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Original Article

Toxoplasma gondii: A Possible Inducer of Oxidative Stress in Reproductive System of Male Rats

Ziba HOSEINY ASL NAZARLU¹, Mohammad MATINI¹, Maryam BAHMANZADEH^{2,3},
*Faeze FOROUGHI-PARVAR¹

1. Department of Medical Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2. Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
3. Department of Anatomical Sciences, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

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*Correspondence

Email:
f.foroughi@umsha.ac.ir

Abstract

Background: Toxoplasmosis is suspected to have adverse effects on the male reproductive system. We aimed to determine the possible role of *Toxoplasma gondii* in oxidative stress in reproductive system of male rats.

Methods: This study was performed from 2018 until 2019 at the Parasitology Research Laboratory of Hamadan University of Medical Sciences, Hamadan, Iran. Eighty male Wistar rats were randomly divided to control and test groups. The animals in the test group were inoculated by 10⁷ *T. gondii* RH strain tachyzoites and the control group were injected by 0.2 ml of phosphate-buffered saline. The both groups were following every 10 days until day 80 post inoculation. Oxidative stress markers (OSMs) including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and non-enzymatic markers including malondialdehyde (MDA), reduced glutathione (GSH) and total antioxidant capacity (TAC) were assessed in testis and serum of *T. gondii* infected rats.

Results: After post inoculation, the variations of the OSMs in the testis tissue of infected rats were as follows: a significant decrease of SOD on day 80 ($P=0.03$), and CAT activity were detected on day 60 and 80 ($P=0.04$ and $P=0.01$) respectively. In addition, GSH ($P=0.01$) and TAC ($P=0.03$) concentration were significantly reduced on day 80. On the contrary, the concentration of MDA ($P=0.01$) was increased 70 days after infection. In addition, consistent changes with the tissue testis were observed in the serum OSMs of infected rats.

Conclusion: *T. gondii* infection caused oxidative stress in testis tissue. Thus, the adverse effects of oxidative stress may affect the male rat reproductive system.



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Introduction

Itoxoplasmosis, widely prevalent parasitic disease, caused by *Toxoplasma gondii*. The Zoonotic protozoan parasite involves most of birds and mammals including humans (1). *T. gondii* as an obligate intracellular parasite could be transmitted from feline species as well as domestic cats to human (2). The final hosts excrete so many oocysts in feces. Mammals including human are infected through ingestion of tissue cysts in raw or undercooked meat or oocyst-contaminated water. The infection in human principally is subclinical but in the patients with impaired immune system causes life-threatening encephalitis (3). Moreover, transplacental transmission of *T. gondii* tachyzoites in maternal infection leads to congenital toxoplasmosis in fetus (4). About 50% of infertility cases are caused by male reproductive system disorders (5, 6). Reproductive function in both male and female could be interfered by infectious agents such as bacteria, parasites, viruses, and fungi (7).

Reports indicated the significant prevalence of *T. gondii* in infertile couples and men with sterility, as well (8-10). Despite the importance of toxoplasmosis in the male genital system, few studies investigated the effects of toxoplasmosis on the male reproductive parameters (8). Congenital toxoplasmosis and abortion due to experimentally inoculation of *T. gondii* infected semen were reported by recent studies (11). It seems that *T. gondii* triggered the oxidative stress reactions in testis through activation of immune system (12). Importantly, during *T. gondii* infection, reactive oxygen species (ROS) increased in testis and serum of infected animal models (13).

Reactive oxygen metabolites are essential for capacitation of differentiated cells involved in apoptotic process of these cells. The imbalance between free radicals production and reactive metabolites is resulting oxidative stress. Therefore, free radicals as double-edged sword, despite their crucial role in cellular

processes, can also damage cells and interfere with essential metabolic processes (14). Also many researchers suggested that sperm cell dysfunction caused by oxidative stress can be a main contributor to the idiopathic male infertility (5, 15).

Thus, we aimed to determine the stress oxidative status related to toxoplasmosis in serum and testis of experimentally infected male rats.

