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HISTOLOGICAL STUDY OF *TOXOPLASMA GONDII* INFECTION IN LIVER OF MICE EMBRYOS

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Abstract

Toxoplasma gondii is a protozoan parasite that infected broad range of animals, The present study aims to follow up the histological changes in the liver of fetuses of laboratory- infected mice with *Toxoplasma gondii* parasite isolated from the placenta of aborted women. A total of 47 placenta samples were collected from Al-Diwaniya hospital for women and children for isolation parasite, the histological changes in mice was performed by intraperitoneal inoculation of 0.3 ml placenta suspension during the period December 2018 until November 2019. The study showed the percentage of affected embryos at age 15th before pregnancy is 46.93%, the affected embryos after pregnancy 78.78%., while the percentage of affected embryos at age 18th before pregnancy is 48.88%, the affected embryos after pregnancy 77.5%, According to the different histopathological changes observed in the liver in the affected embryos from pregnant mice affected before pregnancy, degenerate and necrosis the tissues while the changes in the affected embryos from pregnant mice after pregnancy were in addition to tissue degeneration and necrosis, inflammatory infiltration congestion and hemorrhage within the central veins, dilated sinusoidal capillaries were also very obvious with distortion in the shape of the hepatocytes.

Key words: *Toxoplasma gondii*, Placenta, mice, liver

Introduction

Toxoplasma gondii is one of the most well studied parasites because of its medical and veterinary importance, being readily recognizable by light microscopy and availability of different isolates with varying virulence (Dubey *et al.,* 2016).

Toxoplasmosis is a worldwide disease with most of the infections originating through the oral route and generates various pathological manifestations, ranging from meningoencephalitis to retinochoroiditis and inflammatory bowel disease (Dias *et al.,* 2014).

The parasite can reproduce sexually and complete its life cycle only in felids and they are the final hosts that are able to secrete huge numbers of oocystsinto the environment through feces (Dubey& Frenkel, 1976).

People become infected postnatally mainly by ingesting tissue cysts from undercooked meat, or consuming food or water contaminated with oocysts (Grigg & Sundar, 2009).

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Tissue cysts are most likely viable during the life of the host. During infection, the parasite invades a variety of immune cells and later spreads throughout the body, crossing biological barriers to reach with immune privileges such as the brain where it can cause severe diseases (Barragan & Sibley, 2002).

Mice are a useful model for studying toxoplasmosis due to its similarity to toxoplasmosis in humans (Fux *et al.,* 2000).

So the present study aimed to investigate the histological changes in liver of embryos of mice affected before and after pregnancy.

Materials and methods

Isolation of Parasite

Isolation of the *T. gondii* parasite from the placenta of aborted women infected with the parasite according to Dalgýç, (2008) and after making sure of the presence of the parasite in those samples using the direct smear method (Impression Smear), the placenta tissue was cut

into small pieces and mixed with a quantity of normal saline and crushed then filtered with gauze and then expelled with a centrifuge at 3000 rpm for 10 minutes. Then Removing the suspended matter and add the normal saline to the precipitate, the process is repeated three times and add 1000 units penicillin and 10 mg streptomycin to prevent pollution. A haemocytometer was used to count the tissues cysts.

Each animal injected 0.3 ml of the prepared liquid into the peritoneal cavity. In this study used Swiss albino mice (*Mus musculus*) 60 female from mice were used for this purpose divided into 3 groups, each contained 10 females. aged 8-12 weeks averaged weight 20-30 gm. injected the parasite in intra-peritoneal of experimental animals and a latex test was used to confirm mice were infected after 7 weeks of infection the females were married by setting (2 female + 1 male) in separate cages. Mating was confirmed by observing the vaginal plug and The next day is the first day of pregnancy (Bayat *et al.,* 2013).

Group 1: control non infected (20 mice).

Group 2: Infected with *T. gondii* before pregnancy (20 mice).

Group 3: Infected with *T. gondii* after pregnancy (20 mice) in the second day of pregnancy.

Pregnant mice were anesthetized, stapled and dissected on days 15th and 18th of pregnancy to study the histopathology changes in the brain caused by infection with the *T.gondii* parasite.

