NTU Journal of Agricultural and Veterinary Sciences (2023) 3 (2) : 74-82
DOI: https://doi.org/10.56286/ntujavs.v3i2.508

IRAQI Academic Scientific Journals

P-ISSN: 2788-9890 E-ISSN: 2788-9904
NTU Journal of Agricultural and Veterinary Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JAVS/index

The Histopathological & Behavioral Changes on Mice Experimentally Infected with Toxoplasma Gondii

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Article Informations

Received: 23-5- 2023,
Accepted: 06-06-2023,
Published online: 01-08-2023

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Key Words:
Toxoplasmosis ,Histopathological changes ,Toxoplasma gondii , hippocampus , amygdala

A B S T R A C T

Toxoplasmosis is one of the most important and common zoonotic diseases worldwide with an infection rate ranging between 20-80% of the world's population. The affecting in a wide range of mammals, including humans, causes significant disease effects on human health and economic animals. The parasite has an amazing ability to spread within the host's body and uses various strategies to overcome the blood-brain barrier, with the ability to exist for life within the cells of the infected host. The current study aimed at following up the histopathological changes in the brain of mice experimentally infected with toxoplasmosis. A placenta samples were collected from Al-Salam Teaching Hospital in Mosul, and the parasite was isolated and injection of 100 tissue cyst into the peritoneal cavity of laboratory mice. The animals were divided into three groups, and the mice were dissected after 21, 30 and 40 days of infection period in order to study histopathological changes in the cortex, hippocampus and amygdala. The results of the numbers of parasite cysts in the hippocampus, amygdala and cortex showed an increase in the number of parasite cysts in the cortex compared with the hippocampus and amygdala, the histological sections showed in addition to vacuolar degenerative changes and Apoptosis. After 30 days of infection, the results showed a decrease in body weight in males. The histological sections showed necrosis of the granular cell layer, edema around the nerve axons. The results of the third group, after 40 days of infection, showed a decrease in body weight in males and females compared with the control group and an increase in brain weight in males. The histological sections showed the loss of nuclei in the cells of the basal medial nuclei, vacuolar degeneration, in addition to the presence of vacuolization.

Introduction

Toxoplasma gondii is an obligate intracellular neurotropic parasite worldwide causes a disease called toxoplasmosis which in turn causes economic losses[1]. It has approximately infected one-third of the world's population [2]. The protozoan can nearly infect all warm-blooded animals, including humans [3]. The tachyzoites, converts into bradyzoites, a dormant stage, which mainly forms tissue cysts in brain, heart, and skeletal muscles that persist for several years after infection [4] . The parasite has a complex life cycle [5], including sexual and asexual replication in members of the cat family (Felidae) and in warm-blooded intermediate hosts such as rodents and humans [6]. Three forms in their life cycle, a tissue cysts which is the effective strategies to escape from immune response and remain active in a controlled environment as[7]. Tachyzoites can infect astrocytes, neurons, and microglia cells, and immunological features for bradyzoite development [8]. Toxoplasmosis known to be accompanied by specific behavioral changes it affects. The visceral organs in different degrees according to strain of parasite is based on virulence factors [9]. Infection with the protozoan Toxoplasma gondii induces changes in neurotransmission, neuroinflammation, and behavior [10]. Toxoplasma gondii has been associated with behavioral changes in various hosts but the mechanisms of the effect on host behavior are not well understood. Correspondingly, chronic toxoplasmic encephalitis in mice increase the levels of TLR11 (a specific receptor for T. gondii) in neurons, astrocytes and microglia [11]. T. gondii infection induces functional changes in many areas of the CNS including those involved in essential brain activities such as memory, executive functions, behavior, and motor responses, which are also compromised in AD. Several CNS cell types are affected by T. gondii, altering the physiological action of gliotransmitters and neurotransmitters [12]. Many studies indicated T. gondii infection as a risk factor for the development of behavioral changes and neurodegenerative diseases such as Alzheimer's disease (AD). Toxoplasma gondii has lifelong persistence in the brain and its cysts can affect gene expression and change diverse biological functions of neurons [13].

