Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2023.190273.2738 Volume 30, Issue 1, January 2024

Manuscript ID ZUMJ-2301-2738 (R1)
DOI 10.21608/ZUMJ.2023.190273.2738

ORIGINAL ARTICLE

Histopathological Evaluation of Iron and Iron Chelator Deferoxamine in Experimentally Infected Mice with Toxoplasma Gondi

Enas S Elbahaie[1*], Faten Al-Sayed Mohammed Sayed Ahmed[1], Alia Ibrahim Mohamed Ibrahim Abd El-Atey[1], Eman M Mostafa[1]

[1] Medical Parasitology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

***Corresponding author:**
Enas S. El-Bahaie

E-mail:
enas.elbahaie@gmail.com

Submit Date 2023-02-01
Revise Date 2023-02-04
Accept Date 2023-02-04

ABSTRACT

Background: Toxoplasmosis is a worldwide parasitic disease infecting about one third of human population. With rising prevalence of drug-resistant strains, new therapies for toxoplasmosis are urgently required. Little studies have been managed to evaluate the effect of iron and iron chelators in experimental toxoplasmosis. Therefore, we investigated if the iron chelator, Deferoxamine can affect T.gondii infection outcome in experimentally infected mice.

Methods: Eighty Swiss albino mice were classified into four groups (20 mice each) as follows: (GI): Healthy control group; (GII): infected non-treated control; (GIII): infected iron supplemented group; (GIV): infected deferoxamine treated group. Scarification of mice was performed on day 8 post infection for measuring the serum iron level and for histopathological analysis of intestinal tissue samples.

***Results*:** Our data indicated that infected-iron supplemented group (GIII) was associated with more increase in the mean serum iron level (150µg/dl). However, the mean serum iron level in deferoxamine treated group (GIV) decreased (77.2µg/dl) with highly statistically significant difference ($p<0.001$). Those findings were confirmed by histopathological assessment; the infected iron supplemented group (GIII) exhibited marked histopathological changes in the intestinal tissues of experimentally infected mice that were greatly improved in GIV after chelation of iron using deferoxamine treatment.

Conclusions: Finally, the present work showed that the acquisition of host iron by T.gondii is a critical step in the progression of infection and is determinant in its outcome. Therefore, deferoxamine could be a promising drug to control acute T.gondii infection due to the limitation of iron availability, and further research is necessary to elucidate these points.

Key words: T.gondii, Iron; Deferoxamine; Serum iron; Histopathology

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoa parasite that infects most warm-blooded animals [1]. Toxoplasmosis is an important health problem worldwide, it is one of the principal reasons of foodborne illness in the United States [2]. Nearly, 15-85% of individuals in the world are infected with toxoplasmosis [3]. It is well known that Africa and South America contain a greater number of pathogenic *T. gondii* genotypes [4].

Ingestion of raw or undercooked infected meat, consumption of mature oocysts from the environment,

congenital, blood transfusion, and organ transplantation are considered the main routes of infection with the T.gondii parasite [5].

In healthy people, toxoplasmosis is generally asymptomatic, but it can result in serious illness in pregnant women, immunosuppressed individuals, and recipients of organ transplants [6]. Notably, different studies have revealed a strong correlation between the prevalence of suicide, schizophrenia, Alzheimer's and Parkinson's disorders and toxoplasmosis [7].

The approved medications for the treatment or prevention of toxoplasmosis include pyrimethamine and sulfadiazine. However, they have negative side effects. Other toxoplasmosis medications are poorly tolerated [8]. It is urgently necessary to find novel medications that inhibit T. gondii tachyzoites and prevent their spread to all organs and their transformation into bradyzoites within tissue cysts [9].

Iron is necessary for growing of approximately all living organisms, from prokaryotes to humans. Numerous biological functions, including DNA synthesis and oxygen transport, depend on iron. During infection whenever microorganisms and humans interact, the shared requirement for iron forms these interactions [10]. As a result, there is an ongoing competition for iron throughout infection between the host and the invader, for example, the parasite seeks to access the host's iron, while the host sets up sophisticated iron-withholding systems to fight the iron stealing [10].

Deferoxamine (DFO) is used in clinical practice to remove iron from patients with iron storage diseases. DFO prevents or counteracts the consequences of free radical generation by rendering the iron that it binds, metabolically inactive. Therefore, reduces oxidative damage in vital organs and it may even lead to improved survival in in vivo and in vitro studies [11].

