Study of histopathological changes in brian of mice infected with *Toxoplasma gondii* isolated from domestic rabbit

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Summary

The study was designed to determine the infection rate of *Toxoplasma gondii* in the rabbits and histopathological changes of infected internal organs in mice. A total of 60 blood samples were collected from clinically healthy rabbits from different region of Baghdad city for the serological detection of *T. gondii* infection. Biological assay in mice was performed by intraperitoneal inoculation of 0.1 ml digested organs suspension, (lungs, heart, liver, spleen, kidney, muscles, and brain) during the period from October 2013 until July 2014. The results revealed histopathological changes in bioassay mice infected by *T. gondii* isolated from domestic rabbit, no histopathological changes in the brain of animals of first group1 (control group), but in the group 2 (killed at day 15) the main pathological changes were perivascular perineuronal edema with presence of some degenerated neurons characterized by shrunken dark blue stained (basophilic) cell bodies. In the animals of the group 2 (killed at day 21) the main brain histopathological changes were Sever congestion of meningeal blood vessels with infiltration of inflammatory cells and focal aggregation of microglial cells with diffuse obvious focal gliosis, whereas, in the same group animals that killed at day 28 the main brain histopathological changes were focal encephalomalacia and edema between molecular and granular layer.

Keyword:, Brain histopathological, Mice, *Toxoplasma gondii*, Rabbits.

Introduction

Toxoplasma gondii is a common and significant obligate intracellular pathogen of humans and animals, it is a highly prevalent, intracellular protozoan parasite with indirect life cycles (1). It infects a very broad spectrum of warm- blooded vertebrates, including humans, as intermediate host, but can reproduce sexually only in the feline intestine (2). In the a sexual cycle, the two developmental stages are the rapidly multiplying tachyzoites and into the slowly multiply, causing the cell to rupture and release organisms locally and into the blood stream (3 and 4). As the host develops immunity, the parasite retains its overall size and shape, but transforms into the bradyzoite stage and multiplies more slowly within tissue-cysts establish a persistent infection (5). These microscopic tissue cysts are present most frequently in the brain and skeletal muscle and represent the inactive stage of the parasite within the host. Viable tissue cysts within the muscle (meat) are a significant source of human infection. In animals that submit to acute infection tachyzoite may be demonstrated in ascetic fluid or in lung impression smears as well as in tissue section of the liver and other affected organs (6). Due to poor acknowledgment about the Toxoplasmosis in rabbit (7 - 9). There the present study designed to demonstrate the histopathological changes in bioassay mice infected by toxoplasma isolated from domestic rabbit.

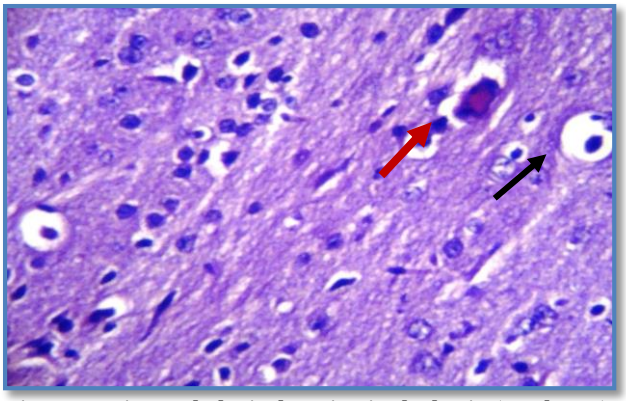
Materials and Methods

Sixty domestic rabbits (30 male, 30 female) aged between 5 to 8 weeks and weighing between 1500 and 2000 gm were collected from different parts of Baghdad and examined for detection and isolation of toxoplasmosis by pepsin digestion methods. Pepsin digestion method: pepsin digestion performed according to Dubey and Beatti (10) briefly by blending of different organs (5 gm of each liver, spleen, kidneys, lungs, heart, and skeletal muscles) with 5 volumes of normal saline until homogeneous, then to each 30 ml of homogenate 25 ml of digestion fluid were added (pepsin 2.6 g; NaCl, 5.0 g; HCl, 7.0 ml; and distilled water to make 500 ml of solution). The homogenates were incubated at

