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# Insight of the Novel Synthetic Quinoline-Based Compound PPQ-6 on Chronic Toxoplasmosis in Immunocompromised Mice

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**Abstract**

Toxoplasma gondii (T. gondii) is a protozoan parasite, which causes toxoplasmosis, a world-wide disease infecting all types of human cells. The efficacy of compound PPQ-6 against Toxoplasma gondii was examined in a murine model infected with a non-virulent (Me49) strain. Forty-eight female Swiss- albino mice (6 weeks old) were inoculated by mouth with 10 cysts/mouse and divided into 6 groups. Group I: was left untreated immunocomptent, group II: untreated immunocompromised, group III: infected immunocomptent, group IV: infected immunocompromised, group V: infected and treated with PPQ-6 and group VI: infected and treated with Pyrimethamine /sulfadiazine, treatment was started eight weeks post-infection and continued for 14 consecutive days. four mice from each group were sacrificed and the rest were observed for 30 days. Results showed that, the median survival duration of group I, II, V were 8.57 weeks, while the median survival duration of group III, IV, VI were 8.41, 7.26 and 8.16 weeks respectively ($P$ <0.05). PPQ-6 induced a significant increase in serum level of IFN-γ more than with Pyrimethamine /sulfadiazine when compared with infected untreated group. Both PPQ-6 and Pyrimethamine / sulfadiazine - treated groups decrease of serum TNF-α level when compared with the untreated group. Conclusively, PPQ-6 shows promising results, hence it could be a potentially used alternatively with Pyrimethamine / sulfadiazine for treatment of reactivated toxoplasmosis.

**Keywords:** *T. gondii*, Cyst, INFγ, TNF, PPQ-6, Survival Rate.

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## 1. Introduction

The protozoan parasite *Toxoplasma gondii* (*T. gondii)* is capable of infecting worm-blooded animal and is approximately found in 30% of human population [1]. Felines are the definitive host for toxoplasmosis and the disease can be transmitted mainly by ingestion of contaminated food by oocysts and ingestion of under-cooked meat containing bradyzoite [2]. In immunocompetent individuals, *T. gondii* infection is mostly asymptomatic or can be manifested as lymphadenopathy and is followed by a lifelong latent infection that may be reactivated because of immune disorders as in patients with AIDS or patients on immunosuppressive drugs or chemotherapy [3-4].

Reactivation of latent infection of toxoplasma is commonly manifested as encephalitis. As reported, *T. gondii* is causing severe encephalitis and death in over 30% of those patients [5]. The recommended treatment for *Toxoplasma* encephalitis (TE) is a combination of pyrimethamine and sulfadiazine, and trimethoprim-sulfamethoxazole (TMP-SMX) plus azithromycin is an alternative therapeutic regimen [6-7]. However, standard treatment is associated with considerable side effects, especially prolonged suppressive treatment is essential to prevent relapse [8-9]. One of the important issues in the treatment of chronic *T. gondii* infection is poor brain penetration of standard treatments. As a part of developing new therapy for toxoplasmosis, anti-*Toxoplasma* properties of synthesized quinolone compounds were tested by Doggett et al., (2012.) who studied the efficacy of quinolone-like agents and documented the high reduction of *T. gondii* brain cysts [10]. Similarly, Kadri et al., (2014) reported that quinolone- like compounds were efficient at inhibiting *Toxoplasma* growth [11]. Previously PPQ-8 showed promising anti-toxoplasma effects in both acute and chronic infection in a murine model [12]. PPQ-6 is another novel quinoline compound, related to PPQ-8. It is a potent heterocyclic agent designed and synthesized using the method reported by Abdel-Fattah et al., (2012) [13]. In previous work, PPQ-6 showed promising results against *Schistosoma mansoni* [14]. This encouraged us to test PPQ-6 against *Toxoplasma* infection. Herein, the aim of this work is to determine the efficacy of PPQ-6 in the treatment of reactivated latent infection of *T. gondii* in a murine model.

## 2. Materials and methods

### *2.1. Study setting*

This is an experimental study, which was conducted at the Parasitology Laboratory (Tropical Health Department) of the High Institute of Public Health, Alexandria University, Parasitology Department, Faculty of Medicine, Mansoura University, Egypt, and Nile center for experimental research (NCER).

