38. Djurkovic-Djakovic O, Milenković V, Nikolić A, Bobić B, Grujić J. Efficacy of atovaquone combined with clindamycin against murine infection with a cystogenic (Me49) strain of *Toxoplasma gondii*. J Antimicrob Chemother 2002; 50:981–987.
39. El-Sayed NM, Aly EM. *Toxoplasma gondii* infection can induce retinal DNA damage: an experimental study. Int J Ophthalmol 2014; 7:431–436.
40. Abdelhameed R, Abdel-Gawad H, Elshahat M, Emam H. Cu–BTC@cotton composite: Design and removal of ethion insecticide from water. RSC Adv 2016; 6:42324–42333.
41. Yahia SH, Etewa SE, Saleh NS, Mohammad SM, Aboulfotouh NI, Kandil AM, *et al*. Histopathological, immunohistochemical and biochemical studies of murine hepatosplenic tissues affected by chronic toxoplasmosis. J Parasitol Res 2022; 2022:2165205.
42. Shirasaki Y, Ito Y, Kikuchi M, Imamura Y, Hayashi T. Validation studies on blood collection from the jugular vein of conscious mice. J Am Assoc Lab Anim Sci 2012; 51(3): 345-351.
43. Stoicov C, Whary M, Rogers A, Lee F, Klucevsek K, Li H, *et al*. Coinfection modulates inflammatory responses and clinical outcome of *Helicobacter felis* and *Toxoplasma gondii* infections. J Immunol 2004; 173:3329–3336.
44. Kim SW, Roh J, Park CS. Immunohistochemistry for pathologists: Protocols, pitfalls, and tips. J Pathol Transl Med 2016; 50:411–418.
45. Heymann F, Tacke F. Immunology in the liver from homeostasis to disease. Nat Rev Gastroenterol Hepatol 2016; 13:88–110.
46. Lee SH, Chu KB, Quan FS. Parasite infiltration and apoptosis in spleen upon *Toxoplasma gondii* Infection. Korean J Parasitol 2019; 57:537–541.
47. Khan IA, Ouellette C, Chen K, Moretto M. *Toxoplasma*: Immunity and pathogenesis. Curr Clin Microbiol Rep 2019; 6:44–50.
48. Tiwari A, Hannah R, Lutshumba J, Ochiai E, Weiss L, Suzuki Y. Penetration of $CD8^+$ cytotoxic T cells into large target, tissue cysts of *Toxoplasma gondii*, leads to its elimination. Am J Pathol 2019; 189:1594–1607.
49. Mahmoud HS, Almallah AA, Gad EL-Hak HN, Aldayel TS, Abdelrazek HMA, Khaled HE. The effect of dietary supplementation with *Nigella sativa* (black seeds) mediates immunological function in male Wistar rats. Sci Rep 2021; 11(1):7542.
50. Shaterzadeh-Yazdi H, Noorbakhsh M-F, Samarghandian S, Farkhondeh T. An overview on renoprotective effects of thymoquinone. Kidney Dis (Basel). 2018; 4(2):74-82.
51. Kulyar MF, Li R, Mehmood K, Waqas M, Li K, Li J. Potential influence of *Nigella sativa* (Black cumin) in reinforcing immune system: A hope to decelerate the COVID-19 pandemic. Phytomedicine 2021; 85:153277.
52. Morshedy SA, Abdelmodather AM, Basyony MM, Zahran SA, Hassan MA. Effects of rocket seed oil, wheat germ oil, and their mixture on growth performance, feed utilization, digestibility, redox status, and meat fatty acid profile of growing rabbits. Agriculture 2021; 11(7):662.