Therefore, this experimental was designed to carry out the pathological lesion caused by T. gondii infection in laboratory mouse to evaluate the behavioral changes with cyst distribution location of cysts in brain (amygdala and hippocampus) dependent on sex and time of infection.

2.Materials & Methods :

2.1 Sample collection and source:

Placenta samples were collected from aborted women at Al-Salam Teaching Hospital in Mosul during September 2022. The samples were kept in clean and sterile containers containing phosphate buffer saline (PBS) and placed in a refrigerated container until the samples arrived at the laboratory to be used for parasite isolation.

2.2 Parasite Isolation:

In order to isolate the parasite from infected placentas, the placenta was cut into small pieces, and the pieces were mashed after that were suspended with 5 ml of pepsin enzyme at 37 °C for 10 minutes and then filtered the mixture using several layers of medical gauze. The mixture was centrifuged at 3000 rpm for 10 minutes, and suspended in a PBS solution with a pH of 7.2 [14].

2.3 Microscopic Examination:

A microscopic examination was carried out by placing 10 microliters of the suspension on a glass slide. The slide was stained with Giemsa stain then microscopically examined with 100x magnification.

2.4 Molecular Diagnosis of the Parasite:

Molecular tests were performed using the polymerase chain reaction (PCR) technique, which was subjected to DNA samples extracted from the placenta to confirm the presence of the parasite by using a special extraction kit to isolate the DNA of T. gondii from the placental tissues. The extraction process was carried out according to the special instructions of the Korean manufacturer Add Bio Extraction kit (add prepGenomic DNA) by using the primer for the B1 gene, as shown in Table (1), and according to the program shown in Table (2).

Table.1 The nucleotide sequence of the primers and the temperature used to detect the B1 gene of the Toxoplasma gondii parasite.

No.	The sequence of the nitrogenous bases	Temperature	Length	Primer
1	TTTTGACTCG GGCCCAGC	60	18	Forward
2	GTCCAAGCCT CCGACTCT	58	18	Reverse

Table. 2 Steps of the PCR program to detect the B1 gene.

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	6 min.	1
2.	Denaturation	95	45 sec.	35
3.	Annealing	56	1 min.	
4.	Extension	72	1 min.	
5.	Final extension	72	5 min.	1

2.5 preparing the injection dose:

The injection dose was prepared by counting the tissue cyst in the placenta suspension previously prepared by taking a drop of 10 microliters of the suspension and stained with Giemsa dye. precipitate until 100 tissue cyst of 0.1 ml were obtained for injection into mice[15].

2.6 Injected animals :

Swiss albino mice, aged 21-25 days, were used, and their weight ranged between 17 to 20 grams. The mice were given an intraperitoneal injection (IP) with a 0.1 ml dose approximately containing 100 tissue cyst with a medical syringe with a capacity of 1 mm and a G21 gauge needle.The following criteria were carried out:

2. 7 Weight measurement

The weights of the mice as well as the weight of the brain and spleen were obtained after the experimental infection during the periods: (3,6,week and after infected as well as the control group [16].

8. 2 Counting the tissue cysts

In order to count the tissue cysts in the brain regions under study, the hippocampus and the amygdala, prints of the tissue of the two aforementioned parts, were made on a glass slide and stained with Giemsa for 10 minutes, then examined by light microscopy [16].

9. 2 Histopathological study

The brains of the mice were preserved in a 10% buffered formalin solution after dissection mice for histopathological study according to the method of [17].

3. Results

3.1 Weighing results:

3.1.1 The results of measuring the body weights of mice after infection with Toxoplasma gondii:

This results showed a significant decrease in the weight of males at a rate of 23.02 ± 1.2 grams and females at a rate of 17.3 ± 0.91 grams after 40 days of infection compared with the control group as shown in Table (3).

Table.3 Average body weight (males, females) in mice experimentally infected with Toxoplasma gondii.

