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Study of some biochemical indicators levels in the people infected by *Toxoplasma gondii*

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Study of some biochemical indicators levels in the people infected by *Toxoplasma gondii*

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Abstract Pregnant women are at risk of contracting *Toxoplasma gondii* due to immunodeficiency, as the increased risk of miscarriage is associated with infected pregnant women or fetal abnormalities**.** Symptoms in children after birth are jaundice, enlarged liver, spleen, lymph nodes, vision and hearing problems, partial encephalitis, microcephaly, as well as motor delay and mild to severe episodes of mental retardation**.** The purpose of this study was to evaluate the effect of *T. gondii* on the biochemical indicators. The current study included the collection of 50 serum samples from pregnant women infected with the parasite *T. gondii*. The results showed that the incidence of infection reached 40% and the highest age group (18-20) reached 16%, followed by the age group (21-25) and the group (26-30) with a percentage of 12%. The results of biochemical tests indicated a significant increase ($p < 0.05$) in cholesterol, Triglycerides, Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), and Alkaline Phosphatase (ALP), ceroplasmin and total iron compared to the control group. The results of this study obtained can be applied to decrease the effect of *T. gondii* in human, by using developed techniques to treat.

Keywords: *Toxoplasma gondii*, cholesterol, triglyceride, liver enzymes, total iron.

INTRODUCTION:

A species of parasite protozoan called *T. gondii* is the primary cause of the dangerous infectious disease toxoplasmosis, which infects both humans and other warm-blooded animals as intermediate hosts before infecting cats as the sole final host [Nelson et al., 2007]. Food contaminated with egg sacs, meat contaminated with tissue bags, contact with cats, or an environment polluted with the disease's infectious stages are all ways that people become infected [Jiang et al., 2015]. The risk of infection during the first months of pregnancy lies in the absence of infection symptoms during this period, which can result in miscarriage, while infection during the middle months of pregnancy can result in the death of the fetus. This makes the transmission of the parasite through the mother's placenta to her fetus one of the most dangerous methods of infection. Infection during the final three months of pregnancy can also cause congenital fetal deformities include hydrocephalus, congenital retardation, and epilepsy [Aabasian et al., 2016]. Chronic toxoplasmosis can harm the liver in tandem with the parasite's observed reproduction, which will impair liver function and change the activity of the liver and ceruloplasmin. One of the

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main signs of liver damage is an increase in liver enzymes like AST and ALT in the blood serum. A higher serum ALT level enhances the ability to diagnose liver damage [Jwad et al., 2020].

T. gondii shapes:

The parasite experiences two periods during its life cycle; sexual phase and the asexual phase. Cats are the parasite's main host as a result. An asexual phase occurs in warm-blooded animals such as cats, mice, humans, and birds [Lozano, 2011].

1. **Bradyzoites**: In this phase the parasite crescent-shaped and cystic in tissues, it grows by internal budding and stays inside the cell, it also has a nucleus centered at the posterior end, and it is less susceptible to enzyme breakdown than the fast phase. Cysts prevalent in the eyes, brain, skeletal muscles and nerve tissue [Dubey, 2010].
2. **Tachyzoites**: The parasite at this stage has an oval form with a convex anterior end and a round posterior end. The nucleus is located close to the center [Montoya & Liesenfeld, 2004]. The cytoskeleton consists of microtubules, apical rings, and at the conical end there are secretory organelles, rhoptries, micronemes, densegranuls, endoplasmic reticulum and golgi apparatus, as this phase moves by rotation because it does not have movement organelles [Dubey & Jones, 2008]. The parasite is present in bodily fluids, including blood plasma fetal and cerebrospinal fluid; as well as heart muscle, brain cells, skeletal muscles, placenta, and eyes [Jiménez-Coello et al., 2012].
3. **Oocyst**: Cats' intestines contain the spherical or semi-spherical sacs, which are encircled by a thick, two-layered membrane. The cat feces contains the mature egg sac each a mature egg sac includes two sporozoites inside which there are divided to four sporozoites in each sac [Dubey, 2004]. Oocysts are characterized by their ability to resist environmental conditions, as they can survive in water and moist soil where they can survive up to 500 days at room temperature [Harker et al., 2012] as shown in Figure (1 A & B).

