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Effect of the Experimental Infection with *Toxoplasma gondii* on some Biochemical aspects and Histological Changes for the Liver and Spleen in Female Rats

Halla Abdul –HadiCHabuk[1]*, Haidar Kamil Zaidan Al-Saadi[1] and Ahmed Khudhair Al-Hamairy[2]

[1]Science College, University of Babylon, Hilla, Iraq
[2]College of Science for Women, University of Babylon, Hilla, 5001, Iraq

Abstract : *Toxoplasma gondii* is an intracellular protozoa, widespread throughout the world, and it has the ability to infection many organs in the body such as the liver and spleen.
Ten rats divided into two groups, first group was control group that treated orally with distilled water and second group was infected with suspension of parasite (tachyzoite) that isolated from placenta, the animals killed after twelve weeks of occurrence of the infection. The results showed significant increase in the levels of enzymes (AST, ALT), and the levels of total blood protein and levels of globulin and bilirubin in the serum of the rats infected when comparing with the control group, but the results showed a significant decrease in the concentration of albumin and total cholesterol level in the serum when compared with the control group. The results also showed for changes in liver tissue represented congestion, degeneration and necrosis of the liver cells with cellular infiltration of the inflammatory cells and sinusoid dilation between the liver plates because of tissue sacs for the parasite, which was also observed changes in the spleen tissue and Occurrence of hypertrophy of lymph nodes. The aim of this study was to detect the effect of toxoplasmosis on some liver functions such as the levels of enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum and the levels of total blood proteins like albumin, globulin, in addition to levels of cholesterol and bilirubin in the serum female rats. This study also included the impact of the parasite on histological structure for each of the liver and spleen.
Key words: *Toxoplasma gondii*, AST,ALT, Albumin, Cholesterol and histological changes in liver and spleen.

Introduction

T.gondii is a single cell protozoa that belongs to the coccidian family. It has the ability to attack the most systems, especially endothelial reticuloendothelial system and central nervous system[1,41], which leads to the destruction of tissues and cells, it causeapoptosis[2]. This parasite causes Toxoplasmosis disease, is a widespread throughout the world[3, 40 , 41], Iraq is representing one of these countries [4, 40 , 41]. Toxoplasmosis affects in both genders (male and female). Human and other mammals are intermediate host for parasite while cats are definitive Host [5].

Most infection of Toxoplasmosis disease comes through the digestive system as a result of eating poorly cooked food or water contaminated with infected cat feces thatcontainoocysts[6,40 ,41] .After that the parasite spread through the lymphatic vessels and the hepatic portal area to reach the liver, spleen , lung, brain ,

eye and other organs, studies indicated that the parasite in the liver accompanied by a number of pathological changes include hepatomegaly granuloma, hepatitis and necrosis[7], therefore it caused order in liver functions such as the liver enzymes level (ALT) & (AST) [8]and liver's ability to synthesize blood proteins, in addition in order to its effect in concentration of cholesterol and bilirubin in the serum[9] with the occurrence of histological changes in the liver and spleen [10].**The aims of this study** were to detect the effect of toxoplasmosis on some liver functions such as the levels of enzyme salanine aminotransferase (ALT) and Aspartate aminotransferase (AST), in serum and the levels of total blood proteins like albumin, globulin, in addition to levels of cholesterol and bilirubin in the serum female rats. This study also included the impact of the parasite on histological structure for each of the liver and spleen.

Material and methods:

- **The parasite isolated from the placenta:** The parasite has been isolated and diagnosed according to [11, 12].

1. The placenta obtained from Al-Hilla Maternity Hospital of pregnant women who are victims of abortion with sterile container and then cut to very small pieces by sharp sterile scalpel, then it crushed by the mortared pestle or using a blender with the addition of an equal amount of physiological salt solution (PBS) until the homogeneity of the solution.
2. Mix the homogenized solution of placental tissue with an equal volume of trypsin solution (0.25 % trypsin in sodium chloride solution pH = 7.2) per gram of tissue.
3. This solution is incubated at 37C° for an hour in a water bath to complete the histological digestion process, the solution filtered through several layers of sterile gauze.
4. After centrifugation of filtered suspension for the first time, and discarding the supernatant, then centrifuged at 300 rpm for 15 min, after that again the supernatant was discarded. the sediment take and add it10 ml of sterile normal saline (0.9 %) Repeat this process several times to remove the effects of the trypsin enzyme, This washing repeated for three times. After that, 10 ml of sterile inoculums was prepared by adding (100 µg) of Streptomycin and (1000 IU) of Penicillin per 1 ml. of inoculum. This solution was used for experimental infection in rats by, as it's considered the best method for inoculation of the parasite from uncontaminated samples and it' sensitive method. After the completion of the preparation suspended placenta tissue and prior to injection in rats take drop of suspense on slide and stained with Giemsastain for the purpose of investigating from parasite

