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Tissue penetration of Toxoplasmosis in experimental rats

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Abstract:

*Toxoplasma gondii*is an intracellular protozoa, widespread throughout the world, and it has the ability to infect many organs in the body such as the brain, eyes, liver and spleen.In this study, 56 rats(28 males and 28 females) were divided into two groups, the first group28 rats(14 male and 14 female) was inoculated with normal saline to serve as control,and the second group 28 rats(14 male and 14 female)was infected intraperitonealy with placental fluid containing1×10^{7}tachyzoites of *Toxoplasma gondii*. After 2 months of infection, the infection was confirmed by using real-time PCR, and the animals were scarified. Brain, liver and spleen tissues were fixed in 10% formalin for histological examination.The results indicated that the parasite invaded many tissues and caused many histopathological changes. The parasite (tissue cyst stage containing bradyzoite and tachyzoite stage) were recorded in the braintissues of both males and females.The brain tissues also revealed many histological changes included inflammatory manifestations and a wide areas of edema. The groups of laboratory rats which infected with *T. gondii* showed several histological changes in the liver tissue, included congestion of the central vein, expansion of the jaundice and accumulation of the inflammatory cells in several sites of the liver. The spleen of infected group showed hemorrhage, extra-medullary mega karyocytosis, necrosis, fibrosis and degeneration of spleen tissues.

Keywords: Toxoplasma gondii, rats, histology, tissue penetration.

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أختراق الأنسجة المصابة تجريبيا بطفيلي المقوسة الكوندية في الجرذان المختبرية

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الخلاصة:

يعد طفيلي المقوسة الكوندية من الا بتدائيات الداخل خلوية، ينتشرعلى نطاق واسع في جميع أنحاء العالم، وله القدرة على إصابة العديد من الأعضاء في الجسم مثل الدماغ والعينين والكبد والطحال. اجريت الدراسة على 56 من الجرذان المختبرية (28 ذكور و 28 إناث) تم تقسيمهم إلى مجموعتين، تم حقن المجموعة الأولى 28 جرذ (14 ذكر و14 انثى) بالمحلول الملحي الطبيعي لتكون بمثابة مجموعة السيطرة، أما المجموعة الثانية 28 جرذ (14 ذكر و 14 انثى) فقد أصيبت داخل البريتون مع سائل المشيمة الحاوي على 1×10^7 الطور السريع التكاثر من طفيلي المقوسة الكوندية. بعد 2 أشهر من الاصابة، تم تأكيد الاصابة باستخدام تقنية real-time PCR ، بعد تشريح الحيوانات جمعت الاعضاء (الدماغ والكبد والطحال) وثبتت في الفورمالين 10٪ للفحص النسجي.أشارت النتائج إلى أن الطفيلي غزا العديد من الأنسجة وتسبب في العديد من التغيرات النسجية. تم تسجيل وجود الطفيلي (طور الكيس النسيجي الحاوي الطور البطيء التكاثر بالاضافةالى وجود الطور السريع التكاثر) في أنسجة الدماغ لكل من الذكور والإناث. كشفت أنسجة الدماغ أيضا العديد من التغيرات النسجية شملت مظاهر التهابية ومناطق واسعة من وذمة. وأظهرت مجموعة الجرذان المختبرية المصابة بالطفيلي العديد من التغيرات النسجية في أنسجة الكبد، وشملت احتقان الوريد المركزي، وتوسيع اليرقان وتراكم الخلايا الالتهابية في العديد من مواقع الكبد. أظهر نسيج الطحال في المجموعة المصابة نزيف، كثرة الكريات الخارج اللب، نخر، والتليف وانحطاط انسجة الطحال.

الكلمات المفتاحية: طفيلي المقوسة الكوندية، الجرذان، علم الانسجة، اختراق الانسجة.

1- Introduction:

Toxoplasma gondiiis an obligate intracellular parasite infecting all warm-blooded animals, with a world-wide distribution. It causes a wide range of clinical manifestations in humans, extended from abortion and congenital infection to eye disease and fatal encephalitis.T.gondiiis penetrated and infected many organs in the host, such as reproductive organs, brain, heart, lung, liver and spleen(Bahia-Oliveira et al., 2003;Dubey, 2004; Zia-Ali et al.,2007).