Materials and Methods

Animals and infection

Eighty male Wistar rats weighing 206 to 275 gr, obtained from Animal center, Hamadan University of Medical Sciences, Hamadan, Iran and housed in standard conditions, at relative humidity ($50 \pm 10\%$) with a controlled 12h:12h light–dark cycle and ambient temperature of 23 °C.

All details were under surveillance of Animal Care and Use Committee of Hamadan University of Medical Sciences, Ethics Code: REC. 1396.125.IR.UMSHA.

T. gondii (RH strain) tachyzoites were achieved through every 4-d peritoneal cavity serial passage of previously infected Swiss Albino mice in Parasitology Research Laboratory, Hamadan University of Medical Sciences, Hamadan, Iran (a gift from Dr. S. Shojaee, School of Public Health, Tehran University of Medical Sciences). Harvested fluid was filtered (0.22 μm Millipore, Sigma-Aldrich Corp) to remove the peritoneal cells and debris, then tachyzoites were counted using a haemocytometer. Adult male Wistar rats divided randomly into two groups: healthy control and *Toxoplasma* infected group. The infected group were intraperitoneally inoculated by 10^7 tachyzoites in sterile phosphate-buffered saline solution. The rats of the control group were inoculated with PBS at the same volume (16). All experimental rats were examined to detect the infection using commercial IgG

ELISA kit, ZellBio (ZB-Q16312S-R9648). Five rats were anesthetized and sacrificed according to ethical considerations every 10 d interval from 10 to 80 post-inoculation days. The blood samples of rats were taken from the inferior vena cava and their testis were removed instantly.

Oxidative stress markers

Determination of lipid peroxidation levels

The level of malondialdehyde (MDA), as a lipid peroxidation product, was determined using commercial colorimetric assay Kit (Kiazist, Iran). In this method the thiobarbituric acid (TBA) reactive substances (TBARS) quantity were detected as a MDA production index in tissue and serum samples based on nmol per ml or mg of tissue. Briefly, according to manufacturer, protocol (Kiazist Co.) 1 mL of TBA-trichloroacetic acid (TCA) solution was added to 0.5 mL of each specimen in test tube and then it was placed in a boiling water bath for 15 min. After cooling, tubes were centrifuged at 1000 g for 10 min and the absorbance of supernatant was measured at 532 nm (nmol/ml or mg).

Determination of total antioxidant capacity (TAC)

Commercial kit (Kiazist, Iran) used to assess the TAC level in serum and tissue samples according to protocol provided by manufacturer. Based on this protocol, the FRAP reagent reduced to produce ferrous tripyridyltriazine with an increase in color which is detectable at 405 nm. Increased absorbance of the samples corresponded the reducing power of total antioxidant capacity of samples. Through this method, TAC could be determined with the sensitivity of 0.1 mM (100 μ mol/L or mg) (17).

Determination of Glutathione Levels (GSH)

GSH were determined through colorimetric method using Ellman's reagent the 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) accord-

ing to protocol provided by a commercial kit (Kiazist, Iran). Briefly, DTNB reacted with reduced form of thiol (-SH) groups and makes complex. The absorbance was read at 405 nm to estimate the GSH levels. The assay sensitivity was 0.1 mM and intra- and inter-assay coefficient of variation were 6.1% and 7.7%, respectively (18).

Determination of Superoxide Dismutase (SOD) enzyme activity

SOD activity in serum and tissue samples, as an important antioxidant enzyme, was recorded according to commercial kit (Kiazist, Iran). In brief, after reduction of 2-(4-iodophenol)-3-(4-nitrophenol)-5-phenyltetrazolium chloride by xanthine oxidase (XO), a red formazan product was produced. This reduction was inhibited by SOD and the produced colored complex absorbance was quantified at 570 nm. The SOD activity was detected by the degree of inhibition of this reaction. The results were expressed as units per milliliter (EU/mL) for serum and units per milligram-tissue (nmol/mg-tissue) for tissue (19).

