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TOXOPLASMA GONDII INFECTION POTENTIATES COGNITIVE IMPAIRMENTS OF ALZHEIMER'S DISEASE IN THE BALB/C MICE

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ABSTRACT: This study tests the hypothesis that in chronic *Toxoplasma gondii* infection communication among immune cells promotes neuroinflammation through cytokine networks and potentiate cognitive impairments in BALB/c mice with Alzheimer's disease (AD). The animal model of *Toxoplasma* infection was established by the intraperitoneal inoculation of 20–25 tissue cysts from the Tehran strain of *T. gondii*. We injected amyloid-beta 1–42 peptide ($A\beta_{1-42}$, 1 and 2 μl) into the hippocampus of BALB/c mice to establish an animal model of AD. The behavioral experiments such as spatial learning and memory were performed using the Morris water maze test. The mRNA levels of TNF-α, IL-1β, IFN-γ, and inducible nitric oxide synthase (iNOS) were examined by real-time PCR. We found that *T. gondii* infection caused AD-like symptoms and impaired learning and memory functions of the infected BALB/c mice. We also found that in *Toxoplasma* infection + $A\beta_{1-42}$ (1 μl) group, *T. gondii* infection could potentiate AD in infected mice receiving subdoses of $A\beta_{1-42}$ (1 μl) and caused considerable impairment in learning and memory functions similar to AD group. Comparison of the results demonstrated that mRNA levels of IL-1β, TNF-α, IFN-γ, and iNOS significantly ($P < 0.001$) increased in *T. gondii* + $A\beta_{1-42}$ (1 μl) in comparison with the other tested groups. The obtained results showed that chronic *T. gondii* infection communication among immune cells promotes neuroinflammation through cytokine networks and induces pathological progression of AD in the mice brain, whereas the presence of neuroanatomical *Toxoplasma* tissue cysts in the brain could also affect the behavioral functions in *T. gondii*–infected mice.

Toxoplasma gondii, a ubiquitous obligatory intracellular protozoan organism, is a neurotropic parasite that is considered one of the world's most successful pathogens. This parasite has remarkable transmissibility and has permanently infected a wide range of warm-blooded animals and approximately one-third of the world's human population (Hill and Dubey, 2002). Humans can normally be infected by 3 main routes of transmission: (1) ingestion of tissue cysts in raw or undercooked infected meat, (2) ingestion of food or water contaminated with sporulated oocysts shed in the feces of an infected cat, and (3) congenitally, vertical transmission from mother to fetus across the placenta when she is formerly infected through one of the above 2 routes during pregnancy (Mahmoudvand et al., 2015a). The clinical spectrum of *T. gondii* infections varies from asymptomatic to serious illness affecting lymph nodes, eyes, and central nervous system (CNS) (Dubey, 2004). During acute infection, tachyzoites can escape from the immune system, leading to the formation of tissue cysts containing bradyzoites, especially in the brain. In addition, during latent infection in the CNS, *T. gondii* cysts can influence neuronal cell biology, including neurotransmitter synthesis and signal transduction, as well as synapse formation and dendritic arborization (Prandovszky et al., 2011; Gatkowska et al., 2013). Previously it has 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 nitric oxide (NO) has both protective and pathological effects (Liesenfeld et al., 2011; Munoz et al., 2011). Although these factors restrict parasite replication and spread, inflammatory responses can also cause considerable injury of uninfected neurons and can additionally influence neurotransmitter functions and synaptic transmission (Dunn, 2006; McCusker and Kelley, 2013; Saito et al., 1991).

Previous studies have suggested that neuronal degeneration induced by neuroinflammation plays a critical role in the pathogenesis of chronic neurodegenerative diseases in general, and in Alzheimer's disease (AD) in particular (Heneka et al., 2010). AD is the most common type of dementia, accounting for 50 to 75% of all cases of dementia (Blennow et al., 2006). AD involves development of a progressive and permanent neuropsychiatric disorder that is characterized by gradual memory and learning impairment and reduction of cognitive abilities and acquired skills (Ferri et al., 2009). The pathogenesis of AD is characterized by widespread neuronal degeneration, involving synaptic and neuronal loss, and extracellular deposits of β-amyloid peptides, so-called neuritic or senile plaques, and intracellular neurofibrillary tangles of hyperphosphorylated tau protein, which have been proven to be the neuropathologic hallmarks of the disease (Querfurth and LaFerla, 2010). Experiments have revealed that inflammatory mediators including cytokines, complement components, various free radicals, and NO may stimulate amyloid precursor protein processing by various means and therefore can create a vicious cycle that could be essential in the pathological progression of AD (Griffin et al., 1998; Griffin, 2000).

