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Sex Hormones can Modulate *Toxoplasma gondii* Infection: An Experimental Study

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Abstract

BECAUSE *Toxoplasma gondii* is a parasite with several developmental stages and strains, anti-*Toxoplasma* medications must be efficient for various stages and strains. The commonly used drugs need long treatment periods and have hazardous side effects. Combining therapies may decrease the pharmaceutical dosages while preserving therapeutic effects and minimizing toxicity. This study evaluated the efficacy of combining spiramycin and metronidazole with testosterone/progesterone and the combination loaded on Zeolitic imidazolate frameworks (ZIF-8) nanoparticles in treating chronic toxoplasmosis in animal models. Mice were orally infected by brain suspension containing *Toxoplasma gondii* cysts. They were sorted into female and male groups, and each group was then divided into five subgroups: negative control (uninfected), positive control (infected untreated), treated with spiramycin and metronidazole, treated with spiramycin and metronidazole in combination with testosterone/progesterone, and treated with the combination-loaded ZIF-8 nanoparticles. Tested compounds' efficacy was determined by parasitological assessment of parasite load in the brain, *Toxoplasma* DNA quantification in brain tissues using real-time PCR, and histopathological evaluation. Treatment of infected mice with spiramycin and metronidazole in combination with testosterone or progesterone significantly decreased the parasite burden. Moreover, mice treated with the drug combination carried on ZIF-8 showed a marked decrease in parasitic load versus the other groups. All treated groups showed a reduction in *Toxoplasma* DNA concentrations in brain tissues, with significant variation compared to positive controls. Likewise, combo therapy successfully cured the histopathological alterations in the infected mice's brains. In conclusion, sex hormones can modulate *Toxoplasma* infection, and their loading on ZIF-8 nanoparticles increased anti-toxoplasmic effect.

Keywords: Toxoplasmosis, Testosterone, Progesterone, Metal–organic frameworks, Drug delivery.

Introduction

Toxoplasma gondii (*T. gondii*) infection affects various tissues and organs in humans and animals. Globally, *T. gondii* infection affects one-third of the population [1]. Toxoplasmosis can be transmitted by consuming contaminated vegetables, fruits, and water with excreted oocysts in infected cats' feces or undercooked meat that contains tissue cysts [2]. Eighty percent of cases in immunocompetent individuals may be asymptomatic. However in immunocompromised patients, fatal encephalitis has been linked to *Toxoplasma* infection [3].

The standard regimen for the treatment of toxoplasmosis is pyrimethamine combined with sulfadiazine. This combination has been effective in treating acute toxoplasmosis, but it has been ineffective in treating congenital toxoplasmosis, chorioretinitis, and toxoplasmic encephalitis. The emergence of resistance, the limited efficacy against *Toxoplasma* tissue cysts, and the occurrence of hazardous complications like teratogenic effects, bone marrow suppression, and hypersensitivity reactions are the main disadvantages of these treatments [4]. Alternatively, the macrolide antibiotic spiramycin has lower toxicity and can cross the

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placenta at high concentrations [5]. Despite exhibiting high tissue penetration, it has low penetration across the blood-brain barrier (BBB). To enhance brain bypass, administering a combination of spiramycin and metronidazole can deactivate the BBB's efflux pumps [6].

Toxoplasma infection has been treated with a variety of nanomaterials. These nanomaterials may have anti-toxoplasmic activity directly or indirectly as drug carriers, allowing for more precise drug delivery and decreasing host toxic effects. Additionally, they can circumvent the drawbacks of anti-toxoplasmic drugs, such as their rapid body elimination, non-specific distribution, low bioavailability, and poor cellular permeability [7]. Metal-organic frameworks (MOFs) are organic-inorganic hybrid porous materials that have extensive research interest in medicinal uses because of their distinct chemical and physical characteristics and low toxicity. They are thought to be promising candidates for drug delivery and diagnosing diseases [8]. A subtype of MOFs known as Zeolitic imidazolate frameworks (ZIF-8) has advantageous physicochemical characteristics, such as pore size, that enable them to load drugs at a high level and effectively control release [9]. ZIF-8 functions as a host, protecting drugs, proteins, and enzymes against destruction caused by proteolytic agents, solvents, and temperature. ZIF-8-encapsulated antimicrobial agents demonstrated higher biological activity than that of free antimicrobial agents [10].

Sex steroids are well recognized for their ability to control many processes, including differentiation, growth, and reproduction. They have drawn attention lately for their ability to control the immunological response against pathogens. Studies on *T. gondii* infection have revealed both positive and negative correlations with the modulation of circulating hormones, such as progesterone and testosterone [11, 12].

Thirty research papers were reviewed by Galvan-Ramirez et al., [13]; eight of the investigations used human subjects, and the remaining 22 involved cell culture and animal models. Among the assessed hormones were testosterone and progesterone. According to this review, hormones affect *Toxoplasma* infection in various animals. Hormones may exacerbate *Toxoplasma* infection as well as stimulate an anti-inflammatory response in cell lines [14]. According to Hegazy et al., [11], combined hormone therapy with anti-*Toxoplasma* medications showed efficacy in infected female mice. On the other hand, Laubach et al., [12] observed no correlation between *Toxoplasma* infection and testosterone levels in the blood of adult male hyenas or female cubs and subadults. So, this study evaluated the efficacy of combining spiramycin and metronidazole with testosterone/progesterone and the combination loaded on ZIF-8 nanoparticles in treating chronic toxoplasmosis in animal models.

Material and Methods

Experimental animals, infectious agent and infectious dose

This study used 100 laboratory-bred Swiss albino mice, both female and male. They were eight weeks old and ~25 grams in weight. Throughout the experiment, the same-sex mice groups were kept inside plastic cages with bedding made from wood shavings in a controlled environment with a temperature of 25±2°C, 12-hour cycles of light and dark, and easy food and water accessibility.

For mice infection, cystogenic ME49 strain of *T. gondii* was provided by the Department of Zoonotic Diseases, National Research Centre, Dokki, Giza, Egypt. It was isolated from mice's brains infected eight weeks earlier. Each brain was homogenized with one ml of phosphate-buffered saline (PBS, pH=7.2). After that, brain suspension was diluted until $1x10^2$cysts/ml [15]. Each mouse was orally infected by a brain suspension (0.1 ml) containing 10 *T. gondii* cysts. On day 40 PI (post-infection), the enzyme-linked immunosorbent assay (MyBioSource, Inc., California, USA) was used in accordance with the manufacturer's instructions to identify *T. gondii* IgG antibodies, confirming the infection in mice.

Tested compounds and schedule of administration

The tested compounds included spiramycin (200mg/kg/day) [16], metronidazole (250mg/kg/day) [17], testosterone (2.8mg/kg/day) [18], and progesterone (2mg/kg/day) [19], which were given for 14 consecutive days for the infection model. All tested compounds were purchased from Pharmaceuticals Co. and Chemical Industries, Egypt. Dosing solutions of spiramycin and metronidazole were mixed with ultra-pure water and given by a 22-gauge blunt feeding needle after an overnight fast. To optimize the spiramycin effect at the BBB, metronidazole was given 30 minutes before the spiramycin dose to improve the dispersion and uptake of tissues [17]. Testosterone and progesterone were administered by subcutaneous injection, either alone or incorporated into ZIF-8 nanoparticles.

ZIF-8 nanoparticles preparation

ZIF-8 nanoparticles were prepared following these steps: 2-methylimidazole (Hmim, 4.10g, 50 mmol) was dissolved in 100 ml of methanol. Methanol solution of Zn(NO3)2·6H2O (2.97g, 10 mmol) was added to a methanol solution of 2-methylimidazole having lignin. The mixture was left at room temperature with constant stirring for 24 hours. The resulting particles were separated via centrifugation, washed in methanol and deionized water, and dried at 60°C for 24 hours under reduced

pressure. All used chemicals were analytical-grade reagents purchased from Sigma.