The previous studies showed that T.gondiiis invaded both reproductive systems in both male and females. The parasite induced sever degenerative changes in the seminiferous tubules, Epididymus and secondary sexual organs. It also induced severe histological changes in ovary and uterus and many other tissues in females(Al-Abadyet al., 2016;Al-Ghezy et al ., 2016a;Al-Ghezyet al ., 2016b;Al-Ghezyet al ., 2016c;Al-Ghezyet al ., 2016d)

This study is aimed to investigate the tissue penetration of T.gondiiand the impact of the parasite on the histological structure of brain, liver and spleen.

2- Material and Methods:

Experimental animals: Fifty six rats (Rattusnorvegicus) (28 males and 28 females, 6-10 weekold,weighing 250 to 300g)were used in the experiment. Theywere maintained on normal diet in an air-conditioned animal room at an ambient temperature of 23 ±2 $^{\circ}$C and in a 12h light / 12h dark cycle. After adaptation period (14 days), they were grouped into two groups: the first group 28 rats (14 male and 14 female) was given normal saline to serve as control, while the second group 28 rats (14 male and 14 female) were inoculated intraperitoneally with placental fluid containing1×10^{7}tachyzoites of Toxoplasma gondii parasite(Dubeyet al., 1998;Al-taie and Abdulla,2008;Alkennay and Hassan,2010).After 2 months of infection, the infection was confirmed using real-time PCR(Lin et al., 2000). Theanimals were scarified, and brain, liver and spleen were fixed in 10% formalin and prepared for histological examination by routine histological techniques(Woods and Ellis, 1994).

3- Results:

Brain:tissue cyst stage of the parasite which containing bradyzoite and tachyzoite stage of the parasite were recorded in the brain tissues of both males and females. The brain tissues also revealed many histological changes included inflammatory manifestations and a wide areas of edema (fig1,2,3). Liver: The microscopic examination of the liver of control group, showed that it consisted of a central vein surrounded by hepatocyteswhich formed a radial arrangement around the central

vein (fig4). The groups of laboratory rats infected with T. gondii showedseveral histological changes in the liver tissue, including congestion of the central vein, expansion of the jaundice and infiltration of the inflammatory cells in several sites of the liver (fig5, 6, 7).Spleen:The spleen of control group, showed normal architecture(fig8). The spleen of infected group showed hemorrhage, extra-medullary mega karyocytosis, necrosis, fibrosis and degeneration of spleen tissues (fig9,10,11).

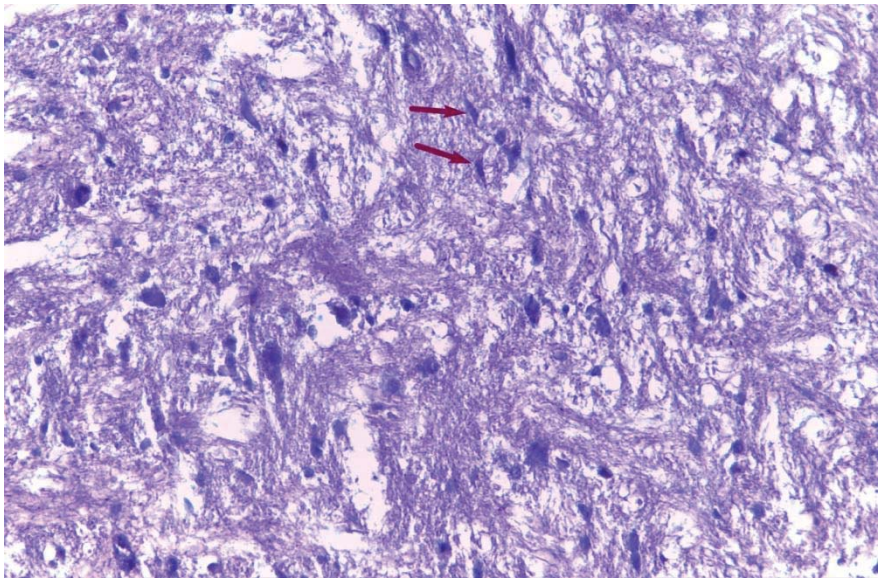


Fig.(1): Cross-section of brain tissue of rat infected with Toxoplasma gondii after 2 months of infection, showed tachyzoite stage (400X, H&E stain).