### *2.2. Animals*

Female Swiss- albino mice, 6-8 weeks old, weighing 25-30 g at the beginning of experiments, were obtained from the Medical Experimental Research Center (MERC). Mice were housed in unisex groups of eight mice in hygienic polypropylene cages in an air-conditioned animal house and offered drinking water and regular mouse feed ad libitum (food product specific for mice) and maintained under controlled conditions of lighting (12 h light/12 h dark cycle) and temperature (20 - 22 °C).

### *2.3. The parasite*

Brain cysts of the Me49 non-virulent strain of *T. gondii* was provided by the department of Medical Parasitology, Faculty of Medicine, Mansoura University, Egypt. The parasite strain was maintained through passage into Swiss-albino mice. Mice were orally inoculated by intraoesophageal gavages with 250 µl brain suspension containing *T. gondii* cysts (10 cysts/mouse).The brain suspension from chronically infected mice was prepared in our laboratory as previously repor*ted* Elgawad et al., (2019) and was used for subsequent experimental infection [12,15].

### *2.4. Drugs*

- **PPQ-6**: is a novel quinoline compound, a potent heterocyclic agent designed and synthesized via combination of both therapeutically active moieties 2-chloroquinoline as lipophilic and diverse heterocyclic ring structure (having a known antiprotozoal activity) directly attached to C3 of quinoline ring. It was dissolved in (Dimethyl sulfoxide) DMSO and given as a single oral dose (20mg/kg/day) for 14 successive days [14].
- **Pyrimethamine and Sulfadiazine**: Pyrimethamine and Sulfadiazine (PYR/SDZ): PYZ/ SDZ were purchased from Sigma- Aldrich (St. Louis, USA) and diluted in polyethylene glycol (PEG) mol. Wt. 200, Sigma Aldrich. The drugs were given orally, in combination with each other at dose of 1 and 100mg/kg/day.
- **Dexamethasone and cortisone:** Dexamethasone (DXM, dexamethasone sodium phosphate), was prepared by dissolving 5 mg DXM per 1-liter drinking water and water was changed 3 times a week, it was given at a dose of 2.5 mg/kg BM/day per mouse. Cortisone acetate (CA, hydrocortisone-21-acetate, given by S.C injection 3 times a week as 50 mg/kg BM per mouse infection [16].

### *2.5. Type of sample and method of selection*

Total sample size was calculated to be 48 mice. To achieve the objectives of this study, two subgroups of experimental animal each of 24 mice (swiss-albino mice) was included and each was subdivided into 6 groups of eight mice each according to the Table 1. Four mice from each group were sacrificed one week after the end of the therapy for assessment of the efficacy of treatment, whereas the rest of mice were observed for 60 days for estimation of the survival rate.

### *2.6. Evaluation of compound PPQ-6 efficacy*

Compound PPQ-6 efficacy was carried out through the following parameters:

#### *2.6.1. Estimation of the survival rate*

Mice were observed daily for 60 days. The survival rate was calculated according to the following equation:

$$\text{SR} = \frac{\text{Number of survival mice at the end of experiment}}{\text{Number of mice at the beginning of the experiment}} \times 100$$

#### *2.6.2. Immunological study (Cytokines assay)*

TNF-α, IFN-γ were measured in serum samples by ELISA, according to the manufacturer's instructions. Data were presented in pg/mL. The limits of detection were determined from standard curves: TNF-α = 8 pg/mL and IFN-γ =15 pg/mL [17].

### *2.7. Splenic index and number of splenocytes*

The relative spleen size was determined as the spleen mass (mg)/BM (g) ratio and total splenocyte numbers are both taken as parameters of immune activation/suppression.

Thus, the spleens were weighed, splenocytes were prepared according to Ferry et al., (1991), first spleen was homogenized and then passed through a strainer with excess PBS, after centrifugation and washing splenocytes were incubate with erythrocytes lysate for 5 min, centrifuged and finally, the splenocytes were resuspended in PBS to make a cell suspension [18]. The viability and relative number were checked using trypan blue [17].

