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# Anti-toxoplasmic and anti-inflammatory activity of haloperidol in mice

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**Abstract**

*Toxoplasma gondii* (*T. gondii*) infection increases the risk of emerging psycho-behavioral disorders, including schizophrenia and epilepsy. The widespread outbreak of *T. gondii* and its relation with psychiatric diseases raises the possibility of applying antipsychotic medications to control this parasite or parasite-based drugs to reduce neurological complications. According to reports, haloperidol prevented *T. gondii* tachyzoites from multiplying in culture. However, animals receiving these two medications did not survive longer or develop fewer cysts during the acute and chronic phases, respectively. In this study, the parasite's repetitive DNA fragment REP529, the bradyzoite-specific protein BAG1, and the tachyzoite-specific protein SAG1 were quantified in mice brains treated with haloperidol using the quantitative real-time PCR (qPCR) technique. Similar to the typical anti-toxoplasmosis drug Trimethoprim/Sulfamethoxazole (TMP/SMZ), the therapy with haloperidol suppressed the Toxoplasma and lowered all BAG1, SAG1, and REP529 copy counts in mouse brains ($P < 0.0001$). In addition, haloperidol reduced brain TNF expression similarly to TMP/SMZ ($P < 0.0001$). Mice brain histology revealed a substantial decrease in lymphocyte perivascular infiltration, glial nodules, and cyst formation, the same as the TMP/SMZ group. Our findings prove the effectiveness of the mood-stabilizing and antipsychotic medication haloperidol in treating persistent Toxoplasma infection. These findings may be used to modify treatment plans for psychotic patients and create new potent anti-Toxoplasma medications.

## 1. Introduction

*Toxoplasma gondii* is a neurotropic parasite with the potential to contaminate all warm-blooded animals. After infection, *T. gondii* converts into the tachyzoite form and spreads throughout the body, especially the central nervous system (CNS). After the immune system response, tachyzoite forms convert into bradyzoite forms, creating whole life remaining cysts in the CNS and muscles (Haroon et al., 2012; Saadatnia & Golkar, 2012; Weiss & Kim, 2011). Vision impairment and neurological defects can also occur due to congenital infection (Roizen et al., 2006; Torgerson & Mastroiacovo, 2013; Wallon et al., 2004). *T. gondii* extensively occupies brain cells, especially microglial cells, as well as neurons and astrocytes (Lüder et al., 1999), inducing

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alternations of immune-related gene expression in the host brain and initiating CNS inflammation and neurotransmitter alternations (Boillat et al., 2020; Carruthers and Suzuki, 2007; Fond et al., 2015; Mendez and Koshy, 2017; Tedford and McConkey, 2017).

Many psycho-behavioral disorders, primarily schizophrenia, may be related to *T. gondii* chronic infection (Tyebji et al., 2019). Evidence, like epidemiological studies and experimental results, proposes a connection between chronic *T. gondii* infection and schizophrenia (Chaudhury & Ramana, 2019; Torrey & Yolken, 2003; J. P. Webster et al., 2013). A significant serological study found similar results of a *T. gondii* and schizophrenia connection (Burgdorf et al., 2019). It appears that among all effective factors, *T.gondii* has a robust link with psychotic disorders (Torrey et al., 2012).

Neuropathological investigations have revealed that both toxoplasmosis and schizophrenia impact microglia and astrocytes. Microglia represent primary CNS innate immune cells, account for 10–15% of total CNS cells, and play a vital role in regulating CNS inflammatory reactions (Comer et al., 2020). The manifestation of schizophrenia appears to be connected to the inflammation regulated by microglia (Mizoguchi et al., 2014). Conversely, *T. gondii* infection induces microglia activation, which secretes inflammatory cytokines and molecules (Hwang et al., 2018). Both toxoplasmosis and schizophrenia affect various neurotransmitter levels (Torrey & Yolken, 2003). Antagonists of dopamine receptors are the principal component of pharmacotherapy of schizophrenia.

Many antipsychotics and mood stabilizers exhibited antiviral and antiparasitic activity (Jones-Brando et al., 2003). Trifluoperazine, an antipsychotic drug and calmodulin antagonist, inhibited cell invasion by *T. gondii* (Pezzella et al., 1997a). In a more comprehensive study, Jones-Brando et al. (2003) investigated whether antipsychotic drugs impede *T. gondii* growth in a cell culture assay. They reported haloperidol, a first-generation antipsychotic drug, and valproic acid displayed anti-toxoplasma activity comparable to or higher than trimethoprim, a frequently prescribed medication for Toxoplasma infection (Fond et al., 2014; D.G. Goodwin et al., 2011; Jones-Brando et al., 2003; Strobl et al., 2007). Other antipsychotics such as zuclopenthixol, fluphenazine, thioridazine, and trifluoperazine have also shown modest to intense Toxoplasma inhibitory activity in cultured cells (Fond et al., 2014; D.G. Goodwin et al., 2011).

In addition to cell culture experiments, findings of some clinical and animal studies have pointed to the possible efficacy of antipsychotics in inhibiting Toxoplasma infection. For example, Webster et al. investigated whether valproic acid or haloperidol's effects are attained or enhanced by their ability to inhibit *T. gondii* infection based on a rat model (J. Webster et al., 2006). The animals that received the medications exhibited a substantial decrease in their attraction to suicidal feline behavior and displayed modified behavioral patterns compared to the infected animals that did not receive any treatment. Interestingly, haloperidol was even more effective than the combination of pyrimethamine and dapsone, a standard Toxoplasma chemotherapy, in reversing the alterations in behavior induced by the infection (Fond et al., 2015). Another study found that CSF and serum IgG levels against *T. gondii* in patients with the recent advent of schizophrenia receiving no antipsychotic treatment were notably higher than in untreated controls without any psychiatric disease (Leweke et al., 2004). The levels of Toxoplasma antibodies in these patients decreased upon treatment with antipsychotic drugs to the same level as the controls (Leweke et al., 2004).

