

# Current and Emerging Therapeutic Strategies for Toxoplasmosis: Conventional Treatments, Herbal Medicine, and Nanotechnology

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## **Abstract:**

Toxoplasmosis is a widespread parasitic infection caused by *Toxoplasma gondii* that may lead to serious complications, particularly in pregnant women, congenitally infected infants, and immunocompromised patients. Current therapeutic options remain limited and are selected according to the clinical form of the disease and the host immune status. The standard first-line therapy is based mainly on pyrimethamine combined with sulfadiazine, which acts synergistically by inhibiting sequential steps in folate metabolism and consequently suppressing parasite proliferation. However, conventional anti-Toxoplasma regimens are frequently associated with adverse effects and toxicity, including bone marrow suppression, which may limit their clinical use. Alternative agents such as clindamycin, azithromycin, atovaquone, trimethoprim-sulfamethoxazole, and spiramycin have therefore been investigated for different clinical settings. More recently, herbal products and nanotechnology-based approaches, particularly metallic nanoparticles, have attracted attention because of their potential antiparasitic activity, improved drug delivery, and ability to reduce treatment-related toxicity. This mini-review summarizes conventional and emerging therapeutic strategies for toxoplasmosis, with consideration of treatment approaches in acquired, congenital, cerebral, ocular, and immunocompromised cases.

**Keywords:** *Toxoplasma gondii*; Toxoplasmosis; Pyrimethamine; Sulfadiazine; Spiramycin; Nanoparticles; Herbal medicine; Treatment.

## **Introduction:**

General anti-parasitic and antibacterial drugs that are used for the treatment of toxoplasmosis remain limited and therapeutic strategies for this disease vary regarding the state of the disease and the host immune response (1).

**Vanichtanankul et al. (2)** stated that most of the studies on *Toxoplasma* treatment were geared toward developing inhibitors of enzymes involved in *Toxoplasma* metabolism, like dihydrofolate reductase(DHFR), (Nicotinamide adenine dinucleotide + hydrogen) NADH dehydrogenases, cysteine proteases, and dihydropteroate synthetase(DHPS).

The recommended first-line treatment for toxoplasmosis is a combination therapy based on pyrimethamine(PYR), sulfadiazine (SDZ), drugs that act synergistically by targeting two steps in folic acid metabolism. Other therapies combine pyrimethamine with clindamycin, azithromycin or atovaquone, or trimethoprim with sulfamethoxazole. Though, all treatments are commonly related with adverse side effects and toxicity (3).

➤ **Pyrimethamine:**

Pyrimethamine is an anti-parasitic medication and it is indicated for the treatment of toxoplasmosis. Pyrimethamine and sulfadiazine are thought to act synergistically to treat toxoplasmosis by inhibiting *T. gondii* proliferation and survival through inhibiting the folate metabolic pathway (4). The drugs inhibit (DHFR), and (DHPS), respectively, and consequently block the synthesis of tetrahydrofolate, which is required by the parasite for DNA synthesis (5).

**Adverse effects:**

Pyrimethamine, and sulfadiazine are inhibitors of DNA synthesis in *T. gondii* tachyzoites but may also inhibit DNA synthesis in tissues with high metabolic activity such as the bone marrow and epithelium. This can be by passed by the addition of folinic acid (FA), and these adverse events are reversed upon cessation of treatment (6).

Bone marrow suppression in treated infants and pregnant women was reported. In treated children, neutropenia, as well as anemia, thrombocytopenia and eosinophilia were reported (6).

The potential severity of adverse events of the PYR-SDZ combination led to considering alternative treatment options such as PYR-clindamycin, PYR-azithromycin, atovaquone, cotrimoxazole (TMP-SMX) is essential (7).

**Dose:**

The dosage of pyrimethamine is 25 mg/day given by the oral route for 3-4 weeks in adults and 2 mg/kg/day for 3 days (to a maximum daily dose of 25 mg) and 1 mg/kg/day for 4 weeks in children. Whereas the dose of sulphadiazine is 4-6 gm/day for 3-4 weeks in adult and 100-200 mg/kg/day for 3-4 weeks in children (8).

➤ **Clindamycin**

Clindamycin is a lincomsamide antibiotic that is often used in combination with Pyrimethamine for the therapy of toxoplasmic retinochoroiditis and encephalitis, especially in patients allergic to sulphonamides (9).

**Side effects:**

Diarrhea, pseudomembranous colitis, nausea, vomiting, abdominal pain or cramps, and/or rash (10).

