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The possible association between *Toxoplasma gondii* infection and risk of anxiety and cognitive disorders in BALB/c mice

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There are conflicting reports concerning the association of *Toxoplasma gondii* infection with increased risk of mental disorders. This investigation will provide a good understanding about defining the possible association between *T. gondii* exposure and risk of anxiety and cognitive alterations. Besides, a secondary objective of this study was to determine the effect of pioglitazone administration on the possible alterations induced by *T. gondii* exposure. Male BALB/c mice were used for this study. The animal model of *Toxoplasma* infection was established by the intraperitoneal inoculation of 20–25 tissue cysts from Tehran strain of *T. gondii.* Pioglitazone (20 mg/kg, i.p.1/day) was administered to the animals for 2 weeks before behavioural tests. Behavioural tests including open-field, elevated plus-maze and passive avoidance learning were evaluated in the groups. Since cytokines were implicated as a contributing factor for mood disorders, the mRNA levels of TNF-α, IL-1β, IL-6 as well as inducible nitric oxide synthase (iNOs) were examined by real-time PCR. Findings demonstrated that *T. gondii* caused anxiety-like symptoms and impaired cognitive functions of the infected BALB/c mice, whereas pioglitazone, a peroxisome proliferator-activated receptor agonist, showed a promising effect against the cognitive impairments induced by *Toxoplasma* infection. The results also revealed that the mRNA levels of the aforementioned cytokines were significantly ($p < 0.05$) increased in the infected mice compared to the uninfected BALB/c ones. Pioglitazone can be offered as a potential neuroprotective agent in the treatment of patients with *T. gondii* infection that manifests anxiety and cognitive impairments; however, further studies are needed to clarify the exact mechanisms.

Keywords: *Toxoplasma gondii*, Pioglitazone, Anxiety, Cognitive function, Learning, Cytokine

Introduction

Toxoplasma gondii is a ubiquitous obligatory intracellular coccidian protozoan organism found throughout the world that infects a wide range of warm-blooded animals and approximately one-third of the world's human population.[1] Humans can be typically infected through three main routes of transmission: (i) ingestion of tissue cysts in raw or undercooked infected meat, (ii) ingestion of food or water contaminated with sporulated oocysts shed in the faeces of an infected cat and (iii) congenitally, vertical transmission from mother to foetus across the placenta when she is infected through one of the above two routes during pregnancy.[1] The clinical spectrum of *T. gondii* infections varies from asymptomatic to serious illnesses, affecting lymph nodes, eyes and central nervous system.[2]

The statistics reported regarding the prevalence of psychological disorders in Iran and other countries around the world has shown the necessity of paying more attention to mental health. According to World Health Organization (WHO), almost 450 million individuals suffer from one of the types of psychological disorders, half of who suffer from depression and anxiety.[3] Anxiety is one of the most frequent diseases among all other psychiatric disorders. Knowledge of disease-specific and non-specific risk

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factors facilitates the early identification of people at risk, which is important for further treatments.[4] So far, several studies have established a genetic contribution to these mental disorders.[5,6] Mapping of direct paths from gene to mental disorders has been slow and inconsistent, since only a few genome-wide association studies have detected risk genes, and many putative gene findings have failed to replicate.[7] However, a large proportion of variation in mental health remains unexplained by genetic factors. Thus, these factors are rendered to the discovery of new risk factors for mental disorders.

With respect to *T. gondii* infection as a possible cause of some mental disorders such as schizophrenia and mood disorders, many works have focused on a large number of epidemiological and serological studies, which have demonstrated association between *T. gondii* infection and these neuropsychiatric diseases.[8–10] For example, Markovitz et al. (2014) reported that *T. gondii* infection may play a role in the development of generalized anxiety disorder (GAD).[11] To allow further evaluation of the potential association between *T. gondii* infection and anxiety disorders, we established a mouse model of acquired *T. gondii* infection. Then, we investigated the relative clinical symptom of anxiety to find some useful information by these mice models.

Pioglitazone (PIO), a peroxisome proliferator-activated receptor (PPAR), is originally used for diabetes mellitus type two; but, due to the presence of neuroprotective properties, it is also used in various diseases including Parkinson's disease, traumatic brain injury, ischaemia, spinal cord injury and Huntington's disease.[12–15] Furthermore, Kemp et al. (2014) reported that pioglitazone as a modulator of mood can reduce the severity of bipolar depression symptom and modulate mood.[16] This proof-of-concept study tested whether administration of a PPARs-γ agonist could reduce cognitive impairments and anxiety symptom severity induced by *T. gondii* infection. Therefore, as a second objective, this study aims to explore the potential role of pioglitazone against anxiety-like conditions in a mice model with *T. gondii* infection.

Materials and Methods

Ethical statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Publication No. 85-23, revised 1985). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Kerman University of Medical Science (Permit Number: 1508).Moreover, all efforts were made to minimize suffering.

Animals

Forty male BALB/c mice (6–8 weeks old) weighing from 18 to 20 g were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Animals were housed in a colony room with a 12:12 h light/dark cycle at 21 ± 2 °C and were handled according to standard protocols for the use of laboratory animals.