Determination of CAT enzyme activity

The assay principles were based on the reaction of catalase to decompose hydrogen dioxide. According to the kit instructions (Kiazist, Iran), samples were mixed with buffer assay (hydrogen peroxide) and the reduction of absorbance as a result of hydrogen peroxidase elimination were recorded at 240 nm (20).

Statistical analysis

The statistical analysis of collected data was carried out using SPSS (Chicago, IL, USA) software version 16. The values were expressed as mean \pm SD, and the *P* values less than 0.05 considered as statistically significant. The one-way ANOVA and the Mann-Whitney tests were employed to analyze the difference between groups. Further analysis performed using Tukey's test.

Results

Evaluation of lipid peroxidation level

MDA concentrations in testis tissue and serum are shown in Fig. 1. MDA level of testis

tissue was significantly elevated on days 70 ($P=0.01$) and 80 ($P=0.02$) post infection (PI), while the MDA level in serum was significantly increased on days 60 ($P=0.03$), 70 ($P=0.001$) and 80 ($P=0.001$) PI.

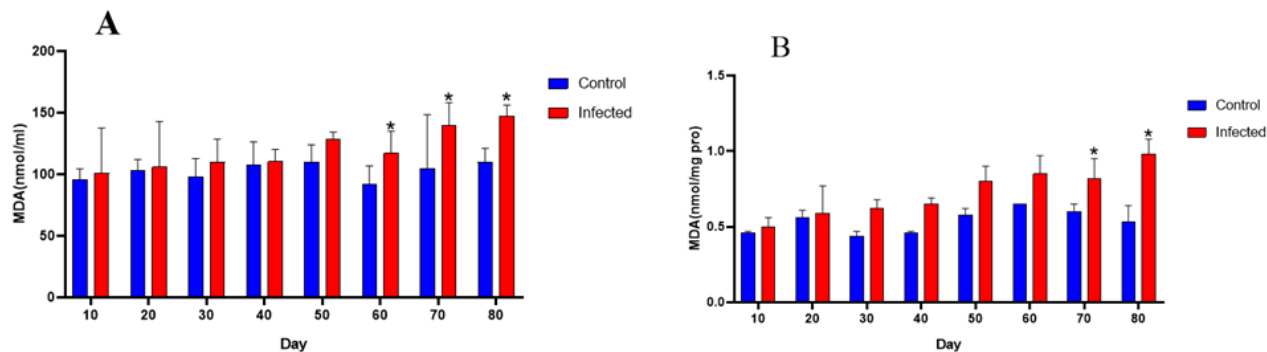


Fig. 1: MDA level in serum (A) and testis tissue (B) in control and *T.gondii*-infected rats. Results are presented as Mean \pm SD; * $P < 0.05$, control versus infected group

Evaluation of total antioxidant capacity (TAC)

The TAC level was remarkably decreased in the testis on days 70 and 80 PI ($P=0.04$ and

0.03 on days 70 and 80 respectively). Remarkable decrease in the level of TAC was detected in the serum, on day 80 PI ($P=0.03$) (Fig. 2).