### *2.8. Statistical analysis*

Data were analyzed using statistical package for social sciences (SPSS) software (SPSS Inc., Chicago, USA), version 16.0. for windows. Quantitative data were described as means, standard deviation (SD) after testing for normality by Shapiro-Wilk test. In the normally distributed variables, parametric tests were used; while in non-normally distributed variables, non-parametric tests were used for comparison between groups. Qualitative data were described as numbers and percentages. Monte carlo test was used for comparison between groups. Overall survival was studied using Kaplan-Meier survival analysis and Log-rank test was used to compare survival distribution across groups. The results were considered significant when the probability of error is equal to or less than 5% ($p \leq 0.05$).

### *2.9. Infection control during the experimental work*

Personal protective equipment including white coat, gloves, safety glasses and face masks were used during handling and scarification of animals or handling and processing of contaminated samples. Hands were washed with soap and water after taking off gloves.

### *2.10. Ethical considerations*

- The use and handling of animals in the study were complied with the Institutional Animal Care and Use Committee (IACUC) Alexandria University Institute of Public Health.
- The study protocol was approved by the (UA-IACUC); code number: (0920022521).
- The International Guidelines for Research Ethics were followed.

All procedures in the present study were met the International Guiding Principles for Biomedical Research Involving Animals, as issued by the International Organizations of Medical Sciences (National Research Council, 2006).

## 3. Results and Discussion

### *3.1. Estimation of the survival rate (SR)*

On 8$^{th}$ week post treatment, mice in group I, II had survival rate of 100%, mice in group IV had survival rate of 50%, while the survival rate was 87.5% ,75% and 75% in immunocomporomized mice infected and treated with PPQ6 (groupV) , pyrimethamine and sulfadiazine (group VI) and (group III) respectively. The median survival duration of group I, II, V were 8.57 weeks, while the median survival duration of group III, IV, VI were 8.41, 7.26 and 8.16 weeks respectively *(P* <0.05) (Table 2). Table 2 showing that there is statistically significant difference between studied groups regarding survival analysis (P < 0.05). Median survival time by weeks decreased in group IV (7.26) according CL (5.54-8.98) followed by group VI was (8.16), CL (7.11-9.21) when all other group have full time period. *Toxoplasma gondii* is an apicomplexan parasite that can invade and replicate intracellularly in virtually all nucleated cell types of warm-blooded animals. Human infections are acquired from contaminated food or water, and it is estimated that this parasite persists chronically in 25–30% of the global human population. Although chronic infections are largely subclinical, they often reactivate in individuals who are immunocompromised resulting in high mortality, especially in those infected with HIV who have progressed to AIDS [19-20]. The most commonly used drugs are DHFR inhibitors, DHPS inhibitors, and macrolides and related derivatives, such as pyrimethamine, sulfonamides, atovaquone, spiramycin, clindamycin, and azithromycin. However, the effectiveness of current drugs is limited due to lack of efficacy against cysts and undesirable side effects (cutaneous intolerance for sulfonamides, teratogenicity for pyrimethamine, etc.). Therefore, more research is needed to develop new anti-toxoplasmosis drugs and combinations that have better long-term efficacy and tolerance [21-22]. Quinolones are bioactive compounds, which have broad remarkable biological actions against bacteria, viruses, fungi and parasites. PPQ-6; 4-(2-Chloroquinolin-3-yl)-2-oxo-6-(p-tolyl)-1,2-dihydropyridine-3-carbonitrile is a new biological active heterocyclic compound synthesized as one hybrid compound composed of two biologically active molecules, thus packaging dual-activity into one molecule. Quinoline as lipophilic compound. PPQ-6 was designed with the aim of enhancing its lipophilicity, which would improve its ability to be absorbed and penetrate cell membrane, permeate the blood brain barrier (BBB) and cyst wall without causing its rupture to destruct parasite either directly or indirectly through disruption of the apicoplast, ultimately leading to better biological activity against parasites [23-25]. Infection with *T. gondii* trigger mainly cellular immune response via macrophages, dendritic cells and T lymphocytes (Th1), which secrete proinflammatory cytokine such as interferon-gamma (IFN-γ), tumor necrosis factor (TNF-α) and IL-6 [3]. It is known that Trimethoprim / sulphadiazine disputes *Toxoplasma* through inhibition of enzymes involved in theparasite's metabolism as dihydrofolate reductase and dihydropteroate synthetase, which block folic acid synthesis in such a way PYR/SDZ interfere directly with the parasite viability without stimulation of the immune system thus the possibility of reactivation of latent infection is more common [26]. Our study supports previous findings where, according to a study by Cristina Meira and Dupont, they emphasize the critical role of IFN-γ in the murine immune system, as it operates during both innate and adaptive immune responses. Additionally, they demonstrate the importance of IFN-γ in human host defense, where individuals with AIDS who have insufficient levels of IFN-γ are more susceptible to chronic *T. gondii* infection [3,27]. This supports the findings of Y. Suzuki and Halonen's study, which also demonstrated that IFN-γ is required to control *T. gondii* infection in the CNS, as shown by antibody ablation treatments that lead to reactivation of chronic infection and severe encephalitis in mice [28-29]. In the same level of agreement with the aforementioned studies, where the changes in the immune system were assessed through TNF-α and interferon gamma (IFN-γ) as markers.