Despite the above findings, administration of antipsychotic drugs to mice did not protect against challenge infection with the parasite (D. G. Goodwin et al., 2008; Saraei et al., 2016; Saraei et al., 2015). Valproic acid did not increase mice’s survival time during acute infection and did not

reduce brain cyst load during chronic infection. Similarly, mice treated with fluphenazine were not protected against chronic Toxoplasma infection (Saraei et al., 2016). Our hypothesis suggests that the unfavorable outcomes observed in the previous studies could be due to incorrect timing of drug treatment and/or imprecise approximation of the parasites' load in the brain, which relied on straight microscopic enumeration of cysts. Consequently, we propose that evaluating the effectiveness of psychiatric drugs against chronic infection should consider the amount of bradyzoites inside the cysts, which could serve as a more reliable standard than merely counting the number of brain cysts. Among the psychiatric medications, haloperidol, a typical antipsychotic, was used in the present study since there are controversial reports regarding its anti-Toxoplasma activity.

To provide direct evidence of the in vivo efficacy of antipsychotic and mood-stabilizing drugs against *T. gondii*, we measured parasite/bradyzoite load in the brains of mice infected with Toxoplasma and treated with haloperidol. A quantitative real-time PCR technique was utilized to measure the transcription level of BAG1 and determine the copy number of REP-529 DNA in the brain homogenate of mice. Additionally, the brain TNF-α expression level was measured as an indicator of inflammation. Furthermore, weight gain and histological examinations of the mice's brains were conducted to observe brain inflammation and the formation of cysts.

## 2. Materials and Methods

### 2.1 Animals, drug preparation, and administration

Fifty-four male NMRI mice (4 to 5 weeks old) were randomly grouped into six experimental batches of 14 mice according to Institutional Animals Ethics Committee rules (approval number: 0201-13085). Exact monitoring was performed for 6 weeks. The *T. gondii* Tehran strain (type II) was used for animal infection (Ghorbani & Sami, 1973). Haloperidol (Caspian Pharmaceutical Co, Iran.) was diluted with phosphate buffer to the required concentration for injection into mice. Drug preparation had a neutral pH close to 7.0. The administered dose of haloperidol was 6 mg/kg, equal to 20% of their $LD_{50}$ (Conceição & Frussa-Filho, 1996; D.G. Goodwin et al., 2008; Lee et al., 2013; Quiñones-Torrelo et al., 2001; Sun et al., 2010; J. Webster et al., 2006) and injected intraperitoneally. Co-trimoxazole (Chemidarou Industrial Co, Iran) was incorporated into the water daily (95 mg/24 h/kg) (Hawk et al., 2005). The daily drug administration commenced four weeks after infection (PI) and continued for 14 days. Apart from the drug-treated groups (haloperidol and co-trimoxazole), the study also included three control groups: the solvent group, the infected group without any treatment, and the uninfected group. The solvent group received phosphate buffer as the solvent/diluent for drugs. The infected group without any treatment consisted of mice infected with *T. gondii* but not given any treatment.

### 2.2 Animal model used to simulate Toxoplasma infection

This research utilized the T. gondii Tehran strain (type II) (Ghorbani & Sami, 1973). To induce Toxoplasma infection in NMRI mice, ten brain cysts suspended in 200 µl of phosphate buffer saline (PBS) were injected intraperitoneally. Mice were observed for one to two months, after which their brains were removed, and brain cysts were estimated under a microscope. Toxoplasma infection was induced by 20 brain cysts intraperitoneally.

### 2.3 Examination of mice brain tissues using histological analysis

Three mice from each group were selected for histological examination. After anesthetizing the mice with CO2, rapid decapitation was performed. The mice brains were collected and fixed in 10% buffered formalin to form paraffin blocks. Five µm thickness brain sections were randomly selected

and stained with hematoxylin and eosin (H&E). The stained slides were imagined using a light microscope (Nikon) connected to a digital camera. Digital photographs were taken at different magnifications (× 40, × 100, × 200, and × 400) using objective lenses (Nikon).

### 2.4 Extraction of DNA and RNA from mice brain tissue and subsequent preparation of cDNA

DNA and RNA were extracted from the brains of six mice in each group. For DNA extraction, half of the frozen brain was homogenized in 500 µl of PBS. From this suspension, a 50 µl sample was centrifuged at 8000 rpm for 5 minutes, and the resulting pellet was purified using the DNAeasy Blood & Tissue Kit from Qiagen. As for RNA extraction, the other half of the brain was homogenized in 500 µl Qiazol reagent from Qiagen. From this homogenate, a 100 µl portion was used for RNA purification, employing the RNAeasy plus universal mini kit from Qiagen. The purified RNA was then utilized for cDNA synthesis using the QuantiTect reverse transcription kit from Qiagen.

### 2.5 Measurement of BAG1 transcript levels and REP-529 copy numbers

As stated earlier, a duplex quantitative real-time PCR (qPCR) was conducted, employing two distinct sets of primers and probes for BAG1 and REP-529 (Babaie, Sayyah, Fard-Esfahani et al., 2017). BAG1 transcripts and REP-529 copy numbers were determined by comparing them to standard curves generated simultaneously.

### 2.6 Quantification of brain TNF-α expression through qPCR analysis

A qPCR approach was employed to estimate the TNF-α transcripts in mice brains (accession number: NM_013693), utilizing specific primers and probes listed in (Table 1) (Babaie et al., 2019). The quantification of TNF-α transcripts was accomplished by creating a standard curve, where known copies of a plasmid containing TNF-α were used in a parallel qPCR analysis (Babaie et al., 2019).

**Table 1:** The primer and probe sequences utilized for quantifying TNF-α.

| Primer name | 5’ to 3’ sequence |
|---|---|
| TNF- α Forward | CACGTCGTAGCAAACCACCA |
| TNF- α Reverse | CAGCCTTGTCCCTTGAAGAGAA |
| TNF- α probe | CAATGCACAGCCTTCCTCACAGAGC CA |

### 2.7 Statistical Examination

Statistical examination was performed by GraphPad Prism software version 8.0.2. The data are expressed as mean ± SD. The normality of the data was assessed using the Shapiro-Wilk test. To determine statistical significance, one-way ANOVA was applied, and P values below 0.05 ($P < 0.05$) were regarded as statistically meaningful.

