Research Article

Therapeutic and Protective Effects of Pyrimethamine Nanoemulsion on Renohistological and Inflammatory Alterations in Rat Embryos Infected with *Toxoplasma gondii*

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Abstract | Infection with *Toxoplasma gondii* (*T. gondii*) during pregnancy can cause serious fetal complications, including renal injury mediated by inflammatory and oxidative stress responses. Although pyrimethamine is widely used for the treatment of toxoplasmosis, its clinical effectiveness is limited by low bioavailability and potential toxicity. Nanoemulsion-based drug delivery systems may enhance pyrimethamine efficacy; however, their effect on fetal renal injury in congenital toxoplasmosis remains insufficiently established. In this study, pregnant rats were randomly allocated into four groups (n = 8 per group): control, infected, pyrimethamine nanoemulsion (PyNE)-only, and infected + PyNE. Infection was induced intraperitoneally on gestational day 7 using 5×10^5 tachyzoites/mL of the highly virulent *T. gondii* RH strain. PyNE was administered orally at a dose of 0.1 mL/day from gestational days 7 to 18. The optimized PyNE formulation was prepared using olive oil, Tween 80, and ethanol, and its stability was confirmed by scanning electron microscopy (SEM). Compared with the control and PyNE-only groups, serum levels of IL-6, IL-8, and IL-17 were significantly elevated in the infected group ($P < 0.05$). PyNE treatment significantly reduced these cytokines in infected rats; however, their levels remained higher than those of the control and PyNE-only groups ($P < 0.05$). Infection also induced significant oxidative stress, as indicated by increased malondialdehyde (MDA) levels and reduced glutathione (GSH) concentrations, which were partially restored following PyNE treatment. Histopathological examination of fetal kidneys from infected rats revealed marked glomerular disruption, tubular degeneration, and interstitial fibrosis, whereas PyNE therapy resulted in a noticeable improvement in renal architecture, with reduced tubular damage and fibrotic changes. In conclusion, pyrimethamine nanoemulsion demonstrated therapeutic potential in attenuating inflammatory, oxidative, and renal histopathological alterations associated with congenital toxoplasmosis.

Keywords | *Toxoplasma gondii,* Nanoemulsions, Olive oil, PyNE therapy, Physiological Parameters, Oxidative Alterations

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INTRODUCTION

Toxoplasma gondii, an intracellular protozoan parasite, poses significant health risks worldwide, affecting both people and animals (Ramírez-Flores and Mondragón-Flores, 2025). Fetal kidney defects related to toxoplasmosis have been reported to be frequently associated with congenital nephrotic syndrome, which is characterised by proteinuria and oedema (Li *et al.*, 2021). Vertical infection is more common in late pregnancy, whereas infection

in early pregnancy is generally associated with severe disease (Shahbazzadegan and Farzizadeh, 2025). These defects can lead to marked renal dysfunction in the fetus, often necessitating urgent medical treatment. Thus, early diagnosis and appropriate treatment during pregnancy are essential to reduce the risk of these complications (Akins *et al*., 2024).

The regulator of *T. gondii* infection by the host is primarily facilitated by the immune response, where innate immune cells (e.g., macrophages and dendritic cells) diagnose pathogen-associated molecular patterns through pattern recognition receptors on their external. This recognition triggers a cascade of intracellular signaling events that culminate in the activation of these immune cells, which are in turn responsible for the activation of adaptive immune cells, especially T helper 1 cells and specific CD8+ T cells (Zhu *et al*., 2019; Lahmar *et al*., 2009). These cells synthesize pro-inflammatory cytokines, such as interleukin (IL)-6, IL-8, IL-17, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha TNF-α (Zhu *et al*., 2019; Mohammed and Taher, 2025). One of the defense mechanisms employed by the body against parasites and other infectious agents is the release of antioxidant enzymes. In eukaryotes, GSH metabolism plays an important role in counteracting the oxidative damage caused by the infectious agents (Wang *et al*., 2017; Salman *et al*., 2024). One of the end products of lipid peroxidation is malondialdehyde, an extremely toxic compound, which is usually determined to estimate the catastrophe of the membrane (Murtaza *et al*., 2025; Ali *et al*., 2025). MDA serves as a critical indicator of oxidative stress and lipid peroxidation in biological specimens (Ijaz *et al*., 2024).

Traditional treatments for toxoplasmosis (like pyrimethamine) frequently face constraints like inadequate bioavailability, medication resistance, and systemic toxicity (Chua *et al*., 2025). Nanotechnology has emerged as a viable approach in the development of novel treatment techniques, including increased drug transport, improved effectiveness, and tailored action against *T. gondii* (Ortiz *et al*., 2025). Nanoparticle-based drug delivery methods have demonstrated significant potential in addressing the limitations of conventional anti-toxoplasmosis therapies (Ismael, 2023).

A novel class of emulsion known as a "nanoemulsion" is characterised by uniformly small droplets, usually between 20 and 200 nm (Che Marzuki *et al*., 2019). Small droplet size that help the active ingredients to deposit uniformly and penetrate effectively into the skin (Sonneville-Aubrun *et al*., 2004). Being consisted of high surface area and low surface tension, nanoemulsions dramatically facilitate the permeability of the components (Saffarionpour, 2019). Nanoemulsion can be produced with lower concentrations of emulsi1er (3–10%) than ME, which needs a high concentration 20% (Preeti *et al*., 2023). Nanoemulsions provide several advantages when utilised as a mechanism for medicine distribution. Nanoemulsions serve as a versatile platform for drug delivery across several therapeutic domains, as they can encapsulate both hydrophilic and hydrophobic pharmaceuticals (Wilson *et al*., 2022). The selection of components and manufacturing methods is essential for tailoring nanoemulsions to specific drug delivery needs, ensuring the stability and effectiveness of the final product (Tanuku *et al*., 2024). The development, analysis, and utilisation of nano emulsions signify a potential strategy for enhancing therapeutic results via efficient and adaptable drug delivery systems, investigate innovative applications, and address current restrictions to improve the effectiveness of nanoemulsion-based drug delivery systems (Huang *et al*., 2024). Therefore, the present study aimed to evaluate the therapeutic and protective effects of pyrimethamine nanoemulsion (PyNE) on inflammatory cytokine levels (IL-6, IL-8 and IL-17), oxidative stress biomarkers (MDA and GSH), and renal histopathological alterations in rat fetuses experimentally infected with *T. gondii*.