Little is known about how DFO affects T.gondii development and few studies have been done to assess the effect of iron and iron chelators in experimental toxoplasmosis [12, 13].

Therefore, the aim of the current work is to evaluate the effect of addition or deprivation of iron on T.gondii infection outcome in mice by serum iron analysis and histopathological study.

METHODS

This case control experimental study was carried out from June 2021 to July 2022 at the Parasitology Department, Faculty of Medicine, Zagazig University, Egypt.

Avirulent (ME49) T.gondii strain:

Avirulent chronic Toxoplasma strain was provided from the Medical Parasitology Department, Faculty of Medicine, Zagazig University, Egypt. Regular oral administration of the mice with diluted brain suspension of previously infected mice containing around (25 cysts in 0.1 ml) every 45 - 60 days allowed us to maintain the strain in our department [14].

Experimental animals

In this experiment, eighty free laboratory-bred swiss albino mice of both sex weighing 20-25 gm and aged 3-4 weeks. Mice were classified into four groups; 20 mice each (GI): Healthy control group. (GII): Infected control group injected intraperitoneally (I.P) with vehicle (0.1 ml phosphate buffered saline) (PBS) one day before infection and for an additional seven days post infection. (GIII): Infected-iron sucrose supplemented group. (GIV): Infected-Deferoxamine treated group.

Mice were housed in well-ventilated cages (20 mice per cage) with daily bedding changes, fed a conventional pelleted food with unrestricted access to water and maintained according to the research protocols following the recommendations of the National Institutes of Health Guidelines for Animal Experimentation. The Zagazig University Faculty of Medicine's ethical committee provided its approval to the experimental protocol. ZU-IACUC/3/F/34/2021.

Drugs

Iron sucrose (iron saccharate) ($C_{12}H_{29}Fe_5Na_2O_{23}$): Scrofer ampoules, manufactured and provided by Amoun pharmaceutical company, Egypt. The selected dose for the present work was 80mg/kg/day [15].

Deferoxamine mesylate ($C_{26}H_{52}N_6O_{11}S$): Desferal vials, provided by Novartis, Egypt. The chosen dosage for this study was 300mg/kg/day [12].

Both drugs were administered intraperitoneally one day before infection and for an additional seven days post infection, according to the drug table of Paget and Barnes [16].

Experimental infection and design

Mice were infected by giving them 25 cysts in 0.1 ml of diluted brain solution orally. The infection was obtained from the brain tissues of another mouse, which was infected 45-60 days earlier. The mouse was sacrificed and the brain was taken, and it was sterilely homogenized in 1ml of saline. Tissue cysts were released by homogenizing glass. A slide was covered with one drop (25µl) of the brain

homogenate for microscopic analysis. To quantify tissue cysts in one ml of the brain suspension, tissue cysts were first counted in four drops and multiplied by 10 [17]. The infective dose was determined to be 25 cysts in 0.1 ml of the brain suspension [14]. All of the mice from the four groups were cervical dislocated-sacrificed on day 8 after infection, and small intestine tissues were removed for histopathological analysis and blood samples were taken to check the serum iron level.

I. Serum iron levels assessment

In non-hemolyzed serum samples, serum iron levels were determined by spectrophotometric measurement using a commercial kit (IRON-CAP, chromazurol B method, for in vitro determination of serum iron, Quimica Clinica Aplicada S.A). Following the manufacturer's instructions, a colorimetric assay was used to measure the iron concentration.

II. Histopathological assessment

Intestinal samples were preserved in 10% formalin for 24 h, rinsed in water for 12 h, and then dried out in ascending ethanol concentrations (70% ethanol for 120 min, then 90% ethanol for 90 min, and finally 100% ethanol (two cycles) for 1 h per cycle. Samples were then cleared by submerging them in a mixture of 50% ethanol and 50% xylene for 1 h, followed by pure xylene for 1.5 h. After that, paraffin wax was used to embed the samples. Paraffin sections (4–5µm) were stained with haematoxylin and eosin [18]. The extent of inflammatory cell infiltrates within intestinal tissue was evaluated. Five histological sections from each animal were examined. The average score of ten low-power fields (100×) from each of the examined sections was then calculated (+1 = mild reaction; +2 = moderate reaction; and +3 = intense reaction) [19].