## 3. Results and Discussion

### 3.1 Haloperidol reduces parasite/cyst quantities in infected mice

One month after being infected with the *T. gondii* Tehran strain cysts (type II), mice received daily intraperitoneal injections of the neuroleptic drug haloperidol for 2 weeks. Another group of mice also received oral administration of co-trimoxazole, a standard anti-Toxoplasma medicine. The researchers then employed qPCR analysis to determine the amount of REP-529, which serves as a measure of parasite load, and the transcription level of BAG1, an indicator of cyst number, in the brains of the mice. The results demonstrated that all drug treatments considerably ($P < 0.0001$) decreased both BAG1 and REP-529 copy numbers in the mice brains. Surprisingly, the marked decrease in parasite/cyst burdens caused by the tested drug was similar to co-trimoxazole ($P > 0.05$). There were some differences in parasite/cyst load reduction among antipsychotic medications, but they were insignificant ($P > 0.05$). Additionally, the infected mice in the solvent group (those receiving the solvent/diluent of the drugs) exhibited a comparable parasite/cyst load to the infected mice in the untreated group (those receiving no treatment) (Fig. 1).

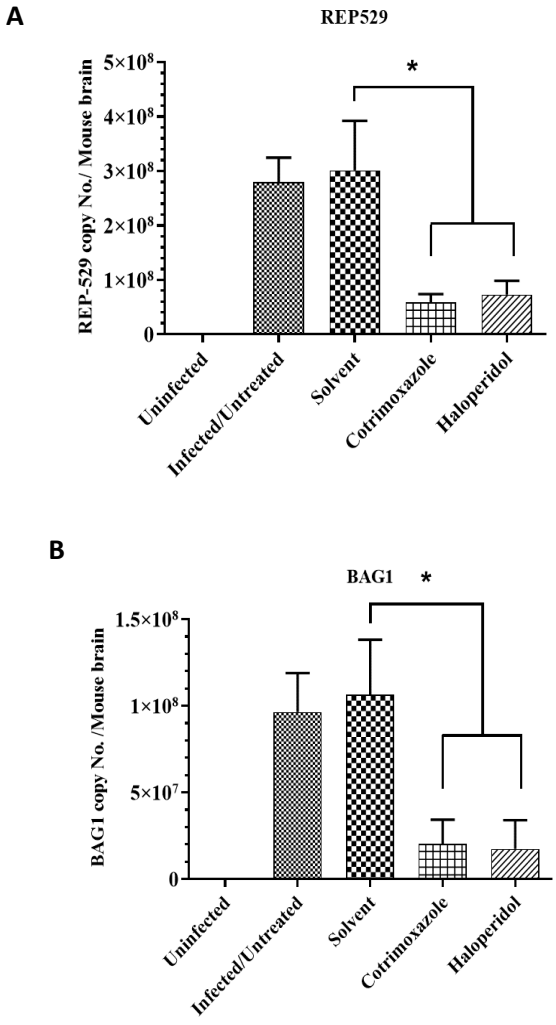


**Figure 1:** Haloperidol administration decreases the parasite/cyst burden in infected mice. A) Quantification of REP-529 copy number served as an indicator of parasite burden. B) Quantification of BAG1 transcripts served as an indicator of cyst burden. Error bars represent the averages and standard deviations for cyst or parasite burden between the examined mice (n=6 per group).

## 3.2 The transcription level of brain TNF-α in haloperidol mice group

TNF-α transcripts in the mice's brains were quantified using a qPCR technique. The results indicated that haloperidol medication led to a major reduction in brain TNF-α transcription ($P < 0.0001$), which was similar to the effects observed with co-trimoxazole ($P > 0.05$). Conversely, mice that received the drugs' solvent displayed similar TNF-α transcript levels as the mice group without any treatment, as depicted in (Fig 2).

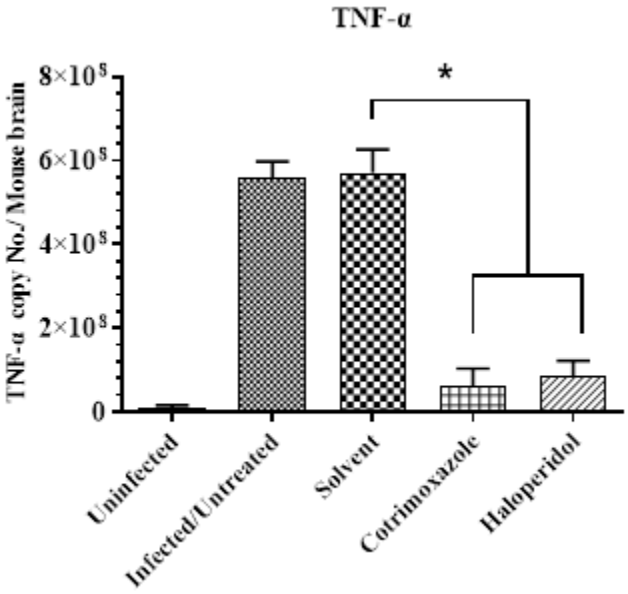


**Figure 2:** A qPCR analysis was used to quantify the TNF-α transcripts in mice treated with haloperidol and the control groups. * ($P < 0.0001$)

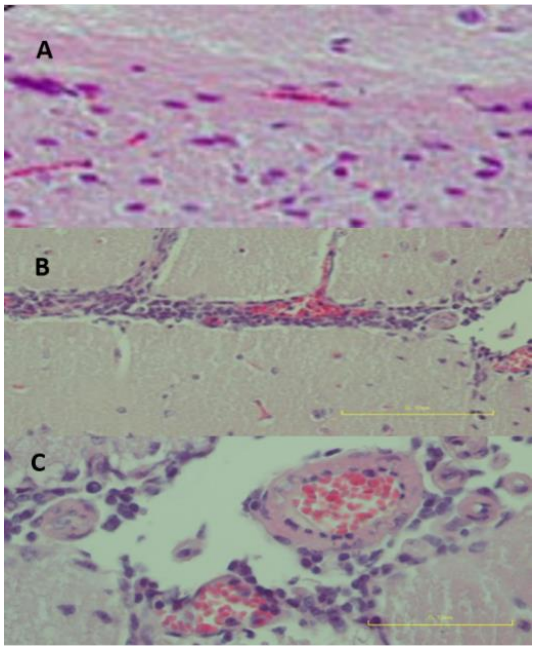


**Figure 3:** Histological examination of mice brains (H&E staining), A) mice that received haloperidol, B) mice from infected/untreated group, and C) representative of mice that received solvent.

## 3.3 Mice brain tissues subjected to histopathological examination

We next examined the histopathology of mice brains for the presence of cysts and inflammation. In addition to cysts observation, infection-related symptoms such as heavy perivascular infiltration of lymphocytes, glial nodules, and encephalitis can be seen in brain sections of infected/untreated mice or the solvent group, fewer histopathologic lesions and brain cysts were found in brain sections of mice that received haloperidol. The lack of noticeable histopathologic lesions was also evident in the mice treated with co-trimoxazole (Fig. 3).