Body weight - gm	Males		Females	
	Control	Infected	Control	Infected
21 days later	28.32±1.01 c	24.4 ± 1.2 b	25.18 ± 0.4 b	21.5 ± 52 a
30 days later	34.18 ± 0.46 c	28.6 ± 0.6 b	22.0 ± 0.7 a	21.47 ± 0.95 a
40 days later	34.39 ± 0.3 c	23.02 ± 1.2 b	22.16 ± 0.2 b	17.3 ± 0.91 a

* Similar letters indicate that there is no significant difference, and different letters indicate that there is a significant difference.
* Values represent the mean ± measurement error for each 5 mice in the group at a significant level $P \leq 0.05$
* The numbers in red indicate the significant difference in the results of the infected group compared with the control group, while the numbers in green indicate that the results are close between the control group and the infected group.

2.1.3 Absolute Brain Weight:

This results recorded that there was a significant increase in the absolute brain weight in males 30 days after infection at a rate of 0.652 ± 0.015 grams, and the increase was also significant in other periods. In females, there was also a significant difference represented by an increase in brain weight 30 days after infection at a rate of 0.577 ± 0.01 grams, while no significant difference was recorded in the other periods of the treatment, as shown in Table (4).

Table.4 Average absolute brain weight (males and females) in mice experimentally infected with *Toxoplasma gondii*.

Absolute brain weight	Males		Females	
	Control	Infected	Control	Infected
21 days later	0.37 ± 0.008 a	0.52 ± 0.04 b	0.506 ± 0.02 b	0.557 ± 0.02 b
30 days later	0.38 ± 0.0009 a	0.65 ± 0.01 d	0.47 ± 0.002 b	0.577 ± 0.01 c
40 days later	0.38 ± 0.001 a	0.45 ± 0.01 b	0.47 ± 0.001 b	0.449 ± 0.01 b

* Similar letters indicate that there is no significant difference, and different letters indicate that there is a significant difference.
* Values represent the mean ± measurement error for each 5 mice in the group at a significant level P ≤ 0.05
* The numbers in red indicate the significant difference in the results of the infected group compared with the control group, while the numbers in green indicate that the results are close between the control group and the infected group.

3. 1. 3 Absolute weight of the spleen:

The results of the absolute weight of the spleen in males showed that there was no significant difference between the control group and the infected group during different periods, while the females recorded a significant increase in the weight of the spleen after 21 and 30 days. The increase in weight was double after 21 days of infection, at a rate of 0.299 ± 0.01 grams, compared with the control group.

Table.5 Average absolute spleen weight (males and females) in mice experimentally infected with *Toxoplasma gondii*.

Absolute weight of the spleen	Males		Females	
	Control	Infected	Control	Infected
21 days later	0.218 ± 0.008 ab	0.137 ± 0.008 a	0.137 ± 0.008 a	0.299 ± 0.01 b
30 days later	0.22 ± 0.003 ba	0.226 ± 0.02 ba	0.159 ± 0.03 a	0.241 ± 0.01 b
40 days later	0.216 ± 0.001 a	0.257 ± 0.09 a	0.126 ± 0.0 a	0.285 ± 0.09 a

* Similar letters indicate that there is no significant difference, and different letters indicate that there is a significant difference.
* Values represent the mean ± measurement error for each 5 mice in the group at a significant level P ≤ 0.05
* The numbers in red indicate the significant difference in the results of the infected group compared to the control group, while the numbers in green indicate that the results are close between the control group and the infected group.

2.3 Number of parasite cysts in the brain after 2, 3 and 6 weeks after infection:

The results of counting the cysts of the parasite in the brain showed that significant differences in the number of tissue cysts among the regions of the brain, the hippocampus, the amygdala and the cortex in the group of infected female mice were represented by a significant increase in the number of cysts in the cortex compared with the hippocampus and the amygdala in females. In males, no significant difference was recorded.

Table.6 Average number of parasitic cysts in different brain regions after 21 days of infection.