Tested compounds loading

To load the tested compounds, metronidazole, spiramycin, progesterone, and testosterone, into ZIF-8 nanoparticles, various concentrations (100–1000 ppm) of each compound were diluted using 100 ml of ethanol. Each drug solution was mixed with one gram of ZIF-8 nanoparticles and stirred at 600×g for ninety minutes at room temperature using a magnetic stirrer. Then, the suspension was kept for a night and followed by centrifugation at 5000×g for five minutes to separate the supernatant and precipitate. The loaded amount of each tested compound was estimated from the variance in its concentration within the solution before and after loading. The tested compounds loading percent were estimated as follows: Drug loading % =[(A−B)/A] × 100; where A and B indicate each compound's initial and final concentrations in the solution.

Characterization of ZIF-8 nanoparticles

X-ray diffraction (XRD) patterns (using an X'Pert MPD Philips diffractometer with Cu Kα monochromate at a wavelength of 1.541 Å and X-ray tube rating maintained at 40 kV and 25 mA) were applied to characterize phase purity and crystallographic structures of the prepared materials. The data were measured in the 2θ with a step width of 0.05° and a range of 3.5° to 30°. The nanostructure morphology of ZIF-8 nanoparticles was tested via a scanning electron microscope (SEM: Hitachi SU-70, JP). KBr pellets of samples were prepared for FTIR spectroscopy and measurements were taken on Varian 670-IR.

Experimental design

Based on their sex, mice were sorted into female (GI) and male (GII) groups, and each group was then divided into five subgroups, each with ten mice. In the female group (GI), GIa was uninfected negative control, GIb was infected untreated (positive control), GIc was infected-treated with spiramycin and metronidazole, GId was infected-treated with spiramycin and metronidazole + progesterone, and GIe was infected and then treated with the loaded spiramycin + metronidazole + progesterone on ZIF-8 nanoparticles. In the male group (GII), GIIa was uninfected negative control, GIIb was infected untreated (positive control), GIIc was infected-treated with spiramycin and metronidazole, GIId was infected-treated with spiramycin and metronidazole + testosterone, and GIIe was infected and then treated with the loaded spiramycin +metronidazole+ testosterone on ZIF-8 nanoparticles.

The mice were given medication starting on day 42 PI and continuing for 14 days. After that, the brains of all the mice were excised, and every brain was divided into two halves. One half was used to count the *Toxoplasma* cysts number. From 2nd half of each brain, 25 mg were suspended in sterile PBS (pH = 7.4) for *T. gondii* DNA quantification using real-time PCR, and the remaining part was preserved in 10% formalin for histopathological examination.

Evaluation of tested compounds' efficacy

Parasitological evaluation was done by:

- Counting *Toxoplasma* cysts number in mice brains: One-half of each mouse brain was homogenized in one ml of PBS (pH=7.2) obtaining a suspension. From each brain suspension, four separate drops (each 25 µl) were put onto a slide, and *Toxoplasma* cysts were counted under a microscope [20]. *Toxoplasma* cysts number per mouse brain=number counted in 4drops x10x2. Thereafter, the mean number in each group was estimated.
- Estimating the reduction percent of *Toxoplasma* brain cysts in treated groups using the following formula: Reduction% = 100 x (mean number of cysts count in controls - mean number of cysts count in treated groups) / mean number of cysts count in controls.

T. gondii DNA quantification

Mice brain tissue samples weighing 25 mg were washed thrice in sterile PBS (pH=7.4). DNA was extracted using a MagMAX™ CORE nucleic acid purification kit (Cat. No. A32700, Thermo Fisher Scientific Inc., USA) according to the manual's instructions. After that, DNA was eluted in 50µl of the kit's elution buffer. Real-time PCR was conducted relying on ViPrime PLUS Taq qPCR Green Master Mix I (SYBR® Green Dye, Cat QLMM12, Vivantis Co., Malaysia). PCR procedure included two primers targeting P29 gene: P29Q-f (forward): CAGCATGGATAAGGCATCTG and P29Q-r (reverse): GTTGCTCCTCTGTTAGTTCC, which were designed by DNASTAR's Lasergene software [21]. Briefly, 2µl DNA were added to 10µl (2X) PCR master mix and 0.1µl (50nmol) from every primer, yielding a 20µl total volume. Thermal cycler (qTOWER³G, Analytikjena, Germany) was programmed as follows: 95°C for 5 minutes as initial denaturation, 40 cycles of amplification at 95°C for 30 seconds and final extension at 60°C for one minute.

Melting curve analyses of the PCR products were performed. Dissociation curves were added upon 40 cycles completion, and data collection coincided with the annealing/extension cycle's end. Cycle threshold value (CT), which represents the TargetGene quantity at which fluorescence exceeds the preset threshold, was estimated, and the mean concentration of *T. gondii* DNA was measured. To obtain standard ranges for a real-time quantitative PCR assay, *T. gondii* DNA (positive control) was extracted from

Toxoplasma tachyzoites (kindly provided) under the same conditions, and then a series of dilutions ranging from $1x10^5$ to 10 ng/µl were made. Then, PCR was performed under the same conditions to establish the standard calibration curve.

Histopathological evaluation

Brain tissue samples were preserved individually in 10% formalin. They were dehydrated at varying alcohol concentrations, cleared using xylol, and embedded in paraffin blocks. Tissue sections of 5µm thickness were stained with hematoxylin and eosin (H&E) staining. Stained slides were inspected microscopically with x100, x400, and x1000 magnifications [22].

Statistical analysis

SPSS software package version 20 was employed to analyze the data. Data was presented as mean ± SD (standard deviation). Student *t*-test was used to compare means between groups. $P<0.05$ was considered significant.

Results

Characterization of nanoparticles & Tested compounds loading

The prepared ZIF-8 was characterized using XRD, FTIR, SEM, and UV. The surface morphology of the ZIF-8 nanocrystals showed uniform nano-sized octahedral crystal structures; the XRD curve showed 2θ values of 7.38° (011), 10.42° (022), 12.76° (112), 14.74° (022), 18.08° (222), 22.16° (114), 24.54° (233), 26.72° (134), and 29.7° (004), the FTIR analysis of ZIF-8 showed bands of 1595 cm^{-1} (C-N stretching); 1458 cm^{-1} (ring stretching), and 421 cm^{-1} (Zn-N stretching), UV-V is absorption spectra showed bands ranged from 200 to 800 nm (Fig. 1).

Using a scanning electron microscope, metronidazole@ZIF-8, spiramycin@ZIF-8, progesterone@ ZIF-8, and testosterone@ZIF-8 exhibited comparable cubic-like morphologies, and their sizes ranged from 80 to 150 nm (Figure 2). Moreover, X-ray diffraction spectra for metronidazole@ZIF-8, spiramycin@ZIF-8, progesterone@ZIF-8, and testosterone@ZIF-8 all exhibited comparable peak positions with varying diffraction intensities (Fig. 3). Based on these diffraction peaks, all loaded ZIF-8 appeared to have good crystallinity. The loading efficacy of ZIF-8 nanoparticles was dependent on the concentration of metronidazole, spiramycin, progesterone, testosterone, and the ratio of ZIF-8 NPs. With an increase in these parameters, loading percentages of metronidazole, spiramycin, progesterone, and testosterone all increased in tandem and stabilized at a certain level. It was demonstrated that variations in drug loading were caused by changes in drug concentration (Fig. 4), and maximum quantity loading amounts were 202, 265, 296, and 383 mg/g for testosterone@ZIF-8, progesterone@ZIF-8, metronidazole@ZIF-8, and spiramycin@ZIF-8, respectively.

