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Evaluation of Local tissue oxidative stress and its possible involvement in the pathogenesis of toxoplasmosis in rats experimentally infected with *Toxoplasma gondii*

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Abstract. Toxoplasmosis caused by the protozoan parasite *Toxoplasma gondii*, is a zoonotic parasitic disease. Oxidative stress plays a dominant role in the host's defense against protozoan infection. In the present study the possible involvement of local oxidative stress in the pathogenesis of *T. gondii* were investigated. Twenty five female Wistar rats were infected with RH strain of *Toxoplasma* tachyzoites and twenty female rats were used as control group that only received sterile PBS. Tissue samples from liver, heart and brain on 0, 3, 5, 8 and 45 days post infection were collected. As biochemical markers of oxidative stress, endogenous concentrations of GSH, GPX and SOD activity, MDA level, protein carbonyl content and total antioxidant capacity were determined from the mentioned tissues of control and infected rats. Based on the results, on day 3, 5 and 8 post infection the level of hepatic glutathione were significantly decreased in infected rats when compared to control. There was a significant rise in hepatic glutathione peroxidase activity and malondialdehyde level on the third day post infection in comparison to uninfected rats. Significant elevation of superoxide dismutase activity and malondialdehyde level on 5 day post infection and protein carbonyls and total antioxidant capacity on 8 day post infection in infected livers were obtained. Significant changes of glutathione level, total antioxidant capacity and protein carbonyls contents were observed in cardiac homogenate on days 3, 5 and 45, respectively. Measured parameters were constant throughout all stages of experiment in brain of infected rats. Indeed increased production of reactive oxygen species accompanies *Toxoplasma* infection in liver and heart tissues of experimentally infected rats. Based on this study, antioxidant defense system can probably play a role in parasitic stage interconversion and shifting the toxoplasmosis into the chronic phase.

INTRODUCTION

Toxoplasmosis is one of the common parasitic infections caused by coccidian protozoon, *Toxoplasma gondii* (Montoya & Liesenfeld, 2004; Bogitsh *et al.*, 2013). Some estimates suggest that about one-third of the world's population has been exposed to this parasite (Hill *et al.*, 2005). Felids such as feral or domestic cats are the only known definitive hosts. Virtually all warm-blooded animals are intermediate hosts; they can become infected by eating undercooked meat containing tissue cysts, consuming contaminated food or water contacted with cat feces and transplacentally from mother to fetus (Dubey, 2002; Dubey, 2016). Toxoplasmosis can cause severe disease when it is reactivated in immunosuppressed patients or when the organism is contracted congenitally (Jones *et al.*, 2001). Reactive oxygen species (ROS) are produced under physiological conditions. Oxidative stress reflects an imbalance between oxidant or/

and antioxidant levels (Birben *et al*., 2012). Recent studies demonstrated that high resistance of the hosts to some parasitic diseases is a result of defense mechanisms which include enhanced generation of ROS (Boczon *et al*., 1996). The production ROS can cause severe damage to biological molecules such as carbohydrates, nucleic acids, lipids, and proteins (Halliwell, 1994). However, reactive oxygen species can be useful, as they are used by the immune system as a way to attack and kill pathogens (Segal, 2005). *T. gondii* modifies its morphology and its metabolism during stage conversion between tachyzoite and bradyzoite forms, including stage-specific antigen expression and alterations to metabolism (Denton *et al*., 1996). *In vitro* research identified that alkaline medium, heat shock protein and acid conditions can induce stage conversion of *T. gondii*. Also Lyons *et al*. (2002) demonstrated that oxidative stress induces *T. gondii* encystment *in vitro*. Since understanding the process of interconversion between tachyzoites and bradyzoites can help in designing new chemotherapeutic agents against *Toxoplasma* (Turrens, 2004), the purpose of this study was to determine probable changes in antioxidant parameters in an *in vivo* model. The present study was designed to assay antioxidant profile in the liver, brain and heart tissue during acute and chronic phases of experimental toxoplasmosis in rats, and to investigate whether oxidative stress can induce *T. gondii* tachyzoie-bradyzoite interconversion.