Regions Groups	Hippocampus and amygdala	Cortex
Males	14.00 ± 0.966 A	18.66 ± 2.01 AB
Females	13.85 ± 0.341 A	19.66 ± 0.76 B

* Similar letters indicate that there is no significant difference, and different letters indicate that there is a significant difference.
* Values represent the mean ± measurement error for each 5 mice in the group at a significant level P ≤ 0.05

By following up the current results, it was observed that there was no significant difference in the number of parasite cysts in different brain regions between males and females in these treated groups after 30 days of infection with Toxoplasma gondii.

Table.7 Average number of parasite cysts in different brain regions after 30 days of infection

Regions Groups	Hippocampus and amygdala	Cortex
Males	17.5 ± 3.354 A	29 ± 2.683 A
Females	20.5 ± 0.223 A	26.5 ± 0.67 A

* Similar letters indicate that there is no significant difference, and different letters indicate that there is a significant difference.
* Values represent the mean ± measurement error for each 5 mice in the group at a significant level P ≤ 0.05.

3. 2. 3 The number of parasite cysts in the brain in the chronic phase, 40 days after infection

The results showed that there was no significant difference between the different brain regions in (males and females).

Table .8 the average number of parasite cysts in the brain regions after 40 days of infection.

Regions Groups	Hippocampus and amygdala	Cortex
Males	33.6 ± 14.1 A	38.6 ± 9.8 A
Females	25.6 ± 5.81 A	45.0 ± 4.6 A

* Similar letters indicate that there is no significant difference, and different letters indicate that there is a significant difference.
* Values represent the mean ± measurement error for each 5 mice in the group at a significant level P ≤ 0.05.

3. 3 Histopathological changes of brain regions in mice infected with Toxoplasma gondii experimentally :

1. Histopathological changes in males:

By following up the histopathological changes in the brain of the hippocampus and the amygdala and cortex during specific periods after 21, 30 and 40 days of infection in brains male regions: hippocampus and the amygdala after 21 years of infection, they showed a thinning of the granular cell layer as well as angioedema and gliosis, in addition to the loss of nuclei in the cells of the nucleus basalis neurons with some vacuolar degenerative changes of the neurons in the granular layer, as well as apoptosis. After 30 days of infection, thinning and necrosis of the granular cell layer appeared in the histological sections, beside loss of nuclei in the cells of the basal medial nuclei, as well as vacuolation as shown in Figures(1,2). Gliosis is an abnormal increase in the number of glial cells (proliferation and hyperplasia). After 40 days of infection, the histological sections showed loss and severe necrosis in the layer of granular cells, gliosis, mononuclear cells, vacuolization and edema. The focal lesion is a tumor or inflammation that occurs in specific areas

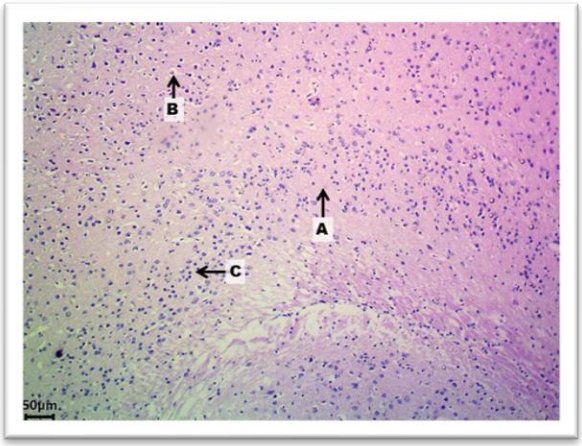

Figure 1: Histological section of a mouse brain from the male group 21 days after experimental infection with Toxoplasma gondii of the amygdala, in which there is loss of nuclei of cells in the basal medial nuclei (A), vasogenic edema (B) and gliosis (C). Hematoxylin and eosin stain 100x.

