Assessment of oxidative stress and lipid peroxidation in cats infected with *toxoplasma*

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Abstract

Toxoplasmosis, caused by the intracellular protozoan *Toxoplasma gondii*, is a widespread zoonotic infection that frequently remains asymptomatic in humans and animals. During parasitic infections, oxidative stress can increase due to excessive free radical production, potentially contributing to disease pathogenesis. The present study investigates oxidative stress biomarkers in cats, the definitive hosts of *Toxoplasma gondii*. Blood samples were collected from 55 cats. Among them, 10 infected and 10 uninfected cats were selected based on serological screening using the Modified Agglutination Test (MAT). Serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and total antioxidant capacity (TAC) were analyzed. Data were evaluated using independent t-tests, with statistical significance set at $P \leq 0.05$. Although serum MDA concentrations were elevated in infected cats (10.68 ± 1.07 µM) compared to controls (9.12 ± 0.56 µM), the difference was not statistically significant. Enzyme activities of SOD showed no significant difference. Infected cats had lower GPx (128.66 ± 35.62 U/mL vs. 235.66 ± 74.13 U/mL), CAT (12.19 ± 1.03 U/mL vs. 13.12 ± 2.60 U/mL), and TAC (0.118 ± 0.004 mM vs. 0.126 ± 0.005 mM), but these reductions were not statistically significant ($p > 0.05$). This study evaluated serum oxidative markers and lipid peroxidation in cats naturally infected with *Toxoplasma gondii*. The lack of significant differences suggests that oxidative stress and lipid peroxidation induced by the infection may be localized within cells, consistent with the intracellular nature of *Toxoplasma gondii*, an obligate intracellular protozoan. Moreover, the absence of overt clinical signs in infected cats might account for the minimal changes in systemic antioxidant parameters. Further research incorporating tissue-specific analyses and experimental infection models is necessary to more accurately elucidate the oxidative stress mechanisms associated with *Toxoplasma gondii* infection.

Keywords: *Toxoplasma gondii*, malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase, total antioxidant capacity

1.Introduction

Toxoplasmosis is a globally prevalent zoonotic disease caused by the intracellular protozoan *Toxoplasma gondii*, which affects a broad range of warm-blooded animals, including humans, mammals, and birds (1,2). Felids, especially domestic cats, are the only known definitive hosts capable of shedding oocysts through their feces, making them key contributors to environmental contamination. Intermediate hosts, such as humans and livestock, become infected through ingestion of tissue cysts in undercooked meat, accidental contact with oocyst-contaminated sources, or congenital transmission (2,3).

The parasite's life cycle includes three infective stages: tachyzoites, bradyzoites, and sporozoites. Sporulated oocysts represent a main source of infection for humans and other intermediate hosts (2,3). All cat breeds, regardless of age or sex, are susceptible to *Toxoplasma gondii* infection. Oocysts excreted in cat feces contaminate soil, water, vegetables, and animal forage. It is estimated that approximately 1% of the cat population is actively shedding oocysts at any given time. The shedding period typically lasts between one to two weeks, which limits the utility of fecal testing as a reliable diagnostic method. Remarkably, under optimal environmental conditions such as moist soil and moderate temperatures, *Toxoplasma gondii* oocysts can remain infectious for over a year, supported by their highly resistant outer wall that provides protection against most common disinfectants (4-6). Consequently, environmental contamination with oocysts represents a significant and persistent source of infection for herbivores, omnivores, carnivores, and humans alike. Like other members of the Apicomplexa phylum, *Toxoplasma gondii* is an obligate intracellular parasite. A critical host defense mechanism is the oxidative burst, in which immune cells rapidly produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) in response to infection. To survive, the parasite must counteract oxidative damage within host cells. Oxidative stress occurs when ROS production surpasses the host's antioxidant defenses, leading to cellular damage. The antioxidant system comprises both enzymatic components such as SOD, CAT, GPx, and glutathione reductase and non-enzymatic elements like reduced glutathione (GSH) and uric acid (7–9). Given the relevance of oxidative stress in parasitic infections, biomarkers such as MDA, SOD, CAT, and GPx provide valuable insights into disease progression (9–11).

