

Original papers

Acute toxoplasmosis and antioxidant levels in the liver, kidney and brain of rats

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ABSTRACT. *Toxoplasma gondii* is a common protozoan parasite which causes toxoplasmosis worldwide. There are limited treatment options against *T. gondii* infection. Once transmitted, *T. gondii* can spread to many organs in the body, including the brain, liver and kidneys. One of the most common signs of toxoplasmosis is a rise in oxidative stress. Therefore, our aim was to determine the antioxidant levels in the brain, liver and kidney of rats infected with this parasite. In the present study, 2–4-months old Wistar albino rats were infected intraperitoneally with 1×10^4 mL of RH strain of *T. gondii* dispersed in 0.9% NaCl. Post-infection after 30 days, the experiment was terminated, the rats were sacrificed, and the blood, brain, liver and kidney tissues were collected for analyses. Catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) levels were determined by ELISA assay. Increased SOD and GSH-Px levels were found in the liver of infected rats compared to controls; however, similar changes were not observed in other tested organs. These results suggest the increased oxidative stress caused by *T. gondii* infection can be efficiently alleviated, at least in the liver, by increased levels of antioxidant enzymes during post-infection. Further research will be required to determine the potential mechanisms of increasing antioxidant levels in the liver at 30 days post-infection, as well as the potential differences in antioxidant enzyme levels during the acute and chronic phases of toxoplasmosis.

Key words: *Toxoplasma gondii*, CAT, SOD, GSH-Px, acute infection

Introduction

Toxoplasma gondii (*T. gondii*) is the intracellular parasite responsible for toxoplasmosis, which leads to many clinical outcomes, especially in immunocompromised individuals [1]. *T. gondii* is divided into three groups based on virulence: high virulence (type I), intermediate virulence (type II) and low virulence (type III) [2]. Of these categories, type I is the most mobile and able to penetrate the blood-brain barrier, which makes it the most lethal form [3,4]. Even though the reproductive life cycle

of *T. gondii* depends solely on felines, it can infect numerous hosts, including humans [5].

The most common form of infection occurs through the consumption of raw or undercooked food or contaminated water containing *Toxoplasma* tissue cysts or oocysts [6, 7]. Even a few hours after oral ingestion, the parasite is able to cross gastrointestinal barriers and penetrate the submucosal tissues of the small intestine [8]. In immunologically healthy individuals, *T. gondii* can be cleared from the body efficiently. However, a small portion of the parasite differentiates into bradyzoites that can

cause the formation of tissue cysts and pathogenesis [9]. After successful entry into the circulation, the parasite can travel to many organs including, but not limited to, the brain, kidneys, liver and retina [6]. In addition, fetal damage and miscarriage can also occur through transplacental *T. gondii* infection [7]; more generally, infection also leads to neurodegenerative diseases [10], epilepsy [11], behavioral alterations [12] and organ damage [13].

Recent studies indicate replication of *T. gondii* is reliant on the carbon and nutrient sources of the host organism [9]. To enable its own intracellular growth, the parasite interrupts the mitochondrial pathway of the host and metabolizes glucose for its own energy production [9]. Moreover, increased oxidative stress and free radical production have been previously reported during the acute period following *T. gondii* infection in the liver, serum and brain of rodent models [14–16]. Altered biochemical parameters indicative of liver damage have been observed in mice in response to acute *T. gondii* infection, such as lipid peroxidation (LPO), and alterations of glutathione (GSH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels [17].

One of the defense mechanisms employed by the body against parasites and other infectious agents is the release of antioxidant enzymes. In eukaryotes, GSH metabolism plays an important role in counteracting the oxidative damage caused by the infectious agents [18]. An increase in GSH levels has been observed in malaria-infected erythrocytes [19,20]. Parasitic infections causes lipid peroxidation in many organs, including the liver and kidney, which leads to production of hydrogen peroxide and oxygen [21]. One of the antioxidant enzyme that prevents the rise of superoxide radicals is SOD, and it has been shown to be active in the liver tissues of sheep infected with fasciola parasites [21]. In addition, catalase is also involved in the first line of defense against oxidants [22]. The role of catalase in parasitic infections has also been shown in previously published studies [23,24].