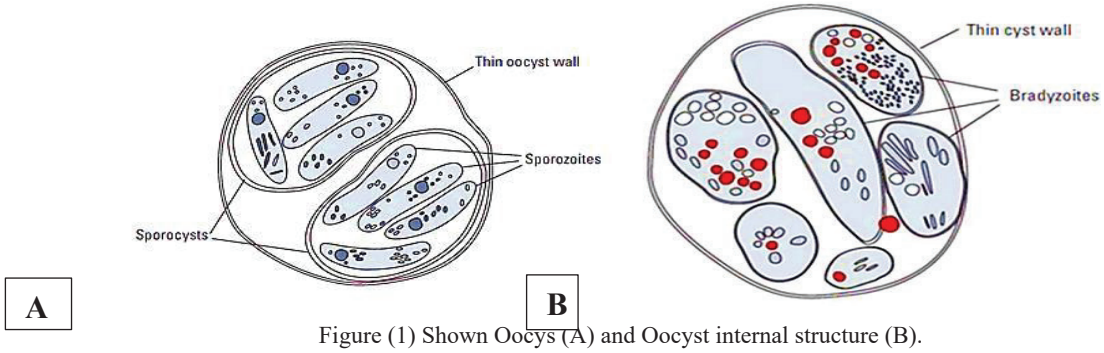

Figure (1) Shown Oocys (A) and Oocyst internal structure (B).

Life cycle:

Sexual and asexual cycle begins in the gastrointestinal tract of cats after eating contaminated foods containing oocyst, after the oocyst is swallowed, its wall begins to decompose by the action of enzymes in the stomach and small intestine to launch the multiply asexually within the intestinal epithelial cells [De Craeye, 2012 & Ahn et al., 2019]. The male gamete containing two flagella fuses with the female gamete to fertilize. The zygote grows into the egg sac, the oocyst egg sacs are released to the outside with the cat's feces [Uzal et al., 2020]. The sac begins to mature when it is released into the environment to be inside the sporozoite, and the egg sac does not become infectious until after the formation of the sporozoites to maintain its ability to infect for a period of up to a year. The rapid phases of Tachyzoites go to infect the different tissues of the cat to form the slow phase of the bradyzoite in which the bursting of these phases causes the parasite to return to the intestine to start a new life cycle [Kheirandish et al., 2019]. After the intermediate host swallows the infective mature egg sac containing the sporozoites, the spores begin to be released as a result of the action of enzymes that penetrate the intestinal epithelium to multiply, forming the rapid phase that attacks tissues by blood and lymph to produce the acute phase of the disease [Mendez & Koshy, 2017], as shown in Figure (2).

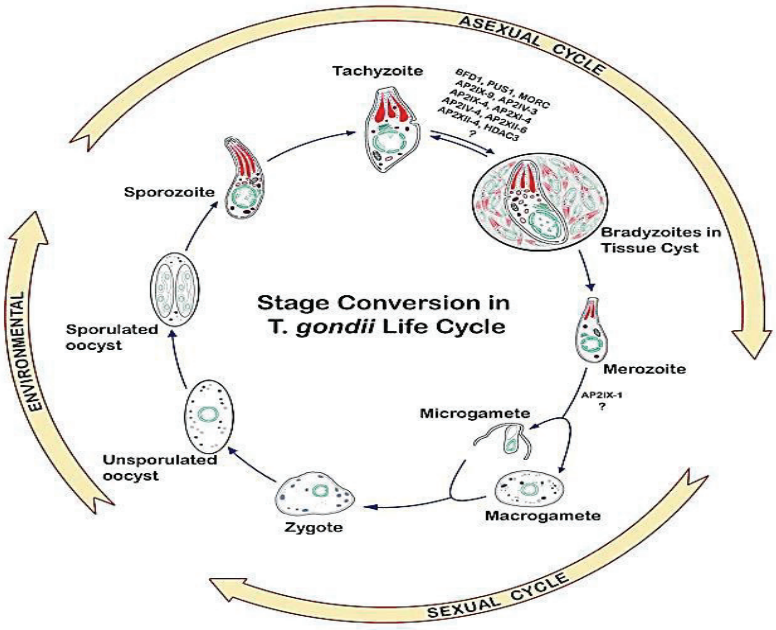

Figure (2) Life cycle of *T. gondii*

Pathogenicity:

The increased risk of miscarriage in infected pregnant women or the occurrence of fetal abnormalities is associated with the presence of this parasite [Khan and Khan, 2018; & Türkoğlu et al., 2018]. The symptoms in children after birth are jaundice, liver, spleen, lymph nodes enlargement, vision and hearing problems, partial encephalitis, microcephaly and hydrocephalus, as well as kinetic delay and mild to severe episodes of mental retardation [Nadwa & Mahmood, 2012]. The presence of the parasite plays a role in influencing the dopamine function, which leads to an increase in the level of dopamine in the brain and thus an increase in the stress hormone [Didiano & Monti, 2020].

METHODOLOGY AND EXPERIMENTAL DESIGN:

The study was conducted from January 2021 to March 2022. The study included samples of female patients arriving at Dujail Surgical Hospital in Dujail and some private laboratories in Samarra. The study included (50) serum samples from women arriving at health centers. The study groups were divided into two groups of women, the first group included (35) serum samples from pregnant women who suffered from previous miscarriages, and the second group was the control group, which included 15 samples from healthy women. Blood samples were collected in a volume of (5) ml in clean dry tubes and placed in an incubator at 37°C for (5) minutes, then the coagulated fraction was separated from the clear solution using a centrifuge at a speed of (2000 cycle/min) for (15) minutes. The clear solution represents the blood serum that was drawn using a micropipette, which was divided into (7) parts using plastic tubes, then the samples were kept at a temperature of 20 °C until the required tests were performed on them. Concentrations of cholesterol, (ALP), (AST) and (ALT), Ceruloplasmin, and total iron were measured by used the equipment of all test.

Statistical analysis:

The statistical analysis was used to analyses the information obtained after the end of the experience period to the aim of reaching right inference, and then to made the suitable recommendations. Statistical analyses of this study data inclusive developed statistical analysis by used ANOVA test (one-way) followed by Duncan's test ($p < 0.05$).

RESULTS:

Biochemical changes and *T. gondii*

Total cholesterol and T. gondii

The results of the serum specimens collected from the women infection by *T. gondii* were analyzed in the end of experiment days to determine the effects of *T. gondii* on total cholesterol. The results show a significant ($p < 0.01$) (Table 1) increase in the blood total cholesterol in the women diagnosed by *T. gondii* compared to the control group.

Table 1: Changes in the blood total cholesterol level in the women diagnosed with *T. gondii*. Each bar represents the Mean ± SD ($n = 50$) at a significant differences ($p < 0.01$) as compared to the respective control value.

TABLE 1. Group 1

	M ± S.d	P value
Control group	**119 ± 50.3**	A
Patient group	**156.3 ± 0.24.2**	B

Triglycerides and T. gondii

The results of the serum specimens collected from the women diagnosed by *T. gondii* were analyzed in the end of experiment days to determine the effects of *T. gondii* on Triglycerides. The results showed significance ($p < 0.01$) (Table 2) raise of the Triglycerides serum in the women infections by *T. gondii* compared to the control group**.**

Table 2: Changes in the blood triglycerides level in the women diagnosed with *T. gondii*. Each bar represents the Mean ± SD ($n = 50$) in a significance differences ($p < 0.01$) as compression to the control groups.

TABLE 2. Group 2

	M ± S.d	P value
Control group	**7.60 ± 2.0**	A
Patient group	**87.5 ± 34.1**	B

ALP enzyme and T. gondii

The results of the serum specimen collection from the women infected by *T. gondii* were analyses at the end of the experiment days for determined the effects of *T. gondii* on (ALP) enzyme. The results show a significant ($p < 0.01$) (Table 3) raise in the blood (ALP) enzyme level of the women infected by *T. gondii* compared to the control group**.**

Table 3: Changes in the blood (ALP) enzyme level in the women diagnosed with *T. gondii*. Each bar represents the Mean ± SD ($n = 50$) at a significance differences ($p < 0.01$) as compression with the control groups.