Statistical Analysis

According to the results of the study, the statistical test used the Chi-square test and the T test to determine the differences between the study groups. Conûdence interval is 95% and the probability level is less than 0.05 (P<0.05) (Motulsky,2003).

Result and Discussion

Congenital toxoplasmosis in the experimental groups

Congenital transmission of *T. gondii* in the experimental groups is summarized in table 1, 2.

Congenital toxoplasmosis in the experimental groups at age 15 day of pregnancy in Group2 Infected before pregnancy 46.93%, 23 affected embryos and at age 18 day of pregnancy 48.88%, 22 affected embryos, These results are in agreement with Vargas-Villavicencio *et al.,* (2016) who observed 18% of the fetuses were infected with the lowest dose and 40% fetuses litter were altered. These data confirm that the severity of Toxoplasma infection in females and embryos depends upon the concentration of parasites.

Table 1: Congenital toxoplasmosis in the experimental groups at age 15 day of pregnancy.

Group	No. of embryos	No. of affected embryos	Perce-ntage %
Control	67	0	0%
Infected before pregnancy	49	23	46.93%
Infected after pregnancy	33	26	78.78%

Table 2: Congenital toxoplasmosis in the experimental groups at age 18 day of pregnancy.

Group	No. of embryos	No. of affected embryos	Perce-ntage %
Control	67	0	0%
Infected before pregnancy	45	22	48.88%
Infected after pregnancy	40	31	77.5%

Whilein Group3 Infected after pregnancy at age 15 day of pregnancy 78.78%, 26 affected embryos and at age 18 day of pregnancy in 77.5%, 31 affected embryos, these results are in agreement with Fux *et al.,* (2000) who observed 60.6% rate of infection in mice pups positive for Tissue cyst and in results of Wang *et al.,* (2011) indicated that a high congenital transmission rate 90%. Difference in results related to the size of the inoculum, the strain used, or be due to the sections examined (Fux *et al.,* 2000).

Maybe it's related to immunity This is consistent with Dubey, (2010) "even when the placenta is infected, the fetus may escape infection".

Histological study in liver of embryos

The histological changes of liver sections in embryo at the age 15th day of pregnancy Infected before

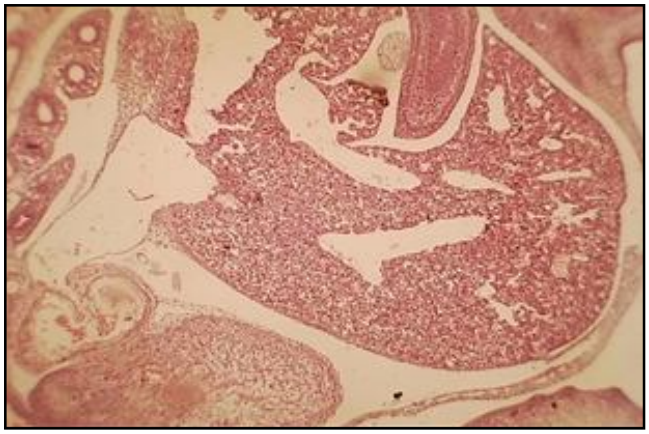

Fig. 1: Section through liver of mouse embryo at 15th day of mice (control group) with no remarkable pathology (H &E,X40).

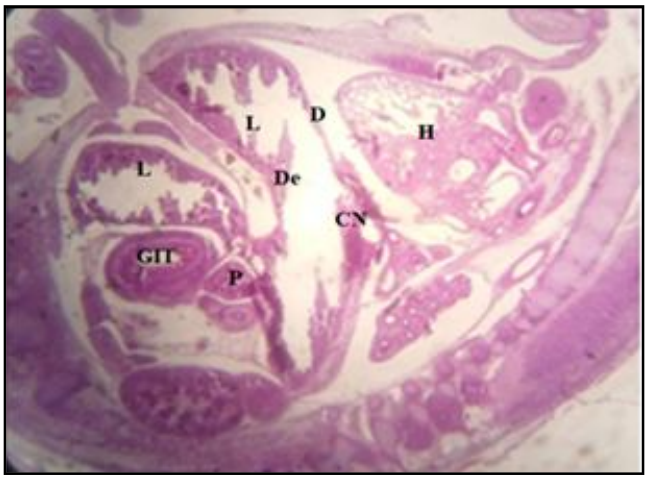

Fig. 2: Section through liver of mouse embryo at 15thday of mice Infected before pregnancy showing heart (H), major blood vessels (BV) with no remarkable pathology, diaphragm (D), Liver (L) with tissue degeneration (De) and coagulative necrosis (CN), proximal intestinal tract (GIT) and pancreatic tissue (P) with no remarkable pathology(H &E,X40).