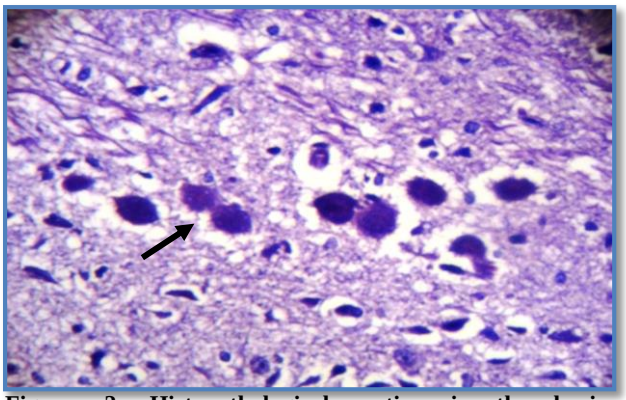
37ºC for 90 min in a shaker, then filtered through gauze and centrifuged at 3,000 rpm. /min for 10 min. The supernatants were poured off while sediments resuspended in 5 ml of normal saline containing 1000IU penicillin and 100 mg streptomycin per 1ml. Mice bio-assay: Twenty mice of both genders with an age range (4 – 6) weeks, adopted at the animal house of College of Veterinary Medicine, Baghdad University, for 2 weeks before starting the experiment by rearing in separated, clean, disinfected cages, they were fed on commercial assorted pellet. They were divided into 2 groups, the first group (as a control group) had 5 mice injected intraperitonally with PBS and were left for 28 days while the animals of group2 had 15 mice were injected I/P with 0.1 suspensions of digested organs solution at zero day and euthanized and dissected at 15, 21, and 28, days respectively. After 0,15,21,28 days post inoculation, randomly selected five mice were sacrificed by chloroform and postmortem examination were done for histopathological examination (11).

Results and Discussion

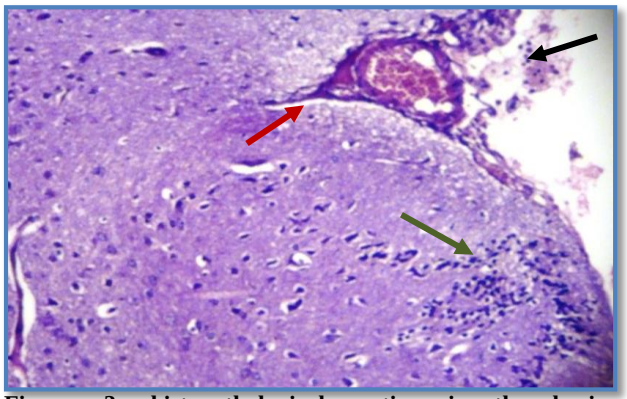
The results revealed no histopathological changes in the brains of mice on the first group1 (control group), but in the group 2 (killed at day 15) the main pathological changes were perivascular perineuronal edema with presence of some degenerated neurons characterized by shrunken dark blue stained (basophilic) cell bodies (Fig. 1 and 2). In the animals of the group 2 (killed at day 21) the main brain histopathological changes were Sever congestion of meningeal blood vessels with infiltration of inflammatory cells and focal aggregation of microglial cells with diffuse obvious focal gliosis (Fig. 3 and 4), whereas, in the same group animals that killed at day 28 the main brain histopathological changes were focal encephalomalacia and edema between molecular and granular layer. With degeneration of Purkinje cells in addition to complete dissolution of the other (Fig. 5 and 6). The present histopathological finding reveals different pathological lesions in internal visceral organs, increasing its severity with time experiment. The main brain lesion at 15 days was congestion of brain, cerebellum and meningeal blood vessels with different microglial aggregation (microglia).



Figure, 1:Histopathological section in the brain (cerebrum) of mice injected I/P with 0.1 ml of suspension of digested organs at day 15 showing perivascular (→) and perineuronal edema (→) H and E 40.



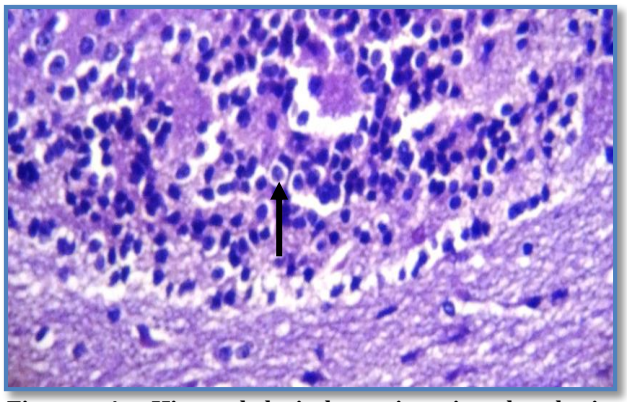
Figure, 2: Histopathological section in the brain (cerebrum) of mice injected I/P with 0.1 ml suspension of digested organs at day 15 showing degenerated neurons characterized by shrunken dark blue stained (basophilic) cell bodie (→) H and E 40x.



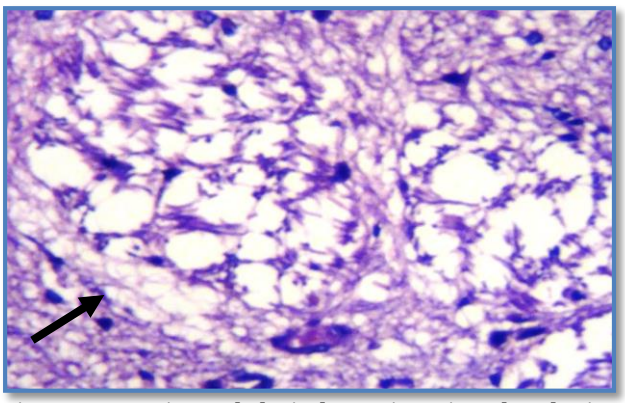
Figure, 3: histopathological section in the brain (cerebrum) of mice injected I/P with 0.1ml suspension of digested organs at day 21 showing sever congestion of meningeal blood vessels (→) with infiltration of inflammatory cells (→) and focal aggregation of microglial cells (→) H and E 10x.