TABLE 3. Group 3

	M ± S.d	P value
Control group	**3.95 ± 1.47**	A
Patient group	**20,8 ± 40.3**	B

AST enzyme and T. gondii

The results of the serum specimen collection from the women infected by *T. gondii* were analyses at the end of the experiment days for determined the effects of *T. gondii* on (AST) enzyme. The results show significance ($p < 0.01$) (Table 4) raise in the blood (AST) enzyme level of the women diagnosed by *T. gondii* compared to the control group.

Table 4: Changes in the blood (AST) enzyme level in the women diagnosed with *T. gondii*. Each bar represents the Mean ± SD ($n = 50$) in a significance differences ($p < 0.01$) as compared with the control group.

TABLE 4. Group 4

	M ± S.d	P value
Control group	**4.53 ± 2.61**	A
Patient group	**4.90 ± 3.45**	B

ALT enzyme and Toxoplasma gondii

The results of the serum specimen collection from the women diagnosed by *T. gondii* were analyses at the end of the experiment days for determined the effects of *T.gondii* on (ALT) enzyme. The results show significance ($p < 0.01$) (Table 5) raise of the blood (ALT) enzyme levels in the women diagnosed by *T.gondii* compared to the control group**.**

Table 5: Changes in the blood (ALT) enzyme level in the women diagnosed with *T. gondii*. Each bar represents the Mean ± SD ($n = 50$) at a significant differences ($p < 0.01$) as compression with the control groups.

TABLE 5. Group 5

	M ± S.d	P value
Control group	**3.92 ± 3.23**	A
Patient group	**7.20 ± 4.80**	B

Ceruloplasmin and T. gondii

The results of the serum specimen collection from the women diagnosed by *T. gondii* were analyses at the end of the experiment days for determined the effects of *T. gondii* on ceruloplasmin. The findings show significance ($p < 0.01$) (Table 6) raise of the blood ceruloplasmin level in the women diagnosed by *T. gondii* compared with the control group.

Table 6: Changes in the blood ceruloplasmin level in the women diagnosed with T. gondii. Each bar represents the Mean ± SD ($n = 50$) in a significance differences ($p < 0.01$) as compare with the control groups

TABLE 6. Group 6

	M ± S.d	P value
Control group	**324 ± 87.5**	A
Patient group	**377.1 ± 83.8**	B

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Total Iron level and T. gondii

The results of the serum specimen collection from the women diagnosed by *T. gondii* were analyses at the end of the experiment days for determined the effects of *T. gondii* on total iron level. The results show a significant ($p < 0.01$) (Table 7) increase in the blood total iron level in the women diagnosed by *T. gondii* compared to the control group.

Table 7: Changes in the blood total iron level in the women diagnosed with *T. gondii*. Each bar represents the Mean ± SD (n = 50) in a significant differences ($p < 0.01$) as compare with the control group.

TABLE 7. Group 7

	M ± S.d	P value
Control group	**71.6 ± 21.2**	A
Patient group	**181.4 ± 32.3**	B

DISCUSSION:

The current study inspected the impact of *T. gondii* infection on some biochemical indicators through examining the biochemical parameters changes in the infected women comparison to healthy typical women. These indicators gives an signal around the impacts of these causative agents on women's' health.

The current findings discovered that T. gondii infections raised blood cholesterol and TG levels in severely infected women. The present findings were in consent with the past studies [Kennany, 2007& Blader et al., 2001]. *T. gondii* used the host metabolism products to its privet metabolisms paths [Nora et al., 2018]. Being widely known that cholesterol is synthesis in the endoplasmic reticulum during the major enzymes of the mevalonate path, hydroxymethylglutaryl-CoA reductase and further utilized by the cell for synthesized of cholesterol derivatives or membrane biogenesis. Past microarray analyzed by used *T. gondii* infections human foreskin fibroblasts appeared regulation of key genes implicated in the mevalonate path and cholesterol syntheses [Amany et al., 2010]. The involve increase in the expression level of hydroxymethylglutaryl- CoA reductase [Al- Kaysi et al., 2010].