### 3.4 Evaluating the weight gain process of mice

Drug administration did not notably decrease weight gain in the treated group compared with the uninfected groups, as shown in (Fig. 4), indicating that the drugs did not have a toxic effect on treated mice. The drugs were administered in the chronic stage of infection, so they had little effect on the weight-gaining process, which aligns with the results in a similar study (Babaie, Sayyah, Choopani, et al., 2017). During the initial 14 days after the injection of cysts, mice exhibited signs of illness, ascites, and irregular weight gain, indicative of acute infection. However, after 2 weeks, both the sickness and ascites showed improvement. The infected mice did not exhibit the usual growth rate and weight gain discerned in healthy, uninfected mice. Mice injected with cysts displayed abscesses and experienced irregular weight gain because of severe ascites. A remarkable increase in weight was observed during the first week after cyst injection (Fig. 4), with significant differences seen on days 6-12 ($p < 0.01$) compared to the corresponding control group. Subsequently, the weight gain chart gradually declined and became comparable to the control group of uninfected mice on day 19. In the chronic phase, it seems that the parasite exerts its effect on the weight gain of mice, so drug administration in the chronic period did not significantly affect the weight gain process. It is evident from the graphs that, due to cyst formation after infection, weight gain in infected groups was higher than in healthy groups till the 14th to 15th day. From the 15th day onwards, weight gain in the healthy group increased, and significant differences with infected groups, even drug-treated groups, can be observed. The mice weight graph is presented in (Fig. 4).

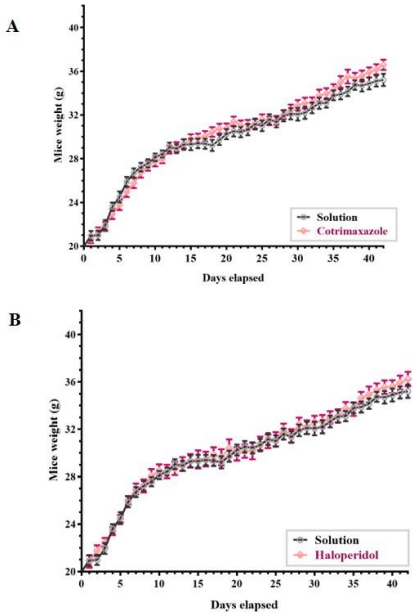


**Figure 4:** A comparison of the daily pattern of the mice's body weight gain in the solvent and treated groups. (A) co-trimoxazole (B) haloperidol.

Since various pieces of evidence point to that there may be a relationship between neuropsychiatric illnesses like schizophrenia and toxoplasma infection, much effort has been made to clarify this association in recent years. Our findings support certain clinical and animal research conclusions that antipsychotic medications effectively prevent *T. gondii* effects in vivo. To our knowledge, several in vitro studies have been done on this topic. According to the studies, however, there are some data concerning in vivo action of such medications against Toxoplasma. It is crucial to understand how different antipsychotics work on *T. gondii* (D. G. Goodwin et al., 2011; Jones-Brando et al., 2003).

Previous studies reported that antipsychotic treatments, while successful in hindering the replication of *T. gondii* in cell culture assays, were

unable to prevent acute or chronic infection in mice. In this study, we showed haloperidol reduced brain cyst/parasite load and TNF-α expression in chronically infected mice. The observed effects were comparable to co-trimoxazole, the standard treatment for *Toxoplasma* infection. These results were consistent with the previous study on the ant-toxoplasmic effect of the mood stabilizer valproic acid in mice brains (Enshaeieh et al., 2021). Clarifying the mechanism(s) of the anti-toxoplasma activity of these drugs might provide a better understanding of the pathogenesis of psycho-behavioral disorders and more effective management of such disorders in affected individuals.

Using qPCR analysis of brain samples, the quantification of Toxoplasma gene indicator levels revealed that all three psychiatric drugs significantly repressed chronic infection ($P < 0.0001$) to a degree comparable to co-trimoxazole. Additionally, histopathological inspection of the mice's brains showed substantially fewer instances of glial nodules, meningitis, and cyst formation in samples treated with psychiatric drugs and co-trimoxazole compared to infected/untreated mice or mice that received the drugs' solvent.

Controversial results were published concerning the in vitro anti-Toxoplasma activity of haloperidol. One study reported the robust action of haloperidol in preventing tachyzoite proliferation in cell culture (Jones-Brando et al., 2003), while others reported weak or no activity (Fond et al., 2014; D.G. Goodwin et al., 2008; D.G. Goodwin et al., 2011; Strobl et al., 2007). Haloperidol was found to reverse the behavioral changes in Toxoplasma-infected rats, implying its potential anti-Toxoplasma activity (J. Webster et al., 2006). Furthermore, some clinical observations have also suggested the in vivo anti-Toxoplasma activity of antipsychotic drugs (Fond et al., 2015; Leweke et al., 2004).

In a previous study (Babaie, Sayyah, Fard-Esfahani, et al., 2017), we found that BAG1 transcripts in the brains of infected mice were significantly reduced by several folds at week 8 compared to week 3. Additionally, microscopic images have indicated bradyzoite reproduction occurring between weeks 3 to 5 post-infection (PI), after which the mean bradyzoite burden stabilizes (Watts et al., 2015). Few studies reported brain cyst reduction when chemotherapy started at week 8 or later. In this regard, we propose that trimethoprim's failure to improve schizophrenia symptoms in *T. gondii*-infected patients might be attributed to the infection long before trimethoprim treatment.

It is important to remember that haloperidol can inhibit calcium transport, especially at the cellular ion channel level. Chemically speaking, haloperidol is distinct from phenothiazine antipsychotics because it has a butyrophenone ring structure. There is strong evidence that this drug acts as a D2 dopamine receptor antagonist, although its ability to block calmodulin is relatively weak. Dopamine in the brain might play a role in *T. gondii's* capacity to proliferate, recruit hosts, infect, or advance through stages (Strobl et al., 2012). Dopamine levels in congenitally infected mice were analyzed at 8 weeks, and no alterations were identified (D. Goodwin et al., 2012).