With respect to *T. gondii* infection as a possible cause of some mental disorders such as AD, 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 (Fekadu et al., 2010; Torrey et al., 2012; Mahmoudvand et al., 2015b). For example, the study conducted by Yilmaz et al. (2011) showed that the seropositivity rates for anti–*T. gondii* IgG antibodies among AD patients and a control group were 44.1

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and 24.3%, respectively, and there was a significant difference between the serum anti–*T. gondii* IgG levels of patients with the control group ($P = 0.005$). These results hypothesize that *Toxoplasma* infection may be involved in the pathogenetic mechanisms of AD. This investigation was carried out to determine whether *T. gondii* infection is involved in the neuroinflammation and cognitive mechanisms of AD. In the present study, we hypothesized that chronic *T. gondii* infection communication among immune cells promotes neuroinflammation through cytokine networks and induces cognitive impairment in BALB/c mice intrahippocampal injected with amyloid-beta peptide ($A\beta_{1–42}$).

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. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Kerman University of Medical Science (No. 1508) and the Kerman Neurosciences Research Center, Kerman, Iran. Moreover, all efforts were made to minimize suffering.

Animals

Sixty male BALB/c mice (6–8 wk old) weighing from 18 to 20 g for establishing an animal model of *T. gondii* and AD were obtained from the Animal Breeding Stock Facility of the Razi Institute of Iran (Karaj, Iran). Animals were housed in a colony room of the Kerman Neurosciences Research Center with a 12:12 hr 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* (type II), kindly provided by Prof. Keshavarz, Tehran University of Medical Sciences (Teharan, Iran), was used throughout the experiment (Ghorbani et al., 1983). It was maintained by intraperitoneal inoculation of cysts (15–20 cysts) from brain tissue of infected BALB/c mice after 3 mo. Cysts were isolated from the brain tissue of infected BALB/c mice, and then the number of cysts was counted under a microscope with a ×10 objective.

Animal model of *Toxoplasma* infection

The animal model of *Toxoplasma* infection was established as described previously elsewhere (Mahmoudvand et al., 2016). Brain homogenized suspension in saline was prepared from the mice infected with the tissue cysts of *T. gondii* 3 mo earlier. Then 0.5 ml of the brain suspension containing 20–25 tissue cysts was inoculated intraperitoneally in each of 10 male BALB/c mice. After 2 mo, all the mice were tested for anti–*T. gondii* antibodies by serological tests.

Serological tests

In order to confirm toxoplasmosis in infected mice, collected serum samples were examined for anti–*T. gondii* IgG antibody via the modified agglutination test using a commercial kit (Toxoscreen DA, Biomérieux, Lyon, France) in accordance with the manufacturer's instructions and starting at a 1/20 dilution.

Hippocampal injection of $A\beta_{1–42}$ to induce an AD model

Hippocampal injection of $A\beta_{1–42}$ to induce an AD model was performed according to the method described elsewhere (Liu et al., 2014). To prepare oligomeric state $A\beta_{1–42}$, freeze-dried $A\beta_{1–42}$ powder (200 μg; Sigma-Aldrich, St. Louis, Missouri) was dissolved in 100 μl of sterile normal saline solution for a stock solution at a concentration of 200 μg/100 μl, which was then aliquoted (20 μg/10 μl) and stored at –20 C. At the time of experimentation, an aliquot was thawed to prepare the working solution (2 μg/μl) and was incubated at 37 C for 24 hr. This allowed aggregation of $A\beta_{1–42}$ to toxic oligomeric Aβ. Mice were anesthetized by intraperitoneal injection of Ketamine (60 mg/kg) and Xylazine (10 mg/kg). The heads were fixed onto a stereotaxic frame, and then the skull was drilled to create a hole at 2.3 mm posterior to bregma and 1.8 mm lateral to the midline, to 1.0 mm depth. A 10 μl microsyringe was inserted 2.0 mm into the brain, 1 and 2 μl $A\beta_{1–42}$ working solution or saline (sham group) was slowly injected bilaterally into the hippocampal CA1. The needles were maintained in place for 5 min and then slowly withdrawn to prevent leakage. The skin was sutured and disinfected with alcohol, followed by intramuscular injections of sodium penicillin (40,000 units) for 3 consecutive days. For the remainder of the experiment, mice were housed in specific-pathogen-free cages.