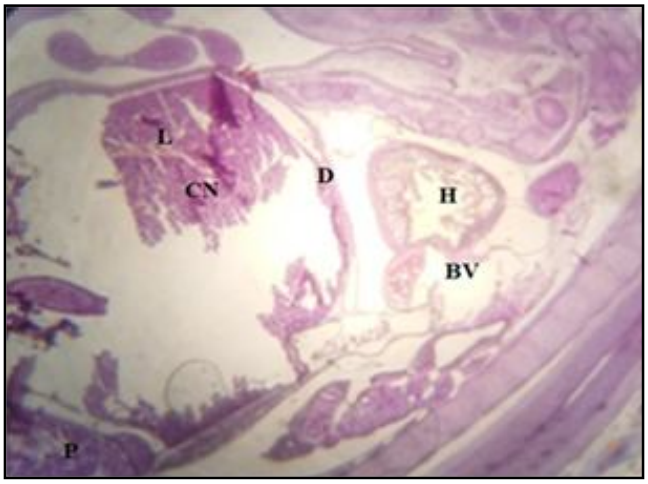

Fig. 3: Section through liver of mouse embryoat 15thday of mice Infected before pregnancy showing heart (H), major blood vessels (BV) with no remarkable pathology, diaphragm (D), Liver (L) with tissue degeneration (De) and coagulative necrosis (CN), and pancreatic tissue (P) with no remarkable pathology(H &E,X40).

pregnancy showing Liver tissue is necrotic and degenerative compared with control groups Fig. 1, 2, 3.

While the histological changes of liver sections in embryo at the age 15th day of pregnancy Infected after pregnancy showing In addition to necrosis and degeneration, bleeding and severe congestion compared with control groups Fig. 4, 5.

Histological study in liver of embryo The histological changes of liver sections in embryo at the age 18th day of pregnancy Infected before pregnancy show The liver

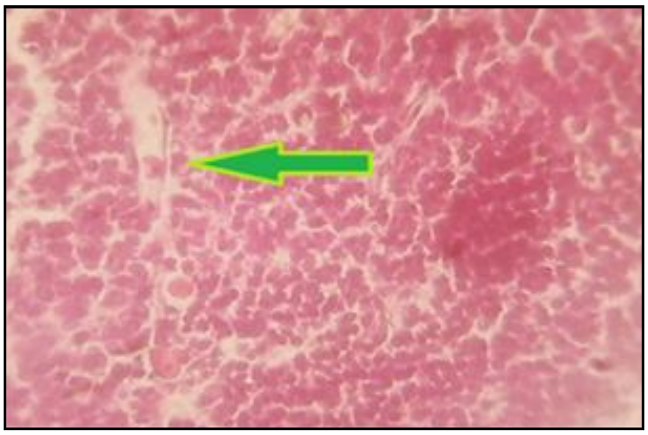

Fig. 4: Section through liver of mouse embryo at 15th day of mice Infected after pregnancy shows Parasite with foci for hemorrhage (H &E,X1000).