At 21days this result agrees with (12). To refer to acute infection with *T. gondii* induce dendritic cell migration, and when infected dendritic cells are introduced to mice, the

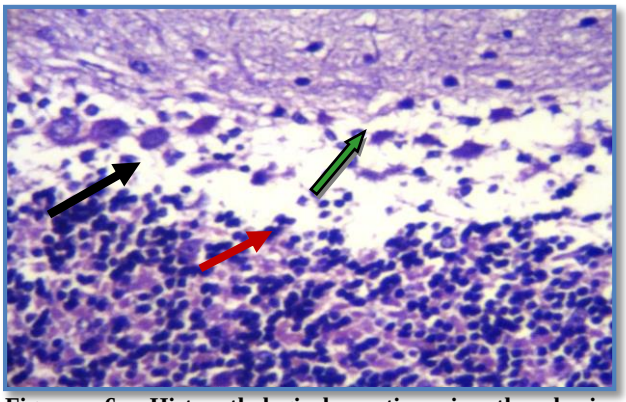
pathogen disseminates more rapidly than with parasite alone, including across the blood-brain barrier (13).While advance lesion characteristic by developed to multiple encephalitis this investigation confirms the results of (14) who explained that chronic toxoplasma infection lesion occur more often in muscle, eye and brain than in visceral tissue, also (15) recorded that the pathological lesion associated with chronic *Toxoplasma* in brain, include congestion of blood vessels in the meninges with numerous mononuclear cell infiltration in the meninges and around the blood vessels. However, variable sized areas of encephalomalacia were recorded at 28 day post infection this may indicate that chronic infection may result in a local degenerative cell loss. Parasites within neurons could directly cause the death of infected neurons or atrophy of their processes and inflammation may contribute, via the production of nitric oxide and other toxic oxygen products, to the death of neighboring neurons (16).



Figure, 4: Histopathological section in the brain (cerebrum) of mice injected I/P with 0.1ml of suspension of digested organs at day 21 showing diffuse gliosis of the submeningeal tissue (⟶) H and E 40x.



Figure, 5: Histopathological section in the brain (cerebrum) of mice injected I/P with 0.1ml of suspension of digested organs at day 28 showing focal encephalomalacia (⟶) H and E 40x.



Figure, 6: Histopathological section in the brain (cerebrum) of mice injected I/P with 0.1ml of suspension of digested organs at day 28 showing edema between molecular and granular layer (⟶) with degeneration of purkinje cells (⟶) in addition to complete dissolution of the other (⟶) H and E 40x.

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دراسة التغيرات النسجية لدماغ الفئران المصابة بطفيلي التوكسوبلازما المعزولة من الارانب

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الخلاصة

استهدفت الدراسة الحالية تحديد نسبة الاصابة بداء المقوسات الكوندية في الارانب، ودراسة التغيرات النسجية الاعضاء المصابة في الفئران، لتحقيق هذين الهدفين تم جمع 60 نموذج دم من ارانب سليمة سريريا من مناطق مختلفة من مدينه بغداد لتحديد الاصابة بداء المقوسات الكوندية مصليا، اما التحليل الحيوي في الفئران فقد تم اجراءه من خلال حقن 0.1 مل من خليط الاعضاء المهضومة (الرئتين، القلب، الكبد، الطحال، الكليتين، العضلات والدماغ) في البريتون وذلك للفترة من تشرين الاول 2013 لغاية تموز2014. واظهرت النتائج عدم وجود اي تغيرات نسجية مرضية في ادمغة فئران مجموعة السيطرة، بينما اظهرت وذمة حول الاوعية الدموية وحول الاعصاب مع وجود بعض الاعصاب المتنكسة تميزت بانكماش الجسيمات الخلوية وتلونها بلون ازرق داكن في الفئران المقتولة في يوم 15 من الحقن، اما الفئران المقتولة في يوم 21 من الحقن فقد كانت التغيرات النسجية الرئيسية في الدماغ عبارة عن احتقان شديد في الاوعية الدموية للسحايا مع ارتشاح للخلايا الالتهابية وتجمع موضعي للخلايا الدبقية الدقيقة وتدبق موضعي واضح ،بينما تمثلت التغيرات النسجية في الفئران المقتولة في يوم 28 بعد الحقن بوذمة بين الطقة الحبيبية والطبقة الجزيئية.

الكلمات المفتاحية: تغيرات نسجية للدماغ، الفئران، طفيلي التوكسوبلازما، الآرانب