Experimental design

Male BALB/c mice were randomly allocated to 6 experimental groups (n =10 per group) for all assays: uninfected mice (control), *T. gondii* infection, $A\beta_{1–42}$ (2 μl), $A\beta_{1–42}$ (1 μl), and sham group. One group was also *T. gondii* + $A\beta_{1–42}$ (1 μl), which was established 3 mo after infection with *T. gondii*.

Morris water maze

The Morris water maze (MWM) task was used to evaluate spatial learning and memory. The MWM consisted of a black circular swimming pool that was painted with nontoxic materials, 160 cm diameter, 80 cm height, filled with water maintained at room temperature to a depth of 40 cm. The pool was geographically divided into 4 quadrants of equal size, and starting points were designated at each quadrant as N, S, E, and W. A square platform (10 cm diameter) was hidden just below (1.5 cm) the surface of the water in the center of the northeast quadrant. The experiments were carried out in a dimly light room with various and fixed extra maze geometric images (e.g., circles, squares, or triangles) attached at different points on the walls around the maze. Performances were recorded by a smart video tracing system (Noldus Ethovision® system, version 5, Noldus Information Technology BV Wageningen, the Netherlands), and animals could be traced on the screen of a computer (Saadati et al., 2015).

Spatial learning: The behavioral experiment was performed during the light cycle (between 0830 and 1200 hr) 10 days after $A\beta_{1–42}$ injection. In the spatial acquisition phase, the mice were allowed to find a submerged hidden platform during a 60-sec interval in 4 training trials (inter-trial interval = 60 sec) repeated in 3 blocks (inter-block interval = 30 min). After finding the platform, the animals were allowed to rest on the platform for 20–30 sec. The mice were dried with a towel and returned to their cages. After 20 to 30 sec of rest, they were once again put in the chamber for the next trial. When mice did not find the platform within 60 sec, the experimenter would put it on the platform. On each trial, mice were randomly released into the water from 1 of the 4 quadrants of the maze with their faces toward the wall of the quadrant where they were released. Each mouse had 4 different releasing points. Parameters such as latency and the traveled distance to find the platform were recorded in each trial.

Spatial memory: Two hours after the acquisition phase, a probe test was performed to evaluate spatial memory retention. For the probe test, the platform was removed and each mouse was allowed to swim for 60 sec. The time and distance spent in the target quadrant (quadrant 4) were analyzed as a measure of spatial memory retention.

Latency to visible platform and swimming speed: Following the probe trial, mice had to complete a visible platform test to determine any possibility of *Toxoplasma* infection and $A\beta_{1–42}$ model interference with sensory and motor coordination or motivation. In this test, the ability of animals to escape to a visible platform was evaluated (the platform was raised 2 cm above the water level and was visible using aluminum foil).

Harvesting the brain tissue

The brain tissue was harvested after behavioral tests. In brief, mice were anesthetized with CO_2 in a desiccator jar with low CO_2 pressure flow (Esmaeili-Mahani et al., 2013). After decapitation, whole brain tissues were rapidly removed and cut into along the middle. The left hemisphere was fixed in 10% formalin and embedded in paraffin for presence of *T. gondii* cysts in the hippocampus region. The right hemisphere was removed and preserved in pre-cooling preservation tubes, then frozen in liquid nitrogen and stored at –80 C for the investigation of cytokine expression.

TABLE I. Sequences of primers used for real-time PCR.

Amplicon	Primers	Sequence (5′–3′)	Size (bp)
IL1 β	F	AACCTGCTGGTGTGTGACGTTC	78
	R	CAGCACGAGGCTTTTTTGTTGT	
iNOs	F	CTGGTGAAGGAACGGGTCAG	120
	R	CCGATCATTGACGGCGAGAAT	
TNF-α	F	CCACCTGCAAGACCATCGAC	91
	R	CTGGCGAGCCTTAGTTTGGAC	
IFN-γ	F	ATGAACGCTACACACTGCATC	182
	R	CCATCCTTTTGCCAGTTCCTC	
β-actin	F	GTGACGTTGACATCCGTAAAGA	245
	R	GCCGGACTCATCGTACTCC	

Confirmation of presence of *T. gondii* cysts in the hippocampus region

To confirm the presence of *T. gondii* cysts in the hippocampus region, especially in the hippocampal CA1, the left hemispheres were examined after blocking and preparing cuts of 3 μm by microtome (Leitz, 1512 Labequip, Markham, Ontario) and stained with hematoxylin-eosin (H&E) to detect any *T. gondii* tissue cysts.