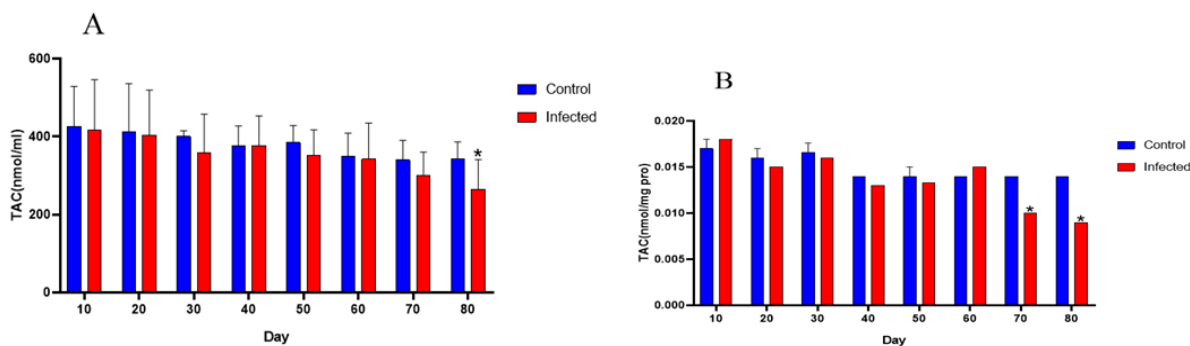


Fig. 2: The TAC level in serum (A) and testis tissue (B) in control and *T.gondii*-infected rats. Results are presented as Mean \pm SD; * $P < 0.05$, control versus infected group

Evaluation of Glutathione (GSH) level

The GSH level was considerably reduced in both serum and testis tissue in the infected rats on day 80 in comparison with control

group ($P=0.01$). Further analysis showed the serum GSH level was remarkably reduced on day 80 PI in infected rats when compared with control ones ($P= 0.02$) (Fig. 3).

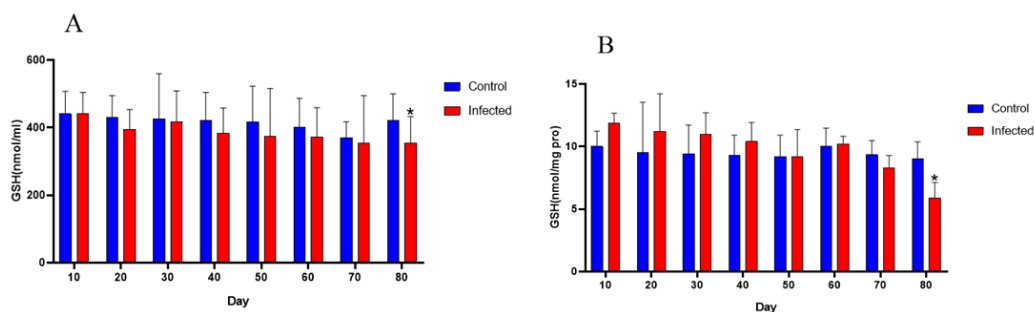


Fig. 3: The GSH level in serum (A) and testis tissue (B) in control and *T.gondii*-infected rats. Results are presented as Mean \pm SD; * $P < 0.05$, control versus infected group

Evaluation of the antioxidant enzymes activity

According to our data, comparing of the activity of SOD in infected and uninfected rats

showed that the SOD activity of both serum ($P=0.04$) and testis tissue ($P=0.03$) were significantly reduced on day 80 PI (Fig. 4).

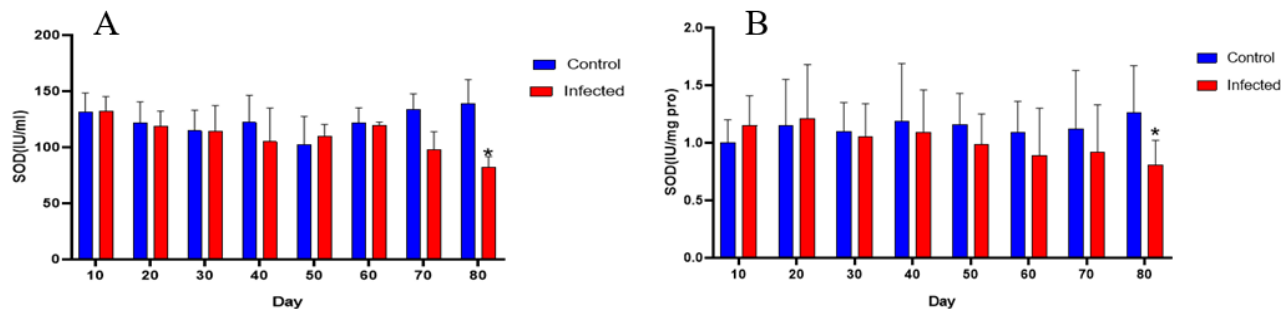


Fig. 4: The SOD level in serum (A) and testis tissue (B) in control and *T.gondii*-infected rats. Results are presented as Mean \pm SD; * $P < 0.05$, control versus infected group

Evaluation of the catalase activity

Reduction of the activity of CAT in the testis of the infected rats compared to uninfected rats was significant on days 60 and 80 PI

($P=0.04$ and 0.01 on d 60 and 80 respectively), while the serum CAT activity was significantly reduced in infected rats on day 80 PI ($P=0.04$) (Fig. 5).