In the literature, although much attention has been focused on the early period of the acute phase of *T. gondii* infection, less is known about *T. gondii* infection later in the acute phase. In the case of human exposure, long-term *T. gondii* infection can result in the development of chronic conditions, including epilepsy, behavioral alterations and even organ failure. Therefore, the aim of the current study is to investigate the impact of the later stages of

acute *T. gondii* infection on certain antioxidant parameters in the brain, liver and kidney of rats.

Following an infection, the immune response triggers specific mechanisms to eliminate the pathogen. Although some intracellular microorganisms, such as *T. gondii*, have developed survival mechanisms, components of the immune system release reactive oxygen species (ROS) as an oxidative burst. In many cases, this ROS production harms various tissues of the host organism, and antioxidant enzymes are produced as a defense mechanism against these molecules. As antioxidative action can be observed in the different tissues and organs of the host organism with the spread of an infectious agent throughout the body, careful evaluation of the enzyme levels in the organs of host organism could improve the diagnosis of infection and allow localization of the parasite. Likewise, such an analysis could also assist in understanding the nature of the immune response to toxoplasmosis.

The present study measures the levels of three antioxidant enzymes in different tissues of rats infected with *T. gondii* RH strain. We hypothesize that increased levels of antioxidant enzymes could be an indicator for detecting the spread of parasitic infection in the host organism.

Materials and Methods

Animal housing. Two to four months old male Wistar albino rats were used. All animals in the current study were obtained from the Abant İzzet Baysal University Animal Research Laboratory colony. Animals were group housed together in 12-hour light:12-hour dark conditions at $22\pm 1^\circ\text{C}$ (room temperature) with food and water provided *ad libitum*. All experimental procedures were approved by the Abant İzzet Baysal University Institutional Animal Care and Use Committee.

Experimental design. Animals were randomly assigned into *T. gondii* infection and control groups ($n=5$ per *T. gondii* infected and control groups). The animals were intraperitoneally (ip) injected with $1\times 10^4/\text{ml}$ of RH strain tachyzoites dispersed in 1 ml 0.9% NaCl or 1 ml of vehicle (0.9% NaCl); the tachyzoites were obtained from routine maintenance passages for Sabin-Feldman dye test in National Public Health Management of Turkey. Thirty days after infection, the experiment was terminated by anesthetizing the animals with ether. The liver, kidney and brain were collected. Half of the tissue samples were used for immunohistochemical staining