STATISTICAL ANALYSIS

Quantitative values of the measured parameters were expressed as mean ± standard deviation (SD). Statistical Package for Social Sciences (SPSS), version 25.0, was used to analyze the data using the ANOVA test to determine the significance of differences between studied groups, version 25.0. All statistical tests were considered significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$.

RESULTS

I. Serum Iron samples results

In the present study, the mean serum iron in the non-infected control group (GI) was 49.3µg/dl. While we found that the mean serum iron increased in infected control group (GII) (100.5µg/dl). It was observed that infected-iron supplemented group (GIII) is associated with more increase in the mean serum iron level (150µg/dl). However, the mean serum iron level in deferoxamine treated group (GIV) decreased (77.2µg/dl) with highly statistically significant difference ($p<0.001$*) between the different study groups as shown in Table (1), Fig. (1).

II. Histopathological results

Group I (GI) showed normal small intestinal villous, mucosal, submucosal and muscular coat structures. However, GII showed intestinal lesions, which were represented by moderate to marked lympho-plasmocytic mucosal infiltration, submucosal aggregations and peyer's patches hyperactivation. Some of the mucosal cells were necrotic and or degenerated and sloughed (Fig. 2). On the other hand, the intestinal lesion of GIII were represented by villous degenerative, necrotic and desquamative changes associated with marked lymphoplasmacytic infiltration, focal epithelial stratification, hyperplastic peyer's patches, focal villous and glandular regenerative changes with presence of many mitotic figures and presence of inter-epithelial large basophilic ovoid structures. Paneth cells were hyper-reactive. Interestingly, lesions of GIV were recognized by mild villous degenerative changes with partial sloughing. Mild lymphoplasmacytic infiltration of the mucosa and hyperplastic peyer's patches were encountered. Villous and glandular mucinous degeneration, focal regeneration and paneth cells hyperactivation were seen (Fig. 3).

Table 1: Serum iron levels among the different studied groups:

Serum iron in µg\dl	GI (healthy control)	GII (infected control)	GIII (iron treated)	GIV(DFO treated)	F	P value
Mean±SD	49.3±15.57	100.5±24.08	150±26.51	77.2±15.48	41.239	<0.001*
Range	29.90 – 70.80	72.60 –143.30	103.90-186.50	52.40 – 96.30		

SD: Standard deviation F: ANOVA test *: highly significant (P<0.001) , GI: Healthy control group, GII: infected, non- treated control group, GIII: infected-iron supplemented group, GIV: infected-DFO treated group.

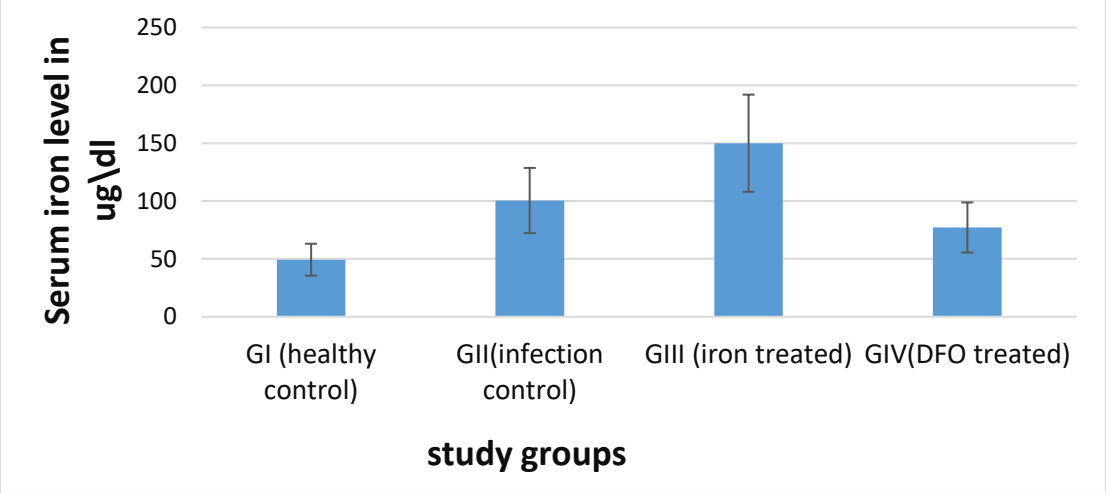

Fig. 1: Comparison between the different study groups as regard serum iron level.