Parasite

The Tehran strain of *T. gondii*, which was kindly provided by Prof. Keshavarz of Tehran University of Medical Sciences (Teharan, Iran), was used throughout the experiment. It was maintained by intraperitonealy inoculation of cysts (15–20 cysts) from brain tissue of infected BALB/c mice after 3 months. Cysts for the infection of BALB/c mice were isolated from the brain tissue of infected mice, and the number of cysts was counted under a microscopy with a 10 × objective.

Animal model of Toxoplasma infection

In this study, the animal model of *Toxoplasma* infection was established as described previously elsewhere.[17] To do this, 0.5 mL of the brain suspension containing 20–25 tissue cysts was inoculated intraperitoneally to each of 10 male BALB/c mice. After 2 months, all the mice were tested for anti-*T. gondii* antibodies by serological tests.

Serological tests

In order to confirm *Toxoplasma* infection in tested mice, collected serum samples were examined for anti-*T. gondii* IgG antibody via the modified agglutination test (MAT) using a commercial kit (Toxoscreen DA, Biom′erieux, Lyon, France) in accordance with the manufacturer's instructions, and starting at a 1/20 dilution. Sera showing an agglutination titre of 1/20 or higher were considered positive and were end-titrated using 2-fold dilutions.

Experimental design

Forty male BALB/c mice were randomly allocated to four experimental groups ($n = 10$ per group) for the behavioural assays. The groups were uninfected (control), *T. gondii* infection (TG), *T. gondii* + pioglitazone (TG + pio) (20 mg/kg, i.p. 1/day) and control + pioglitazone (control + pio). Pioglitazone hydrochloride (Sigma–Aldrich, USA) was dissolved in saline and administered intraperitoneally to the animals for 2 weeks before the behavioural tests.

Behavioural tests

Three months post infection, the behavioural experiments were performed by trained observers who were blind to experimental conditions. The experiments were performed between 8:00 am and 3:00 pm

Open-field test

At first, mice were brought to the laboratory 2 h after last pioglitazone injection. Mice were put in the middle of open-field. The open-field apparatus was made of a square arena (90 × 90 × 30 [H] cm) which was made of Plexiglas; its floor was divided into 16 squares, so the field was divided into central and peripheral squares. The vertical and horizontal

Table 1 Sequences of primers used for real-time PCR

Amplicon	Primers	Sequence (5′–3′)	Size (bp)
IL1 β	F	AACCTGCTGGTGTGTGACGTTC	78
	R	CAGCACGAGGCTTTTTTGTTGT	
IL6	F	ACAACCACGGCCTTCCCTACTT	129
	R	CACGATTTCCCAGAGAACATGTG	
iNOs	F	CTGGTGAAGGAACGGGTCAG	120
	R	CCGATCATTGACGGCGAGAAT	
TNF-α	F	CCACCTGCAAGACCATCGAC	91
	R	CTGGCGAGCCTTAGTTTGGAC	
β-actin	F	GTGACGTTGACATCCGTAAAGA	245
	R	GCCGGACTCATCGTACTCC	

activities of the mice were recorded during a 5-min period and then were analysed using an EthoVision software (version 7.1), a video tracking software for automation of the behavioural paradigms (Noldus Information Technology, the Netherlands). The following parameters were recorded for each animal: total distance moved (TDM, cm), number of grooming and rearing (as a measure of vertical activity) and the time spent in periphery and centre. At the end of each test, animals were removed from the chamber and the field was cleaned using damp cloth.[12]

Elevated plus-maze

The elevated plus-maze comprised a black wooden apparatus with arms having equal dimensions. Two of its arms were enclosed by walls (30 × 15 × 5 cm) and arranged in line with 2 opposite open arms (30 × 5 cm). The maze was elevated 50 cm above the floor. The mice were placed in the centre of the maze, facing the open arms. Two 100 W lamps brightly illuminated the arena. The mice were allowed to explore the maze, and their behaviour was monitored for 10 min using an EthoVision software (version 7.1), a video tracking software for automation of the behavioural paradigms (Noldus Information Technology, the Netherlands). After each test, the apparatus was cleaned with 70% ethanol to eliminate the remaining odours. The time spent in the open arms, the number of entries into the open arms and the total number of entries into the arms were recorded.[18]

Passive avoidance learning

The learning ability of each mouse was evaluated by passive avoidance (PA) learning test, which was described previously elsewhere.[19] Briefly, a shuttle-box device with dimensions of 100 × 25 × 25 which consisted two parts was used. First, animals were placed in the light arena of the shuttle-box apparatus. Then, the door was opened and the animal was allowed to go to the dark sector. Finally, the door was closed without electric shock and the animal was placed in the cage. Thirty minutes later, these steps were repeated and the third time that the animal entered the dark sector, an electric shock was administered to the animal (0.5 Å, 5 ms); then, the animal was returned to the cage. In the retention trials, 24 h after training, the test was performed to evaluate memory; in this step, the animal was placed in the light arena of the shuttle-box apparatus. After 30 s, the door opened and the time required to enter the dark sector was recorded as retention time (step through latency [STL]). Time in dark compartment (TDC) and number of entrance into dark sector were recorded as indicators of contextual memory.