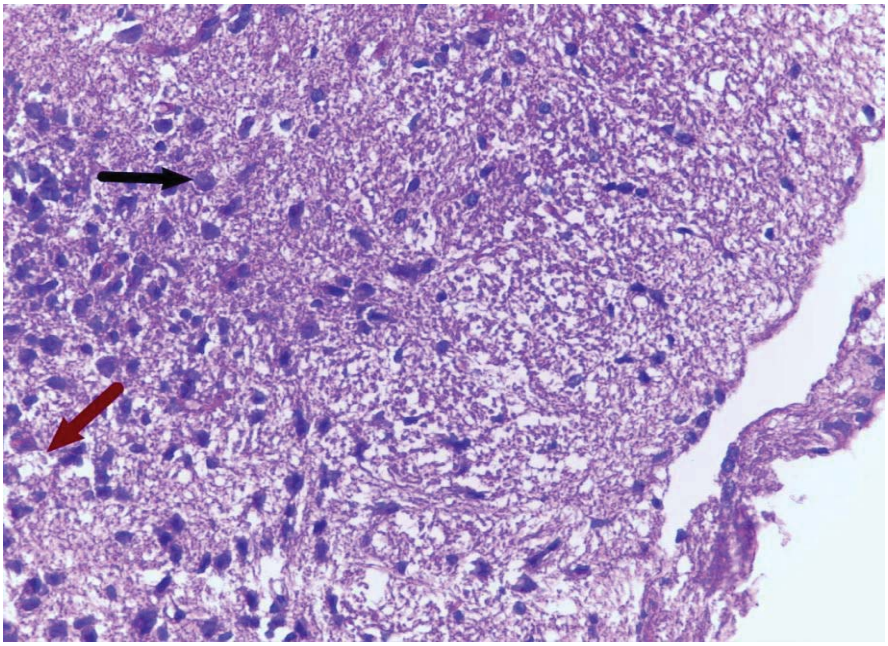


Fig.(2): Cross-section of brain tissue of rat infected with Toxoplasma gondii after 2 months of infection, showed tissue cyst stage (→) and edema (→) (400X , H&E stain)

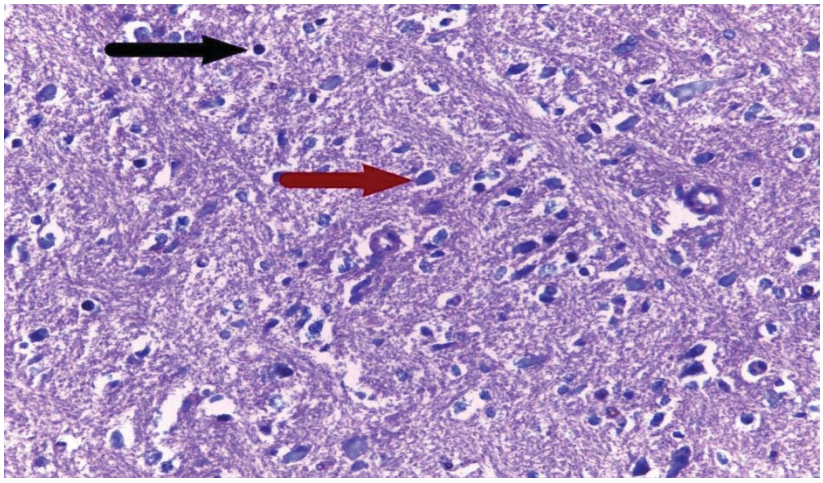


Fig.(3): Cross-section of brain tissue of rat infected with Toxoplasma gondii after 2 months of infection, note migrogilia (→) and neurocytes with central chromatolysis ( ) (400X , H&E stain).