**Dose:**

300mg orally every 6 hours for 3 weeks. It has frequently been used for treatment of toxoplasmic chorioretinitis in humans, with good clinical results (11).

➤ **Atovaquone:**

Atovaquone is a hydroxy-1, 4-naphthoquinone antibiotic. It is a safe and effective treatment against tachyzoites and cyst forms of *Toxoplasma* (12).

Atovaquone acts by targeting mitochondrial respiration to block and collapse the membrane in the organisms. Atovaquone has been shown to protect against maternal and congenital toxoplasmosis as well as inflammatory complications in animal models (13).

Atovaquone can be used as an alternative in patients who are unable to tolerate either sulfonamides or clindamycin (1).

➤ **Spiramycin:**

Spiramycin, a macrolide antibiotic, is another promising less toxic therapeutic substitute for acute toxoplasmosis. The drug can pass through the placenta. The antibiotic action of spiramycin, which inhibits protein synthesis in bacterial cells during translocation, has high antimicrobial activity against Gram-positive bacteria and mycoplasma species, and is widely used to treat toxoplasmosis and other soft-tissue infections in cattle, pigs, poultry, and sheep (14,15).

The exact mechanism of action of spiramycin on toxoplasmosis is not well clarified. However, its action can be explained as it inhibits protein synthesis. (14). Spiramycin is used broadly in Europe. It is exclusively used to prevent maternal-fetal transmission of the organism. However, spiramycin monotherapy is less effective than pyrimethamine or sulfadiazine (15).

Spiramycin produces high tissue concentration, especially in the placenta. It can be given to pregnant women who are primarily infected with *Toxoplasma* without teratogenic effects on the fetus. Spiramycin should be given at a dose of (3 gm/day) throughout the pregnancy to reduce the risk of transplacental passage of the parasite (16).

Spiramycin is often used for treatment while waiting for diagnostic testing for prenatal transmission. In some studies, it is continued for women in whom acute toxoplasmosis is documented even if tests for prenatal transmission are negative (17).

Spiramycin has good bioavailability, ranging from 30% to 40%. Administration away from meals is recommended because food reduces bioavailability by 50% and delays the time-to-peak serum concentration (18).

#### **Side effects:**

Side effects of spiramycin are rare, they include pseudomembranous colitis, cholestatic hepatitis, QT segment prolongation with dysrhythmia, thrombocytopenia, and oxidative hemolysis in glucose-6-phosphate dehydrogenase-deficient patients. Excretion is predominantly through the biliary route, with some enterohepatic recirculation. The drug is relatively free of significant drug interactions (19).

#### **Dose:**

Spiramycin should be taken at a dose of (200 mg/kg body weight /day for 7 days (20).

Spiramycin should be taken at a dose of (3 gm/day) during pregnancy to decrease the risk of transplacental transmission of the parasite (20).

#### **➤ Herbal medicine:**

Many herbs, plants, and natural products are safe, affordable and may have anti-toxoplasmic properties (21).

Farag et al. (22) showed that *Thymus vulgaris* extract was near in effect when compared to spiramycin in reducing the number of tissue cysts and decreasing the inflammatory lesions. El-Shafey et al. (23) showed that curcumin and curcumin metal organic frameworks nano-composite significantly decreased the parasite load (number of cysts) in the brains of the treated rats infected with *T. gondii* ME49 strain.

As regards of *T. gondii* infection, the most commonly used extract for its treatment is *Artemisia*, which has an effective compound present in its flowers and leaves called Artemisinin that participates in the destruction of parasitic cells by the production of reactive oxygen-based free radicals (24).

Elazab et al. (21) suggested that herbal medicines play a vital role in the treatment of many infectious diseases like. *Eurycoma longifolia* plant that has a significantly anti-*T.gondii* activity via inhibiting its growth and also can be used as an alternative to clindamycin for the treatment of toxoplasmosis.

Another example of plants used in the medicinal field is *Allium cepa* (onion), a plant that is widely cultivated in most countries in the world and its juice can be used in the prevention of fungal, bacterial, and parasitic infections (25). Several studies has illusrated the efficacy of this plant against *T. gondii*, In addition, Abdel-Hamed et al. (26) illustrated that the mixture of rosemary extracts can be used as an effective therapy against chronic toxoplasmosis.

#### **➤ Nanotechnology:**

Nanotechnology is the science including particles with a core either polymeric, metallic, or lipidic in nature and size less than 100 nm, with specific physicochemical and biological activities which facilitate transporting drugs into the target tissue due to the large surface area and small size structure (27).