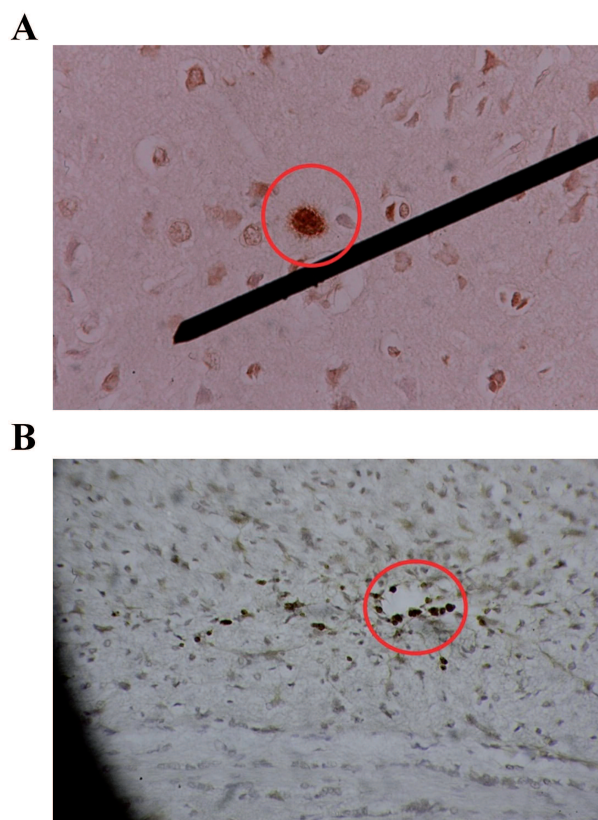


Fig. 1. Representative immunohistochemistry images of brain tissues of *Toxoplasma gondii* infected animals after 30 days post-infection at (A) 1000 \times magnification and (B) 400 \times magnification. Red circled brown dots shows tachyzoites of parasites.

using IHC: Abcam anti-*Toxoplasma gondii* antibody, Cat No: Ab138698 (Abcam, Cambridge, UK). The representative IHC images show successful infection of animals with *T. gondii* in the experimental group (Fig. 1 A and B). The other half of the tissue samples were stored at -80°C until analyzed.

ELISA assay. Catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) levels were measured using commercially-available rat enzyme-linked immunosorbent assay (ELISA) kits as described previously [25]. Tissue samples were homogenized in phosphate buffer (1:1 ratio) and centrifuged at 4000 g for 20 minutes. The supernatant was collected. All ELISA assays were performed according to the manufacturer's instructions (SunRed Biotechnology, Shanghai, China). Samples were measured at 450 nm wavelength using a plate reader (Thermo Fisher Scientific, MA, USA).

Statistical analyses. The differences between CAT, GSH-Px and SOD levels in brain, kidney, and liver were analyzed using one-way ANOVA with Proc GLM in SAS (version 9.2, SAS Institute, Cary,

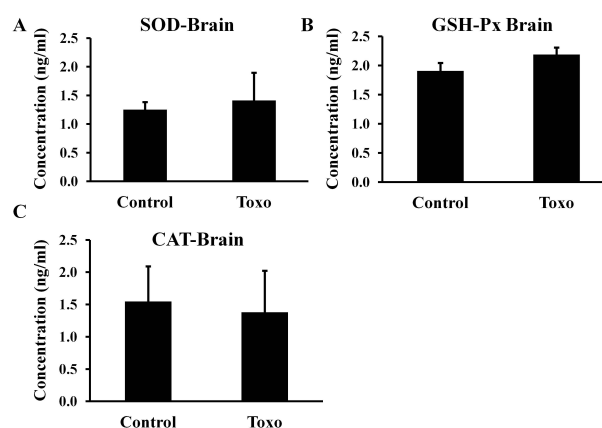


Fig. 2. Antioxidant levels in the brain of rats. (A) SOD, (B) GSH-Px and (C) CAT levels were determined after 30 days post-infection with *Toxoplasma gondii*. No significant difference was found in *T. gondii* infected versus control groups ($p > 0.05$; $n = 5$ per *T. gondii* infected and control groups). Data are presented as mean \pm SEM.

NC, USA). When there was a significant difference, Dunnett's multiple comparison test was applied to determine the differences between the animals infected with *T. gondii* versus controls. $P < 0.05$ was considered statistically significant. All data are presented as mean \pm standard error of the mean (SEM).