MATERIALS AND METHODS

Animals and treatments

Forty young inbred albino rats (*Rattus norvegicus*), weighing between 170 and 200 g, were obtained from the animal house of the College of Veterinary Medicine, Tikrit University. All animals were confirmed seronegative for *T. gondii* antibodies using the latex agglutination test. Rats were housed under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle) with free access to standard pellet diet and water add libitum.

Experimental design and infection protocol

After acclimatisation, the animals were randomly divided into four experimental groups (n = 10 per group; 2 males and 8 females) and housed in separate cages to prevent cross-contamination. The control group received only distilled water orally throughout the experimental period. The second group was infected intraperitoneally on gestational day 7 with 0.5–1.0 mL of a suspension containing 5 × 10^5 tachyzoites/mL of the highly virulent *T. gondii* RH strain. Each female rat was housed overnight with a male for mating. Pregnancy was confirmed the next morning by the presence of a vaginal plug, and reconfirmed by continuing body weight gain gestational days 1–7 (GD 1–7). Only confirmed pregnant rats were included in the study. The third group received oral administration of 0.1 mL of PyNE daily from day 7 to day 18 of pregnancy without infection, to evaluate the formulation's safety and

physiological response. The fourth group was infected with *T. gondii* on day 7 of gestation and concurrently treated with oral PyNE (0.1 mL/day) for 11 consecutive days to assess its therapeutic efficacy. On gestational day 18, all animals were humanely sacrificed under light anesthesia. Blood samples were collected for biochemical analyses, while fetal kidney tissues harvested exclusively for histopathological examination, according to the experimental infection procedures described by Moraes *et al*. (2023).

Blood collection and sampling schedule

All samples were collected on gestational day 18, after infection and treatment, from all experimental groups. Approximately 1–1.5 mL of venous blood was collected from each rat under light anesthesia immediately before euthanasia. Blood samples were allowed to clot at room temperature, centrifuged at 3000 rpm for 15 minutes, and serum was stored at −20 ˚C for biochemical analysis. Serum samples were used for the determination of IL-6, IL-8, IL-17, malondialdehyde (MDA), and glutathione (GSH). On the same day, fetal kidneys were dissected and collected for histopathological examination. No blood or tissue samples were collected before infection.

Chemical materials

Pyrimethamine, triacetin, Cremophor® EL, and Transcutol® were procured from Hyper Chem Company (China). Tween® 80 and Tween® 20 were obtained from Thomas Baker (Chemicals) Pvt. Ltd. (India). Additionally, castor oil, olive oil, coconut oil, and cinnamon oil were sourced locally from markets in Kirkuk, Iraq.

Safety assessment of PyNE

Safety assessment of PyNE (Group III), comprised monitoring of maternal body weight, food consumption, behaviour patterns, mortality and overall health status on daily basis during test period. The dams' organs and the fetal kidneys were grossly examined at necropsy for any toxic changes.

Solubility study of pyrimethamine

The solubility of pyrimethamine was systematically investigated in various oils (castor oil, coconut oil, cinnamon oil, and olive oil), surfactants (Tween® 80 and Tween® 20), and co-surfactants (ethanol and acetone). 5 mL of each solvent was transferred into clean, labelled test tubes and an excess quantity of pyrimethamine powder was added to saturate it in the solvent system. The tubes were sealed to prevent loss of contents and put into an isothermal rotary shaking water bath at 25±0.5˚C for equilibration over a period of 48hr. Samples were centrifuged for 20 min at 3000 rpm after the equilibration phase. The pure supernatant was then decanted and filtered by using a Whatman filter paper (0.45 µm) to eliminate undissolved solid particles. Dilution of filtrates was then carried out with methanol and solubility of pyrimethamine determined spectrophotometrically at λ max 326 nm (UV–visible spectrophotometer). The measurements were performed in triplicate for accuracy and reproducibility (Shriniwas *et al*., 2014).

Pyrimethamine nanoemulsion

Pyrimethamine NEs was formulated with an optimized blend of surfactants and co-surfactants (S_mix), in combination with oil phase selected. Pyrimethamine (2 mg) was weighed precisely and dissolved in the chosen oil at mild stirring temperature (just enough to solubilize completely). The prepared S_mix was added dropwise to oil–drug mixture under stirring and continued for sometimes until a clear, homogenous nanoemulsion is formed. The transparency of the formulation was visually assessed with a light source as described by Gutiérrez *et al*. (2008) and Schertel *et al*. (2021).

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) absorbance spectra were analyzed to determine the compatibility of pyrimethamine and excipients selected in the nanoemulsion formulation. The pure drug, Tween 20 and the optimized PyNE formulation were scanned to observe if any interparticle interaction existed between component I/O and excipient. FTIR spectrum analysis was undertaken for determination of molecular finger-print and confirmation of jaw type specific functional groups and their bonding arrangements. The samples were prepared as KBr pellet and scanned at medium speed in the wave number range of 4000–400 cm^{-1}. The spectra were investigated for any shift or disappearance of characteristic absorption peaks that could be attributed to the drug-excipient interaction (Kumar *et al*., 2017).

Field emission scanning electron microscopy (FESEM)

The surface morphology and droplet configuration of the PyNE were studied by field emission scanning electron microscopy (FESEM). This method provides high resolution imaging, which allows one to visually resolve droplet size, shape, and surface properties. A small amount of the lyophilized nanoemulsion sample was loaded onto an aluminum stub with double-sided adhesive carbon tape and was then coated with a thin layer of gold under high vacuum to ensure electrical conductivity. The samples were examined using an FESEM at 5–10 kV for acceleration voltage. The micrographs obtained were quantitatively analyzed for the uniformity, smoothness and sphericity of PyNE droplets (Lewczuk and Szyryńska, 2021).

Analysis of serum physiological parameters

The levels of interleukins (IL-6, IL-8, and IL-17), MDA, and GSH in the sera were determined by Sandwich ELISA with specific antibodies. Rat IL-6, IL-8, IL-17, MDA and GSH were determined by commercial ELISA kits (SunLong Biotech Co., China) according to the manufacturer' instructions. In brief, 100 µL of each serum sample was transferred to the corresponding antibody-coated wells in microtiter plates and incubated at 37 °C for the indicated times. Following incubation, the wells were washed three times with phosphate-buffered saline (PBS), and a fixed amount of streptavidin–HRP conjugate was pipetted in. The plates were incubated further at 37 °C for 2 hours and then washed to eliminate nonspecifically bound enzymic conjugate. The substrate solutions A and B were finally added to each well to elicit the color reaction, which was stopped by adding 50 µL stop solution. The optical density (OD) was determined at 450 nm of each well with a microplate ELISA reader. The concentrations of the targeted analytes were determined by reference to standard curves included in ELISA kits (Supruniuk *et al.*, 2020; Hassen *et al.*, 2025).