Toxoplasma brain cysts estimation

The findings revealed that all infected mice brains had *T. gondii* cysts. In positive control groups (Tables 1 & 2), female mice were more likely to have *Toxoplasma* cysts (1633.0±66.58) than males (1579.0±85.43), but with no significant difference between them (*t*-test=1.58; P=0.13) (Table 3). Metronidazole and spiramycin combo therapy caused a significant reduction in *Toxoplasma* cysts by 14.85% and 23.6% in infected female and male mice, respectively. Conversely, the mean number of cysts varied significantly between male and female mice (*t*-test=11.35; $P<0.0001$). The fortification of spiramycin and metronidazole in combination with testosterone to treat infected male mice and progesterone to treat infected female mice resulted in a significant decrease in *Toxoplasma* cysts by 22.57% and 49.11% in infected female and male mice, respectively. Moreover, mice treated with the drug combination carried on ZIF-8 exhibited a marked reduction in *Toxoplasma* cysts compared to all other treated groups. It was noted that loading on ZIF-8 nanoparticles increased the combined therapy's potency (Tables 1 & 2). There was a higher drug response in male groups than in female groups (Table 3).

T. gondii DNA quantification in brain tissues

Using real-time PCR, DNA concentrations of *Toxoplasma* P29 gene were quantified in brain tissues, and the results showed significant variance between the studied groups (Tables 4 & 5). The mean concentrations in the positive controls were 581.9±7.03 ng/µl and 578.8±6.50 in female and male mice, respectively (GIb & GIIb). Following spiramycin and metronidazole treatment, *T. gondii* DNA concentrations in brain tissues were significantly reduced to half in both GIc and GIIc groups. When infected mice were given spiramycin and metronidazole along with sex hormones, the levels of parasitic DNA concentration in the female and male groups were 100.5±7.45 and 210.9±5.3 ng/µl, respectively (GId & GIId). Significantly, treatment with the drug combination carried on ZIF-8 (GIe & GIIe) yielded the lowest parasitic DNA concentration, reaching 80 ng/µl in male mice and 90 ng/µl in females.

Histopathological examination of tissue sections from brains

There were severe pathological lesions throughout brain tissues belonging to *T. gondii*-infected mice contrasted with non-infected mice (GIa, GIIa), in which no abnormality was observed. Brain tissues of infected non-treated mice (GIb,

GIIb) revealed *Toxoplasma* cysts within meningeal and cerebral tissues, with infiltration by a higher number of mononuclear inflammatory cells. In treated groups with spiramycin and metronidazole (GIc, GIIc), there were moderate pathological lesions that appeared as mild brain hemorrhage, diffuse gliosis in the cerebral cortex, congestion of meningeal blood vessels, necrosis of neurons, and meningeal inflammatory infiltrates. In mice treated with co-therapy of spiramycin and metronidazole and testosterone or progesterone (GId, GIId), there were minimal pathological lesions, including neuronal necrosis and focal gliosis in the cerebral cortex. Comparatively, mice treated with the drug combination (spiramycin + metronidazole + sex hormone) carried on ZIF-8 (GIe, GIIe) showed more improvement in histopathological features that appeared as focal aggregations of glial cells associated with necrosis of sporadic neurons (Figure 5).

Discussion

The most widely used drugs for toxoplasmosis need long treatment periods and have hazardous side effects. Their ineffectiveness against *T. gondii* tissue cysts accounts for the lengthy treatment regimens and the potential for reactivation of toxoplasmosis [4]. The assessment of drug combinations is necessary to discover novel treatment regimens for latent toxoplasmosis. Combining therapies may result in a lower dosage of medication while preserving the therapeutic effect and minimizing toxicity. So, the current study evaluated the effect of sex hormones combined with metronidazole and spiramycin and also the effect of combination-loaded ZIF-8 nanoparticles in treating chronic toxoplasmosis in the experimental animal model.

In this study, chronic *Toxoplasma* infection in male and female mice demonstrated different parasitological, molecular, and pathological results. According to our findings, females had a higher number of *Toxoplasma* cysts and were less responsive to treatment than males, suggesting that they were more vulnerable to *Toxoplasma* infection. These results are supported by the findings of Alonaizan et al., [23] and Liesenfeld et al., [24], which revealed that female mice were more liable to *Toxoplasma* infection than males as they had more necrotic lesions, parasite burden, and higher mortality rates. Also, Walker et al., [25] showed that male mice exhibited higher resistance to *Toxoplasma* infection than females. Our results also go along with those of Roberts et al., [26], who reported that female mice had higher mortality rates than males in acute infection, and the females who survived to develop chronic infection had more brain cysts than the survived males. The sex difference in vulnerability to *Toxoplasma* infection is correlated to functional variations in the immune response, being that male mice produce more interferon-gamma (IFN-γ) and interleukin-12 (IL-12) earlier than females [25]. These cytokines play a protective role against *Toxoplasma* infection.

This study employed a medication combination to identify novel latent toxoplasmosis treatment protocols. It was found that *Toxoplasma* cysts number in the brains of male and female mice was significantly reduced by co-administration of spiramycin and metronidazole (23.6% and 14.85%, respectively). Co-administration of spiramycin and metronidazole enhanced spiramycin absorption in the brain, increasing *Toxoplasma* cysts eradication. Consistent with this finding, Hegazy et al., [11] observed that administering spiramycin and metronidazole resulted in a remarkable decrease of *Toxoplasma* cyst counts in male and female mice by 78% and 87%, respectively. Likewise, Chew et al., [17] found that when male mice with chronic toxoplasmosis received spiramycin-metronidazole treatment, the number of brain cysts decreased by 93%. The disparity between this study and previous research results regarding the reduction of *Toxoplasma* cysts could be explained by the different methodologies used to count the number of *Toxoplasma* cysts or by the lower dosages of spiramycin and metronidazole that we used.

Hormones have a significant impact on the innate immune system, including macrophages, mast cells, dendritic cells, NK cells, and eosinophils. These cells serve as the initial defense line against many pathogens and contribute to the development of adaptive immunity [27]. In the current research, administering testosterone hormone along with spiramycin and metronidazole to *T. gondii*-infected mice significantly decreased the mean cyst counts by 49.11%. This agrees with Hegazy et al., [11], who showed that the combination of testosterone with atovaquone reduced *Toxoplasma* cyst counts in the brains of infected female mice by 90%. According to Liesenfeld et al., [24], giving testosterone to female mice reduced parasite burden and pathological lesions. Higher numbers of *Toxoplasma* cysts were observed in brain regions relating to fear behaviors [28], and according to Singh et al., [29], rats' fearful behavior was found to decrease upon the administration of testosterone, which targets medial amygdala. Following the *Toxoplasma* infection, the host develops a cellular immunological reaction through T lymphocytes. Specifically, helper T cells (Th1) generate pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IFN-γ, and IL-6 which are crucial in protecting against *Toxoplasma* infection. Testosterone treatment has been shown to enhance immunological responses in both female and male hamsters and stimulate cell-mediated lymphocyte proliferation through *in vitro* and *in vivo* experiments [30]. These confirm our findings about

the beneficial effect of testosterone on parasite burden in mice's brains.