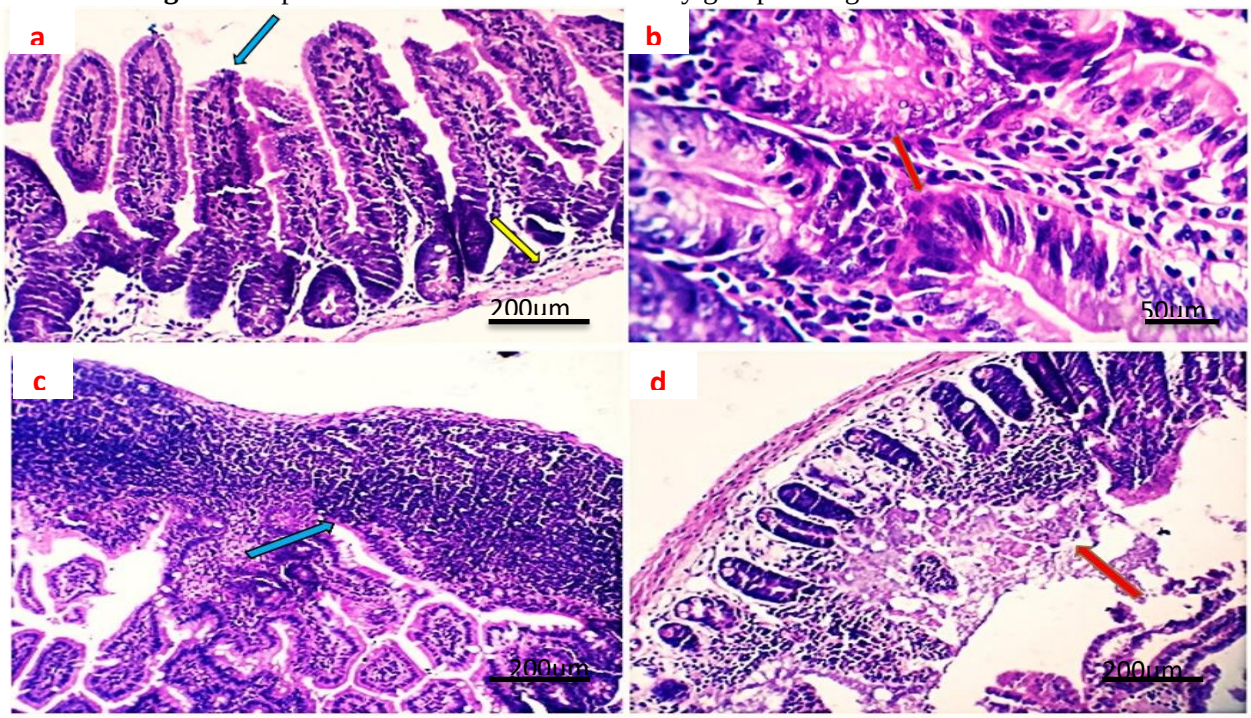

Fig. 2: Photo-micrograph from intestine of GI (a, b) showed normal small intestinal villous (blue arrow), mucosal (red arrow), submucosal and muscular coat structures (yellow arrow). Intestinal sections of GII (c, d) showed submucosal aggregations and peyer's patches hyperactivation (blue arrow). lympho-plasmocytic mucosal infiltration (red arrow), H&E ×200, ×400.