Experimental animals:

This study was conducted in the animal house of the Department of Biology/ College of Science / University of Babylon ,use 10 female rats ranged weights between 250-300 gm. the animals divided to two groups each group of five animals, the first group was inoculated orally with 1 ml of normal saline as a control group while the second group inoculated orally with suspension parasite (3x 10^6tachyzoites for each Rats)that isolated from placenta[13].

When infected animals killed, after twelve weeks of the infection , and removed of the organs (liver and spleen) and it kept in formalin material (10%) for prepare to the study of histological sections, blood drawing directly from the heart using medical syringes five milliliter and it put in Jeltube containing gelatinous substance that helps to increase serum and it put in a centrifuge with speed 3000 rpm / min for 15 minutes for the purpose of separation of serum and conduct the following tests.

1. Measurement of liver enzymes (APT, AST) use the device Reflotron Roche for measure the enzymes by using the device's kit
2. Measurement the concentration of cholesterol using electronic device for measuring cholesterol.
3. Measurement the total globulinand albumin concentration in serum according to the method [14].
4. Measurement the concentration of globulin in the serum=Total Protein concentration minus concentration of albumin[15].

Histological section Preparation: according to [16].

Results and Discussion:

1. Effect toxoplasmosis disease in liver functions.

The results showed a significant change in liver enzymes (AST, ALT) comparison with control group, which revealed an increase in level of enzymes (P <0.05) in all infected animals when compared with control group while the results showed a significant decrease in the concentration of cholesterol(P <0.05) Table (1).

Table 1: **Effect infected female rats with *Toxoplasma gondii* in liver enzymes level AST&ALT (I.U/L)and the concentration of cholesterol (mg / 100ml) in the serum after 12 weeks of inoculation.**

Liver Function / Group	AST (I.U/L) M± SE	ALT (I.U/L) M± SE	Cholesterol mg/100ml M± SE
Control n=5	56.23 ± 2.87	65.55 ± 3.26	175.62 ± 3.6
Infected animals group with toxoplasmosis disease n =5	76.38 ± 3.71	83.44 ± 2.73	139.51 ± 1.92
L.S.D	10.21	9.26	8.9

In the current study was to measure enzymes level AST and ALT to find out the potential damage of the liver as a result of parasite infection. The results showed an increase levels of liver enzymes. This is agreed with observed[17] in the serum of ewes that suffer from abortion at different stages of pregnancy and suspected infected by toxoplasmosis, as well as this result is consistent with [18]they observed an increase in liver enzymes levels in mice infected with parasite Toxoplasmosis . This study also agreed with the study[19] found a rise in liver enzymes levels in serum of women infected with the parasite, which suffer from several abortions. The results agreed with several other studies [20,21]when they noticed an increase in activities of the enzymes ALT, AST when infection different types of fish with different types of parasites. But the current results are not consistent with[22]they not recorded any significant differences in the level of enzymes (AST, ALT), maybe it attributed the cause of the high level of these enzymes in the serum of the rats infected Toxoplasmosis to damage of liver cells due to the parasite penetrate to these cells and the formation of the tissue cysts leading to (Apoptosis) for some cells, necrosis and it analyzes of others since this damage exceeds the plasma membrane to reach the mitochondria and cytoplasm and is the whereabouts of these enzymes which leads to the flow it's into the circulation, thereby increasing its level in the blood serum [23]. also notes a rise in the level of enzyme AST in serum, because the parasite has an effect on the heart and the liver together, and, as the heart produces the biggest part of the enzyme AST therefore rise this enzyme in serum evidence of heart and liver affected by the parasite toxoplasmosis[24] and [25]who said that infection parasite led to atrophy of the heart muscle and necrosis of the liver and spleen with the emergence of multiple necrotic foci scattered them.

The results of the study showed the infection led to a significant decrease of the level of total cholesterol in the blood serum compared with the control group, and this result is not consistent with [25, 17]if note the absence of significant changes in the level of cholesterol in serum rats. But these findings are consistent with[26] observed a decrease in cholesterol concentration when the fish infection with number of parasites. The reason due to the parasite impact in the liver, causing enzymatic changes affect lipid metabolism as it inhibits enzyme Butyryl Cholinesterase which plays an important role in lipid metabolism and manufacture of cholesterol in blood or because of the weakness of the bile secretion thus reduces emulsification of fat and result in a decrease in the susceptibility absorbed or it may be consumption of bloodcholesterol by parasites [27, 40].