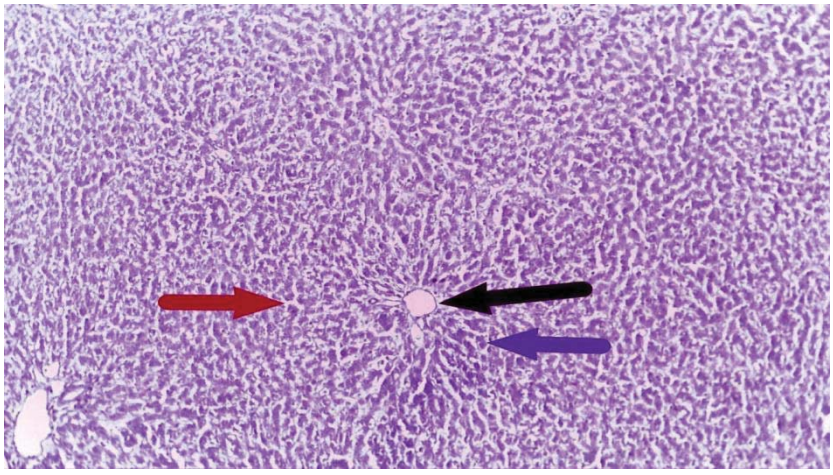


Fig.(4): Cross-section of liver tissue of rat from controle group, showed central vein ( ), hepatocytes ( ) and sinusoids ( ) (100X , H&E stain)

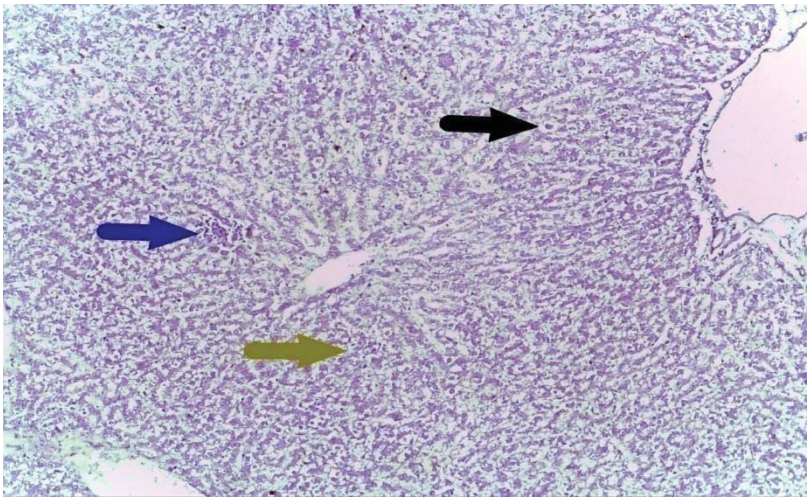
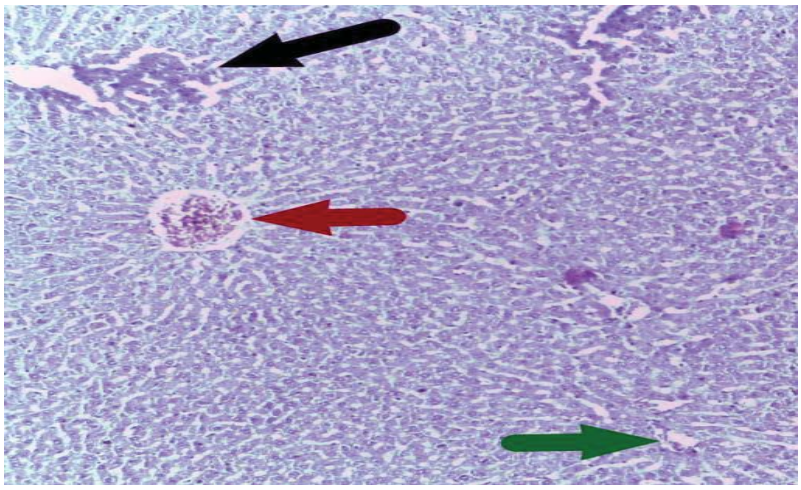


Fig.(5): Cross-section of liver of rat infected with Toxoplasma gondii after 2months, showed hepatocyte vaculation( ), inflamotary cells ( ) ,hyperplasia ( ) (100X , H&E stain)



Fig(6) Cross-section of liver tissue for rat infected with Toxoplasma gondii after 2months, noted inflamotary cells (→), congestion (→) and necrosis (→) (100X, H&E stain)