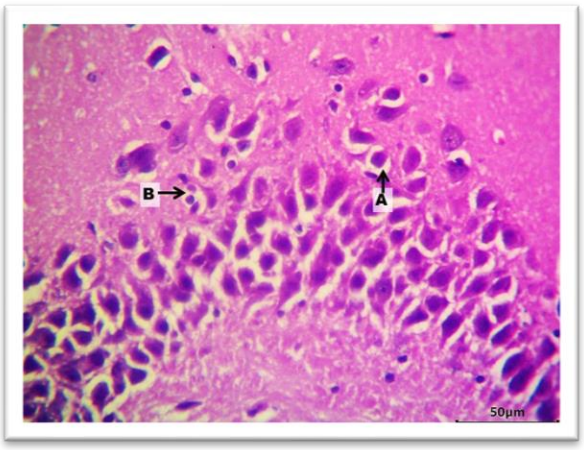

Figure 2: Histological section of a mouse brain from the male group 21 days after experimental infection with Toxoplasma gondii, show the Cornu Ammonis, in which vacuolar degenerative changes neurons in the granular layer (A), Hematoxylin and eosin stain 400x.

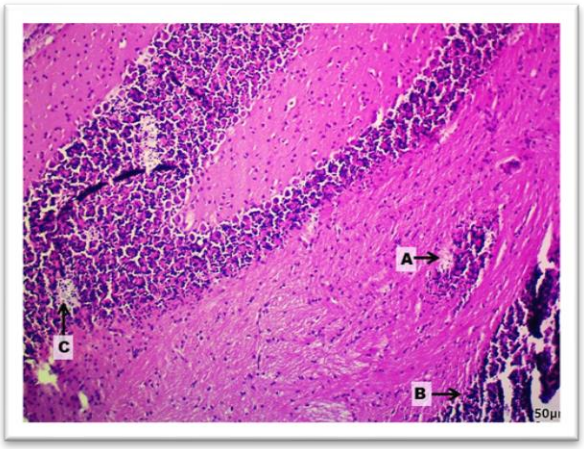

Figure3: Histological section of the brain of a mouse from the male group, 40 days after infection, show the presence of a vacuolization (A) and Gliosis (B) and vacuolization in the hippocampus (C) hematoxylin and eosin stain 100x.

2- Histopathological changes in females:

The histological sections, after 21 days of infection, showed necrosis and degeneration of the granulosa cell layer, and liquefactive necrosis in the form of a cavity, in addition to angioedema, vacuolation and congestion in the blood vessels, beside a vacuolar degeneration and loss of nuclei in the cells of the basal medial nuclei, in addition to a decrease in the thickness of the dentate gyrus layer. After 30 days of infection, the histological sections showed degeneration of the granular cell layer, congestion in the blood vessels, angioedema, in addition to the vacuolar degeneration. And 40 days after the infection, the histological sections showed degeneration and necrosis of the granular cell

layer and loss of cells in the basal medial nuclei, in addition to angioedema, vacuolation, vacuolar degeneration, gliosis, and degenerative changes in the dentate gyrus layeras as shown in Figures(4,5,6).

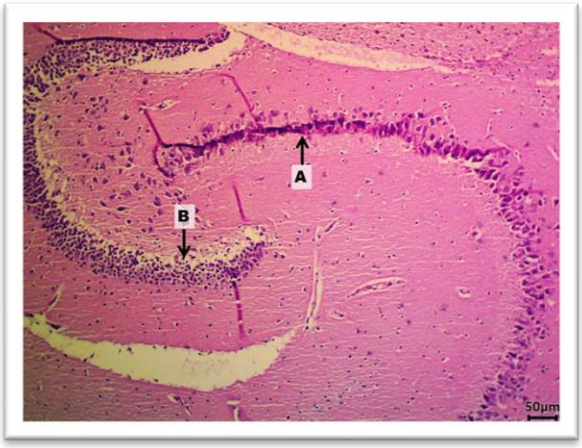

Figure 4: A histological section of the brain of a female mouse, 21 days after experimental infection with Toxoplasma gondii. It shows the hippocampus with thinning of the dentate gyrus layer (A) and degenerative changes in the cornu ammonis layer (B). Hematoxylin and eosin stain 100x.