Analysis of mRNA expression by real-time PCR

Due to cytokines, signaling molecules of the immune system have been implicated as a contributing factor neuroinflammation and neurodegeneration mechanisms; the mRNA levels of of IL-1β, TNF-α, IFN-γ, and inducible nitric oxide synthase (iNOS) were examined in *T. gondii* infection, $A\beta_{1-42}$ (1 μl), *T. gondii* + $A\beta_{1-42}$ (1 μl), control, and sham groups by quantitative real-time PCR. Primer sequences used for IL-1β, TNF-α, IFN-γ, and iNOS are shown in Table I. Total RNAs from brain tissue samples were isolated using RNeasy kits (Qiagen, Hilden, Germany); all samples were reverse transcribed using the RT premix kit (Intron, Sungnam, Korea) according to the manufacturer's protocol. The resulting complementary DNA (cDNA) was subjected either to conventional PCR amplification or to real-time PCR. Real-time PCR was performed using the iQ5 real-time PCR detection system (Bio-Rad, Hercules, California), and SYBR green was used to detect amplification products, as described previously elsewhere (Ha et al., 2010). The reaction conditions used were initial denaturation at 95 C for 10 min, 40 amplification cycles (denaturation at 95 C for 10 sec, annealing at 56 C for 30 sec, and elongation at 72 C for 30 sec), followed by 1 cycle at 72 C for 5 min. Data analysis was performed using $iQ^{TM}5$ optical system software (Bio-Rad). For each gene, PCR reactions were carried out in duplicate. PCR results were normalized to the levels of β-actin genes as reference gene.

Statistical analysis

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

RESULTS

Spatial learning

Figure 1A shows that the distance traveled to reach the platform was significantly increased in the $A\beta_{1-42}$ (2 μl) ($P < 0.01$), Toxo ($P < 0.05$) and Toxo + $A\beta_{1-42}$ (1 μl) ($P < 0.01$), groups compared to the control and sham groups, indicating an impaired learning in $A\beta_{1-42}$ (2 μl), Toxo, and Toxo + $A\beta_{1-42}$ (1 μl) mice. Moreover, the results showed that the distance traveled to reach the platform in Toxo + $A\beta_{1-42}$ (1 μl) group was significantly ($P < 0.01$) higher than $A\beta_{1-42}$ (1 μl) group.