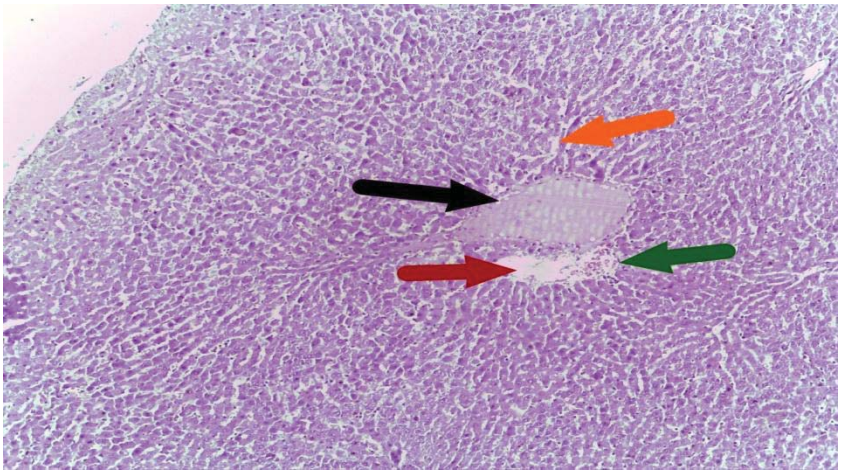


Fig.(7): Cross-section of liver of rat infected with Toxoplasma gondii after 2months, showed edema (→) , necrosis (→), hemorrhages (→) and enlage sinusoids (→) (100X , H&E stain)

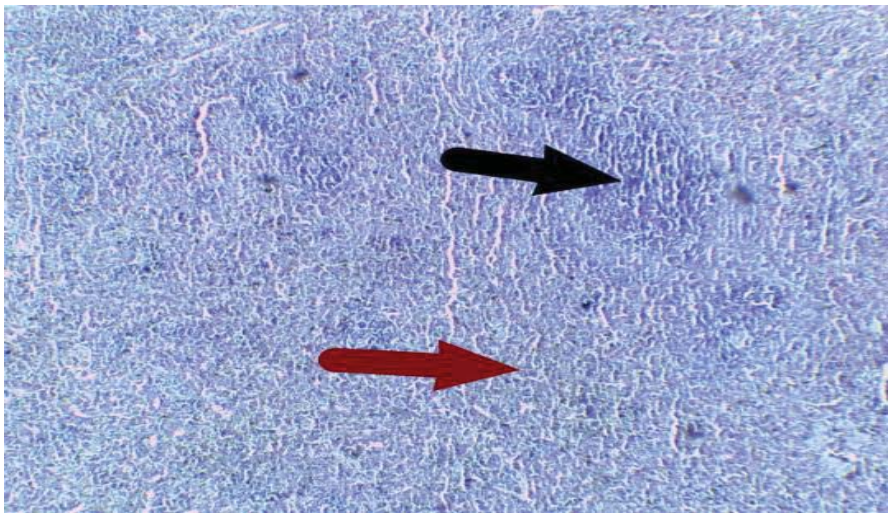


Fig. (8): Cross-section of spleen of rat from control group, showed lymphnode (→) and red pulp (→) (100X , H&E stain).

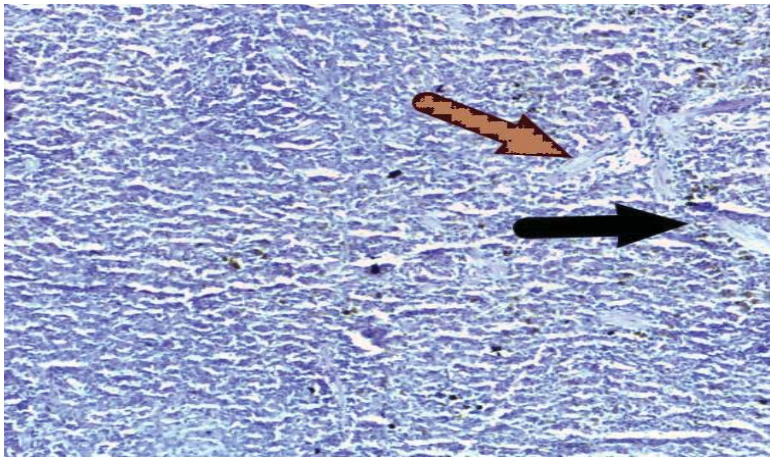


Fig.(9): Cross-section of spleen of rat infected with Toxoplasma gondii after 2months, infiltration (→) and fibrosis (→) (100X , H&E stain).