Our results showed that there were statistically significant differences between each pair of the studied groups, indicating that the effect of PPQ-6 on TNF-α and IFN-γ levels was significant compared to the other groups. This suggests that PPQ-6 treatment had an effect on the immune system, which provides evidence that PPQ-6 has an immunomodulatory effect by reducing the level of TNF-α and increasing IFN-γ levels, suggesting that PPQ-6 may enhance the immune response in infected and immunocompromised mice. Moreover, infected and immunocompromised mice treated with PPQ-6 had the highest mean IFN-γ levels. This finding suggests that PPQ-6 may enhance the immune response in infected and immunocompromised mice by increasing IFN-γ levels. However, the magnitude of the effect on IFN-γ levels was lower than that observed with TNF-α. It is interesting to note that PPQ-6 showed a more significant effect on IFN-γ levels than TNF-α levels. This difference may be due to the different roles played by these cytokines in the immune response to toxoplasmosis. In addition to that, when the effect of PPQ-6 was evaluated using NO and splenic index as markers, the results showed that there were statistically significant differences between each pair of the studied groups, indicating that the effect of PPQ-6 on Nitric Oxide (NO) and splenic index was significant compared to the other groups. The reduced levels of TNF-α obtained after treatment with Trimethoprim/ Sulfadiazine and PPQ-6 is beneficial since it leads to limitation of TE, restriction of parasite growth through tryptophan starvation in addition to activation of parasite killing functions of microglia through iNOS dependent and -independent mechanisms. The lower levels of TNF-α in mice treated with PPQ-6 were probably due to the reported anti-inflammatory effect of quinolines [25]. The current study also supports the findings of Aindrila Biswas and colleagues and Dirk Schlüter and colleagues, which provide additional insights into the immune response against Toxoplasma in the brain. They show that brain-infiltrated inflammatory monocytes produce pro-inflammatory molecules such as TNF, reactive oxygen species (ROS), and NO that directly contribute to killing the parasite [30-32]. This is consistent with Dirk Schlüter and colleagues' findings that microglial cells can also control parasite replication in response to TNF and IL-6 or in response to IFN-γ in vitro [30]. However, in contrast to these findings, another study found that neurons activated with IFN-γ were not able to clear the parasite in vitro, possibly due to decreased or different transcriptional responses to IFN-γ [31]. Our findings are consistent with previous studies that demonstrated the critical role of IFN-γ in the immune response, as well as the importance of TNF-α in controlling parasite replication [33].