Analysis of mRNA expression by real-time PCR

Since cytokines, signalling molecules of the immune system, have been implicated as a contributing factor for mood disorders, the mRNA levels of TNF-α, IL-1β, IL-6, as well as inducible nitric oxide synthase (iNOs) were examined by real-time PCR. Mice were deeply anesthetized with CO_2 in desiccators jar with low pressure flow of CO_2. After decapitation, whole brain tissues were rapidly removed and preserved in pre-cooling preservation tubes, then frozen in liquid nitrogen and stored at −80 °C for the investigation of cytokine expression. Total RNAs from brain tissue samples were isolated using RNeasy kits (QIAGEN, Hilden, Germany); all samples were reverse transcribed using RT premix kit (Intron, Sungnam, Korea) according to the manufacture's protocol. The resulting complementary DNA (cDNA) was subjected either to conventional PCR amplification or real-time PCR. Real-time PCR was performed using the iQ5 real-time PCR detection system (Bio-Rad, Hercules, CA) and SYBR green to detect amplification products, and the reaction conditions were used as described previously elsewhere.[20] Data analysis was performed using iQ^{TM}5 optical system software (Bio-Rad). The housekeeping gene encoding β-actin was used as a normalization control. Primer sequences were shown in Table 1.

Statistical analysis

Obtained results are expressed as the mean ± SEM. Data analysis was carried out using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with Tukey's *post hoc* test was used to assess the differences between experimental groups. In addition, $p < 0.05$ was considered statistically significant.

Results

Establishment of T. gondii infection

MAT showed that all of 20 mice infected with *T. gondii* tissue cysts were seropositive for anti-*T. gondii* IgG antibody with agglutination titres of > 1/20. Figure 1 also

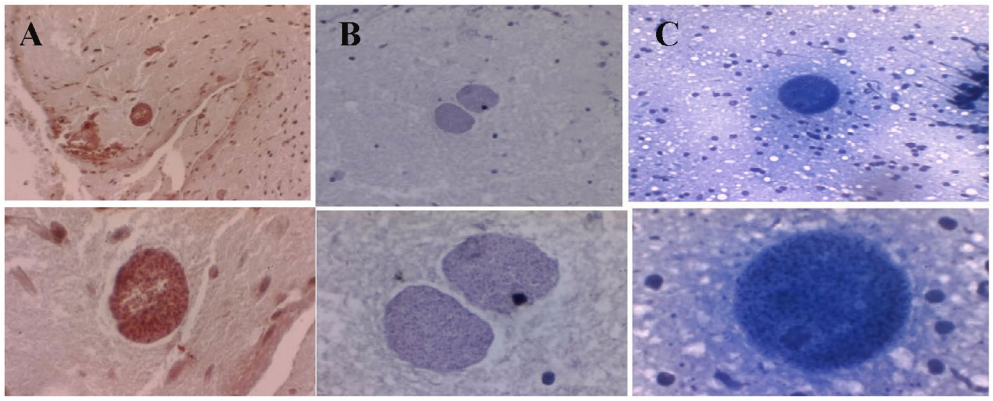

Figure 1 Tissue cysts of *T. gondii* Tehran strain isolated from brain of infected mice (×10 and × 40), A: periodic acid–Schiff (PAS) stain, B: haematoxylin and eosin staining, gimsa staining, C.