These particles are further divided into nanocarriers and nanodrugs. Nanomedicine has been employed to overcome the limitations of traditional drugs (28).

Nanoparticles (NPs) were also used for medical applications as they were proven to have anti-inflammatory and anti-microbial activities against viruses, bacteria, and parasites. The anti-protozoal activity of AgNPs has been confirmed against *Schistosoma japonicum*, *Echinococcus granulosus*, *Giardia intestinalis*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Leishmania* spp, and *Plasmodium* species (29).

In addition, AgNPs can accumulate in tissues, probably reaching cysts of *T. gondii* with increasing concentration of drugs intracellularly and decreasing the side effects of the original drug (30).

NPs compared to conventional treatment used in the clinic, can be considered a good alternative treatment for no presenting cytotoxic results even at higher doses, while classical treatment with pirymethamine induced increased cytotoxicity in low doses.

Many studies reported that nanoparticles decreased the dose of the drugs used in the treatment of acute toxoplasmosis in laboratory mice and could transport the drug through the blood brain barrier (B.B.B.). Also, nanomaterials can decrease the toxicity of herbal components (28).

Nanoparticles can act in a direct mechanism on intracellular pathogens by producing free radicals that can kill infectious agents or as drugs carriers, in such role, they can increase bioavailability, modulate pharmacokinetics and decrease drug toxicity (31)

Treatment of experimentally infected mice with *T. gondii* by biogenic selenium nanoparticles decreased the mean number of brain-tissue cysts with elevation of the inflammatory cytokines compared to the control group (31).

There are two main types of nanomaterials: engineered and non-engineered. Scientists can produce the engineered nanomaterials from several substances like metal oxides ( $\text{CeO}_2$ ,  $\text{AlO}_3$  and  $\text{SiO}_2$ ), metals (Ag, Cu, Zn and Fe), non-metal (quantum dots and silica), polymers (Chitosan and alginate) and lipids (stearic acid and soybean lecithin), but the non-engineered nanomaterials are found in nature resulting from volcanic eruption, forest fires and erosions (32, 33).

Silver-nanoparticles (AgNPs) are one of the most vital metallic nanoparticles that are used in biomedical applications due to its characteristic size and shape (34).

AgNPs play an important role in nanomedicine as it is considered as a promising alternative since they have antifungal, antiviral, antimicrobial, and antiparasitic activity. For example, leishmanicidal activity against *Leishmania amazonensis* and anti-*Toxoplasma* behavior (35).

**da Silva Sanfelice et al. (36)** illustrated that AgNPs could decrease *T. gondii* proliferation in trophoblast cells and villous explants with the induction of inflammatory mediators. So, AgNPs are promising treatment of toxoplasmosis.

Silver-nanoparticles have proved to be the most effective as it has good antimicrobial efficacy against bacteria, viruses, and other micro-organisms. Silver-nanoparticles can act as antiparasitic agents, mainly against *T. gondii* (35).

**Vergara-Duque et al. (37)** evaluated the viability of oocysts of *Cryptosporidium parvum* after the application of AgNPs, and showed that AgNPs strongly reduce the viability of oocysts in addition, the sporozoite/shell ratio was more affected by treatment with AgNPs because sporozoites are destructed by interaction with AgNPs.

**Adeyemi et al. (31)** reported alteration in the conversion of *T. gondii* tachyzoites to bradyzoites, illustrating that AgNPs decrease bradyzoites burden in vitro and affect the mitochondrial membrane potential of the parasite, altering replication, invasion, and possible infectivity processes.

Other mechanisms of AgNPs may involve the release of silver ions (Ag<sup>+</sup>), destruction of membrane structure, damaging DNA structure, inactivating enzymes, and regulating signaling pathways (31).

### **1-Treatment of Acquired Toxoplasmosis:**

Many of these infections are self-limited and resolve without specific treatment or with symptomatic management (i.e. ibuprofen for pain and fever). Treatment with pyrimethamine and sulfadiazine for 4 weeks is effective and is used for moderate to severe cases (38)

### **2-Treatment of Congenital Toxoplasmosis:**

According to Konstantinovic et al. (3), specific anti-T. gondii treatment includes 1) prenatal treatment, aimed at prevention of materno-fetal transmission of parasites (MFTP) and/or reducing fetal damage 2) postnatal treatment for relieving clinical manifestations and/or prevention of long-term sequelae in the infected neonate

- **Prenatal treatment:**

Women infected during pregnancy (or around conception) are generally offered spiramycin (SPI). Due to the low rate of adverse effects; SPI is a comfortable treatment option while awaiting amniocentesis (39).