MATERIALS AND METHODS

Experimental infection

Forty-five adult female Wistar rats were purchased from laboratory animal breeding council (Jundishapur University of Medical Science, Ahvaz, Iran). Bottom of FormThe rats were 8-10 weeks old and weighing approximately 140-200 g each. All animals were maintained in accordance to the Principles of Laboratory Animal Care [NIH]. They were housed in groups of five, kept on a 12 hr light/dark cycle, relative humidity conditions (40–70%) and controlled temperature (24±2ºC). During experiment, all rats had free access to water and standard rat chow. After 1 week acclimation, modified agglutination test was used to ensure rats were not already infected with *Toxoplasma*, and then they were divided randomly into two groups, control (n=20) and experiment (n=25). RH strain of *Toxoplasma* maintained as tachyzoites by passage in Vero cell line. The infected and control groups were injected intraperitoneally with approximately 10^7 tachyzoites of *Toxoplasma* and sterile PBS, respectively.

Blood and tissue Sampling

Blood and tissue samples were collected on 0, 3, 5, 8 and 45 days post infection. In each sampling day, five rats from infected and control group were euthanized in a glass desiccator jar for open-drop anesthesia with chloroform following standard animal ethics guidelines of Iran. Blood samples were obtained by cardiac puncture and collected in *EDTA* and heparin tubes for PCR and biochemical tests, respectively. Hemoglobin concentrations (g/dl) were measured by automated blood cell counter (BC-2800Vet, Mindray). Fresh heparinized blood samples were centrifuged (10 min at 3000 rpm) and the plasma obtained. Erythrocytes were washed three times with normal saline. Packed erythrocytes were hemolysed by adding distilled water. Samples (EDTA blood, plasma, hemolysate) were stored at -70°C until assayed. In addition, necropsy was performed and tissue samples from liver, brain and heart were collected. *Tissue samples were* weighed and placed PBS buffer and subsequent *homogenization was* done with homogenizer. *Homogenized tissues* were centrifuged (three time 10 min at 12000 rpm) and supernatant were collected and stored at -70°C until assayed. It should be mentioned that tissue from different organs were collected for DNA extraction and PCR. During the present study, the animals were handled according to the recommendation of the Ethics Committee, Shahid Chamran University of Ahvaz, Ahvaz, Iran (Ethical Approval No. 29/6/94, 30 Sept. 2014).

DNA extraction and polymerase chain reaction (PCR) amplification

PCR assay was used to confirm acute and chronic phase of infection. In 3, 5 and 8 day post infection (DPI) DNA was extracted from whole blood, As well as at chronic phase (on 45 DPI) DNA was extracted from liver, brain and heart using a genomic DNA purification kit (CinnaGen, Iran). Since 45 DPI, chronic infection of toxoplasmosis happens and bradyzoite stages of *Toxoplasma* is present therefore PCR was used to confirm the infection. *Primers* based on the B_1 region were chosen from the literature (Jalal *et al*., 2004). PCR reactions included a negative control, consisting of the reaction mix and 2 5L of DNase/RNase-free water and a positive control that consisted of a DNA sample from the tachyzoites of *T. gondii*. The following PCR conditions were applied to each assay: 25 µl reaction containing 12.5 µl Taq DNA polymerase master mix Red (Amplicon, Denmark), 1 µM primers and 50 ng DNA templates. PCR Cycling conditions included an initial DNA denaturation at 95°C for 10 min, followed by 30 cycles, denaturation at 94°C for 1 min, annealing at 52°C for 30 s, and an extension at 72°C for 1 min. This was followed by a final extension at 72ºC for 7 min. The presence of amplicons and their size were electrophoresed in 1.5% agarose (SinaClon Bioscience, Iran) in Tris–acetate–EDTA (TAE) buffer, stained with Green Safe stain (SinaClon Bioscience, Iran) and visualized under ultraviolet light. Positive samples showed a band of approximately 469 bp.

Modified Agglutination Test (MAT)

In addition to PCR assay, MAT was also used on 45 *DPI* to ensure that chronic toxoplasmosis occurred in rats. The method described by Desmonts and Rem-ington (1980) and Dubey and Desmonts (1987) was used. The sera were diluted two folds (1:20 to 1:320) with phosphate buffered saline containing 0.2 M 2-mercaptoethanol and 50 µL of each dilution was put in a well of 96 U-bottom ELISA plates. Thereafter, 50 µL of the whole formalin-preserved *T. gondii* tachyzoites were added to each serum dilution. The wells were then mixed thoroughly by pipetting up and down several times, covered, and then incubated at 37°C overnight. The test was considered positive when a layer of agglutinated parasites was formed in wells at di-lutions of 1:20 or higher. Positive and negative controls were included in each test.