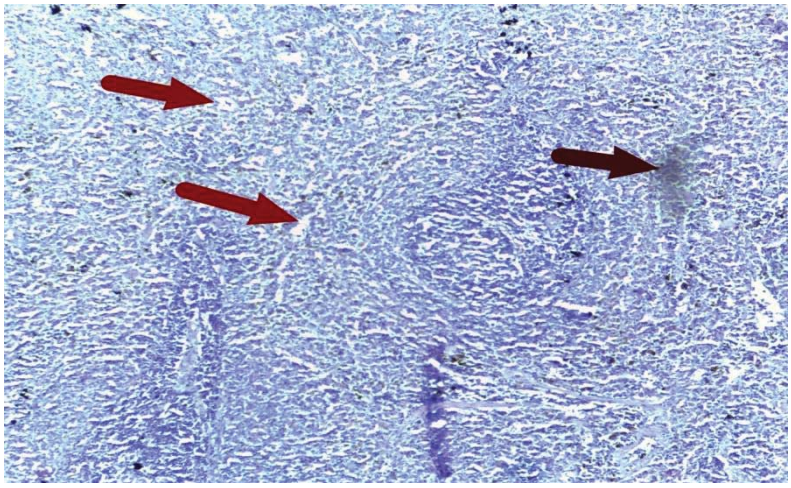


Fig.(10): Cross-section of spleen of rat infected with Toxoplasma gondii after 2 months, showed degeneration (→) and necrosis (→) (100X, H&E stain)

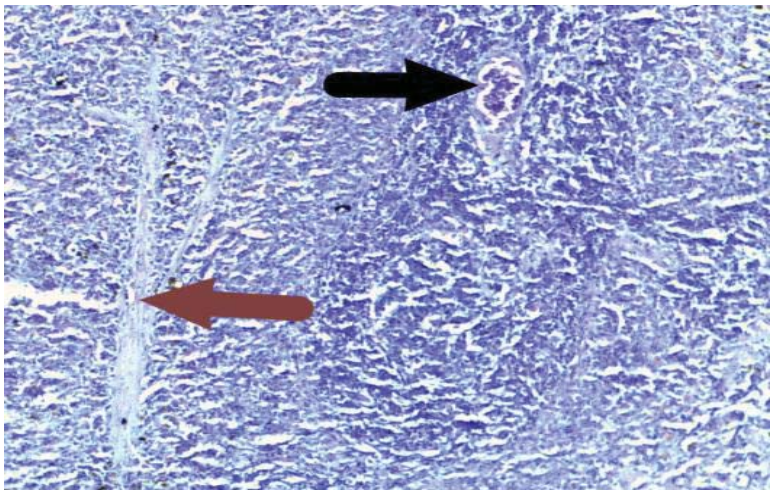


Fig.(11): Cross-section of spleen of rat infected with Toxoplasma gondii after 2months, showed necrosis (←) and fibrosis (←) (100X , H&E stain)

4- Discussion:

The pathogenicityof T. gondii is depended on virulence of the strain of parasite, host susceptibility, immune status of the host and the degree of histopathological change induced by the parasite (Dubey, 2006). The histopathological changes in different organs, due to parasitism, were vary from one species to other and were usually more pronounced in the young animals than adults. These changes occur even in subclinical infection, but clinical manifestations develop when the pathological changes were sufficiently and large enough to cause dysfunction (Frenkel, 1990).

The gliosis (proliferation of glial cells) concomitant to infection by T. gondii was described by Wilson and Hunter(Wilson and Hunter,2004), the study of parasite penetration revealed that the invasion of parasite stimulated linkage of T-cell with molecules on the surface of cerebral epithelium before extravasion, the occurrence of gliosis was an evidence of stimulation of brain immune system by parasite antigen through releasing INF- α and INF- γ (Wilson and Hunter,2004).

The hepatocytes are the active metabolic cells and toxoplasmosis can lead to disturbances in the whole body metabolic activities . The disturbances in the hepatocytes function and histological changes extended to DNA damage in hepatocytes were caused by infection with T. gondii(Eissaet al., 1990; Genget al., 2000;Sukthanaet al., 2003; Ribeiroet al., 2004;.