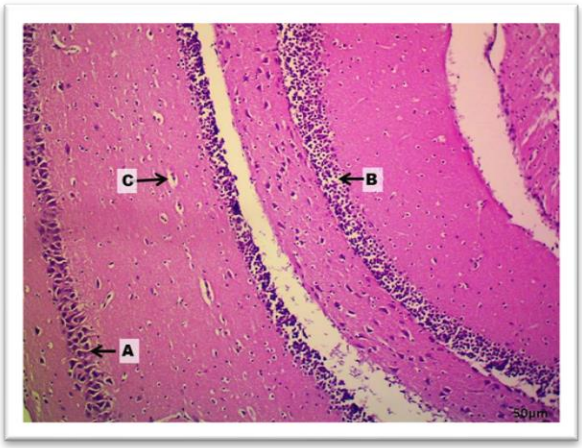

Figure 5: Histological section of the brain of a female mouse, 30 days after experimental infection with Toxoplasma gondii, showing the hippocampus with thinning of the dentate gyrus (A), degenerative changes in the cornu ammonis (B), and angioedema (C). Hematoxylin and eosin stain 100x.

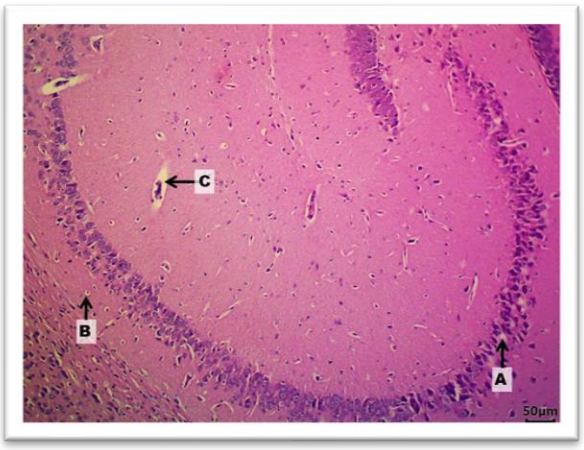

Figure 6: Histological section of the brain of a mouse from the female group 40 days after the experimental infection with Toxoplasma gondii. The hippocampus shows the presence of degenerative changes in the dentate gyrus layer (A), the vacuolation (B) and angioedema (C). Hematoxylin and eosin stain 100x.

4. Discussion :

Toxoplasmosis has gained special attention among researchers, especially in patients with HIV/AIDS. Infection with the parasite occurs within cells throughout life, especially in the central nervous system and within nerve cells causing a change in their function and structure, as well as the changes in neurotransmission and neuroinflammation, which affects behavior. However , it remains difficult to determine how these changes occur [10]. Because of the inflammatory processes, as well as the presence of the parasite and its formation of cysts and the resulting immune response, the infection led to disruption of the nerve circuits and inflammation of the nerves. This indicates a relationship among infection and neurodegenerative disorders, such as disease Alzheimer's and Huntington's disease. Histopathological studies stated that infection of brain cells with the parasite is responsible for causing inflammation in the central nervous system, which in turn contributed to subtle neurological changes [19]. Neurological and behavioral abnormalities were associated with decreased brain weight and inflammation, particularly in the regions around the hippocampus and around the ventricles. [20] and [21] indicated the presence of vacuolization in the rat brain infected with the Toxoplasma gondii parasite, and the inflammatory lesions were present in the brain of most infected mice.