Oxidant/antioxidant assessment

Total protein was determined in tissue samples by Biuret method (Parsazmoon kit, Iran) and using BT-1500 autoanalyzer. Reduced glutathione (GSH) level was determined according to the procedure *described* by Ellman (1959). In this method Ellman's Reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB) reacts with sulfhydryl group of GSH and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). Absorbance of TNB was detected at 412 nm using a microplate reader. Absorbance values were compared with a standard curve generated from known GSH. The activity of glutathione peroxidase (GPX) was measured with a commercial kit (Ransel®-Randox Lab, Antrim, UK). GPX reduces cumene hydroperoxide while oxidizing GSH to GSSG. In the presence of glutathione reductase, GSSG reduced to GSH with concomitant oxidation of NADPH to $NADP^+$. The decrease in NADPH (measured at 340 nm) is proportionate to GPX activity. GPX activity was calculated according to the manufacturer's instruction. Superoxide dismutase (SOD) enzyme activity was measured using a commercial kit (Ransod®-Randox Lab, Antrim, UK). Based on the method, superoxide radicals generated by xanthine oxidase reaction convert 1-(4-iodophenyl)- 3-(-4-nitrophenol)-5-phenyltertrazoliumchloride quantitatively to a formazan dye. Conversion of superoxide radicals to hydrogen peroxide by superoxide dismutase inhibits dye formation and serves as a measure of superoxide dismutase activity. The concentration of Malondialdehyde (MDA) in tissue homogenate was determined as thiobarbituric acid-reactive substances according to Placer *et al.* (1966) with slight modifications. This method is dependent on forming a color complex between the products of lipid

peroxidation and thiobarbituric acid (TBA). The quantification of thiobarbituric acid reactive substances (TBARS) was determined by comparing the absorption to the standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. Protein carbonyls (PC) content in tissue homogenate was performed by the method described by Levine *et al.* (1994) with slight modifications. Based on this method, protein carbonyls react with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-dinitrophenylhydrazone (DNP). The change in absorbance was measured at 370 nm. FRAP method described by Benzie and Strain (1996) was used to measure total antioxidant capacity (TAC) of tissue homogenates. This method depends upon the reduction of ferric tripyridyltriazine complex to ferrous tripyridyltriazine by a reductant at low pH. This ferrous tripyridyltriazine complex has an intensive blue color and can be monitored at 593 nm.

Statistical analysis

Differences between means were analyzed by T-test and one-way analysis of variance (ANOVA) using SPSS version 16.0. Values of $p<0.05$ were considered statistically significant.

RESULTS

After experimental inoculation, acute phase of infection was confirmed by PCR assay. All blood DNA obtained from infected rats on 3, 5 and 8 DPI revealed 469 bp bands. Serological survey was used for verification of chronic phase of experimental infection. In all 5 sampled rats in chronic phase complete carpet of agglutinated organisms were seen and considered as positive. In chronic phase of experimental toxoplasmosis, PCR assay was performed on liver, heart and brain tissues and DNA of *T. gondii* was detected from some tissue samples (Table 1).

The results of hepatic antioxidant assessment in non-infected and *T. gondii* infected rats are shown in Table 2. GSH level in the infected rats on the 3 DPI was significantly decreased comparing to 0 ($p=0.002$), and 45 ($p=0.001$) DPI. Also significant decrease were detected in liver GSH level between the infected rats and the controls on 3 ($p=0.003$), 5 ($p=0.04$) and 8 ($p=0.002$) DPI. Experimental toxoplasmosis increased hepatic GPX activity on 3 ($p=0.02$) DPI as compared to control. In the present study on the 3 DPI, GPX activity was significantly increased comparing to 0 ($p=0.04$), 5 ($p=0.02$) and 45 ($p=0.007$) DPI. Elevations of SOD activity in livers were observed on 5 DPI. On the mentioned day significant differences were detected for SOD activity comparing to control ($p=0.005$), 0 ($p=0.01$) and 45 ($p=0.02$) DPI. On 3 ($p=0.04$) and 5 ($p=0.03$) DPI, MDA was increased as compared to non-infected group while MDA concentration in infected rats in different days was not significantly changed. According to the results PC content in infected groups on different days was not significantly changed while on days 8 post infection ($p=0.02$) PC content significantly decreased as compared to control. In the present study significant increases were detected in hepatic TAC between the infected rats and the controls on day 8 ($p=0.006$) while, on the other days no significant differences were detected between the infected rats and the controls. TAC in different days in infected ($p=0.16$) and uninfected rats ($p=0.98$) was not significant.