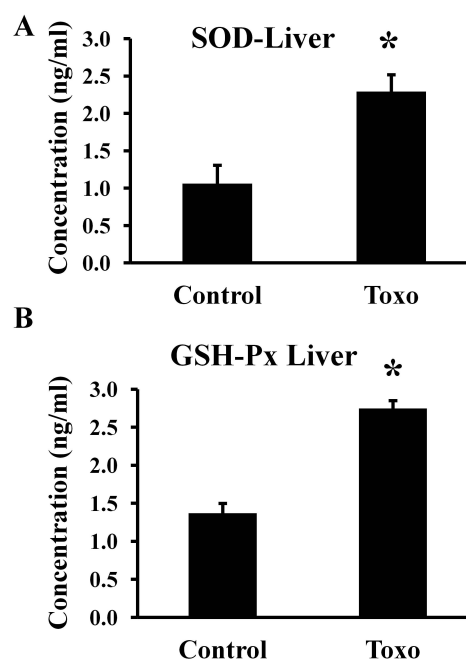


Fig. 3. Antioxidant levels in the liver of rats. (A) SOD and (B) GSH-Px levels were determined after 30 days post-infection with *Toxoplasma gondii*. SOD and GSH-Px levels were significantly higher in the *T. gondii* infected groups compared to controls ($p < 0.05$; $n = 5$ per *T. gondii* infected and control groups). Data are presented as mean \pm SEM.

Results

CAT, GSH-Px and SOD levels in brain

No statistically significant difference was observed between infected and control animals (Fig.2 A-C; $p>0.05$; $n=5$ per *T. gondii* infected and control groups).

CAT, GSH-Px and SOD levels in liver

SOD and GSH-Px levels were significantly elevated in *T. gondii* infected groups compared to controls (Fig.3 A and B; $p=0.0089$ for SOD and $p<0.0001$ for GSH-Px; $n=5$ per *T. gondii* infected and control groups). CAT analyses in liver were excluded from the experiment due to testing errors.

CAT, GSH-Px and SOD levels in kidney

No significant difference was observed between controls and *T. gondii* infected animals (Fig.4 A-C; $p>0.05$; $n=5$ per *T. gondii* treated and control groups).

Comparing GSH-Px and SOD levels between brain, liver and kidney of toxoplasma infected animals

In infected animals, SOD levels were similar between the brain, liver and kidney (Fig. 5 A $p>0.05$; $n=5$); however, GSH-Px levels were significantly elevated in the liver compared to the brain and kidney (Fig. 4 B; $p=0.0030$; $n=5$).

Discussion

The aim of the present study was to determine

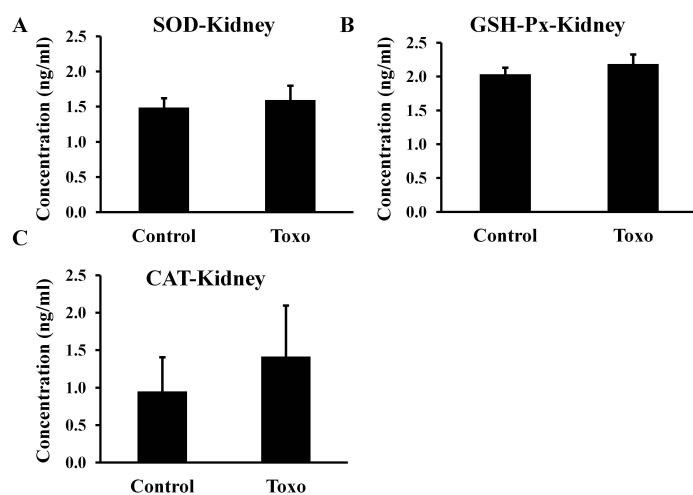


Fig. 4. Antioxidant levels in the kidney of rats. (A) SOD, (B) GSH-Px and (C) CAT levels were determined after 30 days post-infection with *Toxoplasma gondii*. No significant difference was found in *T. gondii* infected versus control groups ($p>0.05$; $n=5$ per *T. gondii* infected and control groups). Data are presented as mean \pm SEM.