As for the parasite effect in blood proteins, it was observed higher significantly (P <0.05) in the total protein and globulin concentration with a significant decrease in the concentration of albumin when compared the control group, but the results showed an increase significantly in the concentration of bilirubin in the serum of infected female animals when compared to the control group table (2).

Table (2): Effect infected female rats with *Toxoplasma gondii* in the concentration of Albumin proteins, Globulin and the concentration of Bilirubin (mg / 100ml) in the serum after 12 weeks of inoculation.

Liver Functions / Group	Albumin Concentration) (mg/100ml) M± SE	Globulin Concentration (mg/100ml) M± SE	Total protein (mg/100ml) M± SE	Bilirubin Concentration (mg/100ml) M± SE
Control n=5	4.62 ± 0.56	3.42 ± 0.49	8.04 ± 0.65	1.70 ± 0.42
Infected animals group with toxoplasmosis disease (n=5)	1.34 ± 0.39	11.8 ± 1.11	13.14 ±0.70	4.39 ± 0.69
L.S.D	1.50	2.66	2.09	1.74

- **Represents values average ± standard error.**
- **Representsn = 5 the number of animals treated.**
- **LSD is least significant difference at the level of significance (P≤0.05).**

The Albumin is the most important proteins, which is produced in the liver, so albumin decrease in blood is considered an indication for the existence of damage in the liver. This result is consistent with [18]and[9]they had noticed an increase in the concentration of liver enzymes and disorder in the concentration of total protein, albumin and globulin in the blood after three days from the infection. But these results are not consistent with [22] they did not record any significant differences in the concentration of blood proteins. The reason due to the liver tissue damage caused by congestion and necrosis of the cells and the appearance of gaps due to it penetrate to the liver cells and multiply there, so it composed tissue cysts. The liver is responsible for most the metabolic events, including plasma proteins manufacturing, so any damage in the liver reflects on its functions such as albumin [28 , 37].

[29]noted a rise globulin in the blood with toxoplasmosis and this is identical to the findings of the current study. It is known that globulin is the second protein of blood components, include: Alpha and Beta, they produce mediated liver, either gama produced by plasma cells in the lymphocytes tissue (spleen and lymph nodes) as a result of the immune response against the parasite and this type is primarily responsible for the globulinrises in the blood because represent the biggest part of it[30, 31].

The results showed above a rise in the concentration of bilirubin in the rats serum, this result matching[19] found a significant increase of the concentration of bilirubin in the women serum infected with parasite Toxoplasmosis. Perhaps due to the bilirubin resulting from the demolition of hemoglobin when old red blood cells are broken down in the liver then come out by the liver with the Bile. As a result of damage caused by the parasite to the hepatic cells that led to changes in the metabolism of the liver, leading to a lack of sufficient ability to link and extraction of bilirubin, so it rise in serum or perhaps because of blockage of the Bile ducts, leading to retrieval direct bilirubin to the liver and ultimately into the blood, leading to a rise in serum [32 , 37, 40] .

Effect of Parasite *T. gondii* on the liver and spleen tissues.

Figure (1): Cross-section spleen tissue for female rats of the control groups are showed white pulp(lymphatic nodule) (←) central vien (←)red pulp(←)There is no histologic changes. (H&E) 10X