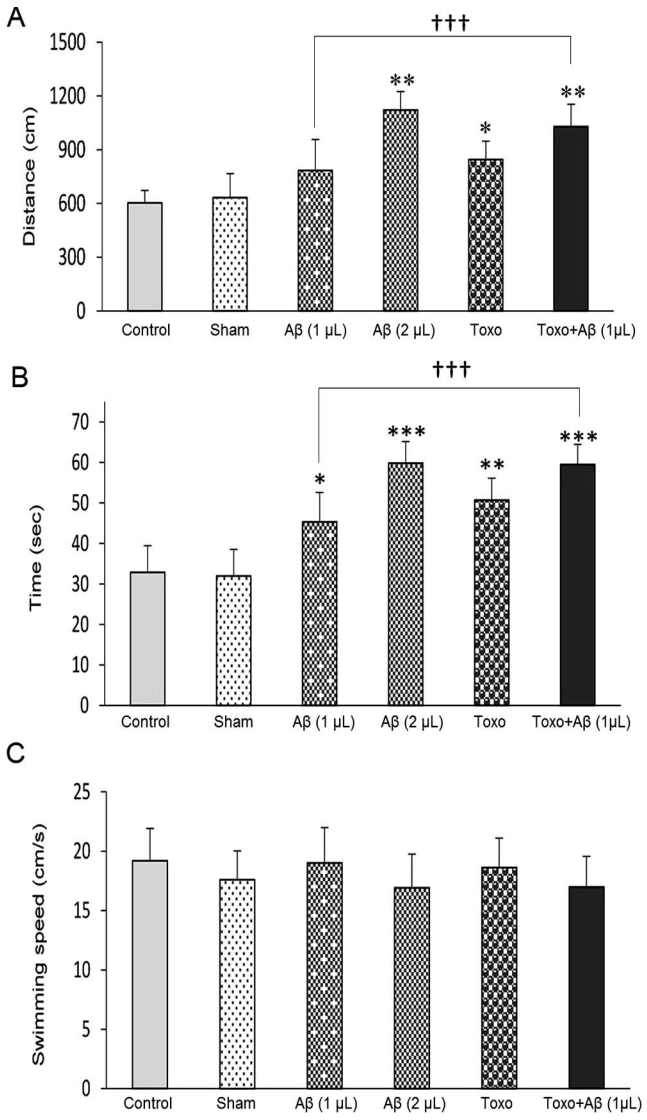

FIGURE 1. Impaired learning observed in the $A\beta_{1-42}$ (2 μl), Toxo, and Toxo + $A\beta_{1-42}$ (1 μl) groups compared to the other groups in Morris water maze task. Increased distance (**A**) to reach the hidden platform were observed in the $A\beta_{1-42}$ (2 μl), Toxo, and Toxo + AD (1 μl) groups in comparison with other groups; the time spent (**B**) to reach the hidden platform was also increased in the $A\beta_{1-42}$ (1 μl), $A\beta_{1-42}$ (2 μl), Toxo, and Toxo + $A\beta_{1-42}$ (1 μl), groups compared to the control and sham groups. There was no alteration in swimming speed of $A\beta_{1-42}$ (2 μl) and Toxo + $A\beta_{1-42}$ (1 μl) mice compared to the other groups (**C**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicating the significant differences with the control and sham groups. ††† $P < 0.001$, the difference was statistically significant.

Analysis of ANOVA demonstrated that the escape latency of Toxo ($P < 0.01$), $A\beta_{1-42}$ (2 μl) ($P < 0.001$), $A\beta_{1-42}$ (1 μl) ($P < 0.05$), and Toxo + $A\beta_{1-42}$ (1 μl) ($P < 0.001$) groups significantly increased in comparison to the control and sham groups (Fig. 1B). Furthermore, the findings revealed that the escape latency of the Toxo + $A\beta_{1-42}$ (1 μl) group was significantly ($P < 0.001$) higher than the $A\beta_{1-42}$ (1 μl) group. The obtained finding also showed no significant difference in the swimming speed among the all tested groups (Fig. 1C).