Interestingly, our results showed that the addition of progesterone to the treatment schedule (spiramycin and metronidazole) led to a significant decrease in *Toxoplasma* cysts number in infected female mice. This is consistent with Wu et al., [31] *in vitro* study, which proved that progesterone inhibited *T. gondii* invasion as well as induced its autophagy. Galvan-Ramirez et al., [32] found the percent of infected astrocytes by *T. gondii* tachyzoites was considerably lower than that of non-treated controls after twenty-four hours following treatment with different concentrations of progesterone. Progesterone's effect on tachyzoites was closely linked to the localization of *T. gondii* progesterone membrane receptor protein in the mitochondrion. By attaching to these receptors, progesterone can control the differentiation, development, and multiplication of *T. gondii* [31]. Progesterone can influence parasite infections by modifying innate and acquired immune responses [27]. It was suggested that elevated progesterone levels may combat *T. gondii* proliferation by increasing eosinophils degranulation, CD30 expression, and the release of IL-5, IL-4, and TNFβ.

In the current research, ZIF-8 served as a drug carrier due to its unique nontoxic and biocompatible properties, along with its great thermal and hydrothermal stability. It is a host material whose good monodispersity, suitable size for cellular intake, ease of synthesis in a moderate environment, and ease of surface modification make it a strong candidate for intracellular drug delivery [33]. ZIF-8 enhanced the drug combination efficacy in treating murine latent toxoplasmosis, as evidenced by the significant decrease in *Toxoplasma* cysts count and *T. gondii* DNA concentrations in the mice's brains, as well as successfully reversed the histopathological alterations seen in the mice's brains compared to all other treatment groups. This is consistent with Abdelhamid [10], who reported that antimicrobial agents encapsulating ZIF-8 demonstrated higher biological activity than that of the free agents. Drug-loaded nanoparticles have been shown to enhance therapeutic effects by regulating drug release, improving drug absorption, delaying drug metabolization, and reducing dosage requirements [7]. According to El-Shafey et al., [34], curcumin loaded on metal-organic framework nanocomposites considerably reduced *Toxoplasma* cyst burden in treated rats' brains that were infected by *T. gondii* ME49 strain. Mohammad et al., [35] showed that wheat germ oil and *Nigella sativa* oil loaded on metal-organic framework nanocomposites markedly increased survivability, decreased *Toxoplasma* cyst burden in brains, as well as alleviated the many organs' pathogenic effects in infected mice with toxoplasmosis. This could explain the beneficial effect of ZIF-8 against *Toxoplasma* infection.

In the current study, *Toxoplasma* DNA concentrations in mice brain tissues were estimated following the treatment of infected mice with spiramycin and metronidazole in combination with testosterone or progesterone, as well as the drug combination carried on ZIF-8. As shown, all treated groups demonstrated a decrease in DNA concentrations with significant variation compared to positive controls. This could be explained by the fact that sex hormones enhanced immunological responses in mouse models of chronic toxoplasmosis. Additionally, loading sex hormones on ZIF-8 increased their anti-toxoplasmic efficacy.

Regarding histopathological evaluation, the current study showed that sex hormone therapy might ameliorate a number of *T. gondii*-induced adverse effects on the brain. Untreated infected mice developed severe inflammatory reactions in their brains, which were mostly manifested as mononuclear cell proliferation, meningeal congestion caused by invaded mononuclear cells, neuronal death, and embedded *Toxoplasma* cysts in the meninges or brain tissues. These observations were more or less reported by several investigators [11, 22]. These findings may be explained by the increased levels of pro-inflammatory cytokines and recruitment of many inflammatory cells. Such components then target the parasite's replication [36]. The present study's finding is that sex hormone therapy, whether given alone or carried on ZIF-8 nanoparticles, significantly improved these pathological lesions. These effects might result from testosterone's ability to regulate inflammatory processes that affect cell differentiation and proliferation and inhibit cytokines production [37]. It was demonstrated that treatment with testosterone inhibited *Toxoplasma* tachyzoites proliferation in the intestine, delaying IFN-γ production and reducing intestinal pathological lesions [24]. Considering the above results, the histopathological findings supported the results obtained from parasitological and molecular testing.

Overall, the current study highlighted the potential advantages of combining parasitological, histopathological, and molecular techniques in evaluating the effects of pharmaceuticals selected for the treatment of chronic toxoplasmosis. To fully understand the potential therapeutic effects of progesterone and testosterone as well as their loading on ZIF-8 nanoparticles in treating chronic toxoplasmosis, more research is required using various dosages of these compounds, different *Toxoplasma* strains, different infection modes and/or dosages, and distinct host characteristics, including variations in genotypes and conditions at the individual level or distinct species. Human clinical trials should be conducted as well.

Conclusion

The current findings provided significant perspectives about the possible medical advantages of sex hormones for the management of chronic toxoplasmosis, and the loading of sex hormones on ZIF-8 nanoparticles increased their anti-toxoplasmic effect. These findings were verified by employing a variety of parasitological, molecular, and histopathological techniques.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Study protocol was approved by the ethical committee of the Faculty of Medicine at Benha University-Egypt (Code No. RC562023). Animal's experiment followed international guidelines for animal care and usage for scientific research.

TABLE 1. Effect of tested compounds on *Toxoplasma* brain cysts burden in female mice

Female mice group (GI)	*Toxoplasma* cyst count	Reduction %	*t*- test	*P*-value
	Mean ± SD			
GIa : normal uninfected (negative control)	0	-	-	-
GIb: infected untreated (positive control)	1633.0±66.58	-	-	-
GIc: infected-treated with Spiramycin + Metronidazole	1390.5±46.99	14.85%	9.41	<0.0001**
GId: infected-treated with spiramycin + metronidazole + progesterone	1264.5±39.05	22.57%	15.09	<0.0001**
GIe: infected-treated with the loaded spiramycin + metronidazole + progesterone on ZIF-8 nanoparticles	894.5±28.43	45.22%	32.25	<0.0001**

** highly significant reduction in *Toxoplasma* cysts in treated groups versus positive control.

TABLE 2. Effect of tested compounds on *Toxoplasma* brain cysts burden in male mice.

Male mice group (GII)	*Toxoplasma* cyst count	Reduction %	*t*- test	*P*-value
	Mean ± SD			
GIIa : normal uninfected (negative control)	0	-	-	-
GIIb: infected untreated (positive control)	1579.0±85.43	-	-	-
GIIc: infected-treated with spiramycin + metronidazole	1206.0±20.79	23.6%	13.41	<0.0001**
GIId: infected-treated with spiramycin + metronidazole + Testosterone	803.5±13.13	49.11%	28.37	<0.0001**
GIIe: infected-treated with the loaded spiramycin + metronidazole + Testosterone on ZIF-8 nanoparticles	525.0±36.21	66.75%	35.92	<0.0001**

** Highly significant reduction in *Toxoplasma* cysts in treated groups versus positive control.

TABLE 3. Comparison of *Toxoplasma* brain cysts burden between male and female groups.

Mice groups	*Toxoplasma* brain cysts		*t*-test	*P*-value
	Male	Female		
	Mean ± SD	Mean ± SD		
Normal uninfected (negative control)	0	0	-	-
Infected untreated (positive control)	1579.0±85.43	1633.0±66.58	1.58	0.13
Infected-treated with spiramycin +metronidazole	1206.0±20.79	1390.5±46.99	11.35	<0.0001**
Infected treated with spiramycin + metronidazole + sex hormone	803.5±13.13	1264.5±39.05	35.39	<0.0001**
Infected treated with the loaded spiramycin + metronidazole+ sex hormone on NPs	525.0±36.21	894.5±28.43	25.38	<0.0001**

** Highly significant response to tested compounds in males versus females

TABLE 4. Effect of tested compounds on *T. gondii* DNA concentration in brain of female mice.