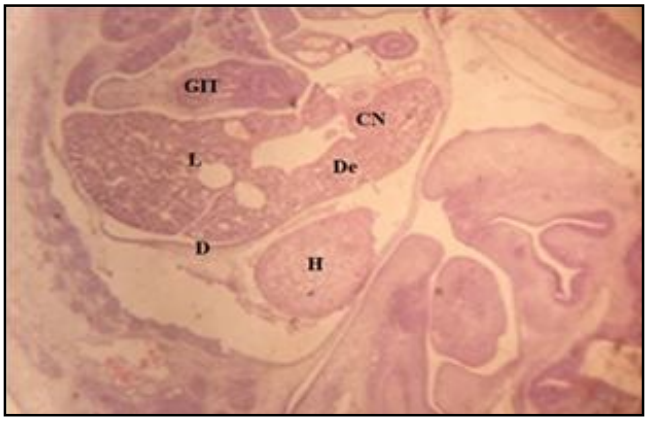

Fig. 5: Section through liver of mouse embryoat 15thday of mice Infected after pregnancy showingunderdeveloped single chamber heart (H, diaphragm (D), Liver (L) with tissue degeneration (De) and coagulative necrosis (CN), and proximal GIT(H &E,X40).

showed areas of slight coagulative necrosis (CN) compared with control groups Fig. 6.

While the histological changes of liver sections in embryo at the age 18th day of pregnancy Infected after pregnancy showing compared with control groups Fig. 7, 8.

The histological changes of liver sections in embryo at the age 15 and 18th day of pregnancy were Infected before pregnancy showing degeneration and necrosis in the liver tissues.

The results agrees with Chen *et al.,* (2018) Notable histological changes in liver, which showed multifocal mononuclear cell aggregations with numerous vacuolated hepatocytes.

The histological changes of liver sections in embryo at the age 15 and 18th day of pregnancy Infected after pregnancy showing degeneration, necrosis, sinus hepatic expansion and hyperemia and inflammatory infiltration.

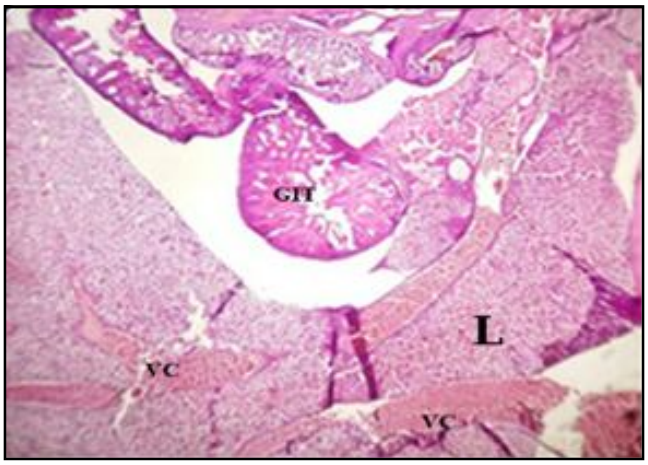

Fig. 7: section of mouse embryo at 18th day of mice Infected after pregnancy the developing Liver (L) and gastrointestinal tract (GIT) with severe vascular congestion (VC)(H &E,X40).

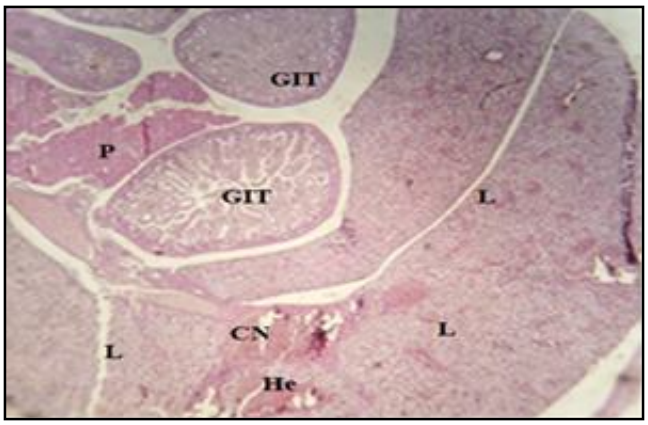

Fig. 8: section through liver of mouse embryo at 18thday of mice Infected after pregnancy showing marked coagulative neccrosis (CN) and severe hemorrhage (He). Both intestinal region and pancreas are free of pathological changes (H &E,X40).

This results agreement Wu *et al.,* (2011) showed the destroyed liver structure, cellular edema, ballooning change, focal necrosis, sinus hepatic expansion and hyperemia and inflammatory infiltration.