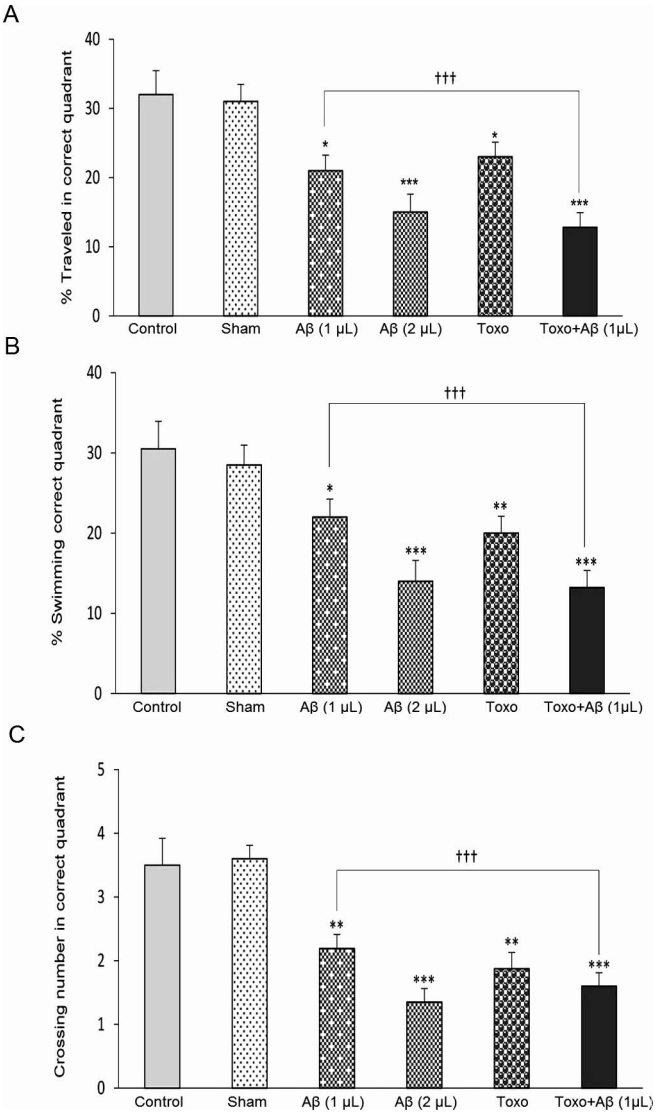

FIGURE 2. The effects of Alzheimer's disease and *Toxoplasma gondii* infection on spatial short-term memory. The distance (**A**) and time (**B**) in the target quadrant decreased significantly in the $A\beta_{1-42}$ (1 μl), $A\beta_{1-42}$ (2 μl), Toxo, and Toxo + $A\beta_{1-42}$ (1 μl), mice compared to the control and sham groups. The number of crossing from the platform region was also significantly decreased in the $A\beta_{1-42}$ (1 μl), $A\beta_{1-42}$ (2 μl), Toxo, and Toxo + $A\beta_{1-42}$ (1 μl) groups compared to the control and sham groups (**C**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicating the significant differences with the sham group. †† $P < 0.001$, ††† $P < 0.001$, the difference was statistically significant.

Spatial memory

In this study, to evaluate short-term spatial memory retention, 2 hr after the spatial learning phase, a probe test was carried out. The obtained results included the mean percentage (%) for time, distance (travel), and the number of crossings in the target quadrant. The findings revealed that the mice in the Toxo ($P < 0.05$ for time and $P < 0.01$ for distance in the target quadrant;), $A\beta_{1-42}$ (2 μl) ($P < 0.001$ for time and distance in the target quadrant), $A\beta_{1-42}$ (1 μl) ($P < 0.01$ for time and distance in the target quadrant), and Toxo + $A\beta_{1-42}$ (1 μl) ($P < 0.001$ for time and distance in the target quadrant) groups significantly spent less distance and time in the target quadrant compared to the control and sham groups (Figs. 2A, B), which indicates short-term memory impairment in these groups. Moreover, analysis of ANOVA demonstrated that the crossing number was significantly different in the Toxo ($P < 0.01$) $A\beta_{1-42}$ (2 μl) ($P < 0.001$), $A\beta_{1-42}$ (1 μl) ($P < 0.01$), and Toxo + $A\beta_{1-42}$ (1 μl) groups in comparison with the control and sham groups (Fig. 2C).

TABLE II. Comparisons of swimming speed and latency to escape onto the visible platform in a Morris water maze among groups using one-way analysis of variance (ANOVA) (the differences were not significant). Data are means ± S.E.M. (10 mice/group).

Group	Swimming speed (cm/s)	Escape latency (s)
Control	19.6 ± 2.51	20.3 ± 2.15
Sham	20.4 ± 3.6	18.8 ± 2.4
$A\beta_{1-42}$ (1 μl)	18.8 ± 2.19	19.4 ± 3.11
$A\beta_{1-42}$ (2 μl)	17.1 ± 1.3	21.2 ± 2.81
Toxo	19.4 ± 1.8	20.1 ± 3.6
Toxo + $A\beta_{1-42}$ (1μl)	17.7 ± 1.6	20.7 ± 2.7

Latency to visible platform and swimming speed

Data analysis demonstrated that escape latency to find the visible platform was 20.3, 18.8, 19.4, 21.2, 20.1, and 20.7 sec for control, sham, $A\beta_{1-42}$ (1 μl), $A\beta_{1-42}$ (2 μl), Toxo, and Toxo+ $A\beta_{1-42}$ (1 μl) group, respectively, where swimming speed was 19.6, 20.4 18.8, 17.1, 19.4, and 17.7 cm/sec for control, Sham, $A\beta_{1-42}$ (1 μl), $A\beta_{1-42}$ (2 μl), Toxo, and Toxo+ $A\beta_{1-42}$ (1 μl) group, respectively. The findings revealed that mice in all groups had a similar escape latency and swimming speed in the MWM test (Table II), which indicates no significant differences between the groups in visual and motor functions.

Confirmation of presence of *T. gondii* cysts in the hippocampus region

Since the hippocampus is one of the main brain structures connected with natural behaviors and learning and memory processing, the presence of *T. gondii* cysts in the hippocampus region, especially in the hippocampal CA1, was examined. Figure 3 confirms the presence of *T. gondii* cysts in the brain and hippocampal CA1 after blocking and preparing cuts of 3 μm of the left hemispheres stained with H&E.