Microscopic examination also revealed intense infiltration of the liver with mixed inflammatory cells, these cells penetrated the hepatic tissues as a part of the immunological response of the animal to T.gondii infection(Ferro et al., 1999;Lawrence et al., 2003). So, the histological changes of T.gondii in the liver occurred either due to a direct effect of the parasite on the tissues leading to cell death and tissue damage or it could be related to indirect effect of infection due to the excessive immunological response to the parasite (Ferro et al., 1999).These results were in agreement with others who mentioned that the infection caused tissue changes in the liver and spleen included vascular congestion inside the red pulp with access hyperplasia in the white pulp (Woods and Ellis, 1994; Buxton, 1998; Greset al., 2003; Fayed et al., 2004; Al-Saidya and Al-Kennany, 2006). Rats infected with T.gondii isolated from farm animals (sheep, cows) has led to pathological lesions in the liver, spleen and lymph nodes(Al-taie and Abdulla,2008; Al-Dulaimiet al., 2015). Furthermore, it was believed that parasites stimulate white pulp of the spleen to produce lymphatic cells that migrate to the inflammatory region, which lead to enlargement of spleen (Hideyuki, 2002; Al-Dulaimiet al., 2015;Manji and Al-Hamairy, 2015;Al-Hamairy,2016;Oliewi Al-Hamairy,2016).

Conclusion:Toxoplasma gondii can be penetrated tissue brain, liver and spleen of male and female rats that infected experimentally with Toxoplasmosis, then caused deterioration the normal histological structure of studied organs of male and female infected rats.

5- References:

Al-Abady, F. A.; Al-Snafi, A. E. and Al-Ghezy, Z. S.(2016). Diagnosis and quantitative detection of Toxoplasma gondiiin male and female laboratory rats using qReal -Time PCR. ThePharmaceutical and Chemical Journal. 3(2):122-130.

Al-Dulaimi, F. H. A.; Al-Hamairy, A. K. and Ageely, A. A. A . (2015). Diagnostic study of Toxoplasma gondii among married and non married women by ELISA and LATEX in Babylon Province. Advances in Environmental Biology. 9(27): 117-123.

Al-Gazi, Z. S.;Al-Abady, F. A. andAl-Snafi, A. E. (2016a). Effect of toxoplasmosis and/ or its treatment (sulpadiazine and pyrimetamine) on female rats reproductive performance. Indian Journal of Pharmaceutical Science & Research. 6(1): 35-40.

Al-Ghezy, Z. S.; Al-Abady, F. A. and Al-Snafi, A. E.(2016b). Effect of Toxoplasma gondii infection in male and female rats on fetal characteristics. European Journal of Biomedical and Pharmaceutical Sciences. 3(5): 692-698.

Al-Ghezy, Z. S.; Al-Abady, F. A. and Al-Snafi, A. E. (2016c). Effect of toxoplasmosis and its treatments on male rats reproductive functions. Asian Journal of Pharmaceutical Science & Technology. 6(2): 82-88.

Al-Ghezy, Z. S.; Al-Abady, F. A. and Al-Snafi, A. E.(2016d). Histological effects of toxoplasmosis and its treatments on male and female rats. American Journal of Pharmacy & Health Research. 4(4): 40-52.

Al-Hamairy, A. H. (2016). Effect of lipopolysaccharide extracted from Escherichia coli on the growth and development of promastigote of Leishmaniatropica (In vitro study). International Journal of PharmTech Research. 9(4): 377-386.

AlKennay,E. and Hassan, S. (2010). Pathological study on some tissues of rat experimentally infected with Toxoplasma gondii. J. Edu. Sci. 23(3):8-17.

Al-Saidya, A. M. A. and Al-Kennany, E. R. (2006). Pathological and histochemical study on cats and mice experimentally infected with Toxoplasma gondii. Iraqi Journal of Veterinary Science. 20 (2) : 263-249.

Al-taie, L. H. and Abdulla, S. H.(2008).Toxoplasma gondii: Experimental infection of isolated local strain in Sulaimani province. Iraqi J Med Sci. 6 (3): 60-71.

Bahia-Oliveira, L. M.; Jones, J. L.;Azevedo-Silva, J.; Alves, C. C.; Orefice, F. and Addiss, D. G. (2003). Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. Emerg Infect Dis. 8**:** 55–62.

Buxton, D. (1998). Protozoan infections (Toxoplasma gondii, Neosporacaninum and Sarcocystis spp.)in sheep and goat: Recent advances. Vet Res Paris. 29: 289-310.

Dubey, J.P.(2006). Comparative infectivity of oocystandbradyzoites of Toxoplasma gondii for intermediate (mice) and definitive host (cat). Vet Parasitol.31: 69-75.

Dubey, J. P. (2004). Toxoplasmosis - a waterborne zoonosis. Veterinary Parasitology. 126(1-2): 57-72.