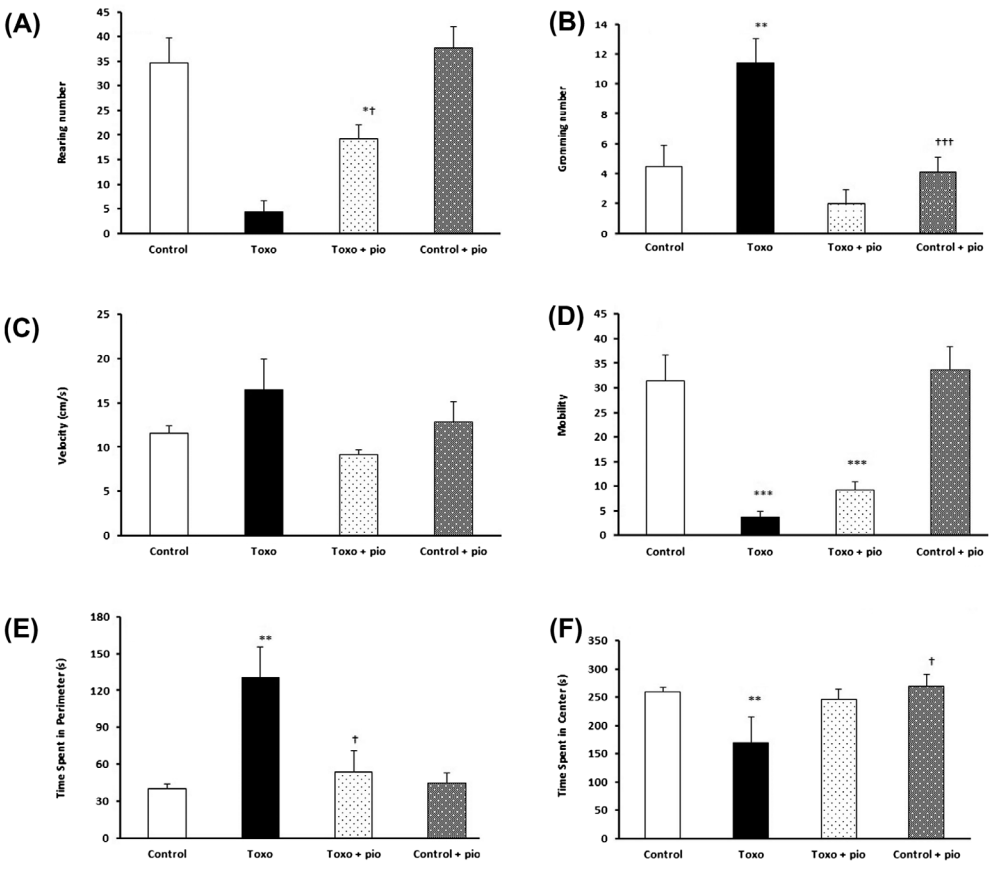

Figure 2 Effect of *T. gondii* and pioglitazone on locomotion and anxiety-like behaviours using with open-field test. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ in comparison with the control groups. †$p < 0.05$ and †††$p < 0.001$ compared with the *T. gondii* group.

shows tissue cysts of *T. gondii* Tehran strain isolated from brain of infected mice.

Effect of T. gondii and pioglitazone on locomotion and anxiety-like behaviours

As shown in Figure 2(A)–(F), locomotion and anxiety-like behaviours such as rearing ($p < 0.001$; A, ANOVA followed by Tukey's *post hoc*), grooming ($p < 0.01$; B, ANOVA followed by Tukey's *post hoc*), mobility ($p < 0.001$; D, ANOVA followed by Tukey's *post hoc*), time spent in perimeter ($p < 0.01$; E, ANOVA followed by Tukey's *post hoc*) and time spent in centre ($p < 0.01$; F, ANOVA followed by Tukey's *post hoc*) were altered in TG mice compared to those in the control groups (Figure 2(A)–(F)), which indicated anxiety-like behaviours in this group; but velocity did not alter ($p > 0.05$; C, ANOVA followed by Tukey's *post hoc*). In TG + piogroup, some locomotion and anxiety-like behaviours were significantly (ANOVA followed by Tukey's *post hoc*) altered compared with the control group, which included rearing ($p < 0.05$) and mobility ($p < 0.001$); compared with TG group, rearing, time spent in perimeter and centre ($p < 0.05$), and grooming ($p < 0.001$) were altered (Figure 2).

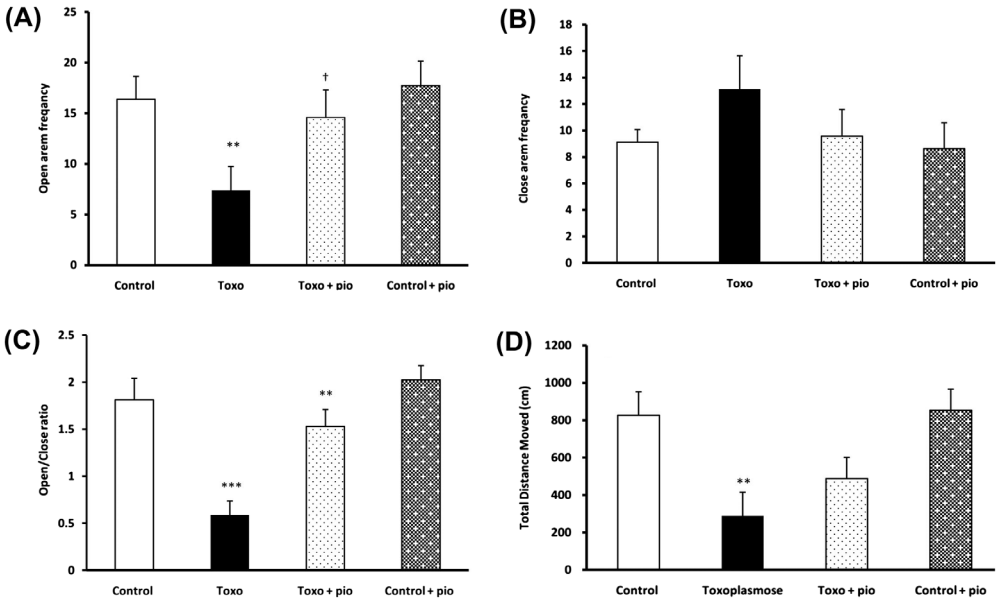

Figure 3 Effect of *T. gondii* and pioglitazone on anxiety-like behaviours using with elevated plus-maze test. **$p < 0.01$ and ***$p < 0.001$ in comparison with the control groups. †$p < 0.05$ compared with the *T. gondii* group.