During infection, the host's immune response intensifies ROS and RNS production to eliminate pathogens. However, these reactive species may simultaneously damage host tissues, contributing to disease pathology (11–13). Thus, oxidative imbalance plays a major role in the pathogenesis of toxoplasmosis in human and animal hosts (14). This study aims to assess serum oxidative stress and lipid peroxidation markers in cats naturally infected with *Toxoplasma gondii*.

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2.Materials and Methods

2.1.Sample Collection

To obtain 10 confirmed *Toxoplasma gondii*-positive samples, 55 blood samples were collected from cats displaying clinical signs such as lethargy and swollen lymph nodes. An additional 10 samples were obtained from healthy cats presented for routine health checks, with no apparent clinical symptoms. Approximately 5 milliliters of whole blood were collected from the cephalic vein of each animal using sterile syringes and transferred into serum-separation tubes pre-coated with clot activators. The samples were centrifuged at 3000 rpm for 10 minutes using a Hettich Rotina 380 centrifuge (Andreas Hettich GmbH & Co. KG, Germany). to separate the serum, which was then aliquoted into microtubes and stored at –80°C until supplementary analysis. Seropositivity was determined using the Modified Agglutination Test (MAT), with a titer of ≥1:20 considered positive. From the symptomatic cats, only those with confirmed seropositivity (n = 10) were included in the infected group. The uninfected group comprised 10 cats with undetectable antibody titers (<1:20).

2.2.Serological Testing: Modified Agglutination Test (MAT)

Serological testing was performed to detect *Toxoplasma gondii* infection by identifying anti-*Toxoplasma* antibodies in serum samples. The presence of antibodies was assessed using the Modified Agglutination Test (MAT) with microplates containing U-shaped wells. For the assay, 50 µL of 0.2 M 2-mercaptoethanol prepared in phosphate-buffered saline (PBS) was added to each well, followed by the serum samples. Serial two-fold dilutions of the sera were prepared, ranging from 1:10 to 1:100. Subsequently, 50 µL of *Toxoplasma gondii* tachyzoite antigen, suspended in an alkaline buffer, was added to each well, introducing approximately 1,000,000 tachyzoites per well. The plates were gently shaken and incubated at 37°C for 24 hours prior to interpretation.

Positive samples were identified by diffuse agglutination, where the precipitate did not settle at the bottom of the well. Negative samples were indicated by the formation of button-shaped sediment at the bottom of the well. Positive and negative controls were included on each plate for validation. Serum dilutions of 1:20 or higher were considered seropositive.(15)

2.3.Measurement of malondialdehyde concentration

MDA concentrations, representing lipid peroxidation, were quantified using a commercially available assay kit (ZellBio GmbH, Germany). In this colorimetric method, samples were acidified and reacted with thiobarbituric acid (TBA) under high-temperature conditions, forming a colored complex. The intensity of the resulting pink product correlates with MDA concentration and was measured spectrophotometrically at 535 nm using a spectrophotometer (Genway Scientific Ltd., UK).

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2.4.Measuring the activity of antioxidant enzymes

2.4.1.Superoxide dismutase enzyme measurement

SOD activity was measured using a commercial assay kit (ZellBio GmbH, Germany). The assay is based on the conversion of superoxide radicals to molecular oxygen and hydrogen peroxide. The final chromogenic compound was detected at 420 nm using an ELISA microplate reader (BioTek ELx808, Agilent Technologies, USA).

2.4.2.Glutathione peroxidase enzyme measurement

GPx activity was assessed with a commercial kit (ZellBio GmbH, Germany), measuring the absorbance of the reaction endpoint at 405 nm via an ELISA reader (BioTek ELx808, Agilent Technologies, USA).

2.4.3.Measurement of catalase enzyme

CAT activity was evaluated employing a commercial kit (ZellBio GmbH, Germany), with absorbance readings taken at 405 nm on an ELISA reader (BioTek ELx808, Agilent Technologies, USA).