### *3.2. Cytokines assay*

#### *3.2.1. Evaluation of serum TNF*

Table 3 demonstrates that there is statistically significant higher mean±SD TNF among group III followed by group V, group II, group IV, VI & I (90.50±1.0, 43.50±1.73, 43.45±1.73 ,37.50±0.58, 27.50±1.91, 17.97±0.05, respectively. Post Hoc Tukey test illustrates statistically significant difference within between each pair of the studied groups. Table 4 illustrates that there is statistically significant higher mean±SD INF -gamma among group III followed by group V, group II, group IV, VI & I (78.25±0.50, 61.50±1.73, 51.43±0.95, 39.50±0.58, 31.25±0.96 and 20.02±0.05 respectively). Post Hoc Tukey test illustrates statistically significant difference within between each pair of the studied groups. Table 5 shows that there is statistically significant higher mean±SD. Splenic index among group IV followed by group III, group VI, group V, II & I (1580.09±37.94, 1382.69±18.79, 1374.47±21.46, 1035.05±37.94, 831.92±40.05 and 796.51±44.28 respectively). Post Hoc Tukey test illustrates a statistically significance between each pair of the studied groups except between group I & II (p=0.277) and between group III & VI (p=0.798). Figure 1 A Cytology examination of splenic smear of non-infected immunocompetent mice showed that easily recognized lymphocytes used as a control for comparison. B, presence of *Toxoplasma gondii* cysts (red arrow, right upper corner) and free trophozoites (left lower corner) in infected immunocompetent mice. C, the lymphocytic cells were decreased in smears from non-infected immunocompromised mice. D, *Toxoplasma* cysts (red arrows) were detected in addition to the decrease in lymphocytes in infected immunocompromised mice (x400). Figure 2 A, Cytologic examination of smears from infected mice treated with sulfadiazine showing slight restoration of lymphocytes in addition to plasma cells with remarkable background cellular debris. B, while those treated with PPQ6 showed plasma cells (red arrow, right upper corner) outnumbered lymphocytes with more clear background. (A & B, Giemsa, magnification x400).

**Table 1:** Type of sample and method of selection

| Serial | Group | Description |
|---|---|---|
| 1 | **Group I** | Non-infected mice, non- treated, immunocompetent |
| 2 | **Group II** | Non-infected mice and immunocompromised |
| 3 | **Group III** | Infected immunocompetent |
| 4 | **Group IV** | Infected immunocompromised |
| 5 | **Group V** | Infected, immunocompromised and treated (PPQ-6) |
| 6 | **Group VI** | Infected, immunocompromised and treated (pyrimethamine and sulfadiazine) |

**Table 2:** Survival rate of studied groups throughout the study period.

| | Overall Survival | | | | |
|---|---|---|---|---|---|
| | **Median Survival time** | **Std. Error** | **95% CI** | **Log Rank test** | **P – value** |
| **Group I** | 8.57 | 0.0 | 8.57-8.57 | 16.51 | 0.011* |
| **Group II** | 8.57 | 0.0 | 8.57-8.57 | | |
| **Group III** | 8.41 | 0.22 | 7.98-8.82 | | |
| **Group IV** | 7.26 | 0.87 | 5.54-8.98 | | |
| **Group V** | 8.57 | 0.0 | 8.57-8.57 | | |
| **Group VI** | 8.16 | 0.53 | 7.11-9.21 | | |
| **Overall survival** | 8.30 | 0.22 | 7.77-8.63 | | |

Log Rank (Mantel-Cox) was used, CI: confidence interval.

**Table 3:** Estimation of serum TNF level among studied groups.

| | Group I | Group II | Group III | Group IV | Group V | Group VI | test of significance |
|---|---|---|---|---|---|---|---|
| TNF TNF-α (pg/ml) | 17.97±0.05 | 43.45±1.73 | 90.50±1.0 | 37.50±0.58 | 43.50±1.73 (16%) | 27.50±1.91 (26.6%) | F=1375.0 P<0.001* |
| P1= | | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | |
| P2= | | | <0.001* | <0.001* | 0.959 | <0.001* | |
| P3= | | | | <0.001* | <0.001* | <0.001* | |
| P4= | | | | | <0.001* | <0.001* | |
| P5= | | | | | | <0.001* | |

F: One Way ANOVA test, *statistically significant, Parameters described as mean ± SD, Values between parentheses refer to the percentage of reduction compared with infected non-treated group. P1: difference between each group & group I, P2: difference between each group & group II, P3: difference between each group & group III, P4: difference between each group & group IV, P5: difference between each group & group V

**Table 4:** Estimation of serum IFN-gamma level among studied groups.

| | Group I | Group II | Group III | Group IV | Group V | Group VI | test of significance |
|---|---|---|---|---|---|---|---|
| | 20.02±0.05 | 51.43±0.95 | 78.25±0.50 | 39.50±0.58 | 61.50±1.73 (55%) | 31.25±0.96 (20.8%) | F=1985.41 P<0.001* |
| P1= | | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | |
| P2= | | | <0.001* | <0.001* | <0.001* | <0.001* | |
| P3= | | | | <0.001* | <0.001* | <0.001* | |
| P4= | | | | | <0.001* | <0.001* | |
| P5= | | | | | | <0.001* | |

F: One Way ANOVA test, *statistically significant, Parameters described as mean±SD. Values between parentheses refer to the percentage of reduction compared with infected non-treated group. P1: difference between each group & group I, P2: difference between each group & group II, P3: difference between each group & group III, P4: difference between each group & group IV, P5: difference between each group & group V.