As mentioned earlier, *T. gondii* tachyzoites rely on calcium to invade host cells, and the prevention of this process can be achieved through calcium channel inhibitors and calmodulin antagonists. This indicates that haloperidol and potentially other antipsychotics' anti-toxoplasmic effect may be linked to their ability to inhibit calcium (Carruthers et al., 1999; Pezzella et al., 1997b).

The results of the present study regarding the effectiveness of haloperidol on toxoplasma activity and replication are consistent with those of a related study that suggested antipsychotic drugs like haloperidol and valproic acid were just as

effective as the traditional anti-toxoplasma medications like pyrimethamine combined with dapsone in preventing *Toxoplasma gondii* proliferation, invasion, and induction of behavioral alternations in chronically infected rats (J. Webster et al., 2006). As previously indicated, the anti-toxoplasmic effects of such medications may partly result from their calcium inhibition properties (Johannessen, 2000). On the other hand, Goodwin found that in human fibroblast cell culture, RH strains of *Toxoplasma gondii* tachyzoites did not appear to be affected by haloperidol or clozapine (D. G. Goodwin et al., 2011), in contrast to the Jones-Brando report regarding the influence of 12 antipsychotic medicines on *Toxoplasma gondii* RH strain activity in human fibroblast cell culture, which stated a strong role of haloperidol in avoiding proliferation of tachyzoites (Jones-Brando et al., 2003).

It is essential to clarify the precise mechanism through which antipsychotics influence *Toxoplasma gondii*. Human brain cells and *Toxoplasma gondii* tachyzoites may use similar harvesting strategies, which alter medication interactions. As a result, cell culture is a valuable model for testing out potential treatments for bipolar illness and schizophrenia. Since pharmaceuticals have an inhibitory impact on Toxoplasma, it is also encouraging to improve the effect of antipsychotic medications in infected people. According to research, toxoplasma-infected individuals with schizophrenia can benefit from drugs like haloperidol and valproic acid. Therefore, it is conceivable to develop more potent pharmacological combinations for treating bipolar illness and schizophrenia (Jones-Brando et al., 2003).

Antipsychotic signals for egressing *T. gondii*, which may entail inhibiting calmodulin or suppressing calcium signals, may cause the release of tachyzoites from parasitophorous vesicles. Atypical antipsychotics may activate ryanodine receptors, causing more calcium to be released from intracellular storage (Qin et al., 2009; Wagner et al., 2004) and tachyzoites to begin egress, which may explain why these medications have cytotoxic effects on the parasite (Lovett & Sibley, 2003).

The connection between *T. gondii* infection, mental illnesses, and psychotic medications with anti-inflammatory and anti-Toxoplasma action is a significant turning point that can fill in numerous gaps and clarify essential milestones in all linked investigations. The literature on the probable anti-inflammatory properties of antipsychotics tends to provide more consistent and less conflicting information. Various studies have shown the anti-inflammatory characteristics of antipsychotic medications, all of which are compatible with the current study's findings (Chien & Pasternak, 1994, 1995; T. Kato et al., 2007; Kowalski et al., 2003; Lü et al., 2004). While antipsychotics impact type 1 and type 2 immunological responses, the overall manner of change is unclear. However, some complex issues can be clarified by considering the relationship between pharmaceutical effects and therapy results (Miller et al., 2011; Potvin et al., 2008). Antipsychotics are included in the list of the most common forms of such drugs due to their anti-inflammatory properties (Sommer et al., 2013).

Studies have shown that external and intracellular $Ca^{2+}$ pools provide the intracellular calcium increase necessary for the lytic processes to be activated or stimulated within the parasite (Nagamune & Sibley, 2006). The *T. gondii* genome was found to include gene sequences that resemble voltage-dependent $Ca^{2+}$ channel sequences (Nagamune & Sibley, 2006; Prole & Taylor, 2011). A previous study found that nifedipine-like dihydropyridine $Ca^{2+}$ channel inhibitors decreased Toxoplasma penetration of host cells (Pezzella et al., 1997a).

In the current investigation, we produced proof-of-concept data demonstrating the effectiveness of haloperidol and toxoplasma infection and

associated neuroinflammation in mice. We propose that the mechanism of action of this medication in preventing toxoplasma infection involves interfering with calcium mobilization in microglia and other brain cells and that dopamine receptors or their canonical signaling may not be directly implicated. Conventional antipsychotics like haloperidol and trifluoperazine are antagonists of calmodulin; atypical antipsychotics have no impact on calmodulin, indicating that the way these medicines lower microglia activity is unrelated to their inhibitory action on calmodulin (Rushlow et al., 2009). Future research must define the precise mechanism(s) through which neuropsychiatric medications work to restrain Toxoplasma. Such knowledge may open up possibilities for the more efficient management of neuropsychiatric illnesses and *T. gondii* infection. According to the findings, several behavioral and neurological issues experienced by patients are caused by parasites. It follows that antipsychotic medications will help limit parasite growth, which will eventually help manage psychotic symptoms.

An increasing body of evidence has revealed that typical and atypical antipsychotics, such as haloperidol, inhibit microglial activation and microglial-induced inflammatory and oxidative responses (Kato et al., 2011; Mizoguchi et al., 2014; Wang et al., 2015). Microglial activation is suggested as an underlying reason for neuroinflammation and psychiatric disorders (A. Kato et al., 2011; Monji et al., 2013; Müller & Schwarz, 2007; Myint & Kim, 2014). Further studies suggested antipsychotics inhibit microglial activation by suppressing elevation in intracellular calcium ($Ca^{2+}$) concentration (Kato et al., 2011; Mizoguchi et al., 2014; Wang et al., 2015), which controls essential functions in microglia (Brawek & Garaschuk, 2013; Sharma & Ping, 2014).

## 4. Conclusion

We administered haloperidol by i.p injection at 6 mg/kg, higher than typical doses used in psychiatric studies. The applied dose, which is equal to 20% of their LD50 when administered by i.p injection (Conceição & Frussa-Filho, 1996; Lee et al., 2013; Quiñones-Torrelo et al., 2001; Sun et al., 2010), did not alter the weight of the animal, matched to control groups (data not shown), and induced no behavioral changes in the animal.