Analysis of mRNA expression

The mRNA levels of IL-1β, TNF-α, IFN-γ, and iNOS were examined in *T. gondii* infection, $A\beta_{1-42}$ (1 μl), *T. gondii* + $A\beta_{1-42}$ (1 μl), control, and sham mice by quantitative real-time PCR. Comparison of the results demonstrated that mRNA levels of IL-1β, TNF-α, IFN-γ, and also iNOS significantly ($P < 0.001$) increased in *T. gondii* + $A\beta_{1-42}$ (1 μl) in comparison with the control and sham groups (Fig. 4). Moreover, the obtained findings revealed that mRNA levels of all the above cytokines and iNOS significantly ($P < 0.001$) increased in *T. gondii* + $A\beta_{1-42}$ (1 μl) compared with the $A\beta_{1-42}$ (1 μl) group (Fig. 4).

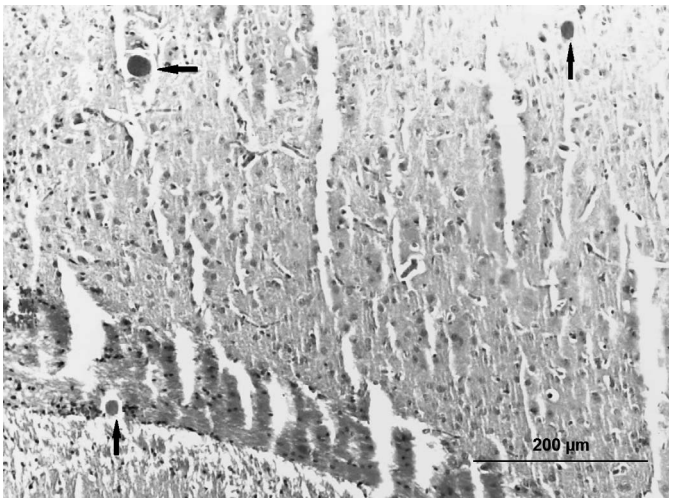

FIGURE 3. Tissue cysts of *Toxoplasma gondii* Tehran strain in the hippocampus region, especially in the hippocampal CA1 of infected mice (×10) using H&E staining.

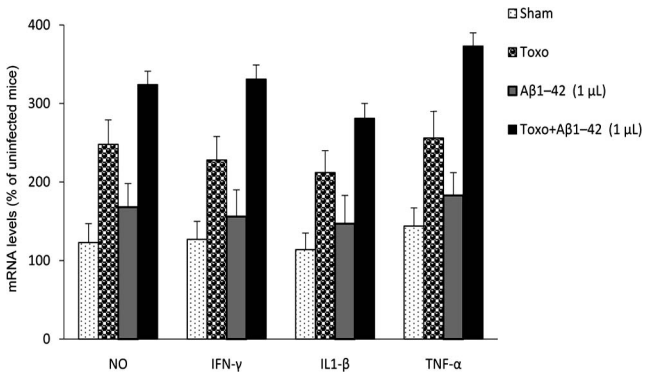

FIGURE 4. The mRNA expressions of some cytokines and also iNOS in *Toxoplasma gondii*, Toxo + $A\beta_{1-42}$ (1 µl), and sham groups in comparison to the uninfected BALB/c mice. mRNA levels are presented as percentages of cytokine levels in tested groups versus uninfected mice.

DISCUSSION

Toxoplasma gondii is a common, global protozoan parasite that has infected approximately one-third of the world's human population (Hill and Dubey, 2002). Epidemiological evidence in humans and experimental studies in rodents have revealed a number of neurological and behavioral disorders such as learning and memory impairment following the establishment of chronic toxoplasmosis (Dalimi et al., 2012). Moreover, associations have been observed between *T. gondii* seroprevalence and some neurodegenerative disorders such as AD, schizophrenia, bipolar, and anxiety disorders (Fekadu et al., 2010; Torrey et al., 2012; Mahmoudvand et al., 2015b).

Recently, Yilmaz et al. (2011) reported that the seropositivity rates for anti–*T. gondii* IgG antibodies among AD patients were significantly higher than a control group, which indicate that *Toxoplasma* infection may be involved in the pathogenetic mechanisms of AD. Thus, it is crucial to examine whether *T. gondii* infection is involved in the neuroinflammation and cognitive mechanisms of AD. To address these questions, the effect of *T. gondii* infection on the progression of AD in BALB/c mice was evaluated using immunological and behavioral tests.