Histopathological studies and microscopy

Fetal kidneys of each group were dissected postmortem and all blood remaining in the organs was removed by perfusion with PBS. The removed kidneys were placed in 10% neutral buffered formalin fixation, and kept tissues to dry at 15 days until completely fixed. Following fixation, the tissues were processed according to standard histological methodology involving dehydration, clearing and paraffin embedding. Five micrometer (5m) thick sections were made by rotary microtome and stained with hematoxylin and eosin (H and E) (Bancroft and Gamble, 2008; Nasar *et al.*, 2025). Under an Olympus photomicroscope (Model CX43, Olympus, Japan), the stained slides were visioned and representative images were taken for morphologic evaluation and comparison.

Ethical approval statement

All animals' procedures in this study were performed strictly by ethical standards issued by the Scientific Research Ethical Committee, Department of Biology, College of Education for Pure Science University of Kirkuk-Iraq. Reference number BCPSKU/003/003Z (20 March 2025) granted approval for the study protocol including animal handling and housing as well as treatment. All of the experimental procedures were performed in accordance with institutional regulations and global standards for animal care (the NIH Guide for the Care and Use of Laboratory Animals and ARRIVE Guidelines). The schematic of the study workflow, including PyNE preparation, *T. gondii* infection and treatment in rats, biochemical and histological assessments, and data analysis, is summarized graphically in Figure 1.

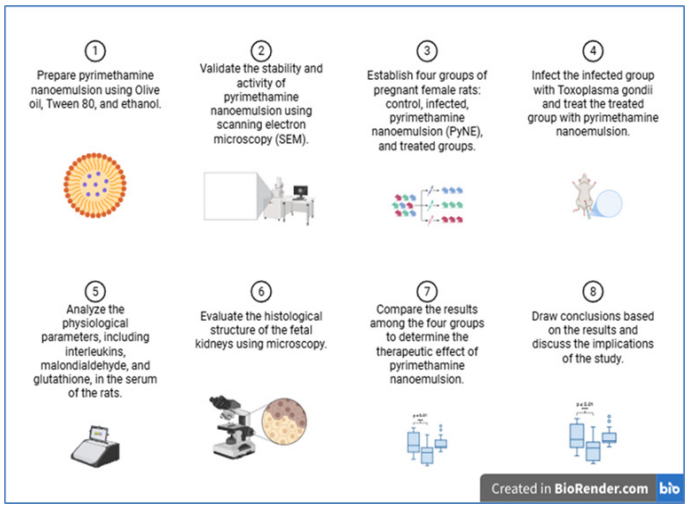

Figure 1: Experimental design and workflow of the study. This figure showing schematic representation showing the order of experimental processes, development of PyNE prepared from olive oil, Tween 80 and ethanol; investigation on stability of nanoemulsion by scanning electron microscopy (SEM); preparation of four rat gestational groups; *T. gondii* inoculation at rats and treatment with PyNE; biochemistry and physiology evaluation; fetal kidneys histology observation among groups; statistical analysis between different cohorts and interpretation based on data determinations to conclude. Generated using BioRender. com.

Statistical analysis

Experimental results were shown as mean ± SD for all measurements. Statistical analysis was performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). The one-way analysis of variation (ANOVA) was used for statistical comparisons of the experimental groups, and to determine significance between treatments on mean values of IL-6, IL-8, IL-17, MDA and GSH in serum were performed. Student's t-test was used for comparison of two variables and when ANOVA showed significance, followed by post hoc pairwise comparison between groups. P-value less than 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

The solubility screening studies for pyrimethamine in different oils, surfactants and co-surfactants are presented in Table 1. Pyrimethamine showed the highest solubility in olive oil (38.91 mg/mL) followed by cinnamon oil (24.95 mg/mL), castor oil (19.04 mg/mL), and coconut oil (17.31 mg/ml). Surfactants showed very higher values of solubility as compared to those showing by Tween 80 was highest (78.64 mg/mL) comparing the solubility with that of Tween 20(51.20 mg/mL). Co-surfactants also increased

the solubilization capacity of the drug, and ethanol (51.84 mg/mL) showed a higher efficiency than acetone (17.22 mg/mL).

Table 1: Pyrimethamine solubility (mg/ml) in different oils, surfactants, and co-surfactants.

Compounds		Solubility mg/dl
Oil	Coconut oil	17.31
	Cinnamon oil	24.95
	Castor oil	19.04
	Olive oil	38.91
Surfactants	Tween 20	51.2
	Tween 80	78.64
Co-surfactants	Ethanol	51.84
	Acetone	17.22

According to these results, olive oil was selected as the oily phase for its excellent solubilising power and biocompatibility. Tween 80, which showed greater solubility among surfactants, was chosen as a surfactant and ethanol as the co-surfactant during the development of PyNE. Choice of these excipients coincides with the earlier reports demonstrating the potential role of olive oil and Tween 80 in improving solubility and bioavailability of poorly water-soluble drugs (Bhalani *et al*., 2022).

No symptoms of toxicity were observed in rats treated with PyNE-only throughout the study. Maternal body weight gain, food consumption, and behaviors did not differ from those of controls. There was no death or abnormal clinical signs found. No macroscopic abnormality was observed in any maternal organ or fetal kidney at necropsy.

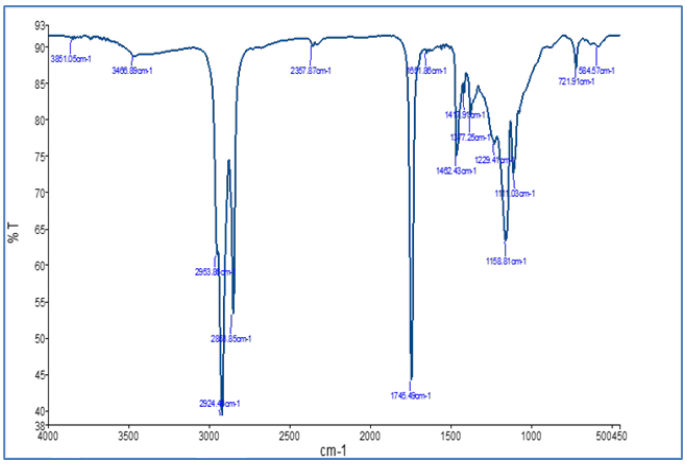

Figure 2: FTIR spectrum of pyrimethamine powder.