In the present study, we presented conclusive evidence supporting the effectiveness of haloperidol in combating neuroinflammation associated with chronic Toxoplasma infection in mice. We suggest that interfering with $Ca^{2+}$ mobilization in microglia and other brain cells is the mechanism of this drug's action in suppressing chronic Toxoplasma infection. Dopamine receptors or their canonical signaling might not be directly involved. Subsequent investigations are necessary to unveil the precise mechanism(s) underlying the anti-toxoplasma activity of neuropsychiatric drugs. Understanding this mechanism could open up possibilities for more efficient management of parasite infection and psychotic disorders.

## Conflict of interest

The authors express that this study was performed without any economic associations that may be perceived as a possible conflict of interest.

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## Ethical approval

Animal experiments were performed according to guidelines and permissions from the Institutional Animals Ethics Committee of Pasteur Institute of Iran (No: 0201-13085).

## Open access

## References

[1] Babaie, J., Sayyah, M., Choopani, S., Asgari, T., Golkar, M., Gharagozli, K. (2017). Toxoplasmosis accelerates acquisition of epilepsy in rats undergoing chemical kindling. *Epilepsy Res.*, *135*, 137-142.

[2] Babaie, J., Sayyah, M., Fard-Esfahani, P., Golkar, M., Gharagozli, K. (2017). Contribution of dopamine neurotransmission in proconvulsant effect of Toxoplasma gondii infection in male mice. *J. Neurosci. Res.*, *95*(*10*), 1894-1905.

[3] Babaie, J., Sayyah, M., Golkar, M. (2019). Effect of co-trimaxazole on REP-529 and TNF-α transcription levels in the brain of Toxoplasma gondii infected-mice. *Iran. J. Physiol.*, *3*(*2*), 107-199.

[4] Boillat, M., Hammoudi, P.-M., Dogga, S. K., Pagès, S., Goubran, M., Rodriguez, I., Soldati-Favre, D. (2020). Neuroinflammation-associated aspecific manipulation of mouse predator fear by Toxoplasma gondii. *Cell Rep.*, *30*(*2*), 320-334. e326.

[5] Brawek, B., Garaschuk, O. (2013). Microglial calcium signaling in the adult, aged and diseased brain. *Cell Calcium*, *53*(*3*), 159-169.

[6] Burgdorf, K. S., Trabjerg, B. B., Pedersen, M. G., Nissen, J., Banasik, K., Pedersen, O. B., Sørensen, E., Nielsen, K. R., Larsen, M. H., Erikstrup, C. (2019). Large-scale study of Toxoplasma and Cytomegalovirus shows an association between infection and serious psychiatric disorders. *Brain Behav. Immun.*, *79*, 152-158.

[7] Carruthers, V. B., Moreno, S. N., Sibley, D. L. (1999). Ethanol and acetaldehyde elevate intracellular [Ca2+] and stimulate microneme discharge in Toxoplasma gondii. *Biochem. J.*, *342*(*2*), 379-386.

[8] Carruthers, V. B., Suzuki, Y. (2007). Effects of Toxoplasma gondii infection on the brain. *Schizophr. Bull.*, *33*(*3*), 745-751.

[9] Chaudhury, A., Ramana, B. (2019). Schizophrenia and bipolar disorders: The Toxoplasma connection. *Trop. Parasitol.*, *9*(*2*), 71.

[10] Chien, C.-C., Pasternak, G. W. (1994). Selective antagonism of opioid analgesia by a sigma system. *J. Pharmacol. Exp. Ther.* , *271*(*3*), 1583-1590.

[11] Chien, C.-C., Pasternak, G. W. (1995). Sigma antagonists potentiate opioid analgesia in rats. *Neurosci. Lett.*, *190*(*2*), 137-139.

[12] Comer, A. L., Carrier, M., Tremblay, M.-È., Cruz-Martín, A. (2020). The inflamed brain in schizophrenia: the convergence of genetic and environmental risk factors that lead to uncontrolled neuroinflammation. *Front. Cell. Neurosci.*, *14*, 274.

[13] Conceição, I. M., Frussa-Filho, R. (1996). Effects of microgram doses of haloperidol on open-field behavior in mice. *Pharmacol. Biochem. Behav.*, *53*(*4*), 833-838.

[14] Enshaeieh, M., Saadatnia, G., Babaie, J., Golkar, M., Choopani, S., Sayyah, M. (2021). Valproic Acid Inhibits Chronic Toxoplasma Infection and Associated Brain Inflammation in Mice. *J. Antimicrob. Chemother.*, *65*(*10*), e01003-01021.

[15] Fond, G., Boyer, L., Gaman, A., Laouamri, H., Attiba, D., Richard, J.-R., Delavest, M., Houenou, J., Le Corvoisier, P., Charron, D. (2015). Treatment with anti-toxoplasmic activity (TATA) for toxoplasma positive patients with bipolar disorders or schizophrenia: a cross-sectional study. *J. Psychiatr. Res.*, *63*, 58-64.

[16] Fond, G., Macgregor, A., Tamouza, R., Hamdani, N., Meary, A., Leboyer, M., Dubremetz, J.-F. (2014). Comparative analysis of anti-toxoplasmic activity of antipsychotic drugs and valproate. *Eur. Arch. Psychiatry Clin. Neurosci.*, *264*(*2*), 179-183.

[17] Ghorbani, M., Sami, A. (1973). Toxoplasmic lymphadenitis in Iran. *Am. J. Trop. Med.*, *76*(*7*), 158-160.

[18] Goodwin, D., Hrubec, T. C., Klein, B. G., Strobl, J. S., Werre, S. R., Han, Q., Zajac, A. M., Lindsay, D. S. (2012). Congenital infection of mice with Toxoplasma gondii induces minimal change in behavior and no change in neurotransmitter concentrations. *J. Parasitol.*, *98*(*4*), 706-712.

[19] Goodwin, D. G., Strobl, J., Mitchell, S. M., Zajac, A. M., Lindsay, D. S. (2008). Evaluation of the mood-stabilizing agent valproic acid as a preventative for toxoplasmosis in mice and activity against tissue cysts in mice. *J. Parasitol. Res.*, *94*(*2*), 555-557.

[20] Goodwin, D. G., Strobl, J. S., Lindsay, D. S. (2011). Evaluation of five antischizophrenic agents against Toxoplasma gondii in human cell cultures. *J. Parasitol. Res.*, *97*(*1*), 148-151.