Well agree with Kassabbashi, (2007) referred to pathological changes in the liver of mice are intense inflammatory cellular infiltrate concentrated mainly around the central veins of the liver lobules, with congestion and hemorrhage within the central veins, dilated sinusoidal capillaries were also very obvious with distortion in the shape of the hepatocytes and foamy appearance of those cells.

Mordue *et al.,* (2001) showed that acute toxoplasmosis induced apoptos is in liver and spleen.

In study Sánchez-Sánchez *et al.,* (2019). A mild portal-reactive hepatitis was found in the liver In the chronically infected mouse group.

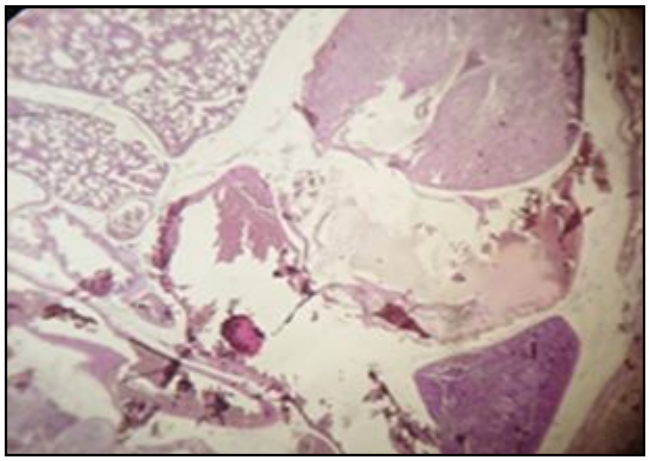

Fig. 9: section of mouse embryo at 18th day of mice Infected before pregnancy showing the thoraci region in which the heart (H) is very well demonstrated, the developing alveolar region of the lung (A) is also very obviuos and the thymus gland (T). Extensive hemorrhage (He) is very clear in addition to vascular congetsion (VC)(H &E,X40).

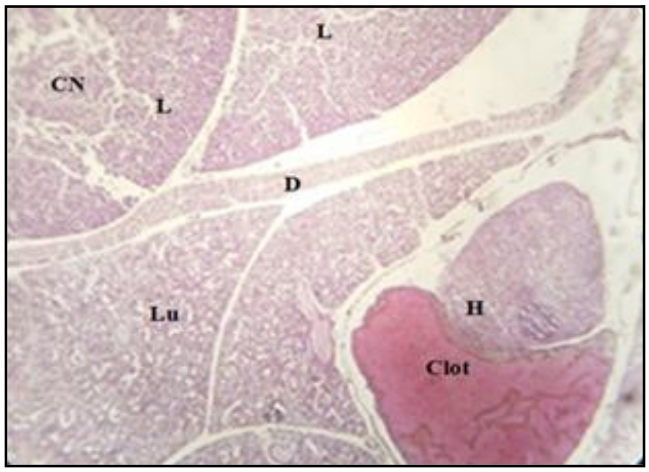

Fig. 6: section through liver of mouse embryo at 18thday of mice Infected before pregnancy showingthe following parts: The heart (H), the lung (Lu), the diaphragm (D) and the liver (L). The heart has a big blood clot resulting from tissue preparation. The lobes of the lung are very clearly demarcated with no remarkable pathology. The liver showed areas of slight coagulative necrosis (CN)(H &E,X40).

During the parasitemia stage of *T. gondii* infection, the liver is one of the most important organs involved and affected (Geng *et al.,* 2000).

Experimental studies observed the presence of tachyzoites and tissue cysts inside the hepatocytes and within the sinusoidal liver capillaries. When such a parasite invades the hepatocyte, it can lead to disturbances in its metabolic activity, shape distortion and "damage in its DNA It is known that the parasite locates the host liver and causes pathological changes that progress to

hepatomegaly, granuloma, hepatitis and necrosis" (Sukthana *et al.,* 2003; Ribeiro *et al.,* 2004).

"The mechanisms of liver damage and the histological changes induced by *T. gondii* infection could be due to a direct proliferative effect of the parasite on the tissues, leading to cell death and tissue damage, or could be related to the indirect effect of infection due to the excessive immunological response to the parasite" (Ferro *et al.,* 1999).

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