The FTIR spectrum of pure pyrimethamine exhibited characteristic peaks at 2953.86, 2924.46, and 2853.85 cm^{-1} (C–H stretching), 3851.05 and 3466.89 cm^{-1} (N–H stretching), 2357.87 cm^{-1} (O–H stretching), 1745.49 and 1651.86 cm^{-1} (C=O stretching), and 1462.43, 1417.91, and 1377.25 cm^{-1} (C=C stretching) (Figure 2). All characteristic peaks of the pure pyrimethamine were found in the FTIR spectrum of the nanoemulsion formulation without notable peak shifts or band disappearance, evincing no chemical interaction between drug and excipients. This confirms the compatibility of all formulation components with the active drug.

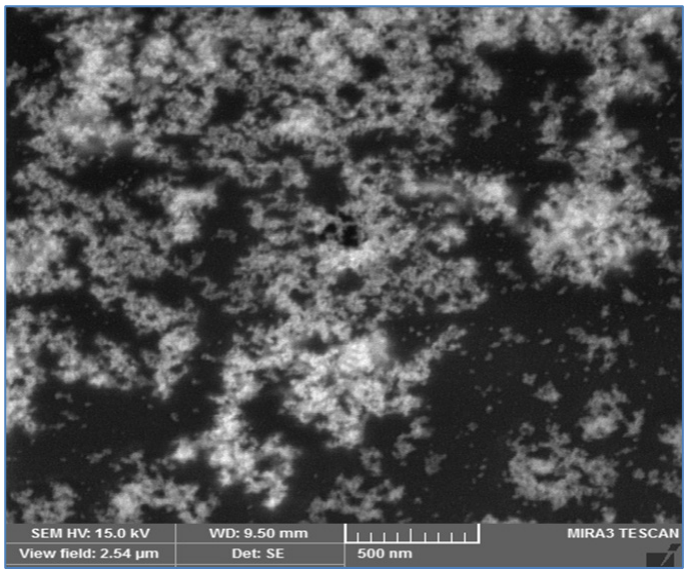

Figure 3: FESEM of optimized formula Py-NE, the optimized formula produces spherical pyrimethamine oil droplets with a collection of smaller oil droplets.

Figure 3 illustrates that while the optimized formula's oil droplets are spherical in shape and accumulate smaller ones, the shape and size of the oil droplets did not significantly alter as a result of the accumulation (De et al.,2000).

Table 2: The concentrations of interleukins in the studied groups.

Groups	IL-6 (ng/L)	IL-8 (pg/ml)	IL-17 (pg/ml)
Control group (I)	18.53±2.74	4.12± 0.42	3.17 ± 0.15
Infected group (II)	167.41±11.52*	137.11±17.64*	184.5 ± 15.63*
PyNE group (III)	16.34±3.47	5.13 ± 1.57	3.94 ± 1.04
Treated group (IV)	73.59±8.1*	36.38 ± 5.18*	53.12 ± 4.71*

* Significantly different from other groups ($P < 0.05$).

The concentrations of selected interleukins in the studied groups are presented in Table 2. The concentration (ng/L) of IL-6 in the serum of the infected group was significantly elevated (167.41 ± 11.52) compared to the PyNE group (16.34 ± 3.47) and the control group (18.53 ± 2.74) ($P < 0.05$). In the group treated with PyNE, IL-6 concentration (73.59 ± 8.1) was markedly reduced compared to the infected group, though still significantly

higher than in the PyNE and control groups (P < 0.05).

Similarly, IL-8 levels (pg/mL) in the serum of the infected group (137.11 ± 17.64) were significantly higher than those in the PyNE group (5.13 ± 1.57) and the control group (4.12 ± 0.42) (P < 0.05). Treatment with PyNE reduced IL-8 concentration (pg/mL) to 36.38 ± 5.18, significantly lower than the infected group but still elevated compared to the PyNE and control groups (P < 0.05).

For IL-17, the infected group exhibited significantly higher serum concentrations (184.5 ± 15.63 pg/mL) compared to the PyNE group (3.94 ± 1.04 pg/mL) and control group (3.17 ± 0.15 pg/mL) (P < 0.05). In the group treated with PyNE, IL-17 concentration (53.12 ± 4.71 pg/mL) was significantly reduced compared to the infected group, yet remained significantly higher than the PyNE and control groups (P < 0.05).

Toxoplasmosis affects both humans and animals causing congenital abnormalities, toxoplasma encephalitis, retinochoroiditis at birth and central nervous system impacts. In addition, infection may cause ocular diseases with late onset (Wallon and Peyron, 2018).

Infected pregnant rats had higher serum IL-6 and IL-8 when compared to the treated and control groups in our study, which shows a more intensifying inflammatory response. IL-6 is essential in immunity by inducing B cell proliferation, increasing natural killer (NK) cell cytotoxicity and promoting acute-phase proteins. It is considered as an early, non-specific and very sensitive marker of inflammation (Rose-John *et al*., 2017; Salomão Lopes *et al*., 2025). It has previously been shown that IL-6 and IL-8 are important components of the response to *T. gondii* infection, consistent with their role as pro-inflammatory cytokines (Pereira *et al*., 2019; Gajewski *et al*., 2025).

Furthermore, increasing evidence highlights the importance of IL-17 in host responses to a variety of infections. Ad hoc experiments performed with IL-17 receptor-deficient ($IL17RA^{-/-}$) mice have demonstrated a critical function of the IL-17 signal transduction pathway in resistance against toxoplasmosis, as its defective expression results in increased susceptibility to infection (Kelly *et al*., 2005). Consistent with these results, the infected pregnant rats in the present study had notably higher bloodstream levels of IL-17 than treated and control groups. This finding is in line with other studies reporting a quick increase of IL-17 in the context of acute *T. gondii* infection (Kelly *et al*., 2005; Moroda *et al*., 2017). Furthermore, IL-17 gene-knockout mice were noted to be more susceptible to oral toxoplasmosis (Kelly *et al*., 2005). IL-17 has been shown to help in the recruitment and activation of neutrophils, which are considered as one of the main host defense mechanisms against *T. gondii* tachyzoites in acute phase (Sana *et al*., 2022).

In this study, PyNE significantly reduced elevated interleukin levels in infected rats, indicating both suppression of *T. gondii* associated inflammatory responses and modulation of host immunity. This immunoregulatory effect highlights the therapeutic potential of PyNE in restoring immune balance and controlling inflammation during toxoplasmosis. Moreover, the absence of behavioral abnormalities, mortality, or gross pathological changes in the PyNE only group indicates that the nanoemulsion formulation was well tolerated during pregnancy, supporting its safety under the experimental conditions. Overall, PyNE demonstrated therapeutic potential in attenuating inflammatory, oxidative, and renal histopathological alterations associated with congenital toxoplasmosis; however, direct comparison with conventional pyrimethamine was not performed and should be addressed in future studies.