[21] Haroon, F., Händel, U., Angenstein, F., Goldschmidt, J., Kreutzmann, P., Lison, H., Fischer, K.-D., Scheich, H., Wetzel, W., Schlüter, D. (2012). Toxoplasma gondii actively inhibits neuronal function in chronically infected mice. *PLoS One*, *7*(*4*), e35516.

[22] Hawk, C., Leary, S., Morris, T. (2005). American College of Laboratory Animal Medicine, European College of Laboratory Animal Medicine. *J. Pharm. Sci.*

[23] Hwang, Y. S., Shin, J.-H., Yang, J.-P., Jung, B.-K., Lee, S. H., Shin, E.-H. (2018). Characteristics of infection immunity regulated by Toxoplasma gondii to maintain chronic infection in the brain. *Front. immunol.*, *9*, 158.

[24] Johannessen, C. U. (2000). Mechanisms of action of valproate: a commentatory. *Neurochem. Int.*, *37*(*2-3*), 103-110.

[25] Jones-Brando, L., Torrey, E. F., Yolken, R. (2003). Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of Toxoplasma gondii. *Schizophr. Res.*, *62*(*3*), 237-244.

[26] Kato, T., Monji, A., Hashioka, S., Kanba, S. (2007). Risperidone significantly inhibits interferon-γ-induced microglial activation in vitro. *Schizophr. Res.*, *92*(*1-3*), 108-115.

[27] Kato, T. A., Monji, A., Yasukawa, K., Mizoguchi, Y., Horikawa, H., Seki, Y., Hashioka, S., Han, Y.-H., Kasai, M., Sonoda, N. (2011). Aripiprazole inhibits superoxide generation from phorbol-myristate-acetate (PMA)-stimulated microglia in vitro: implication for antioxidative psychotropic actions via microglia. *Schizophr. Res.*, *129*(*2-3*), 172-182.

[28] Kowalski, J., Labuzek, K., Herman, Z. S. (2003). Flupentixol and trifluperidol reduce secretion of tumor necrosis factor-α and nitric oxide by rat microglial cells. *Neurochem. Int.*, *43*(*2*), 173-178.

[29] Lee, K. S., Chae, S. W., Park, J. H., Park, J. H., Choi, J. M., Rhie, S., Lee, H. J. (2013). Effects of single or repeated silymarin administration on pharmacokinetics of risperidone and its major metabolite, 9-hydroxyrisperidone in rats. *Xenobiotica*, *43*(*3*), 303-310.

[30] Leweke, F. M., Gerth, C. W., Koethe, D., Klosterkötter, J., Ruslanova, I., Krivogorsky, B., Torrey, E. F., Yolken, R. H. (2004). Antibodies to infectious agents in individuals with recent onset schizophrenia. *Eur. Arch. Psychiatry Clin. Neurosci.*, *254*(*1*), 4-8.

[31] Lovett, J. L., Sibley, L. D. (2003). Intracellular calcium stores in Toxoplasma gondii govern invasion of host cells. *J. Cell Sci.*, *116*(*14*), 3009-3016.

[32] Lü, L., Guo, S., Chen, W., Li, Q., Cheng, J., Guo, J. J. D. j. y. d. x. x. b. A. j. o. t. f. m. c. o. P. (2004). Effect of clozapine and risperidone on serum cytokine levels in patients with first-episode paranoid schizophrenia. *Di 1 jun yi da xue xue bao= Academic journal of the first medical college of PLA*, *24*(*11*), 1251-1254.

[33] Lüder, C. G., Giraldo-Velásquez, M., Sendtner, M., Gross, U. (1999). Toxoplasma gondii in primary rat CNS cells: differential contribution of neurons, astrocytes, and microglial cells for the intracerebral development and stage differentiation. *Exp. Parasitol.*, *93*(*1*), 23-32.

[34] Mendez, O. A., Koshy, A. A. (2017). Toxoplasma gondii: Entry, association, and physiological influence on the central nervous system. *PLoS Pathog.*, *13*(*7*), e1006351.

[35] Miller, B. J., Buckley, P., Seabolt, W., Mellor, A., Kirkpatrick, B. (2011). Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biol. Psychiatry*, *70*(*7*), 663-671.

[36] Mizoguchi, Y., Kato, T. A., Horikawa, H., Monji, A. (2014). Microglial intracellular Ca2+ signaling as a target of antipsychotic actions for the treatment of schizophrenia. *Front. Cell. Neurosci.*, *8*, 370.

[37] Monji, A., Kato, T. A., Mizoguchi, Y., Horikawa, H., Seki, Y., Kasai, M., Yamauchi, Y., Yamada, S., Kanba, S. (2013). Neuroinflammation in schizophrenia especially focused on the role of microglia. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry.*, *42*, 115-121.

[38] Müller, N., Schwarz, M., 2007. The immunological basis of glutamatergic disturbance in schizophrenia: towards an integrated view(pp. 269-280): Springer.

[39] Myint, A.-M., Kim, Y.-K. (2014). Network beyond IDO in psychiatric disorders: revisiting neurodegeneration hypothesis. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry.*, *48*, 304-313.

[40] Nagamune, K., Sibley, L. D. (2006). Comparative genomic and phylogenetic analyses of calcium ATPases and calcium-regulated proteins in the apicomplexa. . *Mol. Biol.*, *23*(*8*), 1613-1627.

[41] Pezzella, N., Bouchot, A., Bonhomme, A., Pingret, L., Klein, C., Burlet, H., Balossier, G., Bonhomme, P., Pinon, J. (1997a). Involvement of calcium and calmodulin in Toxoplasma gondii tachyzoite invasion. *Eur. J. Cell Biol.*, *74*(*1*), 92.

[42] Pezzella, N., Bouchot, A., Bonhomme, A., Pingret, L., Klein, C., Burlet, H., Balossier, G., Bonhomme, P., Pinon, J. (1997b). Involvement of calcium and calmodulin in Toxoplasma gondii tachyzoite invasion. *Eur. J. Cell Biol.*, *74*(*1*), 92-101.

[43] Potvin, S., Stip, E., Sepehry, A. A., Gendron, A., Bah, R., Kouassi, E. (2008). Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biol. Psychiatry*, *63*(*8*), 801-808.

[44] Prole, D. L., Taylor, C. W. (2011). Identification of intracellular and plasma membrane calcium channel homologues in pathogenic parasites. *PLoS One*, *6*(*10*), e26218.