**Table 5:** Comparison of Splenic index between studied groups.

| | Group I | Group II | Group III | Group IV | Group V | Group VI | test of significance |
|---|---|---|---|---|---|---|---|
| Splenic index | 796.51±44.28 | 831.92±40.05 | 1382.69±18.79 | 1580.09±37.94 | 1035.05±37.94 | 1374.47±21.46 | F=211.52 P<0.001* |
| P1= | | 0.277 | <0.001* | <0.001* | <0.001* | <0.001* | |
| P2= | | | <0.001* | <0.001* | <0.001* | <0.001* | |
| P3= | | | | <0.001* | <0.001* | 0.798 | |
| P4= | | | | | <0.001* | <0.001* | |
| P5= | | | | | | <0.001* | |

F: One Way ANOVA test, *statistically significant, Parameters described as mean± SD. P1: difference between each group & group I, P2: difference between each group & group II, P3: difference between each group & group III, P4: difference between each group & group IV, P5: difference between each group & group V.

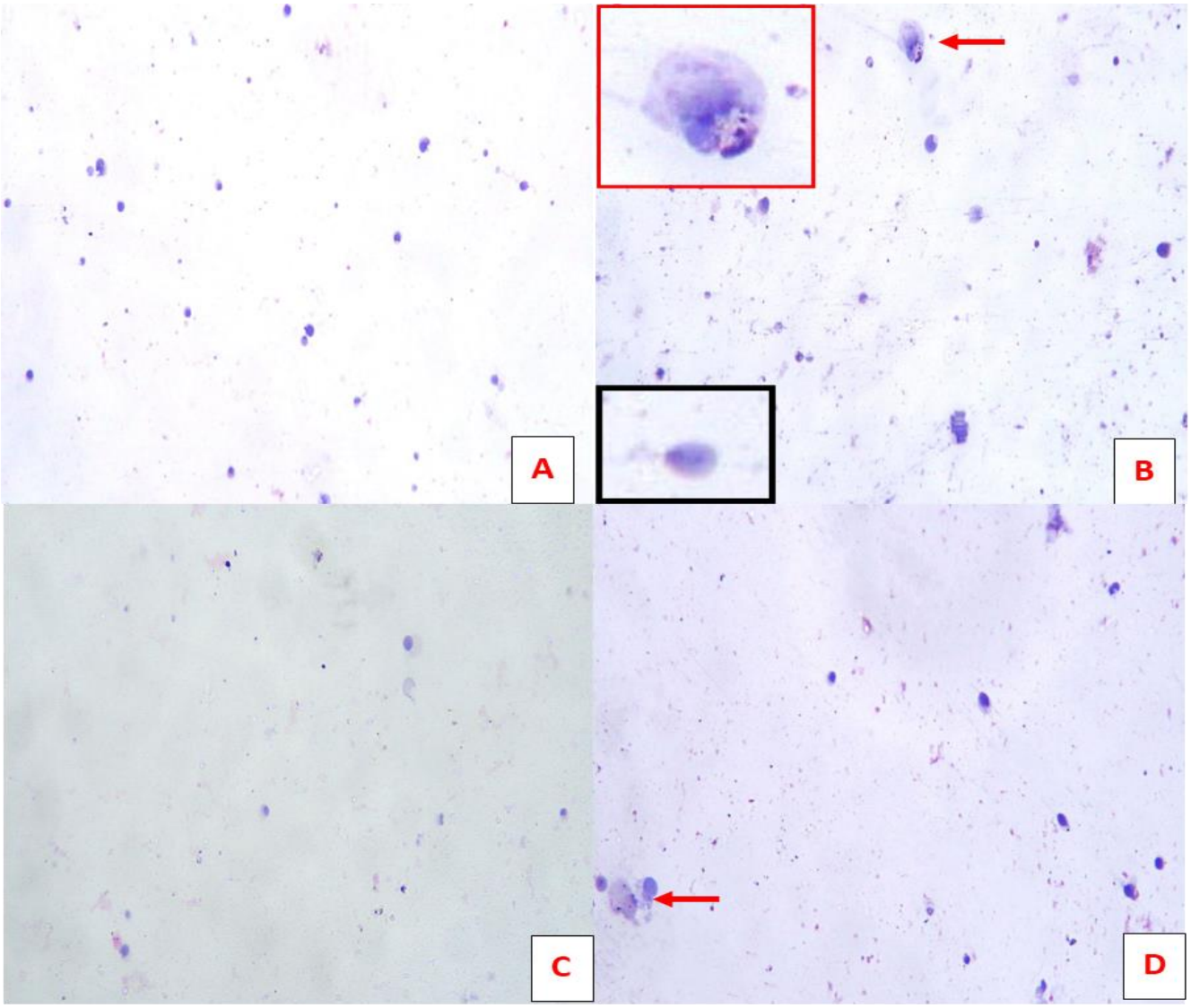