Table 3: The concentrations of oxidative stress in the studied groups.

Groups	MDA ng/ml	GSH ng/L
Control group (I)	12.94 ± 1.29	57.82± 3.17
Infected group (II)	59.13 ± 4.13*	21.78 ± 4.12*
PyNE group (III)	13.04 ± 2.84	59.75 ± 6.21
Treated group (IV)	31.65 ±2.69*	32.05 ± 3.14*

* Significantly different from other groups (P < 0.05).

Oxidative stress biomarkers' levels in the experimental groups are shown in Table 3. MDA concentration in serum of the infected group was (59.13 ± 4.13) ng/ml, demonstrating a significant (P <0.05) increase compared with PyNE (13.04 ± 2.84) ng/ml and control (12.94 ± 1.29) ng/ml groups. In the treated group with PyNE, MDA concentration (31.65 ± 2.69 ng/ml) was lower than in the infected group but still showed a significant (P < 0.05) increase compared with the PyNE and control groups Figure 4.

Correspondingly, serum glutathione (GSH) levels in the infected group (21.78 ± 4.12 ng/ml) were significantly (P < 0.05) reduced compared with the PyNE (59.75 ± 6.21 ng/ml) and control (57.82 ± 3.17 ng/ml) groups. As for GSH, *T. gondii* infection led to decrease GSH level compared with control groups (Figure 4). The therapeutic efficacy of pyrimethamine nanoemulsion (PyNE) had partially recovered GSH, but the levels were significantly lower than those of control.to increase on its GSH concentrations (32.05 ±3.14 ng/mL) that were significantly higher than those obtained in the infected group, but they remained lower than those of both the control and only-PyNE

groups (P < 0.05). The data indicate that *T. gondii* infection leads to oxidative stress, evidenced by increase of MDA levels and decrease of GSH levels; whereas treatment with a pyrimethamine nanoemulsion attenuated the redox imbalance.

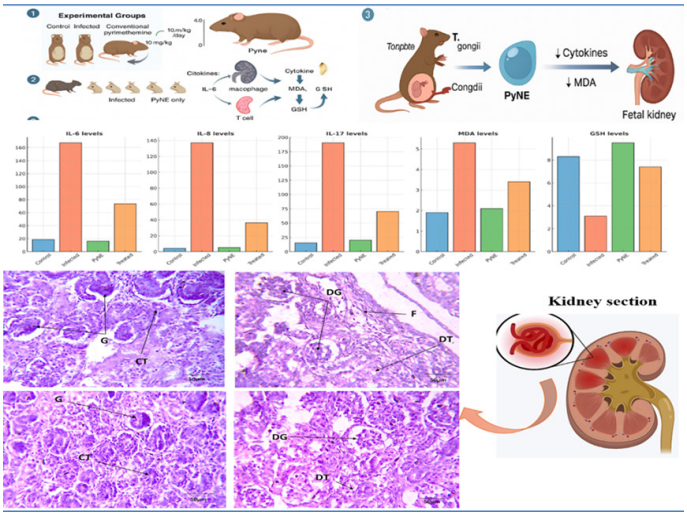

Figure 4: Presents a schematic representation of the experimental design and mechanistic route of PyNE therapy in pregnant rats infected with *T. gondii*. Experimental groups consist of Control, Infected, PyNE alone, and Infected + PyNE, with therapy delivered orally at a dosage of 10 mg/kg. Comparative biochemical and inflammatory tests indicate changes in IL-6, IL-8, IL-17, and MDA levels, alongside an increase in GSH after PyNE therapy. (C) A mechanistic depiction of immune modulation in which *T. gondii* infection stimulates macrophages and increases proinflammatory cytokines (IL-6, IL-8, IL-17) and oxidative markers (MDA), whereas PyNE mitigates these responses, bolstering antioxidant defense (↑GSH) and safeguarding fetal kidney tissue. (D) Representative histological sections of fetal kidney and placenta illustrating significant tissue regeneration in PyNE-treated groups relative to infected controls (HandE, scale bar = 50 µm).

T. gondii infection resulted in a significant increase of serum malondialdehyde (MDA), which were also accompanied with a marked decrease of GSH levels. These findings suggest enhanced lipid peroxidation and a weakened antioxidant defense in infected patients, platelet and tissue MDA was increased at four days after infection, indicating oxidative stress during the acute phase of toxoplasmosis (Engin *et al*., 2012). Additionally, Bahrami *et al*. (2016) reported that *T. gondii* infection disrupts the redox system, as evidenced by decreased GSH levels and increased MDA levels.

In contrast, Choi *et al*. (2014) observed no differences in GSH content between infected and control mice at the same post-infection time, suggesting that the oxidative imbalance may depend on the duration of infection, immune state of host and parasite burden. Taken together, these results suggest that oxidative stress may be involved in the mechanisms of pathogenesis during toxoplasmosis and changes in the MDA level and GSH represent the extent of cellular damage caused by infection. The chronic consequences of acute *T. gondii* infection currently are insufficiently defined. In our present experiments increased activities of hepatic antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase GSH-Px) were detected in the liver after 30 days postinfection in order to ameliorate overwhelming oxidative damage.

The PyNE prepared in this study is designed to improve the solubility, bioavailability and therapeutic potential of the drug against *T. gondii* tachyzoites. Previous studies have evaluated different nanostructured drug carrier systems such as nanoemulsions, solid lipid nanoparticles, polymeric nanoparticle delivery devices, metal particles, and nanosuspensions as possible means to enhance antiparasitic activity *in vivo* and *in vitro* (Azami *et al*., 2018). Synergistic effects of the combined therapeutic strategies of antimicrobials with nanocarriers have been found to be effective in promoting host immune responses and decreasing parasite load.

Nanoparticle-based platforms have emerged as an attractive therapeutic approach which has the ability to cross biological barriers. Hagras *et al*. (2022) showed that combined therapy with silver nanoparticles and spiramycin-loaded chitosan was more effective than a single drug in the treatment of *T. gondii*. These are consistent with our present results in which PyNE treatment significantly attenuated MDA levels and restored GSH content that concomitant with remarkable histopathological observations in fetal kidney tissues of infected rats. Overall, our findings highlight the potential of nanoformulated antiparasitic drugs such as spiramycin and pyrimethamine nanoparticles as promising therapeutic alternatives to reduce oxidative stress and tissue alterations triggered by toxoplasmosis infection, especially those occurring in pregnancy (Hagras *et al*., 2022; Abdel-Wahab *et al*., 2024). In control and PyNE-infected groups, a normal glomerular and tubular histological architecture was noted on microscopic examination with preserved glomerular tufts and the absence of injury to the tubule epithelial lining. However, fetal kidney sections from the infected group (Figure 4) exhibited marked histopathological alterations with glomerular destruction, tubular degeneration, and interstitial fibrosis (Aljaff *et al*., 2023; Al-Moussawi *et al*., 2025). Cellular degeneration and necrosis were also evident, indicating extensive tissue damage from *T. gondii* infection.