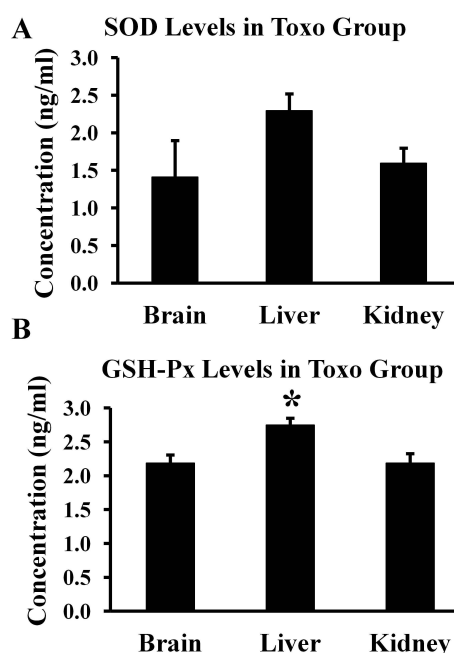


Fig. 5. Comparison of antioxidant levels in the brain, liver and kidney of toxoplasma infected rats. (A) SOD and (B) GSH-Px were determined after 30 days post-infection with *Toxoplasma gondii*. SOD levels were similar between brain, liver and kidney of toxoplasma infected animals ($p>0.05$; $n=5$). GSH-Px levels were significantly higher in the liver compared to brain and kidney of *T. gondii* infected animals ($p=0.0030$; $n=5$). Data are presented as mean \pm SEM.

the antioxidant levels in the organs of rats infected with *T. gondii*. Most previous studies have focused on the impact of *T. gondii* infection early in the acute phase. For instance, it has been previously reported that blood GPX activity and GSH levels in rats are elevated seven days post-infection [14]. Likewise, elevated malondialdehyde (MDA) and reduced glutathione (GSH) levels have also been shown in mice four days post-infection [15], as have elevated levels of tissue MDA in mice [26]. In one study, no significant difference in liver glutathione levels was found between *T. gondii* infected mice four days post-infection and controls [17]. However, less is known about the impact of acute *T. gondii* infection in the weeks following infection. Our present results demonstrate increased antioxidant levels (SOD and GSH-Px) in the liver of *T. gondii* infected rats at 30 days post-infection.

In the literature, parasite infections other than *T. gondii* have been found to cause elevated antioxidant levels in rat models several weeks after post-infection. For instance, rats infected with *Fasciola hepatica* displayed elevated hepatic SOD activity 87 days post-infection [27]. Likewise, the

levels of blood GPx and SOD activity in rats were found to be increased eight weeks post-infection with *Trichinella spiralis* [28]. Similar to rats, increased antioxidant levels were reported for mice models in the later stages of acute parasite infection. In a study with mice infected with *Trypanosoma cruzi*, levels of glutathione-S-transferase and SOD were elevated compared to controls 15 days post-infection [29]. Elevated CAT activity in the liver and SOD activity in the kidney have also been observed in mice 45 days post-infection with *Schistosoma mansoni* [30]. However, to our knowledge, no such data exists regarding the antioxidant levels at the later stages of acute *T. gondii* infection.

The increased antioxidant levels in the liver of treated rats observed in the present study could be caused by the presence of parasitic cysts in the other organs. These cysts could increase the levels of inflammatory infiltrates passing throughout the liver. However, an alternative mechanism might also exist independent of the damage in other organs. Increased oxidative stress in the liver caused directly by the parasite infection could also increase antioxidant levels. Recent studies in the literature also demonstrate that *T. gondii*-derived enzymes trigger the immune system to fight *T. gondii* infection [31]. From this perspective, together with the observed elevation of antioxidant enzymes in the liver, it can be promising to use *T. gondii* infection, at least later on in the acute phase, as a potential trigger for the immune defense system, or similar bodily processes.

In conclusion, our results demonstrate increased antioxidant (SOD and GSH-Px) levels in the liver of rats 30 days post-infection with *T. gondii*. However, similar results for the elevation of antioxidant enzymes were not observed in other organs of tested animals. Future studies will be required in order to determine the potential mechanisms of acute *T. gondii* infection at later stages which cause elevated antioxidant enzymes in the liver. Moreover, the increased antioxidant levels persisting later on in the acute phase, at least in the liver, can be used to diagnose *T. gondii* infection. This may be helpful, especially in complex cases where the diagnosis of *T. gondii* infection is difficult earlier on in the acute phase.

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