Table 3 summarizes the changes of antioxidant parameters in brain of control and infected groups. In the brain tissue of infected rats no significant results were observed in

Table 1. Results of PCR assay in liver, heart and brain tissues of infected rats

Animals	Tissues		
	Liver	Heart	Brain
Rat 1	Positive	Positive	Negative
Rat 2	Positive	Positive	Positive
Rat 3	Positive	negative	Negative
Rat 4	Positive	Negative	Negative
Rat 5	Negative	Negative	Positive

Table 2. Results of hepatic oxidant/antioxidant assessment as mean ± standard error in *T. gondii* infected and non-infected rats

Parameter	Group	Days Post Infection				
		0	3	5	8	45
GSH (µmol/g tissue)	Infected	$18.58 \pm 2.61^{A,a}$	$8.76 \pm 1.47^{A,b}$	$13.44 \pm 0.7^{A,ab}$	$13.01 \pm 0.94^{A,ab}$	$18.92 \pm 1.05^{A,a}$
	Non-infected	$18.58 \pm 2.61^{A,a}$	19.89 ± 2.15^{Ba}	20.19 ± 2.7^{Ba}	20.52 ± 1.43^{Ba}	22.32 ± 2.76^{Aa}
GPX (U/g Protein)	Infected	$73.97 \pm 5.33^{A,a}$	$105.93 \pm 8.16^{A,b}$	$70.12 \pm 8.06^{A,a}$	$88.43 \pm 4.47^{A,ab}$	$62.33 \pm 7.8^{A,a}$
	Non-infected	$73.97 \pm 5.33^{A,a}$	$80.5 \pm 3.49^{B,a}$	$76.99 \pm 5.21^{A,a}$	$82.76 \pm 3.82^{A,a}$	$74.21 \pm 5.29^{A,a}$
SOD (U/g Protein)	Infected	$165.61 \pm 16^{A,a}$	$184.76 \pm 10^{A,ab}$	$221.94 \pm 5^{A,b}$	$195.44 \pm 10^{A,ab}$	$160.56 \pm 7^{A,a}$
	Non-infected	$165.61 \pm 16^{A,a}$	$169.09 \pm 7^{A,a}$	$181.26 \pm 9^{B,a}$	$173.59 \pm 6^{A,a}$	$174.29 \pm 5^{A,a}$
MDA (nMol/g Protein)	Infected	$145.12 \pm 22^{A,a}$	$171.87 \pm 11^{A,a}$	$180.5 \pm 8^{A,a}$	$161.54 \pm 9^{A,a}$	$137.66 \pm 4^{A,a}$
	Non-infected	$145.12 \pm 22^{A,a}$	$140.24 \pm 7^{B,a}$	$151.51 \pm 6^{B,a}$	$149.59 \pm 15^{A,a}$	$147.49 \pm 6^{A,a}$
Protein carbonyl (nmol/g tissue)	Infected	$94.7 \pm 4.46^{A,a}$	$119.32 \pm 18.76^{A,a}$	$113.9 \pm 17.05^{A,a}$	$129.55 \pm 6.46^{A,a}$	$99.62 \pm 10.4^{A,a}$
	Non-infected	$94.7 \pm 4.46^{A,a}$	$90.53 \pm 28.55^{A,a}$	$95.07 \pm 4.3^{A,a}$	$89.39 \pm 9.37^{B,a}$	$89.39 \pm 23.58^{A,a}$
Total antioxidant capacity (mmol/g tissue)	Infected	$17.98 \pm 0.82^{A,a}$	$19.12 \pm 1.05^{A,a}$	$17.13 \pm 1.1^{A,a}$	$20.60 \pm 0.27^{A,a}$	$18.44 \pm 0.7^{A,a}$
	Non-infected	$17.98 \pm 0.82^{A,a}$	$17.47 \pm 1.2^{A,a}$	$17.46 \pm 1^{A,a}$	$17.39 \pm 0.55^{B,a}$	$17.60 \pm 0.7^{A,a}$

Values in columns and rows with different upper and lower- cases superscripts are significantly different ($p<0.05$).