This study found that *T. gondii* infected leads to raise the action of blood liver enzymes. In previous study examined of blood samples, where noted that ALP activity was increased clearly in the *T. gondii* infected groups compression with the monitoring group. As well as, there are a significance raise in serum AST, ALT activity in the women samples infected, and a significant increase in AST activity in the specimen with chronic infection of *T. gondii*. These results were consent with the studies indicated by [Alekish et al., 2017; Al-khamesi et al., 2016; El-Sayed et al., 2016& Calderaro et al., 2009] who found that the activities of AST, ALP and ALT were raised in the animals infected by *T. gondii*. In humans, the results of *Toxoplasma* patients show significance increased of the liver enzymes AST, ALT, and ALP compression to healthy individual [Donahoe et al., 2015 & Linder, 2016]. These changes perhaps due to liver damages caused by *T. gondii* infected, which elevated the releasing liver enzymes in the serum. Past research recorded that *T. gondii* infected leads to comprehensive damages in the liver cells [Liu et al., 2022 & Adamczyk-Sowa et al., 2016].

Ceruloplasmin CP is a multi-copper oxidase and antioxidant that is fundamentally producing in the liver [El-deeb et al., 2022] it has been found in glial cells [Mitsunaga et al., 2018]. CP has many physiological functions, where CP carries 40–70% of Cu in the plasma and plays importance roles in Cu transfer, Fe regulating, free radical scavenging, and antioxidant processes. CP also stimulates the oxidation of types of substrates, including Cu, Fe, and other organic materials [Mitsunaga et al., 2019]. CP level is changing in inflammation, infections, and shocks, which is mostly, attributed to its antioxidant characteristics [El-Sayed et al., 2016]. The mentioned results previous studies

pointed that it must be expectant that Cp levels would raise with disease activity, in agreement with results that show increased Cp levels were observed in the women infected by *T. gondii* [Oliveira et al., 2020].

The current study found an increase of total iron level in women infected by *T. gondii* parasite. This study was consistent with a study conducted on mice, where the accumulation of large amounts of iron was observed in the intestines, lungs and liver of mice infected with *T. gondii* parasite compared with the control group, and the reason for the high level of iron may be due iron could be involved in the nutritional need for iron by the parasite [36 & 37].

CONCLUSION

There is a relationship between vital signs and infection with different pathogens. Therefore, taking the necessary precautions to prevent infection with pathogens, especially parasites of the reproductive system, can help improve the performance of the reproductive system. The present study showed that the *T. gondii* have remarkable effect in serum Ch, TG, ALP, AST, ALT, CP, and Total iron levels than healthy controls. These biomarkers can be used to investigate the effectiveness of the predominating medicines and to enhance effect the new medicines which it's used to eradication of *T. gondii*.

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REFERENCE

1. P. Nelson, Kenrad E and Carolyn Masters Williams, “INFECTIOUS DISEASE Theory and Practice,” *World Hqrs. Jones Bartlett Learn.*, pp. 1–18, 2007.
2. C. Jiang, Z. Li, P. Chen, and L. Chen, “The seroprevalence of Toxoplasma gondii in Chinese population with cancer a systematic review and meta-analysis,” *Med. (United States)*, vol. 94, no. 50, pp. 1–7, 2015, doi: 10.1097/MD.0000000000002274.
3. L. Aabasian, S. Shirbazou, M. Shamsi, S. Damghani, and A. Delpisheh, “Hormonal changes in women with breast cancer infected with Toxoplasma gondii,” *J. Basic Res. Med. Sci.*, vol. 3, no. 1, pp. 16–21, 2016, [Online]. Available: http://jbrms.medilam.ac.ir/article-1-143-en.html.
4. B. A. A. Jwad, Z. A. M. Al-Haboobi, and N. M. Al-Khafaji, “Biochemical and hormonal study in women infected with Toxoplasma gondii,” *EurAsian J. Biosci.*, vol. 14, no. 1, pp. 515–519, 2020.
5. D. F. ARANDA LOZANO, “Modeling of parasitic diseases with vector of transmission: toxoplasmosis and babesiosis bovine,” *Riunet*, pp. 1–62, 2011, [Online]. Available: https://riunet.upv.es/handle/10251/11539.
6. J. P. Dubey, *Toxoplasmosis of Animals and Humans*. 2010.
7. M. JG Montoya, MD & Liesenfeld, “Toxoplasmosis,” vol. 363, no. 9425, p. 16412, 2004.
8. J. P. Dubey and J. L. Jones, “Toxoplasma gondii infection in humans and animals in the United States,” *Int. J. Parasitol.*, vol. 38, no. 11, pp. 1257–1278, 2008, doi: 10.1016/j.ijpara.2008.03.007.
9. Jiménez-Coello P.-S. M. and O.-P. A. Jiménez-Coello M, Acosta-Viana K. Y, Guzmán-Marín E, “Toxoplasmosis: A relevant zoonotic food borne disease in tropical conditions,” *African J. Microbiol. Res.*, vol. 6, no. 12, pp. 2956–2964, 2012, doi: 10.5897/ajmr11.1548.
10. K. S. Harker, N. Ueno, and M. B. Lodoen, “Toxoplasma gondii dissemination: A parasite’s journey through the infected host,” *Parasite Immunol.*, vol. 37, no. 3, pp. 141–149, 2015, doi: 10.1111/pim.12163.