[45] Qin, J., Zima, A. V., Porta, M., Blatter, L. A., Fill, M. (2009). Trifluoperazine: a rynodine receptor agonist. *Pflug. Arch. Eur.*, *458*(*4*), 643-651.

[46] Quiñones-Torrelo, C., Sagrado-Vives, S., Villanueva-Camañas, R., Medina-Hernández, M. (2001). An LD50 model for predicting psychotropic drug toxicity using biopartitioning micellar chromatography. *Biomed. Chromatogr.*, *15*(*1*), 31-40.

[47] Roizen, N., Kasza, K., Karrison, T., Mets, M., Noble, A. G., Boyer, K., Swisher, C., Meier, P., Remington, J., Jalbrzikowski, J. (2006). Impact of visual impairment on measures of cognitive function for children with congenital toxoplasmosis: implications for compensatory intervention strategies. *Pediatrics*, *118*(*2*), e379-e390.

[48] Rushlow, W., Seah, C., Sutton, L., Bjelica, A., Rajakumar, N. (2009). Antipsychotics affect multiple calcium calmodulin dependent proteins. *J. Neurosci.*, *161*(*3*), 877-886.

[49] Saadatnia, G., Golkar, M. (2012). A review on human toxoplasmosis. *Scand. J. Infect. Dis.*, *44*(*11*), 805-814.

[50] Saraei, M., Ghaderi, Y., Mosavi, T., Shahnazi, M., Nassiri-Asl, M., Jahanihashemi, H. (2016). The effect of fluphenazine and thioridazine on Toxoplasma gondii in vivo. *Iran. J. Parasitol.*, *11*(*2*), 226.

[51] Saraei, M., Samadzadeh, N., Khoeini, J., Shahnazi, M., Nassiri-Asl, M., Jahanihashemi, H. (2015). In vivo anti-Toxoplasma activity of aripiprazole. *Iran. J. Basic Med. Sci.*, *18*(*9*), 938.

[52] Sharma, P., Ping, L. (2014). Calcium ion influx in microglial cells: physiological and therapeutic significance. *j. Neurosci. Res.*, *92*(*4*), 409-423.

[53] Sommer, I. E., van Westrhenen, R., Begemann, M. J., de Witte, L. D., Leucht, S., Kahn, R. S. (2013). Efficacy of anti-inflammatory agents to improve symptoms in patients with schizophrenia: an update. *Schizophr. Bull.*, *40*(*1*), 181-191.

[54] Strobl, J. S., Cassell, M., Mitchell, S. M., Reilly, C. M., Lindsay, D. S. (2007). Scriptaid and suberoylanilide hydroxamic acid are histone deacetylase inhibitors with potent anti–Toxoplasma gondii activity in vitro. *J. Parasitol. Res.*, *93*(*3*), 694-700.

[55] Strobl, J. S., Goodwin, D. G., Rzigalinski, B. A., Lindsay, D. S. (2012). Dopamine stimulates propagation of Toxoplasma gondii tachyzoites in human fibroblast and primary neonatal rat astrocyte cell cultures. *J. Parasitol.*, *98*(*6*), 1296-1299.

[56] Sun, F., Su, Z., Sui, C., Zhang, C., Yuan, L., Meng, Q., Teng, L., Li, Y. (2010). Studies on the acute toxicity, pharmacokinetics and pharmacodynamics of paliperidone derivatives–comparison to paliperidone and risperidone in mice and rats. *Basic Clin. Pharmacol. Toxicol.*, *107*(*2*), 656-662.

[57] Tedford, E., McConkey, G. (2017). Neurophysiological changes induced by chronic Toxoplasma gondii infection. *J. Pathog.*, *6*(*2*), 19.

[58] Torgerson, P. R., Mastroiacovo, P. (2013). The global burden of congenital toxoplasmosis: a systematic review. *Bull. World Health Organ.*, *91*, 501-508.

[59] Torrey, E. F., Bartko, J. J., Yolken, R. H. (2012). Toxoplasma gondii and other risk factors for schizophrenia: an update. *Schizophr. Bull.*, *38*(*3*), 642-647.

[60] Torrey, E. F., Yolken, R. H. (2003). Toxoplasma gondii and schizophrenia. *Emerg. Infect. Dis.*, *9*(*11*), 1375.

[61] Tyebji, S., Seizova, S., Hannan, A. J., Tonkin, C. J. (2019). Toxoplasmosis: a pathway to neuropsychiatric disorders. *Neurosci. Biobehav. Rev.*, *96*, 72-92.

[62] Wagner, R., Fink, R., Stephenson, D. (2004). Effects of chlorpromazine on excitation–contraction coupling events in fast-twitch skeletal muscle fibres of the rat. *Br. J. Pharmacol.*, *141*(*4*), 624-633.

[63] Wallon, M., Kodjikian, L., Binquet, C., Garweg, J., Fleury, J., Quantin, C., Peyron, F. (2004). Long-term ocular prognosis in 327 children with congenital toxoplasmosis. *Pediatrics*, *113*(*6*), 1567-1572.

[64] Wang, H., Liu, S., Tian, Y., Wu, X., He, Y., Li, C., Namaka, M., Kong, J., Li, H., Xiao, L. (2015). Quetiapine inhibits microglial activation by neutralizing abnormal STIM1-mediated intercellular calcium homeostasis and promotes myelin repair in a cuprizone-induced mouse model of demyelination. *Front. Cell. Neurosci.*, *9*, 492.

[65] Watts, E., Zhao, Y., Dhara, A., Eller, B., Patwardhan, A., Sinai, A. P. (2015). Novel approaches reveal that Toxoplasma gondii bradyzoites within tissue cysts are dynamic and replicating entities in vivo. *MBio.*, *6*(*5*), e01155-01115.

[66] Webster, J., Lamberton, P., Donnelly, C., Torrey, E. (2006). Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on Toxoplasma gondii's ability to alter host behaviour. *Proc. R. Soc. B: Biol. Sci.*, *273*(*1589*), 1023-1030.

[67] Webster, J. P., Kaushik, M., Bristow, G. C., McConkey, G. A. (2013). Toxoplasma gondii infection, from predation to schizophrenia: can animal behaviour help us understand human behaviour? *J. Exp. Biol.*, *216*(*1*), 99-112.

[68] Weiss, L. M., Kim, K., 2011. Toxoplasma gondii: the model apicomplexan. Perspectives and methods: Elsevier.