Table 3. Results of brain oxidant/antioxidant assessment as mean ± standard error in *T. gondii* infected and non-infected rats

Parameter	Group	Days Post Infection				
		0	3	5	8	45
GSH (µmol/g tissue)	Infected	$3.42 \pm 0.45^{A,a}$	$3.75 \pm 0.13^{A,a}$	$2.96 \pm 0.21^{A,a}$	$3.3 \pm 0.19^{A,a}$	$3.83 \pm 0.24^{A,a}$
	Non-infected	$3.42 \pm 0.45^{A,a}$	$3.39 \pm 0.13^{A,a}$	$3.62 \pm 0.26^{A,a}$	$3.02 \pm 0.19^{A,a}$	$3.97 \pm 0.47^{A,a}$
GPX (U/g Protein)	Infected	$250.49 \pm 60^{A,a}$	$245.06 \pm 51^{A,a}$	$154.92 \pm 35^{A,a}$	$172.45 \pm 39^{A,a}$	$177.49 \pm 21^{A,a}$
	Non-infected	$250.49 \pm 60^{A,a}$	$233.43 \pm 28^{A,a}$	$210.63 \pm 16^{A,a}$	$196.46 \pm 63^{A,a}$	$234.27 \pm 43^{A,a}$
SOD (U/g Protein)	Infected	$655.74 \pm 62^{A,a}$	$576.88 \pm 6^{A,a}$	$587.4 \pm 61^{A,a}$	$623.86 \pm 126^{A,a}$	$679.21 \pm 122^{A,a}$
	Non-infected	$655.74 \pm 62^{A,a}$	$663.14 \pm 30^{A,a}$	$739.55 \pm 47^{A,a}$	$669.89 \pm 85^{A,a}$	$234.27 \pm 43^{A,a}$
MDA (nMol/g Protein)	Infected	$925 \pm 48^{A,a}$	$811.45 \pm 32^{A,a}$	$855.53 \pm 174^{A,a}$	$889.9 \pm 69^{A,a}$	$847.36 \pm 73^{A,a}$
	Non-infected	$925 \pm 48^{A,a}$	$923.93 \pm 49^{A,a}$	$1092.9 \pm 84^{A,a}$	$1011.3 \pm 73^{A,a}$	$959.51 \pm 82^{A,a}$
Protein carbonyl (nmol/g tissue)	Infected	$104.92 \pm 11.25^{A,a}$	$131.82 \pm 31.98^{A,a}$	$134.85 \pm 8.85^{A,a}$	$115.91 \pm 5.71^{A,a}$	$92.8 \pm 6.44^{A,a}$
	Non-infected	$104.92 \pm 11.25^{A,a}$	$108.33 \pm 2.48^{A,a}$	$112.88 \pm 16.36^{A,a}$	$113.26 \pm 11.81^{A,a}$	$91.66 \pm 5.73^{A,a}$
Total antioxidant capacity (mmol/g tissue)	Infected	$5.45 \pm 0.1^{A,a}$	$6.22 \pm 0.3^{A,a}$	$5.61 \pm 1^{A,a}$	$6.42 \pm 0.1^{A,a}$	$5.3 \pm 0.2^{A,a}$
	Non-infected	$5.45 \pm 0.1^{A,a}$	$5.28 \pm 0.2^{A,a}$	$5.54 \pm 0.4^{A,a}$	$5.29 \pm 0.3^{A,a}$	$5.44 \pm 0.3^{A,a}$

Values in columns and rows with different upper and lower- cases superscripts are significantly different ($p<0.05$).