PCR analysis of the amniotic fluid (AF) samples from the 16th gestational week (gw) onwards allows for a treatment switch to Pyrimethamine (PYR)-based combinations, primarily PYR – sulfadiazine (SDZ) combination (PYR-SDZ) when a positive PCR result is obtained. However, the PYR-SDZ combination is teratogenic and hence should be avoided during the first 14 gw, although this cut-off varies between countries (38). Anyway, prenatal diagnosis is never performed before 14 gw, thus SPI treatment is the rule during the first trimester of gestation (3).

Treatment with pyrimethamine and sulfadiazine during at least the first year of life reduces parasite burden and may help prevent adverse sequelae such as retinal damage and loss of vision (38).

Sulphadiazine is given at a dose of (50- 100 mg/kg per day), and pyrimethamine is given at a dose of (0.5- 1.0 mg/kg/day) with which vitamin supplement is given for 3 weeks. This regimen is followed by a further 3 weeks therapy consisting of spiramycin (3 gm/day). Alternating 3 weeks drug courses are given until delivery. If bone marrow toxicity is demonstrated, the frequency of pyrimethamine dosing is reduced (40).

- **Postnatal treatment:**

Postnatal treatment is started when the diagnosis of congenital infection is confirmed and aims at preventing or reducing clinical manifestations at birth and alleviating possible long-term sequelae or clinical relapses, mainly eye sequelae. A classical study that changed the general approach to post-natal treatment of congenital toxoplasmosis was in the 1994 Chicago Collaborative Treatment Trial (CCTT), which revealed an encouraging outcome of a year-long PYR-SDZ treatment in 120 infected neonates followed up between 1981 and 2004, significantly better than in untreated (or sub-optimally treated) historical controls. Even among children with severe presentations at birth, 80% had normal motor function, 64% did not develop new eye lesions, and none developed sensorineural hearing loss (41).

The CCTT also standardized the PYR-SDZ treatment regimen, and recommended that it should be administered continuously throughout the entire first year of a congenitally infected child (41).

### **3-Treatment of Immunocompetent Toxoplasmosis:**

Many of these infections are self-limited and resolve without specific treatment or with symptomatic management (i.e. ibuprofen for pain and fever). However, treatment should be considered if a patient continues to have persistent and discomforting symptoms or displays severe symptoms. This may be particularly relevant to areas of the world, such as South America, where virulent strains of T. gondii have been reported to cause a severe illness with multivisceral involvement, sometimes with a fatal outcome. Treatment with pyrimethamine and sulfadiazine for 4 weeks is effective and is used for moderate to severe cases (42).

#### **4-Treatment of Immunocompromised patients:**

In organ transplant recipients, pyrimethamine (2.5 mg/day) is given as prophylaxis for 6 weeks after the operation to prevent primary infection especially in the case of seronegative recipients of a seropositive liver or heart donor. When an obvious disease is detected in any organ graft recipient, high dose therapy similar to that given for AIDS is indicated **(43)**.

In AIDS patients with toxoplasmosis, treatment includes acute therapy with sulphadiazine (4-8 gm/day). Pyrimethamine (50-75 gm/day) with vitamin supplements are given for 6 weeks continuously. Clindamycin (2.4-4.8 gm/day) can be substituted if severe sulphonamide toxicity occurs **(44)**.

#### **5-Treatment of Cerebral Toxoplasmosis:**

Toxoplasmic encephalitis is the most common clinical manifestation which is observed with a high prevalence in HIV-infected patients, but the spectrum of the disease has changed with the increasing number of non-HIV immunocompromised patients **(45)**.

The treatment of toxoplasmic encephalitis requires combination of: sulfadiazine 1000 mg four times a day (or 1500 mg four times a day if weight is more than 60 kg) and pyrimethamine 200 mg loading dose followed by 50 mg of pyrimethamine daily (or 75 mg daily if weight if more than 60 kg) **(46)**.

Leucovorin (folinic acid) 10 mg daily is given to prevent hematological toxicity. If sulfadiazine is not tolerated, it may be replaced by clindamycin 600 mg four times a day. Alternative regimens based variously on trimethoprim and sulfamethoxazole combination, atovaquone, and dapsone have been reported **(47)**.

Steroids may be useful in cases with significant cerebral edema. Neurological imaging should be done 2 weeks after initiating therapy to assess the efficacy of treatment **(48)**.