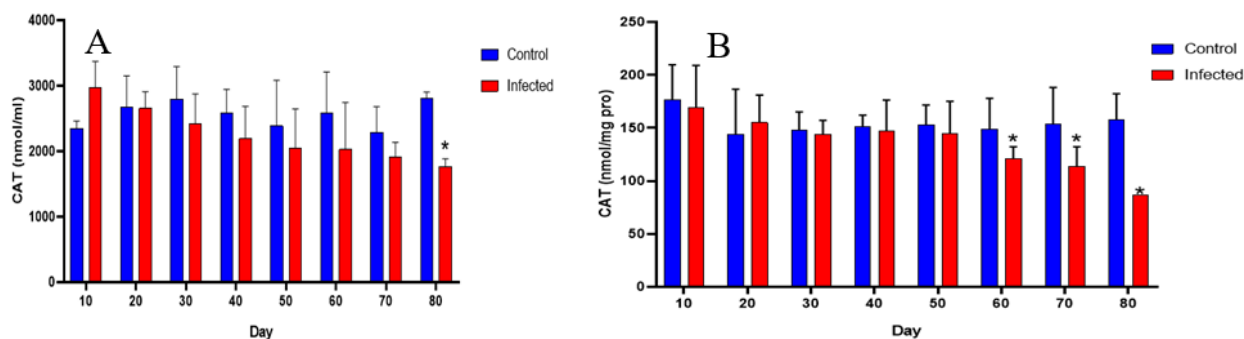


Fig. 5: CAT level in serum (A) and testis tissue (B) in control and *T.gondii*-infected rats. Results are presented as Mean \pm SD; * $P < 0.05$, control versus infected group

Discussion

Oxidative stress induced by immune system is one of the vital strategies of host defense against intracellular parasites. As previously has been investigated, lymphokine activated macrophages play major role in defense against *T. gondii* and production of ROS (12, 21). Antioxidant system, which include enzymatic and nonenzymatic antioxidants, interfere against harmful effects of ROS (22). According to study, which conducted oxidative stress on apicomplexan parasites, as well as the study of Abd Ellah, catalase and superoxide dismutase are the first enzymes that reacted directly with oxidants (23, 24). In the present study, SOD decreased in the serum and testis of infected rats on day 80 PI. SOD as an important defense against oxygen radicals involved in dismutation of the superoxide radicals to H₂O₂ which can cause subsequent oxidative damage to various types of macromolecules including lipids, proteins and DNA (25, 26). In a study, SOD and CAT decreased in the liver of *Toxoplasma* infected mice. The excessive accumulation of radicals was suggested in the tissue as the main cause of decreased activity of enzymes (27). Based on explanation discussed above and other studies in England and Turkey, the severity of the toxoplasmosis was demonstrated as the main cause of oxidative stress along with the decrement of SOD activity in *T. gondii* infection (22, 28).

CAT activity of testis and serum were significantly reduced on days 60 and 80 PI, respectively in this study. CAT activity adversely affected by oxidative stress caused by various diseases such as toxoplasmosis (29). There was no significant change in CAT activity in rats infected with *T. gondii* until day 45 after infection in Bahrami et al. study (12). The probable reason for this difference might be due to the seeking of activity of enzyme level after 45 d of *Toxoplasma* infection until day 80 in current study. In fact, the activation of the host immune system against *T. gondii*

and the respiratory burst caused by macrophages lead to the production of active oxygen species by extending the study (30). CAT enzyme by the detoxification of reactive oxygen species produced by immune cells prevents the possible damage (31, 32). It seems that *T. gondii* reduced the ROS-encoding proteins activity. Also, with the excessive production of these active oxygen species, important enzymes such as CAT and SOD lose their effective function and became inactive (33).