Treatment with PyNE produced a noticeable improvement in renal histology (Figure 4). The kidneys displayed partial

restoration of normal glomerular and tubular structures, reduced fibrosis, and fewer degenerative changes. However, mild residual lesions persisted in some areas, including focal glomerular damage and limited interstitial inflammation. These findings confirm the protective and reparative potential of PyNE against *T. gondii* induced renal injury.

Kidney injury caused by *T. gondii* can lead to hypoalbuminemia due to protein loss in urine, ultimately progressing to renal failure. Such renal impairment results from glomerular damage and structural abnormalities of the urinary system, with a consequent reduction in glomerular filtration rate (Gharadaghi *et al*., 2012; Ahmed *et al*., 2023). In the current study, hematoxylin and eosin–stained sections of fetal kidney tissue from infected rats revealed extensive inflammatory infiltrates, vascular congestion, necrotic foci, and numerous Toxoplasma tachyzoites. These findings are consistent with those reported by Mady *et al*. (2016) and El-Temsahy *et al*. (2016).

In contrast, rats treated with PyNE exhibited marked histological improvement, evidenced by reduced inflammatory infiltration, decreased vascular congestion, and partial restoration of normal renal architecture. These results suggest that PyNE effectively mitigates *T. gondii* induced renal pathology through its antiparasitic and anti-inflammatory actions. Comparable findings were observed by Anand *et al*. (2015) and Mady *et al*. (2016), who demonstrated similar therapeutic outcomes using nanocarrier-based formulations, including bovine lactoferrin nanocapsules and pyrimethamine combined with clindamycin or *Nigella sativa* oil. Collectively, the current results confirm that pyrimethamine-loaded nanoemulsion is a potent therapeutic alternative that enhances drug efficacy, reduces tissue damage, and improves tolerance in the treatment of acute toxoplasmosis.

CONCLUSIONS

The present study demonstrated that *T. gondii* infection induces severe histopathological alterations in the fetal kidneys, including glomerular destruction, tubular degeneration, and inflammatory infiltration. The current observed protective effect of PyNE against inflammatory and oxidative stress parameters and improvement in renal histopathology changes could be attributed to its previously mentioned antioxidant properties; which are known to play a role as an anti-inflammatory agent. These results indicate the strong therapeutic potential of PyNE in congenital toxoplasmosis, although further studies are needed.

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AUTHOR'S CONTRIBUTION

All authors contributed substantially to the conception and design of the study, data acquisition, analysis, and interpretation. They jointly participated in drafting, revising, and critically reviewing the manuscript for important intellectual content and approved the final version to be published.

Consent for publication

Not applicable. This study does not include any individual person's data in any form (including individual details, images, or videos).

Availability of data and material

The data that support the findings of this study are not publicly available due to [ethical/legal/privacy] restrictions but are available from the corresponding author on reasonable request.

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Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdel-Wahab A A, Shafey DA, Selim SM, Sharaf SA, Mohsen KK, Allam DM, Gouda MA (2024). Spiramycin-loaded maltodextrin nanoparticles as a promising treatment of toxoplasmosis on murine model. Parasitol. Res., 123(7): 286. https://doi.org/10.1007/s00436-024-08280-4.

Ahmed QA, Abdullah SI, Taher HM (2023). Effects of the alcoholic extract of ginseng roots and carob fruits in comparison with vitamin E in improving the efficiency of the male reproductive system of albino rabbits. Casp. J.