Elevated plus-maze

Mice with *T. gondii* infection showed a significant difference in terms of anxiety behaviours using elevated plus-maze in comparison with the other groups ($p > 0.05$, ANOVA followed by Tukey's *post hoc*; Figure 3(A)–(D)). *T. gondii* infection group had a reduced number of entrances into the open arms ($p < 0.01$, ANOVA followed by Tukey's *post hoc*), implicating an increased anxiety-like behaviour in these mice. Treatment with pioglitazone counteracted the effect of *T. gondii* infection, because the TG + pio mice showed significant enhancement with TG group in the number of entrances into the open arms ($p < 0.05$, ANOVA followed by Tukey's *post hoc*; Figure 3(A)). There was no difference in the number of entries into the closed arms in all the groups (Figure 3(B)), but open/close arm ratio significantly decreased in the TG group compared with the control groups ($p < 0.001$, ANOVA followed by Tukey's *post hoc*). Treatment with pioglitazone counteracted the effect of *T. gondii* infection, because the TG + pio mice showed significant enhancement compared to the TG group in the open/close arm ratio ($p < 0.01$, ANOVA followed by Tukey's *post hoc*), but no difference was found with the control groups (Figure 3(C)). Furthermore, total distance moved was significantly reduced in the TG group compared with the controls ($p < 0.01$, ANOVA followed by Tukey's *post hoc*); TG + pio group had an increased TDM compared to the TG group, while there was no significant difference between TG + pio and controls or TG group, implicating a protective role for pioglitazone (Figure 3(D)).

Effect of T. gondii infection and pioglitazone treatment on PA learning

As shown in Figure 4(A), TG group received significantly a higher number of shocks compared to the control groups ($p < 0.01$, ANOVA followed by Tukey's *post hoc*), implicating an impaired learning. Treatment with pioglitazone counteracted the effect of *T. gondii* infection, because the TG + pio mice showed a significant redaction with the TG group in the number of shocks received ($p < 0.05$, ANOVA followed by Tukey's *post hoc*, Figure 4(A)).TG mice demonstrated a significantly decreased STL compared to the control groups ($p < 0.05$, ANOVA followed by Tukey's *post hoc*), indicating an impaired memory function in the PA paradigm. Treatment with pioglitazone partially reversed the effect of TG, because there was no significant difference between TG + pio compared with the TG and control groups (Figure 4(B)). TG mice showed an increased TDC compared with the control groups ($p < 0.01$, ANOVA followed by Tukey's *post hoc*, Figure 4(C)). TG + pio group had a decreased TDC compared to the TG group, while there was no significant difference between TG + pio and control or TG group, implicating a protective role for pioglitazone.

Analysis of mRNA expression

The mRNA levels of TNF-α,IL-1-β, IL-6 and iNOs were examined in *T. gondii*-infected BALB/c mice by quantitative real-time PCR. The results demonstrated that mRNA levels of TNF-α ($p < 0.001$), IL-1-β ($p < 0.01$), IL-6 ($p < 0.01$) and iNOs ($p < 0.01$) significantly increased (at least two fold) in infected mice in comparison with the uninfected BALB/c ones (ANOVA followed by Tukey's *post hoc*, Figure 5).

Discussion

In this study, *Toxoplasma* infection caused anxiety-like symptoms and impaired cognitive functions of the infected BALB/c mice, whereas pioglitazone, a PPAR agonist, showed a promising effect against the cognitive