2.4.4.Total Antioxidant Capacity Measurement

TAC was evaluated with a commercial kit (ZellBio GmbH, Germany). Absorbance of the resultant product was read between 460–490 nm using an ELISA microplate reader (BioTek ELx808, Agilent Technologies, USA).

2.5.Statistical Analysis

All results were expressed as mean ± standard error (SE). Data analysis was conducted using SPSS software (Version 24). Independent samples t-tests were applied to compare the mean values between infected and uninfected groups. A p-value of ≤ 0.05 was considered statistically significant for all comparisons.

3.Results

The study identified 10 cats as positive for *Toxoplasma gondii* infection, based on antibody titers ≥1:20. Additionally, 10 cats with undetectable antibody titers, indicating no evidence of *T. gondii* infection, were included in the uninfected (control) group.The statistical data on CAT, SOD, and GPx enzyme activities, as well TAC, are presented in Table 1. Although the activities of all enzymes were lower in infected cats compared to uninfected cats, the decrease was not statistically significant ($P > 0.05$).

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Table 1: Mean values ± SE for total antioxidant capacity, catalase , superoxide dismutase and glutathione peroxidase activities in infected and uninfected cats

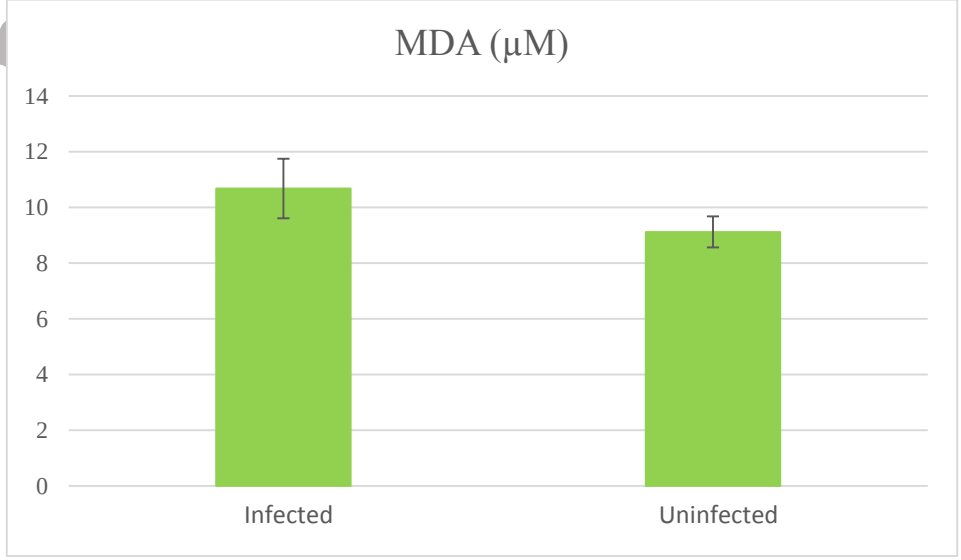
parameter	Infected Mean ± SE	Uninfected Mean ± SE
TAC (mM)	0.118 ± 0.004	0.126 ± 0.005
SOD (U/mL)	34.47 ± 1.32	30.82 ± 3.82
GPx (U/mL)	128.66 ± 35.62	235.66 ± 74.13
CAT (U/mL)	12.19 ± 1.03	13.12 ± 2.6

There were no statistically significant differences between the groups ($p > 0.05$).

3.1.Malondialdehyde level

Figure 1 illustrates the MDA concentrations in both infected and uninfected groups. Although MDA levels appeared elevated in the infected group relative to controls, statistical analysis indicated that the difference did not reach significance. ($P > 0.05$).

Figure 1. Mean for malondialdehyde concentration in infected and uninfected cats.



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4.Discussion

In recent years, the role of oxidative stress in the development and progression of parasitic infections has garnered increasing attention. Several investigations have demonstrated elevated levels of ROS following parasitic infections, suggesting that oxidative imbalance may contribute significantly to disease mechanisms. This study evaluated the status of oxidative stress and lipid peroxidation in cats naturally infected with *Toxoplasma gondii*, comparing results with uninfected controls.