Environ. Sci., 21(4): 815-826.
Akins GK, Furtado JM, Smith JR (2024). Diseases caused by and behaviors associated with *Toxoplasma gondii* infection. Pathogens, 13(11): 968-993. https://doi.org/10.3390/pathogens13110968
Al Moussawi RI, Razooqi QA, Subhi RN, Al-Hayawi AY (2025). Analysis of some physiological and histological effects of repaglinide on liver and lung tissues in rats. Tikrit J. Agric. Sci., 25(2): 234-248. https://doi.org/10.25130/tjas.25.2.19
Ali RH, Hussein FK, Nasar DM, Abd Al salam Salem A (2025). The relevance of mitochondrial DNA mutation in human diseases and forensic sciences. Al-Nahrain J. Sci., 28(1): 96-106. https://doi.org/10.22401/ANJS.28.1.11
Aljaff DAA, Ahmed QA, Salih AA (2023). Comparison of the effect of olive oil and its derivatives with atorvastatin in mitigating physiological and histological disorders due to hyperlipidemia in male rats. Biomedicine, 43(3): 886-892. https://doi.org/10.51248/.v43i3.2821
Anand N, Sehgal R, Kanwar RK, Dubey ML, Vasishta RK, Kanwar JR (2015). Oral administration of encapsulated bovine lactoferrin protein nanocapsules against intracellular parasite *Toxoplasma gondii*. Int. J. Nanomed., pp. 6355-6369. https://doi.org/10.2147/IJN.S85286
Azami SJ, Amani A, Keshavarz H, Najafi-Taher R, Mohebali M, Faramarzi MA, Shojaee S (2018). Nanoemulsion of atovaquone as a promising approach for treatment of acute and chronic toxoplasmosis. Eur. J. Pharm. Sci., 117: 138-146. https://doi.org/10.1016/j.ejps.2018.02.018
Bahrami S, Shahriari A, Tavalla M, Azadmanesh S, Hamidinejat H (2016). Blood levels of oxidant/antioxidant parameters in rats infected with *Toxoplasma gondii*. Oxid. Med. Cell. Longev., 2016(1): 8045969. https://doi.org/10.1155/2016/8045969
Bancroft JD, Gamble M (2008). Theory and practice of histological techniques. Elsevier health sciences.
Bhalani DV, Nutan B, Kumar A, Singh Chandel AK (2022). Bioavailability enhancement techniques for poorly aqueous soluble drugs and therapeutics. Biomedicines, 10(9): 2055. https://doi.org/10.3390/biomedicines10092055
Che Marzuki NH, Wahab RA, Abdul Hamid M (2019). An overview of nanoemulsion: Concepts of development and cosmeceutical applications. Biotechnol. Biotechnol. Equip., 33(1): 779-797. https://doi.org/10.1080/13102818.2019.1620124
Choi HJ, Yu ST, Lee KI, Choi JK, Chang, BY, Kim, SY, Park H (2014). 6-trifluoromethyl-2-thiouracil possesses anti-*Toxoplasma gondii* effect *in vitro* and *in vivo* with low hepatotoxicity. Exp. Parasitol., 143: 24-29. https://doi.org/10.1016/j.exppara.2014.05.002
Chua YW, Chow SC (2025). Bug as a drug: Unveiling anti-cancer properties of *Toxoplasma gondii* and its therapeutic prospects in cancer immunotherapy. Acta Trop., 267: 107684. https://doi.org/10.1016/j.actatropica.2025.107684
De, B.C., (2000). Characterization and shape analysis of spherical oil droplets in optimized emulsion systems. Journal of Colloid and Interface Science, 224(2), 215–223.
El-Temsahy MM, El-Kerdany ED, Eissa MM, Shalaby TI, Talaat IM, Mogahed NM (2016). The effect of chitosan nanospheres on the immunogenicity of *Toxoplasma lysate* vaccine in mice. J. Parasit. Dis., 40(3): 611-626. https://doi.org/10.1007/s12639-014-0546-z
Engin AB, Dogruman-Al F, Ercin U, Celebi B, Babur C, Bukan N (2012). Oxidative stress and tryptophan degradation pattern of acute *Toxoplasma gondii* infection in mice. Parasitol. Res., 111(4): 1725-1730. https://doi.org/10.1007/s00436-012-3015-6
Gajewski PD, Bröde P, Claus M, Golka K, Hengstler JG, Reinders J, Getzmann S (2025). Changes of cognitive functions and proinflammatory cytokines across the lifespan in latent *Toxoplasma gondii* infection. Brain Behav. Immun. Health, pp. 101105. https://doi.org/10.1016/j.bbih.2025.101105
Gharadaghi Y, Shojaee S, Khaki A, Fathiazad F, Khaki A, Ghdamkheir E, Rouhaninia M (2012). Antiprotozoal effect of Allium cepa on acute renal failure caused by *Toxoplasma gondii*. Afr. J. Pharm. Pharmacol., 6)10(: 771-777. https://doi.org/10.5897/AJPP12.086
Gutiérrez JM, González C, Maestro A, Solè IM, Pey CM, Nolla J (2008). Nano-emulsions: New applications and optimization of their preparation. Curr. Opin. Colloid Interface Sci., 13(4): 245-251. https://doi.org/10.1016/j.cocis.2008.01.005
Hagras NA, Mogahed NM, Sheta E, Darwish AA, El-Hawary MA, Hamed MT, Elwakil BH (2022). The powerful synergistic effect of spiramycin/propolis loaded chitosan/alginate nanoparticles on acute murine toxoplasmosis. PLoS Negl. Trop. Dis., 16(3): e0010268. https://doi.org/10.1371/journal.pntd.0010268
Hassen MD, Mousa NO, Radwan SM, Gabre RM (2025). The ameliorative effect of interleukin-17A neutralization on doxorubicin-induced cardiotoxicity by modulating the NF-κB/NLRP3/caspase-1/IL-1β signaling pathway in rats. Inflammation, pp. 1-14. https://doi.org/10.1007/s10753-024-02187-z
Huang Y, Guo X, Wu Y, Chen X, Feng L, Xie N, Shen G (2024). Nanotechnology's frontier in combatting infectious and inflammatory diseases: prevention and treatment. Signal Transduct. Target. Ther., 9(1): 34. https://doi.org/10.1038/s41392-024-01745-z
Ijaz M, Khan AU, Ullah S, Khan A, Ibenmoussa S, Sitotaw B, Dawoud TM, Khan A, Iqbal F (2024). *Toxoplasma gondii* infection affects the complete blood count and disturbs the markers of oxidative stress from the vital organs of wild rodents. Sci. Rep., 14(1): 22716. https://doi.org/10.1038/s41598-024-73265-3
Ismael SS (2023). Role of nanotechnology in treating of *Toxoplasma gondii*. Sci. Publ., 2: 202-212. https://doi.org/10.47278/book.zoon/2023.64
Kelly MN, Kolls JK, Happel K, Schwartzman JD, Schwarzenberger P, Combe C, Moretto M, Khan IA (2005). Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against *Toxoplasma gondii* infection. Infect. Immun., 73(1): 617-621. https://doi.org/10.1128/IAI.73.1.617-621.2005
Kumar R, Soni GC, Prajapati SK (2017). Formulation development and evaluation of Telmisartan Nanoemulsion. Int. J. Res. Dev. Pharm. Life Sci., 6(4), 2711-2719. https://doi.org/10.21276/IJRDPL.2278-0238.2017.6(4).2711-2719
Lahmar I, Abou-Bacar A, Abdelrahman T, Guinard M, Babba H, Ben Yahia S, Kairallah M, Speeg-Schatz C, Bourcier T, Sauer A, Villard O (2009). Cytokine profiles in toxoplasmic and viral uveitis. J. Infect. Dis., 199(8): 1239-1249. https://doi.org/10.1086/597478
Lewczuk B, Szyryńska N (2021). Field-emission scanning electron microscope as a tool for large-area and large-volume ultrastructural studies. Animals, 11(12): 3390. https://doi.org/10.3390/ani11123390
Li J, Zhao J, Yang X, Wen Y, Huang L, Ma D, Shi J (2021). One

severe case of congenital toxoplasmosis in China with good response to azithromycin. BMC Infect. Dis., 21(1): 920. https://doi.org/10.1186/s12879-021-06619-1

Mady RF, El-Hadidy W, Elachy S (2016). Effect of Nigella sativa oil on experimental toxoplasmosis. Parasitol. Res., 115(1): 379-390. https://doi.org/10.1007/s00436-015-4759-6

Majeed ZH, Hasan SA, Ismail RM (2022). Evaluate the benefit effects of *Dodonaea viscosa* in kidney of infected rats with Staphylococcus aureus. J. Pharm. Negat. Results, 13(3): 291. https://doi.org/10.47750/pnr.2022.13.03.046

Mohammed HH, Taher HM (2025). Cytokines and kidney function biomarkers in entamoeba histolytica-infected patients. ICAIIT, 13(2): 753–759.