Female mice group (GI)	*T. gondii* DNA concentration (ng/µl) Mean ± SD	*t*- test	*P*-value
GIa : normal uninfected (negative control)	0	-	-
GIb: infected untreated (positive control)	581.9 ±7.03	-	-
GIc: infected-treated with spiramycin + metronidazole	264.6±7.39	98.37	<0.0001**
GId: infected-treated with spiramycin + metronidazole + progesterone	100.5±7.45	148.61	<0.0001**
GIe: infected-treated with the loaded spiramycin + metronidazole + progesterone on ZIF-8 nanoparticles	90.8±3.86	193.64	<0.0001**

**Highly significant reduction in *T. gondii* DNA concentration in brain tissues of treated groups versus positive control

TABLE 5. Effect of tested compounds on *T. gondii* DNA concentration in brain of male mice.

Male mice group (GII)	*T. gondii* DNA concentration (ng/µl) Mean ± SD	*t*- test	*P*-value
GIIa : normal uninfected (negative control)	0	-	-
GIIb: infected untreated (positive control)	578.8±6.50	-	-
GIIc: infected-treated with spiramycin + metronidazole	267.3±10.82	78.04	<0.0001**
GIId: infected-treated with spiramycin + metronidazole + Testosterone	210.9±5.3	138.71	<0.0001**
GIIe: infected-treated with the loaded spiramycin + metronidazole + Testosterone on ZIF-8 nanoparticles	80±3.09	219.16	<0.0001**

**Highly significant reduction in *T. gondii* DNA concentration in brain tissues of treated groups versus positive control

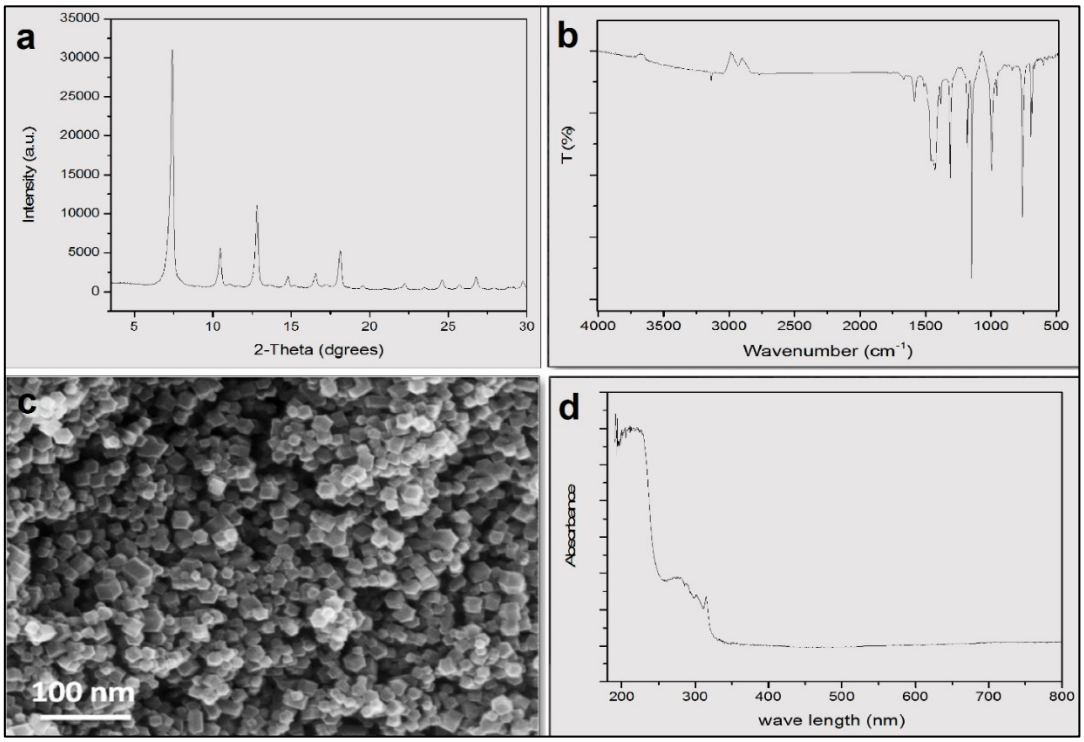

Fig. 1. [a] PXRD of ZIF-8, [b] FTIR of ZIF-8, [c] SEM of ZIF-8, [d] UV-vis absorption spectra of ZIF-8

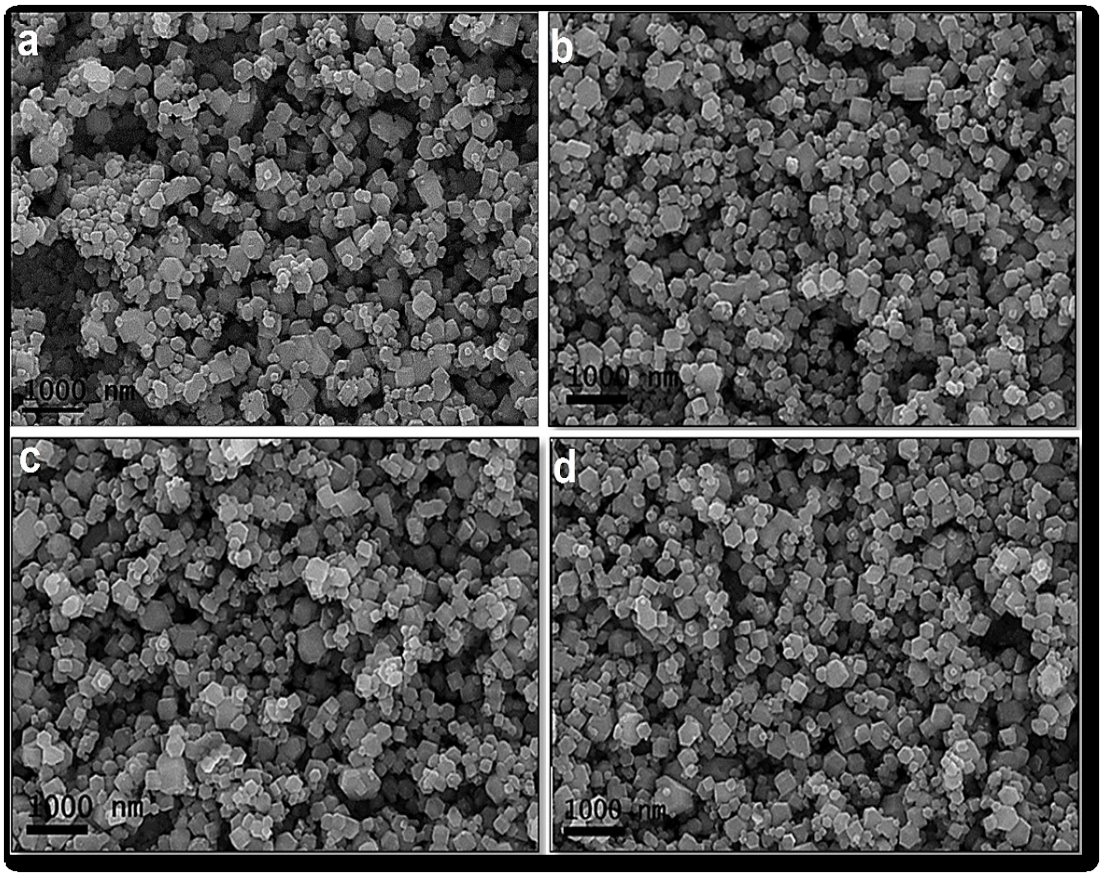

Fig.2. SEM micrographs: [a] metronidazole@ZIF-8, [b] spiramycin@ZIF-8 [c] progesterone@ZIF-8, [d] testosterone@ZIF-8