Table 4. Results of cardiac oxidant/antioxidant assessment as mean ± standard error in *T. gondii* infected and non-infected rats

Parameter	Group	Days Post Infection				
		0	3	5	8	45
GSH (µmol/g tissue)	Infected	$10.09 \pm 0.33^{A,ab}$	$6.35 \pm 0.92^{A,b}$	$11.93 \pm 1.15^{A,a}$	$12.29 \pm 0.78^{A,a}$	$11.89 \pm 0.84^{A,a}$
	Non-infected	$10.09 \pm 0.33^{A,a}$	$10.34 \pm 0.39^{B,a}$	$12.18 \pm 0.74^{A,a}$	$10.75 \pm 0.5^{A,a}$	$12.2 \pm 0.74^{A,a}$
GPX (U/g Protein)	Infected	$560.91 \pm 179^{A,a}$	$540.14 \pm 15^{A,a}$	$445.5 \pm 70^{A,a}$	$665.07 \pm 82^{A,a}$	$481.14 \pm 10^{A,a}$
	Non-infected	$560.91 \pm 179^{A,a}$	$565.96 \pm 83^{A,a}$	$546.82 \pm 45^{A,a}$	$574.05 \pm 56^{A,a}$	$524.95 \pm 27^{A,a}$
SOD (U/g Protein)	Infected	$668.23 \pm 79^{A,a}$	$619.11 \pm 32^{A,a}$	$510.98 \pm 30^{A,a}$	$630.24 \pm 82^{A,a}$	$454.23 \pm 18^{A,a}$
	Non-infected	$668.23 \pm 79^{A,a}$	$659.44 \pm 45^{A,a}$	$610.67 \pm 37^{A,a}$	$706.16 \pm 79^{A,a}$	$560.98 \pm 56^{A,a}$
MDA (nMol/g Protein)	Infected	$534.19 \pm 23^{A,a}$	$535.04 \pm 11^{A,a}$	$378.66 \pm 20^{A,a}$	$422.31 \pm 57^{A,a}$	$381.15 \pm 70^{A,a}$
	Non-infected	$534.19 \pm 23^{A,a}$	$529.49 \pm 39^{A,a}$	$420.31 \pm 29^{A,a}$	$501.24 \pm 36^{A,a}$	$405.75 \pm 72^{A,a}$
Protein carbonyl (nmol/g tissue)	Infected	$50.76 \pm 13.61^{A,a}$	$88.63 \pm 13.12^{A,ab}$	$93.18 \pm 11.15^{A,ab}$	$60.22 \pm 16.5^{A,ab}$	$119.32 \pm 12.46^{A,b}$
	Non-infected	$50.76 \pm 13.61^{A,a}$	$58.33 \pm 11.48^{A,a}$	$52.27 \pm 15.25^{A,a}$	$54.16 \pm 26.32^{A,a}$	$54.45 \pm 15.46^{B,a}$
Total antioxidant capacity (mmol/g tissue)	Infected	$7.04 \pm 0.1^{A,a}$	$6.18 \pm 0.23^{A,a}$	$10.45 \pm 0.5^{A,b}$	$8.8 \pm 0.41^{A,a}$	$7.14 \pm 0.7^{A,a}$
	Non-infected	$7.04 \pm 0.1^{A,a}$	$7.22 \pm 0.68^{A,a}$	$7.09 \pm 0.4^{B,a}$	$7.51 \pm 0.65^{A,a}$	$8.07 \pm 0.4^{A,a}$

Values in columns and rows with different upper and lower- cases superscripts are significantly different ($p<0.05$).

GSH level, GPX activity, SOD activity, MDA level, PC content and TAC as compared to non-infected ones. As well as these parameters in infected rats in different days was not significantly changed.

As shown in Table 4 no significant changes was observed in GPX, SOD activity and MDA level in heart of rats infected with *T. gondii*. On 3DPI, GSH level in the heart of the infected group was significantly lower than control (p=0.01), 5 (p=0.009), 8 (p=0.005) and 45 (p=0.004) DPI. In chronic phase (45 DPI) PC content (p=0.03) in heart of the infected rats was higher than the control group. On this day PC content significantly increased comparing to 0 (p=0.03) DPI. Significant increase was detected in TAC between the infected rats and the control ones on 5 *DPI* (p=0.001). TAC was significantly increased comparing to 0 (p=0.002), 3 (p=0.00), 8 (p=0.03) and 45 (p=0.00) DPI.

DISCUSSION

Oxidative stress of host can take part in the defense mechanism against toxoplasmosis. In response to infection, the innate immune system is activated with involvement of macrophages and neutrophils. These cells generate large amounts of active oxygen and nitrogen species, causing oxidative stress (Miller & Britigan, 1997). *In vitro* studies have demonstrated the ability of oxidative stress in interconversion between tachyzoites and bradyzoites in *Toxoplasma* infection (Lyons *et al.*, 2002). Furthermore, some studies have shown that inhibitors of mitochondrial function and inducers of oxidative stress can induce *Toxoplasma* encystment *in vitro* (Bohne *et al.*, 1994; Soete *et al.*, 1994). The aim of the present study was to investigate probable role of oxidative stress in acute and chronic experimental toxoplasmosis.