In the present study and according to the obtained findings in behavioral experiments (Morris water maze) we found that *T. gondii* infection caused AD-like symptoms and impaired learning and memory functions in the infected BALB/c mice. Consistent with our results, Zhou et al. (2011) have reported that chronic toxoplasmosis induced by the *T. gondii* Prugniaud strain impaired learning and memory functions in Kunming mice. 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.

To address the effect of *T. gondii* infection on the progression of AD in BALB/c mice, we established an animal model AD using hippocampal injection of $A\beta_{1-42}$ (2 µl, 2 µg/µl) according to the method described by Liu et al. (2014), whereas injection of $A\beta_{1-42}$ at the sub-dose of 1 µl (2 µg/µ;) revealed nearly 50% of cognitive impairments in BALB/c mice. We found that in Toxo + $A\beta_{1-42}$ (1 µl) group, *T. gondii* infection could potentiate AD in infected mice receiving a sub-dose of $A\beta_{1-42}$ and caused considerable impairment in learning and memory functions similar to the AD group. In contrast to our findings, Jung et al. (2013) have demonstrated that *T. gondii* infection in the brain inhibits neuronal degeneration and learning and memory impairments in a mice model of AD. This difference in the reported impacts of *T. gondii* infection on AD and cognitive functions can be attributed to the type of establishment of AD, rodent species, route of infection, parasite strain, and dosage (Haroon et al., 2012; Worth et al., 2013). It is well known that changes in behavior observed during chronic *T. gondii* infection can be the consequence of a range of indirect and/or direct effects. Whereas indirect effects may involve immune response to infection (Novotná et al., 2015), direct effects are likely to include the presence of the parasite in the brain or parasite-elicited effects or products (Webster, 2007). Since the hippocampus is one of the main brain structures connected with natural behaviors and learning and memory processing we evaluated the *T. gondii* cysts present in this brain region of infected mice. In the present study, the parasite cysts were found in the hippocampus, especially hippocampal CA1 regions. Similarly, Gatkowska (2012) reported the presence of parasite cysts in both the hippocampus and the amygdala regions of *T. gondii*–infected mice. This anatomical analysis indicated *T. gondii* cysts among main anatomical regions of brain could directly affect neuronal function and thus explain neuropsychological deficits.

Regarding indirect effects, it has been previously proven that parasites evoke innate and TH_1 adaptive immune responses in the CNS, where the expression of inflammatory cytokines alters to keep *T. gondii* dormant, which could then subsequently influence neuromodulator levels and host behavior (Liesenfeld et al., 2011; Munoz et al., 2011). While this alteration in cytokines levels is 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 (Saito et al., 1991; Dunn, 2006; McCusker and Kelley, 2013). Here the results of analysis of mRNA expression by quantitative real-time PCR showed that the mRNA levels of IL-1β, TNF-α, IFN-γ, and iNOS significantly increased in infected mice in comparison with the

uninfected BALB/c ones, which indicates higher expression of these cytokines and mediators of inflammatory after stimulation with infection. Interestingly, the mRNA levels of IL-1β, TNF-α, IFN-γ, and iNOS in the Toxo + $A\beta_{1-42}$ (1 μl) group was much more than *T. gondii* infection, which indicates that intrahippocampal injection $A\beta_{1-42}$ exacerbates mRNA expression in *T. gondii*–infected mice. Similarly, Liu et al. (2014) have shown that hippocampal injection of Aβ peptides significantly increased expression levels of IL-1β and TNF-α in BALB/c mice. It has been reported previously that inflammatory mediators including cytokines, complement components, various free radicals, and particularly NO can stimulate amyloid precursor protein processing by various means and therefore can create a vicious cycle that could be essential in the pathological progression of AD. Therefore, we could suggest that chronic *T. gondii* infection communication among immune cells promotes neuroinflammation through cytokine networks and induces progression of AD in the mice brain.

CONCLUSION

The obtained results showed that chronic *T. gondii* infection communication among immune cells promotes neuroinflammation through cytokine networks and induces pathological progression of AD in the mice brain, whereas anatomical *Toxoplasma* tissue cysts presence in the brain could also affect the behavioral functions in *T. gondii*–infected mice. Therefore, the present findings suggest that *T. gondii* infection induces pathological progression of AD in the mice brain via both indirect and direct effects.

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