Moraes RD, Araujo E, Castelo Filho A, Peixoto AB, Amed AM (2023). Action of enfuvirtide on the pregnancy of albino rats (Rattus norvegicus albinus, Rodentia, Mammalia): Biological assay and functional and histological analyses of exposed maternal-fetal organs. Einstein (São Paulo), 21: eAO0230. https://doi.org/10.31744/einstein_journal/2023AO0230

Moroda M, Takamoto M, Iwakura Y, Nakayama J, Aosai F (2017). Interleukin-17A-deficient mice are highly susceptible to *Toxoplasma gondii* infection due to excessively induced *T. gondii* HSP70 and interferon gamma production. Infect. Immun., 85(12): 10-1128. https://doi.org/10.1128/IAI.00399-17

Murtaza G, Rizwan M, Usman M, Ahmed Z, Iqbal J, Ahmad S, Alwahibi MS, Rizwana H, Iqbal R, Deng G, Lackner M (2025). Physiological and biochemical alterations in soybean by banana peel biochar under different degrees of salt stress. Sci. Rep., 15(1): 30532. https://doi.org/10.1038/s41598-025-98701-w

Nasar D, Saleh H, Al-Rikabi H, Said R, Saleh H (2025). Assessment potential mechanism similarity in epigenetic action and functional status between estradiol and phytoestrogenic supplements. Iraqi J. Vet. Sci., 39(4): 781-788.

Ortiz JO, Potter AK, Benmerzouga I (2025). Novel drug targets for the bradyzoite form of *Toxoplasma gondii*. Res. Rep. Trop. Med., pp. 25-30. https://doi.org/10.2147/RRTM.S431290

Pereira AC, Silva RJ, Franco PS, de Oliveira Gomes A, Souza G, Milian IC, Ribeiro M, Rosini AM, Guirelli PM, Ramos EL, Mineo TW (2019). Cyclooxygenase (COX)-2 inhibitors reduce *Toxoplasma gondii* infection and upregulate the pro-inflammatory immune response in Calomys callosus rodents and human monocyte cell line. Front. Microbiol., 10: 225. https://doi.org/10.3389/fmicb.2019.00225

Preeti, Sambhakar S, Malik R, Bhatia S, Al Harrasi A, Rani C, Saharan R, Kumar S, Geeta, Sehrawat R (2023). Nanoemulsion: An emerging novel technology for improving the bioavailability of drugs. Scientifica, 2023(1): 6640103. https://doi.org/10.1155/2023/6640103

Ramírez-Flores CJ, Mondragón-Flores R (2025). Comprehensive analysis of *Toxoplasma gondii* migration routes and tissue dissemination in the host. PLoS Negl. Trop. Dis., 19(7): e0013369. https://doi.org/10.1371/journal.pntd.0013369

Rose-John S, Winthrop K, Calabrese L (2017). The role of IL-6 in host defence against infections: Immunobiology and clinical implications. Nat. Rev. Rheumatol., 13(7): 399-409. https://doi.org/10.1038/nrrheum.2017.83

Saffarionpour S (2019). Preparation of food flavor nanoemulsions by high-and low-energy emulsification approaches. Food Eng. Rev., 11(4): 259-289. https://doi.org/10.1007/s12393-019-09201-3

Salman HA, Hussein FK, Abdulrahman SJ (2024). A comparison of glutathione and malondialdehyde concentrations in athletes engaged in certain sports. Thamar Uni. J. Nat. Appl. Sci., 9(1): 39-42. https://doi.org/10.59167/tujnas.v9i1.2053

Salomão Lopes C, Carvalho RJ, da Silva TL, Barros HL, Costa LV, Mota DC, Barbosa BF, Vieira LS, de Araújo TM, Costa AR, Awoyinka RO (2025). Pregnant women chronically infected by *Toxoplasma gondii* with depressive disorder: Differential modulation of pro-inflammatory and anti-inflammatory cytokines. Pathogens, 14(4): 330. https://doi.org/10.3390/pathogens14040330

Sana M, Rashid M, Rashid I, Akbar H, Gomez-Marin JE, Dimier-Poisson I (2022). Immune response against toxoplasmosis some recent updates RH: *Toxoplasma gondii* immune response. Int. J. Immunopathol. Pharmacol., 36: 03946320221078436. https://doi.org/10.1177/03946320221078436

Schertel S, Salar-Behzadi S, Zimmer A (2021). Impact of surface properties of core material on the stability of hot melt-coated multiparticulate systems. Pharmaceutics, 13(3): 366. https://doi.org/10.3390/pharmaceutics13030366

Shahbazzadegan S, Farzizadeh N (2025). Association between maternal *Toxoplasma gondii* IgG seropositivity and sex ratio at birth. Sci. Rep., 15(1): 35383. https://doi.org/10.1038/s41598-025-19298-8

Shriniwas Y, Sumit P, Pankaj S, Anurag B (2014). Design and development of solid self micro-emulsifying osmotic drug delivery system for isradipine. J. Drug Deliv. Ther., pp. 28-41.

Sonneville-Aubrun O, Simonnet JT, L'alloret F (2004). Nanoemulsions: A new vehicle for skincare products. Adv. Colloid Interface Sci., 108: 145-149. https://doi.org/10.1016/j.cis.2003.10.026

Supruniuk E, Maciejczyk M, Zalewska A, Górski J, Chabowski A (2020). Blood profile of cytokines, chemokines, growth factors, and redox biomarkers in response to different protocols of treadmill running in rats. Int. J. Mol. Sci., 21(21): 8071. https://doi.org/10.3390/ijms21218071

Tanuku S, Velisila D, Thatraju D, kumar Vadaga A (2024). Nanoemulsion formulation strategies for enhanced drug delivery. J. Pharma. Insight. Res., 2(4): 125-138. https://doi.org/10.69613/3f8m9151

Wallon M, Peyron F (2018). Congenital toxoplasmosis: A plea for a neglected disease. Pathogens, 7(1): 25. https://doi.org/10.3390/pathogens7010025

Wang XN, Zhang CJ, Diao HL, Zhang Y (2017). Protective effects of curcumin against sodium arsenite-induced ovarian oxidative injury in a mouse model. Chin. Med. J., 130(09): 1026-1032. https://doi.org/10.4103/0366-6999.204927

Wilson RJ, Li Y, Yang G, Zhao CX (2022). Nanoemulsions for drug delivery. Particuology, 64: 85-97. https://doi.org/10.1016/j.partic.2021.05.009

Zhu W, Li J, Pappoe F, Shen J, Yu L (2019). Strategies developed by *Toxoplasma gondii* to survive in the host. Front. Microbiol., 10: 899. https://doi.org/10.3389/fmicb.2019.00899