By following up the results of this study, the effect of parasite infection on body weight, brain, and spleen was observed due to the effect and increase of inflammatory

processes.[22] mentioned that after the third week of infection, body weight increased after parasite invasion, as the loss of body weight was statistically significant in acute toxoplasmosis compared with uninfected mice. He also indicated that there was a significant decrease in weight in the infected group compared with the control group after two weeks of infection and the weight returned after four weeks of infection [23]. While [24] indicated that the weights of infected mice did not differ from those of the uninfected group in any time period after infection. [25] reported in his experiment on experimentally infected female mice that there was a decrease in body weight in the acute stage of infection. After this stage, the body weight loss stopped. [26] also observed a transient decrease in body weight in infected micef from 21 to 29 days after infection. When conducting their study on C57BL/6 mice, they observed continued gradual body weight loss, as well as the inability of the mice to reduce the inflammatory response provoked by the parasite [27]. The development of progressive wasting syndrome has also been observed with respect to other strains of mice [28]. [16] reported an experiment on experimentally infected male mice at the age of 12 and indicted eks anthat there was a significant increase in brain weight in the acute phase (21 days after infection), and the chronic phase was characterized by a decrease in organ weight compared with the control group. Decreased brain weight was associated with neurological disorders and inflammation in mice, as chronic innate and acquired chronic infection in adults caused secondary neurological and behavioral abnormalities [20]. The results showed an increase in both brain and spleen weights during the acute phase of the infection. The spleen, as the predominant secondary lymphoid organ, was involved in the development of a specific immune response to parasitic antigens with the aim of eliminating the parasite from the host organism. The heavy accumulation of inflammatory cells was reflected by a clear increase in spleen weight, and the host immune response contributing to the conversion of fast-proliferating to slow-proliferating stages, which was accompanied by a slow decrease in both the inflammatory response and spleen weight in chronic toxoplasmosis. The transient increased in brain mass can also be attributed to inflammatory processes led by several pro-inflammatory cytokines such as IL-1β and IL-6 that were produced in the brain during acute infection [29] and the accumulation of inflammatory cells [30]. The decrease in brain weight in mice with chronic toxoplasmosis may result from an inflammatory response in the brain. The effect of the parasite on the central nervous system through its effect on brain cells, especially during chronic infection, was due to the position of parasite cysts in areas of the brain that included the areas of processing emotions and controlling behavior. [20] stated that there is a clear decrease in brain weight with parasite invasion, although neuronal loss or myelination could not be demonstrated. Studies have also confirmed neuroinflammation as critical to the establishment and progression of Alzheimer's disease. Age-related inflammatory status and severe inflammatory response are considered part of the pathologic scenario as well as glial interaction [31]. Excessive activation of inflammation in the brain can also lead to neuronal apoptosis in chronically infected mice[32]. Many neuropsychiatric disorders such as schizophrenia whose behavioral symptoms can be associated with parasite-mediated inflammation, antipsychotic drugs with activity against T.gondii help reducing behavioral symptoms and inflammation in patients with schizophrenia [33].

The results obtained by [16] in his study to evaluate the number of parasite cysts in the hippocampus and amygdala (areas involved in processing emotions and controlling behavior) showed the presence of parasite cysts in both the hippocampus and the amygdala in infected mice. However, no clear distribution was observed depending on the region, as the presence of cysts in a greater number in the acute phase was in the hippocampus region, while in the chronic phase, cysts tended to be present in a greater number in the amygdala. Some studies indicate that certain regions of the brain are more consistently affected than others, with a density of cysts up to twelve times higher [34]. Cerebral cortical regions always showed higher cyst density than subcortical regions [26].[24] reported no difference in the average cyst density for each region (the cyst distribution is random).

From following-up the pathological changes in the brains of infected mice at various stages, cases of necrosis and apoptosis were recorded. Chronic infection for a year or more after the initial infection in adulthood was noted to cause behavioral and neurological abnormalities and ventricular dilatation on magnetic resonance imaging of the brain.

5. Conclusion :

By following up the results of the current study, it was found that the parasite has a clear

effect on body weight, as well as on brain, spleen and the greatest effect was on females. It also recorded the presence of cysts of the parasite between the regions (hippocampus, amygdala, and cortex) as well as the rest of the brain.

We suggest conducting a study that focuses on the neurological and behavioral effect in human infected with T. gondii and ,the effect of the immune response in the brain.

Acknowledgment:

The researchers greatly thank the department of Biology in the College of Education for Girl/ University of Mosul for their support of this study.

Competing interest:

The researchers declare that there has no conflict on interest about this research.

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