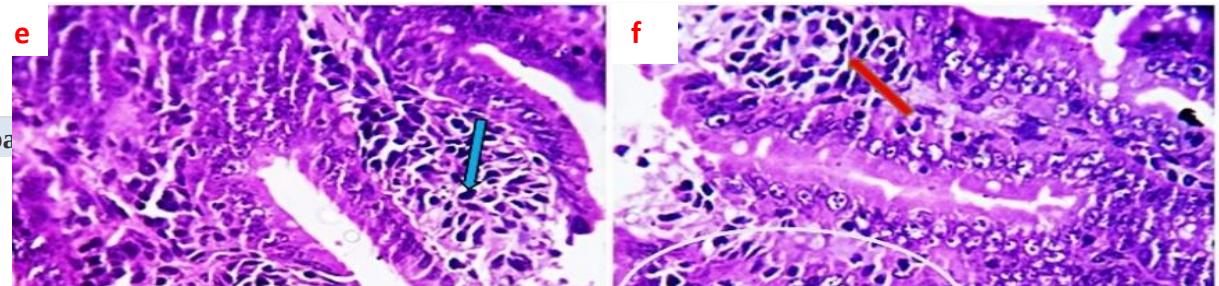

Fig. 3: Photo-micrograph of the intestinal section of GIII (e,f) showed lymphoplasmacytic infiltration (blue and red arrows). Focal epithelial stratification is also seen (white circle). Intestinal sections of GIV (g,h) showed hyperplastic Peyer's patches are seen (blue arrow). Paneth cells hyperactivation is seen (white circle). Large ovoid basophilic structures with ill-distinct internal details are seen among cells of the intestinal glands (red arrow) H&E ×200, ×400.

DISCUSSION

Toxoplasma gondii is an intracellular parasite of humans and animals which produces life-threatening conditions in immunocompromised people [20]. Current therapeutics are associated with numerous side effects [21]. Additionally, because of the parasite's developing resistance, the current anti-Toxoplasma medications have become less effective due to the evolving genetic mutations [22].

Numerous biological activities involve iron. For instance, proteins necessary for redox processes, mitochondrial respiration, and the production of nucleic acids. All forms of life, from single-celled organisms to humans, retain these evolutionary conserved functions. Additionally, experimental research has shown that a greater availability of iron promotes the proliferation of a number of pathogenic protozoa [23].

Deferoxamine is effective in treating a variety of protozoa infections, including Plasmodium berghei [24], Leishmania infantum [25] and Trypanosoma cruzi [26] due to limitation of iron availability. Few studies have been reported to determine the interactions between iron metabolism and the pathogenesis of T.gondii [12, 13, 27].

In the present study, serum iron levels of all mice were measured on day 8 after infection to determine the role of iron in parasite growth during oral Toxoplasma infection. In relation to serum iron, it was discovered that there was a statistically significant difference between all of the studied groups (Table 1). When T.gondii infection was treated with iron saccharate ($C_{12}H_{29}Fe_5Na_2O_{23}$) or phosphate buffer saline, it was found that the iron serum levels in the treated mice were higher than those in the uninfected animals. The fact that deferrioxamine mesylate ($C_{26}H_{52}N_6O_{11}S$) therapy was able to reduce the iron levels systemically ($p < 0.001$) suggesting that iron is a nutrient that T. gondii needs for growth and reproduction. Additionally, a rise in iron was also noted by Oliveira et al. [12] and Rishi et al. [28] as well as an accumulation of iron in the intestinal epithelium, lung, and liver was detected on day 8 of the infection when mice were orally infected with T. gondii.

Regarding histopathological examination, intestinal lesions in GII were represented by moderate lympho-plasmocytic mucosal infiltration submucosal aggregations and Peyer's patches hyperactivation (Fig.2 c,d). Trevizan et al. [28] found that acute T.gondii infection resulted in morphological changes in the intestinal wall and epithelial cells of the duodenum of rats, including an increase in villi and crypt width, an increase in goblet cell number, and an increase in intraepithelial lymphocyte number. Additionally, the infected groups demonstrated atrophy in the submucosa, muscle layers, and the whole wall.

On the other hand, in GIII, pathological changes in intestinal sections showed higher degree of inflammatory infiltration and degenerative changes compared to GII (Fig.3 e,f). In comparison to other groups, the infected-iron supplemented group is associated with greater inflammatory reactions, according to Olivera and his colleagues [12]. T.gondii needs iron for survival and multiplication in the host cells, according to Dziadek et al. [29]. Furthermore, a variety of evidence may point to the existence of particular parasite receptors for human lactoferrin that are involved in delivering host iron to the tachyzoites. As regard, DFO-treated mice (GIV), inflammatory and degenerative changes were significantly lower in the small intestine (Fig.3 g,h) when compared with infected control group (GII). Our findings are supported with that of Olivera et al. [12], who mentioned that DFO is associated with decreasing inflammatory score in experimentally infected mice with T.gondii. Interestingly, Dimier and Bout [30] stated that by employing the iron chelator, deferoxamine, the parasite's intracellular growth was inhibited due to a lack of iron availability, which was then overcome by the addition of iron saturated transferrin, holotransferrin. Also, Almeida et al. [13] stated that deferoxamine improve the cellular viability. They added that DFO controls T.gondii proliferation in human trophoblastic cells as well as villous explants.