11. S. De Craeye, “Toxoplasma Gondii, a Successful and Underestimated Foodborne Parasite: Development of Detection Methods and Their Use for the Screening of Animal Reservoirs,” no. 1, p. 190, 2012.
12. K. S. Ahn, A. J. Ahn, S. I. Park, W. M. Sohn, J. H. Shim, and S. S. Shin, “Excretion of toxoplasma gondii oocysts from feral cats in Korea,” *Korean J. Parasitol.*, vol. 57, no. 6, pp. 665–670, 2019, doi: 10.3347/kjp.2019.57.6.665.
13. F. A. Uzal, B. L. Plattner, and J. M. Hostetter, “CHAP TER 1 Alimentary System,” pp. 2–443, 2020.
14. F. Kheirandish *et al.*, “Toxoplasma serology status and risk of miscarriage, a case-control study among women with a history of spontaneous abortion,” *Int. J. Fertil. Steril.*, vol. 13, no. 3, pp. 184–189, 2019, doi: 10.22074/ijfs.2019.5740.
15. O. A. Mendez and A. A. Koshy, “Toxoplasma gondii: Entry, association, and physiological influence on the central nervous system,” *PLoS Pathog.*, vol. 13, no. 7, pp. 1–12, 2017, doi: 10.1371/journal.ppat.1006351.
16. K. Khan and W. Khan, “Congenital toxoplasmosis: An overview of the neurological and ocular manifestations,” *Parasitol. Int.*, vol. 67, no. 6, pp. 715–721, 2018, doi: 10.1016/j.parint.2018.07.004.
17. Ş. A. Türkoğlu, K. Yaman, H. Orallar, C. Camsari, Ş. Karabörk, and E. Ayaz, “Acute toxoplasmosis and antioxidant levels in the liver, kidney and brain of rats,” *Ann. Parasitol.*, vol. 64, no. 3, pp. 241–247, 2018, doi: 10.17420/ap6403.159.
18. M. N. D. Nadwa A. J. Mahmood, “Liver function tests in toxoplasmosis,” *Ann. Coll. Med. Mosul*, vol. 38, no. 2, pp. 68–72, 2012, doi: 10.33899/mmed.2012.64600.
19. A. Didiano, P. Monti, O. Taeymans, and G. B. Cherubini, “Canine central nervous system neosporosis: Clinical, laboratory and diagnostic imaging findings in six dogs,” *Vet. Rec. Case Reports*, vol. 8, no. 1, 2020, doi: 10.1136/vetreccr-2019-000905.
20. E. R. . A.- Kennany, “Pathological study on the capability of toxoplasma gondii to induce oxidative stress and intiation a primary lesion of Atherosclerosis experimentally in broiler chickens.” pp. 938–942, 2007.
21. I. J. Blader, I. D. Manger, and J. C. Boothroyd, “Microarray Analysis Reveals Previously Unknown Changes in Toxoplasma gondii-infected Human Cells,” *J. Biol. Chem.*, vol. 276, no. 26, pp. 24223–24231, 2001, doi: 10.1074/jbc.M100951200.
22. M. B. Nora, A. . Adel, M. A. AhMed, and M. I. HaNy, “Biochemical Effects of Toxoplasma gondii and Neospora caninium Infection on Diary Bovine Models in Menoufia Province, Egypt,” *Adv. Anim. Vet. Sci. Biochem.*, vol. 4, no. 4, pp. 527–534, 2018.
23. B. G. . Amany, M. Al- Kaysi, Eid, R.A.A, and Fahmy, “Biochemical studies on the effect of Toxoplasma infection on liver and kidney functions in mice,” *Egypt. J. Comp. Path. Clin. Path.*, vol. 23, no. 1, pp. 174–185, 2010.
24. N. A. J. Al- Kaysi.-H. and A. S. . M. A.- Zuhairy, “Effect of toxoplasmosis and brucellosis on some biochemical parameters in ewes,” *Iraqi J. Vet. Sci.*, vol. 24, no. 2, pp. 73–80, 2010, doi: 10.33899/ijvs.2010.5609.
25. M. O. Alekish, A. Q. Talafha, M. A. Alshehabat, and Z. A. B. Ismail, “Relationships between certain metabolic diseases and selected serum biochemical parameters in seropositive dairy cows against Neospora caninum infection in different stages of lactation,” *Ann. Parasitol.*, vol. 63, no. 2, pp. 99–103, 2017, doi: 10.17420/ap6302.91.
26. M. B. Al-khamesi, Z. N. Al-sibahi, L. H. A. Al-obaidy, and Z. H. Hilal, “Studying of Kidney , Liver Functions and Some Blood Ions In Toxoplasmosis Patients,” *Al-Mustansiriyah J. Sci.*, vol. 27, no. 1, pp. 43–46, 2016.
27. N. M. El-Sayed, M. E. Ramadan, and M. E. Ramadan, “Toxoplasma gondii Infection and Chronic Liver Diseases: Evidence of an association,” *Trop. Med. Infect. Dis.*, vol. 1, no. 1, pp. 1–8, 2016, doi: 10.3390/tropicalmed1010007.
28. A. Calderaro *et al.*, “Laboratory diagnosis of Toxoplasma gondii infection,” *Int. J. Med. Sci.*, vol. 6, no. 3, pp. 135–136, 2009, doi: 10.7150/ijms.6.135.
29. S. L. Donahoe, S. A. Lindsay, M. Krockenberger, D. Phalen, and J. Šlapeta, “A review of neosporosis and pathologic findings of Neospora caninum infection in wildlife,” *Int. J. Parasitol. Parasites Wildl.*, vol. 4, no. 2, pp. 216–238, 2015, doi: 10.1016/j.ijppaw.2015.04.002.