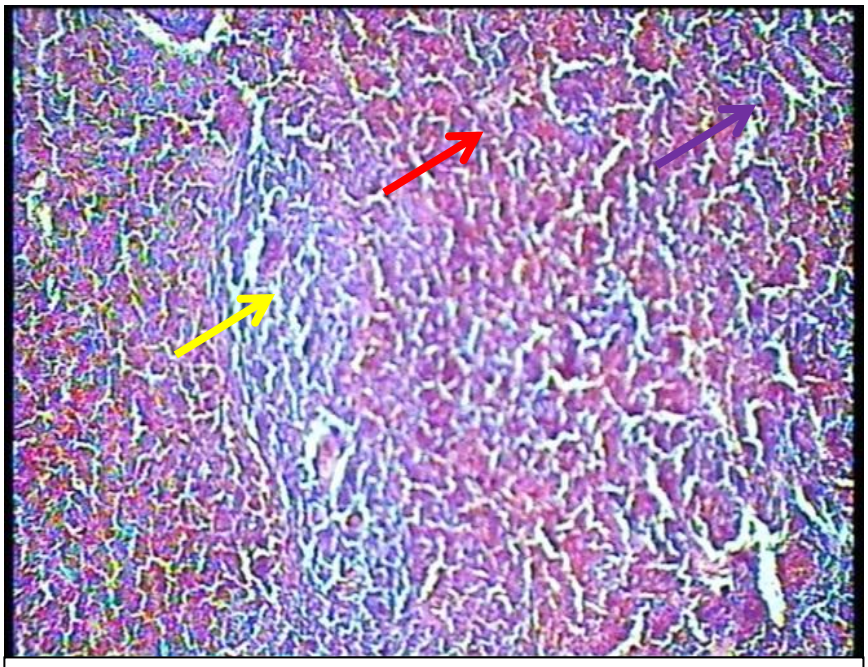


Figure (2):Cross-section of spleen tissue for female rats infected parasite Toxoplasmosis after 12 week, noted the severe congestion (←) in the red pulp and sharp inflation in the white pulp hypertrophy (←)with a cellular infiltration of inflammatory cells (←)(H&E) 10X

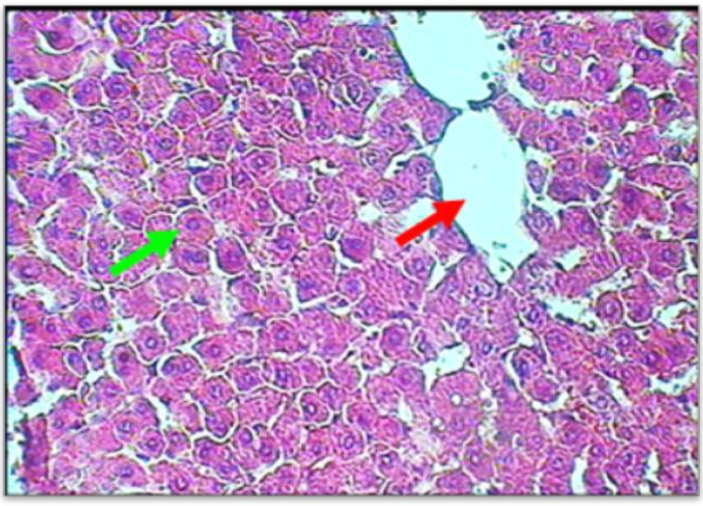


Figure (3):Cross-section of liver tissue for female rats of the control groupsobserved is not affected liver tissue. Central vein (←) and hepatocytes plates (←). (H&E) 10X

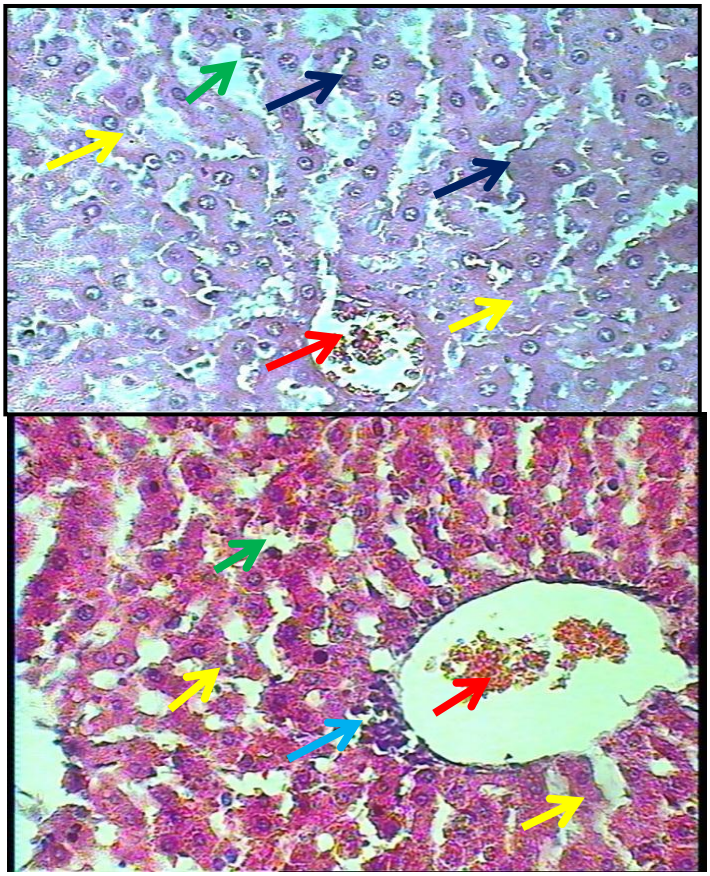
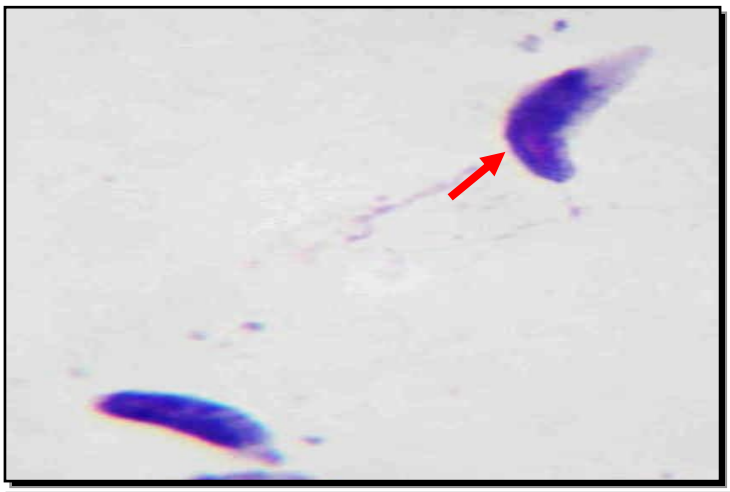