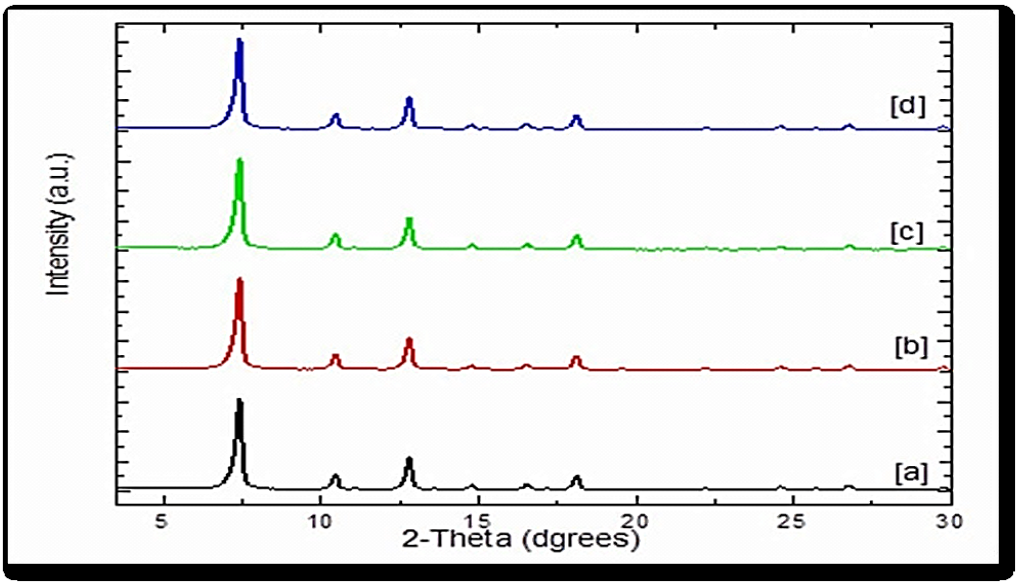

Fig. 3. X-ray diffraction patterns of [a] metronidazole@ ZIF-8, [b] spiramycin@ZIF-8, [c] progesterone@ZIF-8, [d] testosterone@ZIF-8

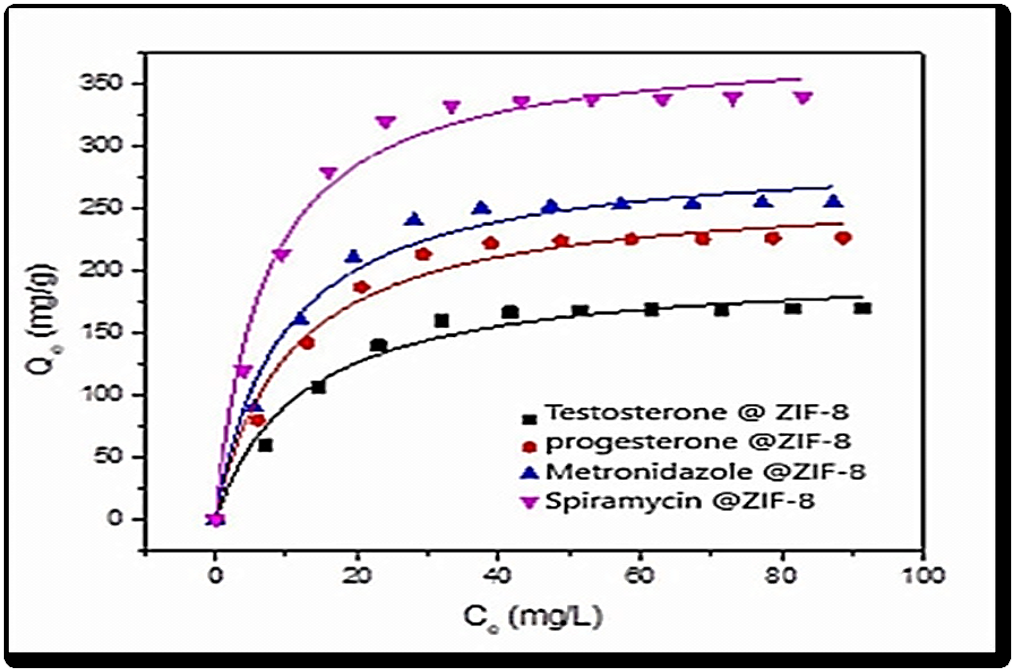

Fig.4. Equilibrium between the different concentrations of metronidazole, spiramycin, progesterone, testosterone and the loading amount.

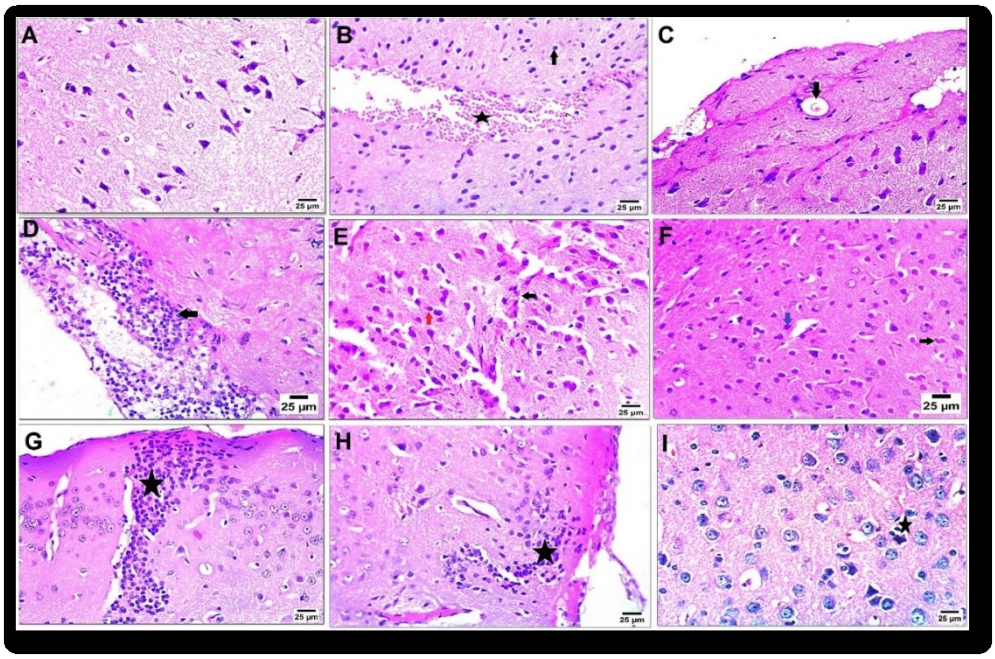

Fig.5. Brain tissues of experimental mice stained by H&E: (A) Normal histological structure of the brain (negative control group). **(B)** Severe hemorrhage at cerebral cortex (star) with diffuse gliosis (arrow) (female positive control group). **(C)** *Toxoplasma* cyst in cerebral cortex (arrow) (male positive control group) **(D)** Meningitis with infiltration by a higher number of mononuclear inflammatory cells (arrow) (female positive control group) **(E)** Mild brain hemorrhage (black arrow) with diffuse gliosis (red arrow) (treated with spiramycin and metronidazole) **(F)** Pyknotic nuclei in some neurons (blue arrow), while others showed necrosis with acidophilic nuclei and cytoplasm (black arrow) (treated with spiramycin and metronidazole + progesterone). **(G)** Focal gliosis (star) in cerebral cortex (treated with spiramycin and metronidazole + testosterone). **(H)** Focal aggregations of glial cells (star) in cerebral cortex (treated with spiramycin and metronidazole + progesterone loaded on nanoparticles). **(I)** Necrosis of sporadic neurons (treated with spiramycin and metronidazole + testosterone loaded on nanoparticles) (star).