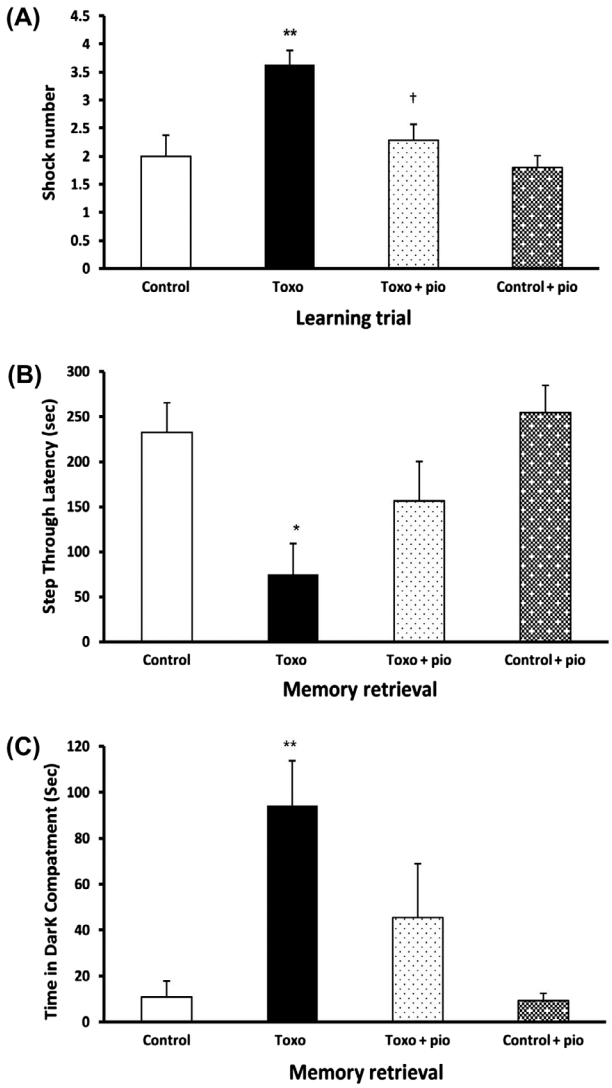

Figure 4 Effect of *T. gondii* and pioglitazone on PA learning and memory using shuttle-box test.
***$p < 0.05$ and **$p < 0.01$ in comparison with the control groups. †$p < 0.05$ compared with the *T. gondii* group.**

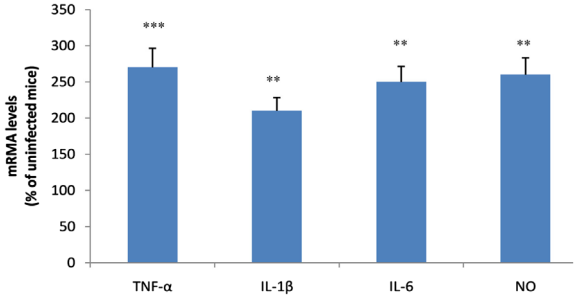

Figure 5 The mRNA expressions of some cytokines and also iNOs in *T. gondii*-infected BALB/c mice in comparison with the uninfected BALB/c mice. mRNA levels are presented as percentages of cytokine levels in infected mice vs. uninfected mice.
****$p < 0.01$, and ***$p < 0.001$ in comparison with the control group.**

impairments induced by *Toxoplasma* infection. In addition, our findings revealed that the mRNA levels of some cytokines and iNOs significantly increased in the infected mice in comparison with the uninfected BALB/c mice. Epidemiological evidence in humans and experimental studies in rodents has shown a number of neurological and behavioural disorders such as learning and memory impairment following the establishment of chronic toxoplasmosis.[21] Skallová et al. (2006) have reported that *Toxoplasma* tissue cysts in the brain could affect mouse behaviour through changes in the dopaminergic neuromodulatory system.[22] Zhou et al. (2011) have demonstrated that chronic toxoplasmosis induced by *T. gondii* Prugniaud strain impaired learning and memory functions in Kunming mice.[23] In the other study conducted by Daniels et al. (2015), it has also been reported that latent toxoplasmosis contributes to neurocognitive symptoms especially memory impairment in infected rats.[24] In addition, Hodkova et al. (2007) have shown that the differences between *T. gondii*-infected and control mice were a result of impaired ability to recognize novel stimuli rather than of impaired learning capacity in animals with latent toxoplasmosis.[25] However, the difference in the reported impacts of *T. gondii* infection on these impairments can

be attributed to rodent species, route of infection, parasite strain and dosage.[26,27]

Despite compelling evidence that *T. gondii* infection profoundly affects the manner in which rodents perceive and respond to stressful stimuli, only few studies have shown the possible relationship between *T. gondii* infection and human anxiety. They have found that *Toxoplasma* antibodies are significantly higher in anxiety disorders.[11,28] However, mechanisms by which *T. gondii* infection alters the host behaviour are not well understood, but neuroanatomical cyst presence and the localized host immune response to cysts are the potential candidates.[29] Previously, Evanse et al. (2014) reported that, while cysts are randomly distributed throughout the forebrain, individual variation in cyst localization can explain individual variation in the effects of *T. gondii* on behavioural test.[29] However, recent studies have demonstrated that cysts may preferentially persist and increase in number in limbic regions known to mediate anxiety, including the amygdala and hypothalamus.[26–28]

Our results also demonstrated a protective role for pioglitazone on anxiety and cognitive alterations induced by *T. gondii* in BALB/c mice. In the study conducted by Zeinoddini et al. (2015) on the safety and efficacy of pioglitazone in the patients with bipolar depression, it was proven that pioglitazone could be a tolerable and effective adjunctive therapy for improving depressive symptoms in bipolar disorder without type 2 diabetes or metabolic syndrome.[30] In addition, Kemp et al. (2014) reported that open-label administration of the PPAR-γ agonist pioglitazone was associated with improvement in depressive symptoms and reduced cardio-metabolic risk.[16] Kumar et al. (2010) have shown that 3 weeks of pioglitazone (5 and 10 mg/kg) pretreatment significantly attenuated the chronic fatigue-like condition as compared to the one in the control (chronic fatigue) animals.[31] Although the exact mode of action of PIO in the improvement of these symptoms is poorly understood, recent studies have indicated that PIO may act in several ways: (i) attenuating oxidative damage (decreased lipid peroxidation, nitrite concentration, restored reduction in glutathione and catalase levels), (ii) altering mitochondrial enzymes' complex (I, II and IV) activities and mitochondrial redox activity and (iii) reducing inflammation modulates mood.[12,31] It has been previously proven that cytokines, signalling molecules of the immune system through the neuroendocrine and central neurochemical changes, have been implicated as a contributing factor for mood disorders such as depression and anxiety.[32] Moreover, the evidence has supported that, in humans and animals, exposure to cytokines induces depressive-like mood and behavioural alterations.[32,33] Among cytokines, IL1, IL6, TNF-α, also iNOs are the most common ones related to anxiety and depression disorders.[28] Reviews have been proven that *T. gondii* elicit robust innate and TH_1 adaptive immune responses in the CNS, where the expression of inflammatory cytokines and mediators such as TNF-α, IL-6, IL-1 and NO has both protective and pathological effects. While vital for restricting parasite replication and spread, inflammatory responses can cause bystander injury of uninfected neurons and can additionally influence neurotransmitter functions and synaptic transmission.[34–38] Consistent with previous studies, we found that the mRNA levels of these cytokines significantly ($p < 0.05$) increased in infected mice compared with the uninfected ones. Thus, it could be suggested that chronic *T. gondii* infection communication among immune cells promotes neuroinflammation through cytokine networks and induce anxiety-like symptoms in the BALB/c mice brain; whereas pioglitazone, a PPAR agonist, showed a promising effect against the cognitive impairments induced by *Toxoplasma* infection