**Figure 1:** (A) Cytologic examination of smears from spleen of non-infected immunocompetent mice showing easily recognized lymphocytes used as a control for comparison. (B) presence of toxoplasma gondii cysts (red arrow, right upper corner) and free trophozoites (black arrowhead, left lower corner) in infected immunocompetent mice. (C) the lymphocytic cells were decreased in smears from non-infected immunocompromised mice. (D) toxoplasma cysts (red arrows) were detected in addition to the decrease in lymphocytes in infected immunocompromised mice. (A, B, C & D, Giemsa, magnification x400).

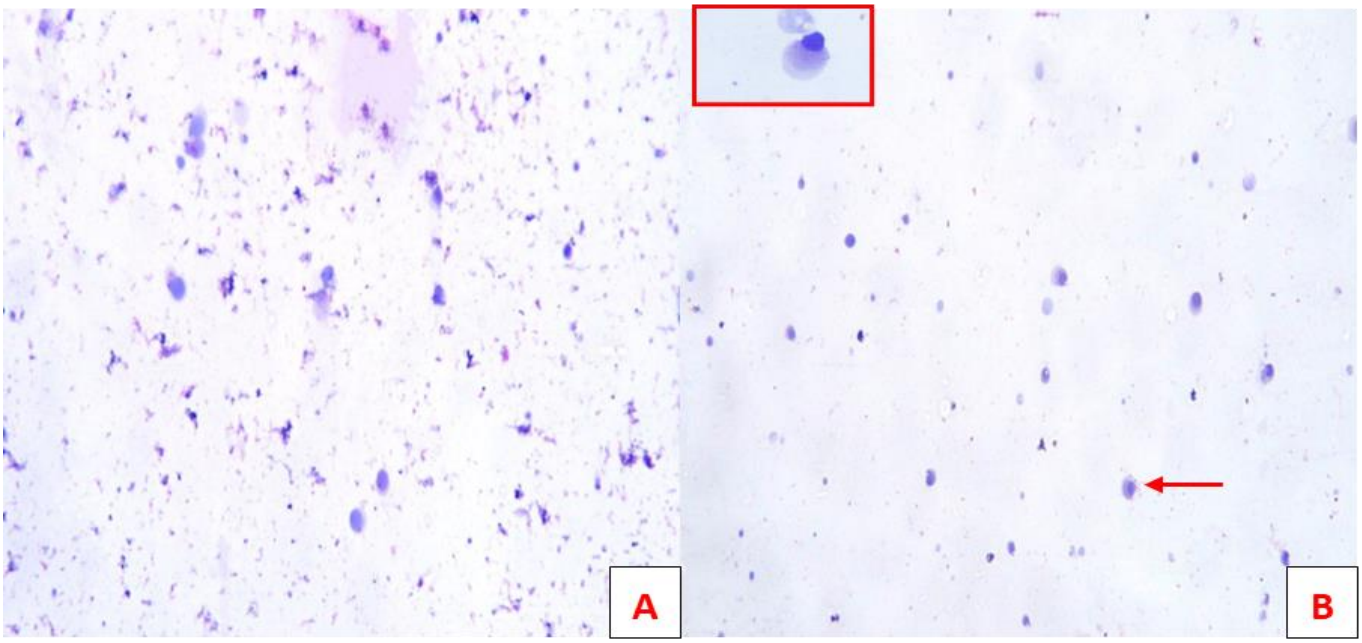


**Figure 2:** (A) Cytologic examination of smears from infected mice treated with sulfadiazine showing slight restoration of lymphocytes in addition to plasma cells with remarkable background cellular debris. (B) while those treated with PPQ6 showed plasma cells (red arrow, right upper corner) outnumbered lymphocytes with more clear background. (A & B, Giemsa, magnification x400).