There was a marked depletion of GSH on day 80 in both serum and testis. Glutathione, an important non-protein thiol source, can act as substrate for glutathione peroxidase and served as functional protective molecule for oxidative damage in various tissue (34). In agreement with present study, toxoplasmosis could decrease the blood level of GSH as an important antioxidant agent in *Toxoplasma* seropositive pregnant women (26). The decrease might be due to elevation of oxidative stress in infected cells, that is why the significant difference between enzymatic markers occurred in the last days of the study. Additionally, decreased serum and testis GSH level in chronic toxoplasmosis could change the detoxified capacity of reproductive tissue and then result in oxidative damage of reproductive organs which can adversely affects the fertility capacity (35, 36). On the other hand, *Toxoplasma* infection in pregnant women could induce oxidative stress which resulted in marked increase in the blood level of MDA and decrease in the blood level of GSH (37).

The MDA level was considerably increased in serum and testis up to days 60 and 70 after infection, respectively. Detection of TAC level in serum and tissue may represent the environmental, physiological factors of the oxidative stress in various tissue (12). It may also show the cumulative function of all the antioxidant agents existing in tissue and serum, therefore TAC measurement provided a helpful parameter rather than a simple total antioxidant (12, 38).

The MDA level was remarkably raised while the TAC level was significantly decreased. The significant drop in this parameter were determined in the final days of the study (70 and 80 day PI). In agreement with current result, Bahrami et al. reported that the *Toxoplasma* infection resulted in decreased TAC level during 45 d of study (12). TAC concentration indicated the whole action of the antioxidant agents. Therefore, determining the concentration of TAC could be introduced as a useful parameter for analyzing the antioxidant status in different tissue (38). It could be concluded that oxidative stress by *Toxoplasma* is one of the factors that resulted to the depletion of antioxidants and ultimately leads to lipid oxidation in the cell membrane (39). It is in accordance with our result, which due to depletion of antioxidants TAC level decreased in 70 and 80 d PI. MDA as an end-product of lipid peroxidation was increased through this process which developed the cross link between membrane contents especially, ion channels that prompted to impact on the membrane charges and enzymes activity (35).

MDA as lipid peroxidation marker significantly increased in serum *Toxoplasma* infected individuals (35). *T. gondii* Chinese genotype 1 caused apoptosis of trophoblast due to oxidative stress and impairment of mitochondrial function in pregnant mice (2). In Parallel to current results, the serum MDA level was markedly enhanced in mice infected with *T. gondii*. The MDA level also increased in the *T. gondii*-infected mice spleen, liver, and brain (40). Increased level of MDA as an important lipid peroxidation marker was elevated in the study in *Toxoplasma* infected chickens (13). In fact, following the destruction of the tissue, lipid peroxidation took place in a large volume, MDA as the most important product of this process elevated and fascinated demolition of cell structure, inflammation and necrosis (41).

Toxoplasmosis in rats is usually sub-clinical and thus rats are the best model for studying human *T.gondii* infection (42). However, toxoplasmosis had been considered asymptomatic

in rats (43, 44). The persistence of the RH strain in rats is variable and depends on the infection dose (45). Studies indicated that rats were infected with *T. gondii* so, just like as human can develop high levels of detectable antibodies by dye test (42). This statement can support the fact that biochemical interactions that occur in rat tissue can also be generalized to human and lead to further researches.

Conclusion

Toxoplasmosis induced SOD, CAT, GSH and lipid peroxidation (MDA) increase in serum and testis of *T. gondii*-infected rats. Toxoplasmosis induced oxidative stress in tissue and it might be affecting the reproductive susceptibility of male rats.

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Conflict of interest

The authors declare that there is no conflict of interest.

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