Dubey, J. R.; Lindsy, D. S. and Speer, C. A. (1998). Structures of Toxoplasma gondiitachyzoites, bradyzoites and sporozoites biology and development of their tissue cysts. Clinical Microbiology Res. 11(2): 267-299.

Eissa, M. H.; Antonious, S. N.;Salama, M. M.Fikry, A. A. and Morsy, T. A.(1990).Histopathological studies of acute, chronic and congenital infections of toxoplasmosis in mice. J. Egypt. Soc. Parasitol. 20(2): 805-816.

Fayed, H. M.;Allam, K. A. M. and Ali, N. S. (2004).Merogony of Toxoplasma gondii (Apicomplexia: coccidian) and its effect on the mortality and histopathology in the house mouse Musmusculus. J. Egypt. Soc. Parasitol. 34: 45-64.

Ferro, E. A. V.;Bevilaqua, E. S.; Silva, D. A.; Mortara, R. A. and Mineo, J. R.(1999).Trophoblast cells as a host cells to Toxoplasma gondii. Parasitol Res. 85(8-9): 647-654.

Frenkel, J. K. (1990). Transmission of toxoplasmosis and the role of immunity in limiting transmission and illness. J. Am. Vet. Med. Assoc. 196:233-240.

Geng, Z. H.; Shi, Y.; Fang, Y. Q.; Li, S. H. and Liu, L. (2000). Analysis of trace elements in liver, spleen and brain of rats infected with Toxoplasma gondii. ZhongguoJi Sheng Chong Xue Yu Ji Sheng Chong Bing ZaZhi. 18(6):347-349.

Gres, V.;Voza, T.;Chabaud, A. and Landau, I. (2003).Coccidiosos of the wild rabbit (Oryctolaguscuniculus) in France. Parasitology. 10**:** 51-57.

Hideyuki, N. A. G. (2002). Host Immune system against Toxoplasma infection, national research center for protozoan diseases. Jpn. J.Protozool. 35 (1) : 3-9.

Lawrence, L. J.;Kiera, N. B.; Frank, M. S.;Wangxue, C. and Stephen, T. S. (2003). Fibrin mediated protection against infection-stimulated immunopathlogy JEM . 197(6): 801-806.

Lin, M.; Chen, T.; Kuo, T.; Tseng, C. and Tseng, C. (2000). Real-Time PCR for Quantitative Detection of Toxoplasma gondii. Journal of Clinical Microbiology. 4121–4125.

Manji, Z. F. and Al-Hamairy, A. K. (2015). Sero-epidemiological and molecular study of toxoplasmosis in the blood donors and applicants for marriage peoples in the Babylon Province, Iraq. Res. J .Pharm. Bio. Chem. Sci. 6(6): 1106-1114.

Oliewi,M. K. and Al-Hamairy, A. K. (2016). Epidemiological and diagnostic study for diarrheic parasites (Entamoebahistolytica, Giardia lamblia, and Cryptosporidium sp.) among diarrheic infected patients by using multiplex polymerase chain reaction in the Babylon Province, Iraq. Res. J. Pharm. Bio. Chem. Sci. 7(1): 438-447.

Ribeiro, D. A.; Pereira, P. C.; Machado, J. M.; Silva, S. B.; Pessoa, A. W. and Salvadori, D. M. (2004). Dose toxoplasmosis causes DNA damage? An evaluation in isogenic mice under normal diet or dietary restriction. Mutat. Res. 11:559 (1-2): 169-176.

Sukthana, Y.;Waree, P.; Pongponratn, E.;Chaisri, U. and Riganti, M.(2003). Pathologic study of acute toxoplasmosis in experimental animals. Southeast Asian J. Trop. Med. Public Health. 34(11): 16-21.

Wilson, E. H. and Hunter, C. A. (2004). The role of a strocytes in the lmmunopahtogensis of toxoplasmic encephalitis. Int. J. Parasitol. 34: 543-548.

Woods, J. and Ellis, M. (1994). Laboratory histopathology: A complete Reference. Churchil, Livingstone, 348pp.

Zia-Ali, N.;Fazaeli, A.;Khoramizadeh, M.; Ajzenberg, D.; Darde, M. and Keshavarz-Valian, H. (2007). Isolation and molecular characterization of Toxoplasma gondii strains from different hosts in Iran. Parasitol Res. 101(1): 111-115.