## 4. Conclusions and implications for practice

In conclusion, our experimental study provides evidence that PPQ-6 is a novel and promising drug that has significant action against experimental toxoplasmosis produced by *T. gondii*. Histopathological and parasitological advancements were seen in infected, immunocompromised mice treated with PPQ-6, reducing the number and size of brain cysts and ameliorating brain pathology. Furthermore, PPQ-6 demonstrated an immunomodulatory effect by reducing the level of TNF-α and increasing IFN-γ levels, suggesting that it may enhance the immune response in infected and immunocompromised mice. The implications of this study for clinical practice suggest that PPQ-6 has the potential to be a promising alternative treatment option for toxoplasmosis in immunocompromised patients. Further studies are needed to investigate the efficacy and safety of PPQ-6 in human subjects. The findings of this study may also contribute to the development of new treatment options for other parasitic infections. Overall, the results of this study provide a foundation for further research on the potential of PPQ-6 as a therapeutic option for toxoplasmosis.

## 5. The strength of the study

Our experimental study appears to have several strengths. First, the study utilizes a rigorous experimental design by including a control group and randomly assigning mice to treatment groups. Additionally, the study uses multiple outcome measures to assess the effect of PPQ-6 on the survival rate, brain pathology, number of brain cysts, and immune system response. The use of multiple outcome measures increases the reliability and validity of the study's findings. Furthermore, the study demonstrates the effectiveness of PPQ-6 in reducing the mean cyst count and brain cyst size compared to untreated mice and mice treated with pyrimethamine and sulfadiazine. This suggests that PPQ-6 may be a promising alternative to conventional treatment options for toxoplasmosis. Finally, the study assesses the impact of PPQ-6 on the immune system through multiple markers such as TNF-α, IFN-γ, NO, and splenic index, providing further evidence of its immunomodulatory effects. Overall, the study's rigorous design, use of multiple outcome measures, and promising results suggest that PPQ-6 could be an effective treatment option for toxoplasmosis in immunocompromised individuals.

## 6. Limitations of the study

Despite the promising outcomes in the current study, the following limitations have to be taken into consideration*:*

- This study certainly has some limitations. First, the study was conducted on mice, and the results may not necessarily translate to humans. Therefore, further studies are needed to determine the efficacy and safety of PPQ-6 in humans.
- Second, the study did not investigate the long-term effects of PPQ-6 treatment on mice. Therefore, it is unclear whether the treatment is safe for long-term use.
- Third, the study did not investigate the mechanism of action of PPQ-6. Further research is needed to understand how PPQ-6 works to control and treat toxoplasmosis.
- Fourth, the study did not investigate the potential side effects of PPQ-6 treatment. Further research is

needed to determine the safety of PPQ-6 in the long-term and its potential side effects.

- Finally, the study used a small sample size of mice, which may not be representative of the entire population. A larger study with a more significant sample size would be required to confirm the findings of this study.

## 7. Recommendations

PPQ-6 is a promising alternative to conventional treatment options for toxoplasmosis, as it demonstrated significant parasitological and histopathological improvement, with a significant reduction in mean cyst count and size compared to untreated mice. Further studies are necessary to investigate the long-term effects of PPQ-6 on the immune system and the development of resistance in the parasite. PPQ-6's immunomodulatory effect in reducing TNF-α levels and increasing IFN-γ levels suggests that it may enhance the immune response in infected and immunocompromised mice. Additional research is needed to determine the optimal dosage and duration of PPQ-6 treatment for toxoplasmosis and its safety profile in humans. PPQ-6's effect on NO and splenic index suggests that it may have a potential therapeutic benefit in other diseases that involve inflammation and immune dysregulation. Further studies are needed to explore this potential.

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