Finally, the present work showed that the acquisition of host iron by T.gondii is a critical step in the progression of infection and is determinant in its outcome. The chelation of iron using

deferoxamine treatment showed histopathological improvement in intestinal tissues of experimentally infected mice with T.gondii parasite. Therefore, deferoxamine could be a promising drug to control acute T.gondii infection due to the limitation of iron availability and additional experiments are necessary to clarify these points.

DECLARATION OF INTEREST

The authors report no conflicts of interest.

FUNDING INFORMATION

None declared

REFERENCES

1. Lindsay DS, Dubey JP. Chapter 6-Toxoplasmosis in wild and domestic animals. In Toxoplasma Gondii, 3rd ed.; Weiss, L.M., Kim, K., Eds.; Academic Press: Cambridge, MA, USA. 2020: 293–320.

2. CDC. Retrieved From CDC: Centers for Disease Control Prevention. In: Parasites-Toxoplasmosis (Toxoplasma Infection): Epidemiology and Risk 2021 https://www.cdc.gov/parasites/toxoplasmosis/epi.html

3. Daryani A, Sarvi S, Aarabi M, Mizani A, Ahmadpour E, Shokri A et al. Seroprevalence of Toxoplasma gondii in the Iranian general population: a systematic review and meta-analysis. Acta Trop 2014; 137:185–94. https://doi.org/10.1016/j.actatropica.2014.05.015

4. Hamidović A, Etougbétché JR, Tonouhewa ABN, Galal L, Dobigny G, Houémènou G, et al. A hotspot of *toxoplasma gondii* Africa 1 lineage in Benin: how new genotypes from West Africa contribute to understand the parasite genetic diversity worldwide. PLoS Negl. Trop. Dis. 2021;15 doi: 10.1371/journal.pntd.0008980.

5. Dumetre A, Darde ML. How to detect Toxoplasma gondii oocysts in environmental samples? FEMS Microbiol Rev. 2003; 27:651-61. https://doi.org/10.1016/S0168-6445(03)00071-8

6. Dubey J, Jones J. Toxoplasma gondii infection in humans and animals in the United States. Int J Parasitol. 2008; 38:1257-78. https://doi.org/10.1016/j.ijpara.2008.03.007

7. Abo-Al-Ela, HG. Toxoplasmosis and Psychiatric and Neurological Disorders: A Step Toward Understanding Parasite Pathogenesis. ACS Chem. Neurosci. 2019; 11 (16), 2393–406. https://doi: 10.1021/acschemneuro.9b00245

8. Shammaa AM, Powell TG, Benmerzouga I. Adverse Outcomes Associated With the Treatment of Toxoplasma Infections. Sci. Rep.2021; 11 (1), 1–8. doi: 10.1038/s41598-020-80569-7.

9. Black MW, Boothroyd JC. Lytic Cycle of Toxoplasma Gondii. Microbiol. Mol. Biol. Rev. 2000; 64 (3), 607–23. doi:10.1128/MMBR.64.3.607-623.

10. Arroyo R, Ochoa T, Tai JH, de la Garza M. Iron and Parasites. Biomed Res Int. 2015; doi: 10.1155/2015/291672.

11. Simeonova PP, Luster MI. Iron and reactive oxygen species in the asbestos-induced tumor necrosis factor-a response from alveolar macrophages. Am J Resp Cell Mol Biol.1995; 12:676-83.

12. Oliveira MC, Coutinho LB, Almeida MPO, Briceño MP, Araujo ECB, Silva NM. The availability of iron is involved in the murine experimental Toxoplasma gondii infection outcome. Microorganisms. 2020; 8(4), 560.

13. Almeida MPO, Ferro EAV, Briceño MPP, Oliveira MC, Barbosa BF, Silva NM. Susceptibility of human villous (BeWo) and extravillous (HTR-8/SVneo) trophoblast cells to Toxoplasma gondii infection is modulated by intracellular iron availability. Parasitology research. 2019; 118(5), 1559-72.

14. Fuentes-Castro BE, Reyes-García JG, Valenzuela-Vargas MT, Martínez-Gómez F. Histopathology of murine toxoplasmosis under treatment with dialyzable leukocyte extract. Mem Inst Oswaldo Cruz. 2017; 112 (11): 741-7.