Conclusion

The obtained findings demonstrated that *T. gondii* infection caused anxiety-like symptoms and impaired cognitive functions in BALB/c mice; also, some of these impairments were reversible by pioglitazone as a PPAR agonist. Previously, neuroprotective effects of pioglitazone have been proven. However, further investigations are required to evaluate the effect of different dosages and durations of pioglitazone treatment on cognitive impairments induced by *T. gondii* infection and to elucidate the possible underlying mechanisms.

References

1 Dubey JP. Toxoplasmosis-a waterborne zoonosis. Vet Parasitol. 2004;126:57–72.
2 Mahmoudvand H, Saedi Dezaki E, Soleimani S, Baneshi MR, Kheirandish F, Ezatpour B, Zia-ali N. Seroprevalence and risk factors of *Toxoplasma gondii* infection among healthy blood donors in southeast of Iran. Parasite Immunol. 2015;37(7):362–367.
3 World Health Organization (WHO). *A public health approach to mental health*. Geneva: 2001. Available from: http://www.who.int/whr/2001/en/whr01_ch1_en.pdf
4 Hermanns N, Kulzer B, Krichbaum M, Kubiak T, Haak T. Affective and anxiety disorders in a German sample of diabetic patients: prevalence, comorbidity and risk factors. Diabet Med. 2005;22(3):293–300.
5 Hettema JM, Neale MC, Kendler KS. A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am J Psychiatry. 2001;158:1568–1578.
6 Xian H, Chantarujikapong SI, Scherrer JF, Eisen SA, Lyons MJ, Goldberg J, et al. Genetic and environmental influences on posttraumatic stress disorder, alcohol and drug dependence in twin pairs. Drug Alcohol Depend. 2000;61:95–102.
7 Hamer D. Genetics. Rethinking behavior genetics. Science. 2002;298:71–72.
8 Fekadu A, Shibre T, Cleare AJ. Toxoplasmosis as a cause for behavior disorders–overview of evidence and mechanisms. Folia Parasitol. 2010;57:105–113.
9 Miman O, Mutlu EA, Ozcan O, Atambay M, Karlidag R, Unal S. Is there any role of *Toxoplasma gondii* in the etiology of obsessive–compulsive disorder? Psychiatry Res. 2002;177:263–265.
10 Torrey EF, Bartko JJ, Yolken RH. *Toxoplasma gondii* and other risk factors for schizophrenia: an update. Schizophr Bull. 2012;38:642–647.
11 Markovitz AA, Simanek AM, Yolken RH, Galea S, Koenen KC, Chen S, et al. *Toxoplasma gondii* and anxiety disorders in a community-based sample. Brain Behav Immun. 2015;43:192–197.
12 Aghaei I, Shabani I, Doustar N, Nazeri M, Dehpour A. Peroxisome proliferator-activated receptor-γ activation attenuates motor and cognition impairments induced by bile duct ligation in a rat model of hepatic cirrhosis. Pharmacol Biochem Behav. 2014;120:133–139.