#### **6-Treatment of Ocular toxoplasmosis:**

Considering the natural history of this disease and the side effects of medications, the risk of treatment secondary to the potential toxicity of antiparasitic medications may be greater than any benefit provided. However, treatment is recommended for lesions within the vascular arcades, adjacent to the optic disk to reduce the chance of vision loss **(49)**.

In immunocompetent patients, Toxoplasma –related chorioretinitis is usually a self-limited infection and generally resolves spontaneously in a period of 4–8 weeks **(50)**.

When active inflammation is noted, sulphadiazine (2.0 gm/day) or clindamycin (1.2 gm/day) is given with pyrimethamine (25 mg/day) and vitamin supplementation is advised. Treatment is continued for 10 days after inflammation relieves **(51)**.

Azithromycin (250-1,000mg/day for three weeks) is synergistic with pyrimethamine with equivalent results and fewer side effects as compared with classic treatment in one small prospective study **(52)**.

There are many advantages of giving the treatment by intravitreal injection like clindamycin. This allows high concentrations of therapeutic agents to be delivered into the vitreous cavity and retina with no systemic side effects **(53)**.

Corticosteroids may be indicated in diminished vision, severe vitreous inflammation, and proximity of lesions to the optic disc. The preferred drug for oral corticosteroid is prednisone at a dose of 0.5-1.0 mg/kg/day. Corticosteroid therapy without antiparasitics may lead to large retinal lesions even in immunocompetent patients **(54)**.

#### **Prevention and control of toxoplasmosis:**

**Wehbe et al. (55)** illustrated that health education plays an important role in the prevention of toxoplasmosis by advising people to wash their hands and their tools like knives with soap and water when come in contact with raw meat as they contain viable tissue cysts which is considered a source of infection with toxoplasmosis.

These viable tissue cysts can be killed by heating the meat up to 67°C or by cooling it at less than 12°C for at least 3 days (56).

In addition, proper washing of fruits and vegetables plays an important role in the prevention and control of the disease (57).

Infection in domestic animals is a threat to public health from food-borne outbreaks and causes a great economic loss as it may lead to abortion, stillbirth, and neonatal loss, so pregnant women should avoid contact with raw meat, cats, and soil. Domestic cats should be fed dry or cooked food with wearing gloves during dealing with cats or their faeces during gardening (58).

Recommendations specifically for pregnant women include wearing gloves when gardening or being in contact with soil or sand, followed by thorough hand-washing. In addition, pregnant women should avoid changing cat litter if possible (59).

Early detection with repeated serological examination and the treatment of pregnant women can reduce the risk of infection by *T. gondii*. Serum monitoring throughout pregnancy, to detect early maternal seroconversion (60).

A lack or incomplete prenatal treatment was identified as an important risk factor for congenital toxoplasmosis, reinforcing the need for improvement of prenatal care in Brazil (61).

Universal prenatal screening procedures have been adopted. In France, for example, screening of pregnant women, in utero diagnosis and treatment are routinely performed. Routine prenatal treatment has been advocated given the suggested improved clinical outcome in reducing transplacental transmission (62).

#### **Toxoplasma Vaccination:**

In recent years, studies have focused on finding safe and effective vaccines against *T. gondii*. So, it is essential to evaluate antigens and identify vaccine candidates that could produce extreme and protective immune responses against toxoplasmosis (62).

For immunization against toxoplasmosis, many vaccination trials, including subunit, protein, DNA, and live attenuated vaccinations, have been performed, but, they only led to partial protection. Although different antigens from *T. gondii* micronemes, rhoptries, dense granules organelles, and surface antigens have been tested, the researchers could not find a suitable vaccine for controlling *T. gondii* infection in humans (61).

Considering the role of rhoptry antigens in virulence and satisfactory immunogenicity, they could be promising vaccine candidates against *T. gondii* (63).

Remarkable advances have been reported in the evaluation of vaccine candidates that can stimulate strong immunity against this infection. *T. gondii*, surface antigens and excretory-secretory antigens (ESA) have been known as possible vaccine candidates (64).

The commercial vaccine "Toxovax" based on a live attenuated S48 strain has been licensed for use to avoid congenital infection in sheep but, this vaccine may revert to a pathogenic strain, expensive with several side effects, so it is not suitable for human use. From this point, numerous vaccination trials against toxoplasmosis have been performed for their immunological effects in animal models, including attenuated and inactivated vaccines, subunit vaccines, genetically engineered vaccines and DNA vaccines (65).

Vaccination of farm animals is important to decrease risk of abortions in goats and sheep as well as decrease the risk of human infection from eating the infected meat (66).

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