Figure (4):Cross-section of liver tissue for female rats infected with Toxoplasmosis disease after 12 a week notes congestion of central vein (←),loss of form and arrangement of the hepatic cells with necrosis in some of them and show nuclei of cells either small in size or fragmented or decadent(Karyorrhexis and karyolysis in the nuclei of necrotic hepatocytes) and occur Apoptosisin the number of tissue cells (←), notes the tissue cysts in liver (←) , cellular infiltration (←) and dilation in blood sinusoid(←)(H & E, 40X)



Figure(5): Tachyzoite of *T. gondii* from peritoneal fluid ,for female Rats (after 12 weeks) giemsa stain. (100 X)

A microscopic examination of spleen tissue showed negative effects in the infected group compared with the control group which appeared normal spleen and did not show any changes in the spleen tissue while infected group showed existence of hypertrophy in the white pulp and sever congestion in the red pulp with cellular infiltration of inflammatory cells (Figure 1 and 2).This result are compatible to [10]noted existence of tissue changes in the liver and spleen cats when infected it with toxoplasmosis noting vascular congestion inside the red pulp with access hyperplasia in the white pulp. As signal[33, 41] that rats injured with toxoplasmosis that isolated from farm animals (sheep, cows) has led to the emergence of a satisfactory lesions in the liver, spleen and lymph nodes. Perhaps the reason for that is immune reactions, Furthermore, spleen is the important organ of the defense of the body because it contains lymphoid B andT cellsand macrophages, So it is believed that parasites stimulate white pulp of the spleen to produce lymphatic cells that migrate to the inflammatory region , which lead to an enlarged spleen, in addition to ingestion of parasites, germs and infected cells by macrophages in splenic sinusoid [34, 38-41].

A microscopic examination of liver tissue infected Showed presence of tissue changes compared to a group of control, Representing Congestion of central vein and infiltration of cells inflammatory, especially lymphocytes and neutrophil multiple forms of nuclei PMNs and loss of form and arrange plates hepatocytes with appearance dilation blood sinusoid, as well as the spread of Kupffer's cells in large numbers, in addition to complete necrosis in some liver cells with the occurrence of cellular programmer death (Apoptosis) in other liver cells, Figure (3, 4) this study are consistent with [35, 39, 40]they found necrosis and death programmer hepatic cells with cellular infiltration and congestion with the advent of the tissue cysts in rats infected with toxoplasmosis . As mentioned [25, 41]appearance of tissue changes in both the liver , Spleen and Brain in Rabbits infected with parasite, The reason due to the phagocytic cells(Kupffer s cell) is one of the most important defensive cells in the liver, it release high concentrations of Cytokines and many of free oxygen roots that help in occurrence of necrosis of the liver tissue, or perhaps because of excessive production of nitric oxide NO produced from Kupffer cells and liver cells caused severe inflation in the blood vessels (Blood sinusoid) Affect decrease of blood flow, in the fact that this molecule contributes to necrosis ofhepatocytes.Decrease blood inside blood sinusoid affects the liver cells, which require a high concentration of oxygen, leading to a crash in the liver cells [36-47].

Conclusion :

The toxoplasmosis affects liver functions include an increase level of total protein and ALT,AST activates and decrease of most blood parameters for infected rats. The *T. gondii* was isolated successfully by trypsin enzyme digestion procedure and the most affected organs are spleen and liver.

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