13 Fruchart J, Bastide M. PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases. Biochem Soc Trans. 2006;34:1341–1346.
14 Swanson CR, Joers V, Bondarenko V, Brunner K, Simmons HA, Ziegler TE, et al. The PPAR-g agonist pioglitazone modulates inflammation and induces neuroprotection in parkinsonian monkeys. J Neuroinflammation. 2011;8:91–115.
15 Kemnitz JW, Elson DF, Roecker EB, Baum ST, Bergman RN, Meglasson MD. Pioglitazone increases insulin sensitivity, reduces blood glucose, insulin, and lipid levels, and lowers blood pressure, in obese, insulin-resistant rhesus monkeys. Diabetes. 1994;43:204–211.
16 Kemp DE, Schinagle M, Gao K, Conroy C, Ganocy SJ, Ismail-Beigi F, et al. PPAR-γ agonism as a modulator of mood: proof-of-concept for pioglitazone in bipolar depression. CNS Drugs. 2014;28(6):571–581.
17 Mahmoudvand H, Ziaali N, Ghazvini H, Shojaee S, Keshavarz H, Esmaeilpour K, Sheibani V. in Press*Toxoplasma gondii* infection promotes neuroinflammation through cytokine networks and induced hyperalgesia in BALB/c mice. Inflammation 2015 Oct 21.
18 Nazeri M, Shabani M, Ghotbi Ravandi S, Aghaei I, Nozari M, Mazhari S. Psychological or physical prenatal stress differentially affects cognition behaviors. Physiol Behav. 2015;142:155–160.
19 Aghaei I, Nazeri M, Shabani M, Mossavinasab M, Mirhosseini FK, Nayebpour M, Dalili A. Erythropoietin ameliorates the motor and cognitive function impairments in a rat model of hepatic cirrhosis. Metab Brain Dis. 2015;30(1):197–204.
20 Ha TY, Chang KA, Kim JA, Kim HS, Kim S, et al. S100a9 knockdown decreases the memory impairment and the neuropathology in Tg2576, AD animal model. PLoS One. 2010;5:e8840.
21 Dalimi A, Abdoli A. Latent toxoplasmosis and human. Iranian J parasitool. 2012;2(1):1–17.
22 Skallová A, Kodym P, Frynta D, Flegr J. The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethologicaland ethopharmacological study. Parasitology. 2006;133(5):525–535.
23 Zhou YH, Wang XB, Jiang SF, Xu YL, Tao JP, Zhang XP, et al. Impairment of learning and memory ability in mice with latent infection of *Toxoplasma gondii*. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2011;29(5):333–338.
24 Daniels BP, Sestito SR, Rouse ST. An expanded task battery in the Morris water maze reveals effects of *Toxoplasma gondii* infection on learning and memory in rats. Parasitol Int. 2015;64(1):5–12.
25 Hodkova H, Kodym P, Flegr J. Poorer results of mice with latent toxoplasmosis in learning tests: impaired learning processes or the novelty discrimination mechanism? Parasitology. 2007;134(10):1329–1337.
26 Haroon F, Händel U, Angenstein F, Goldschmidt J, Kreutzmann P, Lison H, et al. *Toxoplasma gondii* actively inhibits neuronal function in chronically infected mice. PLoS ONE. 2012;7:e35516.
27 Worth AR, Lymbery AJ, Thompson RC. Adaptive host manipulation by *Toxoplasm gondii*: fact or fiction? Trends Parasitol. 2013;29: 150–155.
28 Gale SD, Brown BL, Berrett A, Erickson LD, Hedges DW. Association between latent toxoplasmosis and major depression, generalised anxiety disorder and panic disorder in human adults. Folia Parasitol (Praha). 2014;61(4):285–292.
29 Evans AK, Strassmann PS, Lee IP, Sapolsky RM. Patterns of *Toxoplasma gondii* cyst distribution in the forebrain associate with individual variation in predator odor avoidance and anxiety-related behavior in male Long-Evans rats. Brain Behav Immun. 2004;37:122–133.
30 Zeinoddini A, Sorayani M, Hassanzadeh E, Arbabi M, Farokhnia M, Salimi S, et al. Pioglitazone adjunctive therapy for depressive episode of bipolar disorder: a randomized, double-blind, placebo-controlled trial. Depress Anxiety. 2015;32(3):167–173.
31 Kumar A, Vashist A, Kumar P. Potential role of pioglitazone, caffeic acid and their combination against fatigue syndrome-induced behavioural, biochemical and mitochondrial alterations in mice. Inflammopharmacology. 2010;18(5):241–251.
32 Anisman H, Merali Z. Cytokines, stress and depressive illness: brain-immune interactions. Ann Med. 2003;35(1):2–11.
33 Pollak Y, Yirmiya R. Cytokine-induced changes in mood and behaviour: implications for 'depression due to a general medical condition', immunotherapy and antidepressive treatment. Int J Neuropsychopharmacol. 2002;5(4):389–399.
34 Liesenfeld O, Parvanova I, Zerrahn J, Han SJ, Heinrich F, Muñoz M, et al. The IFN-γ-inducible GTPase, Irga6, protects mice against *Toxoplasma gondii* but not against *Plasmodium berghei* and some other intracellular pathogens. PLoS ONE. 2011;6:e20568.
35 Munoz M, Liesenfeld O, Heimesaat MM. Immunology of *Toxoplasma gondii*. Immunol Rev. 2011;240:269–285.
36 Dunn AJ. Effects of cytokines and infections on brain neurochemistry. Clin Neurosci Res. 2006;6:52–68.
37 McCusker RH, Kelley KW. Immune-neural connections: how the immune system's response to infectious agents influences behavior. J Exp Biol. 2013;216:84–98.
38 Saito K, Markey SP, Heyes MP. Chronic effects of γ-interferon on quinolinic acid and indoleamine-2,3-dioxygenase in brain of C57BL6 mice. Brain Res. 1991;546:151–154.