15. Kuo KL, Hung SC, Lee TS, Tarng DC. Iron Sucrose Accelerates Early Atherogenesis by Increasing Superoxide Production and Upregulating Adhesion Molecules in CKD: Journal of the American Society of Nephrology. (JASN). 2014; 25 (11):2596-606.

16. Paget GE, Barnes JM. Evaluation of results: Qualitative application in different species. In: Laurence DR, Backarach AL (eds.), Evaluation of drug activities. Pharmacometrics. Academic press, London and New York.1964; 1: 160-7.

17. Dubey JP and Beattie CP. Toxoplasmosis of Animals and Man. 1988; CRC Press Boca Raton, Florida.

18. Kiernan JA. histological and histochemical methods: Theory and practice, 3rd edition. Shock. 1999; 12(6): p 479.

19. Elgendy D, Othman A, Hasby Saad M, Soliman N, Mwafy S. Resveratrol reduces oxidative damage and inflammation in mice infected with Trichinella spiralis. Journal of Helminthology 2020; 94, E140. doi:10.1017/S0022149X20000206.
20. Daher D, Shaghlil A, Sobh E, Hamie M, Hassan ME, Moumneh MB, El Hajj H. Comprehensive Overview of Toxoplasma gondii-Induced and Associated Diseases. Pathogens. 2021; 10(11): 1351.
21. Allam AF, Shehab AY, Fawzy Hussein Mogahed NM, Farag HF, Elsayed Y, Abd El-Latif NF. Effect of nitazoxanide and spiramycin metronidazole combination in acute experimental toxoplasmosis. J Parasit Dis. 2020; 6(4):e03661.
22. Montazeri M, Mehrzadi S, Sharif M, Sarvi S, Tanzifi A, Aghayan SA. Drug resistance in Toxoplasma gondii. Front Microbiol. 2018; 9: 2587.
23. Weinberg ED. Iron availability and infection, Biochim Biophys Acta. 2009;1790(7):600-605. doi: 10.1016/j.bbagen.2008.07.002.
24. Hershko C, Peto TE. Deferoxamine inhibition of malaria is independent of host iron status. J. Exp. Med.1988; 168, 375–87.
25. Malafaia G, Marcon Lde N, Pereira Lde F, Pedrosa, ML, Rezende SA. Leishmania chagasi: effect of the iron deficiency on the infection in BALB/c mice. Exp Parasitol. 2011; 127:719-23.
26. Paiva CN, Feijó DF, Dutra FF, Carneiro VC, Freitas GB, Alves LS et al. Oxidative stress fuels Trypanosoma cruzi infection in mice. J. Clin. Investig.2012; 122, 2531–42.
27. Portes JA, Souza TG, dos Santos TA, da Silva LL, Ribeiro TP, Pereira MD et al. Reduction of Toxoplasma gondii Development Due to Inhibition of Parasite Antioxidant Enzymes by a Dinuclear Iron (III) Compound. Antimicrob Agents Chemother. 2015; 59(12):7374-86. doi: 10.1128/AAC.00057-15.
28. Rishi G, Subramaniam VN. The liver in regulation of iron homeostasis. Am. J. Physiol. Gastrointest. Liver Physiol. 2017; 313:157–65.
29. Trevizan AR, Vicentino-Vieira SL, da Silva, Watanabe P, Góis MB, de Melo Gde A, Garcia JL et al. Kinetics of acute infection with Toxoplasma gondii and histopathological changes in the duodenum of rats. Exp Parasitol. 2016; 165:22-9. Doi: 10.1016/j.exppara.2016.03.015.
30. Dziadek B, Dziadek J, Dlugonska H. Identification of Toxoplasma gondii proteins binding human lactoferrin: a new aspect of rhoptry proteins function. Experimental parasitology. 2007; 115(3), 277-82.
31. Dimier IH, Bout DT. Interferon-gamma-activated primary enterocytes inhibit Toxoplasma gondii replication: a role for intracellular iron. Immunology. 1998; 94(4):488-95.

To Cite :

Elbahaie, E., Mohammed, F., Ibrahim, A., Mostafa, E. Histopathological Evaluation of Iron and Iron chelator Deferoxamine in Experimentally Infected Mice with Toxoplasma gondii. *Zagazig University Medical Journal*, 2024; (241-247): -. doi: 10.21608/zumj.2023.190273.2738