References

1. Bisetegn, H., Debash, H., Ebrahim, H., Mahmood, N., Gedefie, A., Tilahun, M., Alemayehu, E., Mohammed, O. and Feleke, D.G. Global seroprevalence of *Toxoplasma gondii* infection among patients with mental and neurological disorders: A systematic review and meta-analysis. *Health Science Reports*, **6**(6), e1319 (2023). Doi: 10.1002/hsr2.1319
2. El-Sayed, N.M., Ramadan, M.E. and Masoud, N.G. A step forward towards food safety from parasite infective agents. In: Babalola, O.O., (eds) Food Security and Safety. Springer, Cham. Chap. **40**, 807–832 (2021). https://doi.org/10.1007/978-3-030-50672-8_40
3. Khan, K. and Khan, W. Congenital toxoplasmosis: An overview of the neurological and ocular manifestations. *Parasitology International*, **67**(6), 715–721, (2018). https://doi.org/10.1016/j.parint.2018.07.004
4. El-Sayed, N.M. Recent advances in the treatment of toxoplasmosis. In: Frontiers in Clinical Drug Research: Anti-Infectives, Atta-ur-Rahman (Ed.) Bentham Science, Vol **6**, pp. 127–164, (2020). https://doi.org/10.2174/9789811425745120060006
5. Konstantinovic, N., Guegan, H., Stäjner, T., Belaz, S. and Robert-Gangneux, F. Treatment of toxoplasmosis: Current options and future perspectives. *Food and Waterborne Parasitology*, **15**, e00036(2019). https://doi.org/10.1016/j.fawpar.2019.e00036
6. Allam, A.F., Shehab, A.Y., Mogahed, N.M.F.H., Farag, H.F., Elsayed, Y. and El-Latif, N.F. Effect of nitazoxanide and spiramycin metronidazole combination in acute experimental toxoplasmosis. *Heliyon*, **6**(4), e03661(2020). Doi: 10.1016/j.heliyon.2020.e03661.
7. Masoud, N.G., El-Sayed, N.M. and Ramadan, M.E. Role of nanomedicine in ocular parasitic infections, Applications of Nanomaterials in Medical Procedures and Treatments Frontiers in Nanomedicine, Vol **4**, pp. 116–147, (2023). https://doi.org/10.2174/9789815136951123040006
8. Al Sharabati, M., Sabouni, R. and Husseini, G.A. Biomedical applications of metal-organic frameworks for disease diagnosis and drug delivery: A review. *Nanomaterials*, **12**(2), 277 (2022). https://doi.org/10.3390/nano12020277
9. Ramos, V.C., Reyes, C.B.G., García, G.M., Quesada, M.I.S., Barrero, F.J.M-C., Rábago, J.J.S. and Polo, M.S. ZIF-8 and its magnetic functionalization as vehicle for the transport and release of ciprofloxacin. *Pharmaceutics*, **14**, 2546(2022). Doi: 10.3390/pharmaceutics14112546
10. Abdelhamid, H.N. Zeolitic imidazolate frameworks (ZIF-8) for biomedical applications: A review. *Current Medicinal Chemistry*, **28**(34), 7023–7075 (2021). Doi: 10.2174/0929867328666210608143703
11. Hegazy, M.M., Elmehankar, M.S., Azab, M.S., El-Tantawy, N.L. and Abdel-Aziz, A. Sex dichotomy in the course of experimental latent toxoplasmosis. *Experimental Parasitology*, **202**, 15–21(2019). https://doi.org/10.1016/j.exppara.2019.05.003
12. Laubach, Z.M., Gering, E., Yang, E., Montgomery, T.M., Getty, T. and Holekamp, K.E. Associations between *Toxoplasma gondii* infection and steroid hormone levels in spotted hyenas. *International Journal for Parasitology: Parasites and Wildlife*, **17**, 53–59 (2022). https://doi.org/10.1016/j.ijppaw.2021.11.007
13. Galvan-Ramirez, Mde L., Gutierrez-Maldonado, A.F., VerduzcoGrijalva, F. and Jimenez, J.M. The role of hormones on *Toxoplasma gondii* infection: a systematic review. *Frontiers in Microbiology*, **5**, 503 (2014). https://doi.org/10.3389/fmicb.2014.00503
14. Galván-Ramírez, M.L., Ramírez De, Arellano A., Rodríguez-Pérez, L.R., Lopez-Pulido, E.I., Muñoz-Valle, J.F. and Pereira-Suárez, A.L. Hormonal modulation of *Toxoplasma gondii* infection: Regulation of hormonal receptors and cytokine production in THP-1 cells. *Experimental Parasitology*, **204**, 107721(2019). https://doi.org/10.1016/j.exppara.2019.107721
15. El-Sayed, N.M. and Aly, E.M. *Toxoplasma gondii* infection can induce retinal DNA damage: an experimental study. *International Journal of Ophthalmology*, **7**(3), 431–36 (2014). Doi: 10.3980/j.issn.2222-3959.2014.03.08
16. Grujić, J., Djurkovic-Djakovic, O., Nikilic, A., Klun, I. and Bobic, B. Effectiveness of spiramycin in murine models of acute and chronic toxoplasmosis. *International Journal of Antimicrobial Agents*, **25**, 226-30 (2005). https://doi.org/10.1016/j.ijantimicag.2004.09.015
17. Chew, W.K., Segarra, I., Ambu, S. and Mak, J.W. Significant reduction of brain cysts caused by *Toxoplasma gondii* after treatment with spiramycin coadministered with metronidazole in a mouse model of chronic toxoplasmosis. *Antimicrobial Agents and Chemotherapy*, **56**(4), 1762–1768 (2012). Doi: 10.1128/AAC.05183-11
18. Putnam, S.K., Sato, S., Riolo, J. and Hull, E. Effects of testosterone metabolites on copulation, medial preoptic dopamine, and NOS-immunoreactivity in castrated male rats. *Hormones and Behavior*, **47**, 513–522 (2005). https://doi.org/10.1016/j.yhbeh.2005.01.007
19. Escobedo, G., Camacho-Arroyo, I., Nava-Luna, P., Olivos, A., Pérez-Torres, A., Leon-Cabrera, S., Carrero, J.C. and Morales-Montor, J. Progesterone induces mucosal immunity in a rodent model of human taeniosis by *Taenia solium*. *International Journal of Biological Sciences*, **7**(9), 1443–1456 (2011). Doi: 10.7150/ijbs.7.1443
20. El-Sayed, N.M., Ismail, K.A., Badawy, A.F. and Elhasanein, K.F. In vivo effect of anti-TNF agent (etanercept) in reactivation of latent toxoplasmosis. *Journal of Parasitic Diseases*, **40**(4), 1459–1465 (2016). https://doi.org/10.1007/s12639-015-0712-y
21. Barakat, A.M., Fadaly, H.A.M.E., Gareh., A, Abd El-Razik, K.A., Ali, F.A.Z., Saleh, A.A., Sadek, S.A.S., Dahran, N., El-Gendy, A.E.-N.G., El-Khadragy, M.F. and Elmahallawy, E.K. Wheat germ oil and propolis decrease parasite burden and restore marked histopathological changes in liver and lung in mice

with chronic toxoplasmosis. *Animals*, **12**, 3069, (2022). https://doi.org/10.3390/ani12223069

22. Eraky, M.A., El-Fakahany, A.F., El-Sayed, N.M., Abou-Ouf, E.A. and Yaseen, D.I. Effects of *Thymus vulgaris* ethanolic extract on chronic toxoplasmosis in a mouse model. *Parasitology Research*, **115**(7), 2863–2871 (2016). https://doi.org/10.1007/s00436-016-5041-2

23. Alonaizan, R., Woods, S., Hargrave, K.E. and Roberts, C.W. An exaggerated immune response in female BALB/c mice controls initial *Toxoplasma gondii* multiplication but increases mortality and morbidity relative to male mice. *Pathogens*, **10**(9), 1154, (2021). https://doi.org/10.3390/pathogens10091154

24. Liesenfeld, O., Nguyen, T.A., Pharke, C. and Suzuki, Y. Importance of gender and sex hormones in regulation of susceptibility of the small intestine to peroral infection with *Toxoplasma gondii* tissue cysts. *Journal of Parasitology*, **87**, 1491–1493(2001).