30. M. C. Linder, “Ceruloplasmin and other copper binding components of blood plasma and their functions: An update,” *Metallomics*, vol. 8, no. 9, pp. 887–905, 2016, doi: 10.1039/c6mt00103c.
31. Z. Liu, M. Wang, C. Zhang, S. Zhou, and G. Ji, “Molecular Functions of Ceruloplasmin in Metabolic Disease Pathology,” *Diabetes, Metab. Syndr. Obes. Targets Ther.*, vol. 15, pp. 695–711, 2022, doi: 10.2147/DMSO.S346648.
32. M. Adamczyk-Sowa *et al.*, “Changes in serum ceruloplasmin levels based on immunomodulatory treatments and melatonin supplementation in multiple sclerosis patients,” *Med. Sci. Monit.*, vol. 22, pp. 2484–2491, 2016, doi: 10.12659/MSM.895702.
33. W. El-deeb, M. A. Abdelghani, A. Alhaider, and M. Fayez, “Oxidative stress , ceruloplasmin and neopterin biomarkers in dromedary camels with clinical endometritis,” *Anim. Reprod.*, vol. 19, no. 3, pp. 1–12, 2022.
34. T. Mitsunaga, K. Norose, F. Aosai, H. Horie, N. Ohnuma, and A. Yano, “Infection dynamics of Toxoplasma gondii in gut-associated tissues after oral infection: The role of Peyer’s patches,” *Parasitol. Int.*, vol. 68, no. 1, pp. 40–47, 2019, doi: 10.1016/j.parint.2018.08.010.
35. M. C. Oliveira, L. B. Coutinho, M. P. O. Almeida, M. P. Briceño, E. C. B. Araujo, and N. M. Silva, “The availability of iron is involved in the murine experimental toxoplasma gondii infection outcome,” *Microorganisms*, vol. 8, no. 560, pp. 1–18, 2020, doi: 10.3390/microorganisms8040560.