Our findings indicate that lipid peroxidation, as assessed by MDA levels, was higher in infected cats ; however the increase did not reach statistical significance Similarly, SOD levels in infected cats did not show significant differences compared to healthy cats. While CAT, GPx, and TAC levels showed a downward trend in infected cats, these changes were also not statistically significant. These findings suggest that oxidative alterations induced by *Toxoplasma gondii* infection may be primarily localized within tissues rather than reflected in serum enzyme levels. It is possible that, during the acute phase of toxoplasmosis, reactive ROS are intensively produced, and oxidative stress is induced in the tissues of infected animals as the host defense against the infection (16,17).

Comparable studies have reported similar findings. For instance, Engin et al. noted that mice infected with *Toxoplasma gondii* exhibited increased MDA concentrations in liver, brain, and spleen, while no significant elevation was detected in serum levels (18). Bahrami et al. also documented a non-significant rise in serum lipid peroxidation markers in infected rats on the eighth day post-infection (19).

In contrast, Al-Kennany reported a significant increase in MDA levels in placental tissues of ewe infected with *Toxoplasma gondii*, suggesting that localized oxidative stress may vary depending on the specific tissue affected (20). Atmaca et al. demonstrated a significant rise in MDA levels in gerbils infected with *Toxoplasma*, attributing this to excessive production of free radicals and oxidative stress following infection (21).

With respect to SOD activity, our findings align with prior reports indicating no significant alterations in serum SOD levels in *Toxoplasma gondii* infection (20,22). Nevertheless, some studies have shown reduced SOD activity in infected animals (21). Nazarlu's research on experimental *Toxoplasma gondii* infection in male rats demonstrated a significant reduction in SOD activity by day 80 post-infection, suggesting that prolonged infection may have a more pronounced impact on antioxidant defense mechanisms (23).

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In terms of glutathione peroxidase and catalase activity, our results demonstrated a reduction in infected cats, although these changes were not statistically significant. These results are consistent with previous investigations by Al-Kennany and Machado et al., who documented decreased GPx activity in infected tissues (9,20). Nazarlu also identified a significant reduction in catalase levels in rats infected with *Toxoplasma gondii* (23). Mohammed et al. reported reduced GPx activity in the serum of seropositive pregnant women (24). In contrast, Delavari et al. observed increased GPx in liver tissue at an early stage of infection, suggesting time-dependent and tissue-specific differences in enzyme responses (25).

In addition, Turkoğlu et al. reported a significant increase in GPx activity in the liver tissue of infected rats, whereas no significant changes were reported in brain and kidney tissues (22). The TAC trends observed in our study are consistent with findings by Mohammed and Bahrami, who reported no significant reduction in serum catalase levels in infected samples (19,24). However, Delavari et al. observed an increase in TAC in liver tissue on the eighth day post-infection, indicating that antioxidant responses may fluctuate depending on the phase of infection (25).

The present study suggests that *Toxoplasma gondii* infection affects antioxidant enzyme activity and lipid peroxidation in cats. However, these changes were not statistically significant in serum samples. Given that *T. gondii* is an obligate intracellular protozoan, oxidative stress may be more pronounced at the tissue level rather than in systemic circulation, which could explain the lack of significant variations in serum antioxidant markers. Further research focusing on tissue-specific analysis and experimental infections is necessary to better understand the oxidative stress mechanisms associated with *Toxoplasma* infections and their potential impact on host pathophysiology.

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Not applicable.

Authors' contributions

Study concept and design: S.P.Y Acquisition of data: M.H and S.P.M Analysis and interpretation of data: S.P.Y and M.H and M.K Drafting of the manuscript: S.P.Y and M.H Revision of the manuscript: S.P.M and M. H. Statistical analysis: S.P.Y and S.P.M

Ethics

It is hereby asserted that all ethical standards have been observed in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest

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Data Availability

The data that support the finding of this study are available on request from the corresponding author.

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