25. Walker, W., Roberts, C.W., Ferguson, D.J., Jebbari, H. and Alexander, J. Innate immunity to *Toxoplasma gondii* is influenced by gender and is associated with differences in interleukin-12 and gamma interferon production. *Infection and Immunity*, **65**(3), 1119–1121(1997). https://doi.org/10.1128/iai.65.3.11191121.1997

26. Roberts, C.W., Cruickshank, S.M. and Alexander, J. Sex-determined resistance to *Toxoplasma gondii* is associated with temporal differences in cytokine production. *Infection and Immunity*, **63**(7), 2549–2555 (1995). https://doi.org/10.1128/iai.63.7.2549-2555.1995

27. Roberts, C.W., Walker, W. and Alexander, J. Sex-associated hormones and immunity to protozoan parasites. *Clinical Microbiology Reviews*, **14** (3), 476–488(2001). https://doi.org/10.1128/cmr.14.3.476-488.2001

28. Vyas, A., Kim S-K., Giacomini, N., Boothroyd, J.C. and Sapolsky, R.M. Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *PNAS*, **104**, 6442–6447 (2007).

29. Singh, D.K., Hari Dass, S.A., Abdulai-Saiku, S. and Vyas, A. Testosterone acts within the medial amygdala of rats to reduce innate fear to predator odor akin to the effects of *Toxoplasma gondii* infection. *Frontiers in Psychiatry*, **11**, 7–12(2020). Doi: 10.3389/fpsyt.2020.00630

30. Bilbo, S.D. and Nelson, R.J. Sex steroid hormones enhance immune function in male and female Siberian hamsters. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*, **280** (1), R207–13 (2001). https://doi.org/10.1152/ajpregu.2001.280.1.R207

31. Wu, Y., Zhang, X., Fu, Y., Liu, J., Xue, Y. and Liu, Q. Progesterone can directly inhibit the life activities of *Toxoplasma gondii* in vitro through the progesterone receptor membrane component (PGRMC). *International Journal of Molecular Sciences*, **23**(7), 3843(2022). Doi: 10.3390/ijms23073843.

32. Galván-Ramírez, Mde L., Gutiérrez-Maldonado, A.F., Dueñas-Jiménez, J.M., Rodríguez-Pérez, L.R. and Troyo-Sanromán, R. Effect of 17β-estradiol and progesterone on astrocytes infected with *Toxoplasma*. *The Journal of Advances in Parasitology*, **3**, 22–31 (2016).

33. Sun, Y., Zheng, L., Yang, Y., Qian, X., Fu, T., Li, X., Yang, Z., Yan, H., Cui, C. and Tan, W. Metal-organic framework nanocarriers for drug delivery in biomedical applications. *Nano-Micro Letters*, **12**(1), 103(2020). https://doi.org/10.1007/s40820-020-00423-3

34. El-Shafey, A.A.M., Hegab, M.H.A., Seliem, M.M.E., Barakat, A.M.A., Mostafa, N.E., Abdel-Maksoud, H.A. and Abdelhameed, R.M. Curcumin@metal organic frameworks nano-composite for treatment of chronic toxoplasmosis. *Journal of Materials Science: Materials in Medicine*, **31**(11), 90(2020). https://doi.org/10.1007/s10856-020-06429-y

35. Mohammad, O.S., El Naggar, H.M., Abdelmaksoud, H.F., Barakat, A.M., Abdelhameed, R.M. and Shehata, M.A.S. The effect of *Nigella sativa* oil- and wheat germ oil-loaded metal organic frameworks on chronic murine toxoplasmosis. *Acta Tropica*, **239**, 106823(2023). https://doi.org/10.1016/j.actatropica.2023.106823

36. Mukhopadhyay, D., Arranz-Solis, D. and Saeij, J.P.J. Influence of the host and parasite strain on the immune response during *Toxoplasma* infection. *Frontiers in Cellular and Infection Microbiology*, **10**, 580425(2020). Doi: 10.3389/fcimb.2020.580425

37. Bianchi, V.E. The anti-inflammatory effects of testosterone. *Journal of the Endocrine Society*, **3**(1), 91–107 (2019). https://doi.org/10.1210/js.2018-00186

تستطيع الهرمونات الجنسية تعديل عدوى التوكسوبلازما جوندي: دراسة تجريبية

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الملخص

نظرا لأن التوكسوبلازما جوندي هو طفيل له العديد من مراحل النمو والسلالات ، يجب أن تكون الأدوية المضادة للتوكسوبلازما فعالة لمختلف المراحل والسلالات. تحتاج الأدوية إلى فترات علاج طويلة ولها آثار جانبية خطيرة. قد يؤدي الجمع بين الأدوية إلى انخفاض الجرعات مع الحفاظ على التأثير العلاجي. قامت هذه الدراسة على الجمع بين السبيراميسين والميترونيدازول مع هرمون التستوستيرون / البروجسترون وكذلك تركيبة الأدوية محملة على جسيمات ZIF-8 النانوية في علاج داء التوكسوبلازما في النماذج الحيوانية. وقد أصيبت الفئران بالطور المعدي عن طريق الفم بعد تصنيفها إلى مجموعتين من الإناث والذكور ، وكل مجموعة قسمت إلى خمس مجموعات فرعية تضمنت: مجموعة غير مصابة (سيطرة) ، ومجموعة مصابة وغير معالجة ، ومجموعة تم علاجها بالسبيرامايسين والميترونيدازول ، ومجموعة تم علاجها بالسبيراميسين والميترونيدازول بالاشتراك مع هرمون التستوستيرون / البروجسترون ، ومجموعة تم علاجها بتركيبة الأدوية المحملة علي جسيمات ZIF-8 النانوية. وقد حُدِدَتْ فعالية المركبات المختبرة من خلال عد أكياس التوكسوبلازما في أنسجة المخ، وقياس حمضها النووي في أنسجة المخ باستخدام تفاعل البلمرة المتسلسل، وفحص الأنسجة الدماغية. وقد أدى علاج الفئران المصابة بالسبيراميسين والميترونيدازول بالاشتراك مع هرمون التستوستيرون أو البروجسترون إلى تقليل عدد حويصلات التوكسوبلازما الموجودة بمخ الفئران بشكل كبير. أظهرت الفئران التي عولجت بتركيبة الدواء المحملة على ZIF-8 انخفاضا ملحوظا في الحمل الطفيلي مقارنة بالمجموعات الأخرى .أظهرت جميع المجموعات المعالجة انخفاضا في تركيزات الحمض النووي للتوكسوبلازما في أنسجة المخ. وقد نجح العلاج في تحسين التغيرات النسيجية التي شوهدت في الفئران المصابة. ونستنتج ان الهرمونات الجنسية تستطيع تعديل عدوى التوكسوبلازما، وتحميلها على الجسيمات النانوية ZIF-8 يزيد من التأثير المضاد للمرض.

الكلمات الدالة :التوكسوبلازما، التستوستيرون، البروجسترون، الهياكل المعدنية العضوية، موصل الأدوية.