According to the results, acute phase of *T. gondii* infection induces production of ROS and oxidative stress in liver of infected rats, because of Gpx and SOD activities elevation, glutathione depletion, and also increasing lipid peroxidation, protein carbonyl contents and total antioxidant capacity. The changes in GSH levels as well as the protein carbonyls content alteration and total antioxidant capacity in hearts, provided evidence of impaired antioxidant status and the occurrence of oxidative stress. In contrast, brain antioxidant parameters remained unchanged.

Hydrogen peroxide (H_2O_2) is one of the main ROS leading to oxidative stress (Halliwell & Gutteridge, 1999). GPX catalyzes the reduction of hydrogen peroxide to water by using reduced glutathione. This function of glutathione peroxidase is important in antioxidant defense and in maintenance of the health of cells and organisms (Gallo & Martino, 2009). A significant depletion of glutathione and increased GPX activity were noted in the present study in liver of infected rats. On 3 DPI, GPX activity in liver significantly increased, also on this day hepatic and heart GSH level significantly decreased which was the result of high oxidative stress and glutathione over-use by the cells. As the infection progressed, it is possible that glutathione reductase activity was increased in response to oxidative stress. (GSH can be regenerated by the enzyme glutathione reductase) (Couto *et al.*, 2013). Therefore on day 5 and 8 post infection hepatic and heart GSH level started to improve. Although on this days hepatic GSH level were significantly lower than the control group but heart GSH level was normal in comparing to control group. Our results were in agreement with the previous reports in which the infection with *T. gondii* depleted glutathione in host's different tissues (Al-Kennany, 2007; Xu *et al.*, 2012). The presence of local oxidative stress in liver of rats suffering from *T. gondii* is also suggested by the increase in the SOD enzyme on 5 DPI. Superoxide dismutase is an enzyme participating in the systemic antioxidant defense for deletion and neutralization of toxic ROS. In our study only hepatic SOD activity on 5 DPI was significantly elevated. The elevation of SOD may be due to higher production of oxidants, but its activity was constant throughout all stages of experiment in heart of infected rats.

Based on our study, lipid peroxidation, as determined by MDA concentrations, was significantly increased in liver during acute phase of *Toxoplasma* infection. The higher MDA levels in liver of infected rats were due to the oxidative stress occurring after the *Toxoplasma* infection. These results showed that *T. gonadii* infection-induced ROS are not totally scavenged by the antioxidant enzymes in liver. However in infected rat's hearts no significant results were observed for MDA level, as compared to non-infected. Overall it seems that in heart on 3 DPI oxidative stress has been occurred but it was not terminate to end product of lipid peroxidation elevation.

Significant increase was detected for total antioxidant capacity between the infected rats and the control ones on 5 and 8 *DPI* in heart and liver, respectively. Probably total antioxidants were expended to compensate oxidative stress and to prevent injuries from free radicals and effective infection control. Protein carbonyls are commonly used as a marker of protein oxidation in biological fluids. Elevated production of ROS during infection could pose a threat to biomolecules by oxidation of proteins (Halliwell and Gutteridge, 1999).

All measured parameters in hepatic and heart homogenate except cardiac protein carbonyl content returned to normal values during chronic phase of infection (45 DPI) as comparing to control ones. In fact, it indicates that innate immune system generates normal amounts of active oxygen in dormant stage. Carbonyl groups are relatively stable which are useful for both their detection and storage (Dalle-Donne *et al.*, 2003) therefore, on 45 DPI cardiac protein carbonyl content was still significantly higher than controls. According to the results obtained in the present study, all the parameters were constant throughout all stages of experiment in brain of infected rats. It may be assumed that oxygen-derived species were produced at a low level in brain and normal cellular defense mechanisms destroyed most of

them. Likewise, any damage to cells was constantly repaired.

In conclusion, based on the results it seems that antioxidant defense system can be one of the effective mechanisms in tachyzoite- bradyzoite interconversion in *Toxoplasma* infection, although further studies are needed to confirm the hypothesis.

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