NTU Journal of Agriculture and Veterinary Sciences (NTU-JAVS)
P. ISSN: 2788-9890 e. ISSN: 2788- 9904

Study of Histopathological and Histochemical Changes Caused by *Toxoplasma gondii* in Vision Systems of Mice Congenitally Infected and the Synergistic Effect of Malarone and Clindamycin

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Received: 5-9-2021, **Accepted:** 20-9-2021, **Published online:** 29-9-2021

Abstract. The present work was conducted aiming at isolating and diagnosing *Toxoplasma gondii* from the placentae of infected women and inducing infection in female Swiss albino mice, through intraperitoneal injection resulted in 100% infection rate. Evaluation of malarone and clindamycin, single and synergistic between them in infection of female mice on the tenth day of gestation and delivery and confirmation of congenital infection, treatment was started at the age of one month with the above mentioned drugs. The criteria considered were the number of dead mice, the percentage of survival, and the average number of brain tissue cysts. Treatment with the synergized malarone and clindamycin were used to treat congenitally infected mice, number of death was not reported and the survival rate was 100%. Average numbers of brain tissue cysts are reduced to 5.55 in adult infected mice and the cure rate was 100%. Histopathological effects that occurred in the congenitally affected females, they were the appearance of hyperplasia and introversion of the retina several folds and the presence of scars that connect the retina to the choroid, and the reduction of the visual papilla, and it was noted for the first time by using the Congo red stain, signs of amyloid deposits in the vitreous fluid and in the debris of the lens that appeared irregular and seemed filled with fluid. And fatty materials and debris residues of lentil fibers. It was observed for the first time that the optic nerve was doubled using Mallory's triple stain. As for the brains, the same changes were observed, and amyloidosis appeared using the toludine stain. When using the treatment malaron and clindamycin at a dose of 100 mg/kg synergistically, the effect of infection in the eyes was slight and a noticeable improvement appeared in the brains. As for the histochemistry of stains and their components, and brain, different response were noted between strong +ev control and –ev for those treated with the two drugs malarone and clindamycin at a dose of 200mg/kg with PAS stain and Alician blue stain pH 2.5%. For Von kossa stain and Pearl`s stain, different responses also appeared in the eyes and the brains.For Feulgen stain, used to detect apoptosis good results was obtained in eyes, brains.

Keywords: Congenital Toxoplasmosis , Malarone , Clindamycin , Histochemistry.

Introduction

Toxoplasmosis is one of the most important and common parasitic diseases in the world, with an estimated infection rate of 25-30% in humans. It is caused by *Toxoplasma gondii*, an obligate intracellular parasites that can infect many types of intermediate hosts such as rodents, birds and animals. More than 350 species have been described in wild and aquatic species [1,2]. Cats and other species of the family Felidae are obligatory and intermediate specialized final hosts in the parasite's life cycle [3].

Congenital infection during pregnancy can be divided into three stages, as infection in the first three months of pregnancy leads to abortion, repeated abortion, neonatal death, or congenital abnormalities in the fetus, but if the mother is infected in the middle months of pregnancy, it leads This leads to the occurrence of congenital malformations and the appearance of symptoms of jaundice and hepatosplenomegaly, while the infection in the last three months of pregnancy is not accompanied by early clinical symptoms of the fetus, but the infection will develop with the age of the child to lead to Chorioretinitis and then ocular

toxoplasmosis that appears 2-3 years after delivery [4,5].

The drugs Pyrimethamine and Sulfadiazine, which show a synergistic effect in inhibiting the proliferation of Toxoplasma, may be used in treatment, but these two drugs have a high toxicity to the mother and fetus if they are given before the 20th week of pregnancy, so Spiramycin is replaced if the infection is during pregnancy [5,6].

Studying the effect of using two types of drugs alone and in combination together, malarone and clindamycin, in experimentally infected and congenitally infected adult female mice with *Toxoplasma gondii*, and the effect of these drugs on treating the visual system and brain of adult female albinos.

Materials and Methods

In this study, Swiss albino mice of *Mus musculus* were used, obtained from the animal house of the College of Dentistry at the University of Mosul. These animals were visually examined to ensure that they are free from visible impairments and diseases and confirm that they are free from toxoplasmosis by a latex test. Latex test. At the beginning of the work, the mice were distributed to groups for breeding purposes, to obtain mice at the age of two months, with an average weight of about 25-28 g for subsequent experiments during the study period for this purpose, the mice were distributed in groups that included 10 plastic cages with metal mesh lids, with 5 females and two males in each A cage, supplied with water and food.

Placenta samples were collected from Al-Batool and Al-Khansaa Teaching Hospitals in Mosul, specifically the maternity halls, to obtain placentas from abortions and natural births of sick women who are hospitalized in these hospitals to receive medical care. The information obtained, such as the age of the patients, the duration of pregnancy and the number of abortions, were recorded in special forms for this purpose.

Exposed placentas were collected in clean and sterile plastic containers with tight fitting lids and the necessary information was written on them, containing physiological saline (0.9%) (NaCl). This placenta was brought to the Parasitology Postgraduate Research Laboratory at the College of Education, Department of Life Sciences / University of Mosul for the purpose of parasite isolation and study , This was accompanied by collecting blood samples from the patients themselves as long as possible to obtain the serum and conducting a latex test on them to confirm the presence of infection.

Isolation and diagnosis of the parasite in the placenta:

The following steps were followed to isolate the parasite from samples of placentas of infected women, according to [7].

1. A piece of tissue was taken from each placenta, weighing between 40-50 g.
2. This tissue sample was cut into small pieces with sterile scissors and a scalpel.
3. homogeneity the tissue sample after mashing it with a mortar and a ceramic pestle or an electric mixer and adding an equal amount of physiological saline solution (1:1 weight:volume) until the mixture is completely homogeneous.
4. Then filter the mixture through several layers of medical gauze to get rid of large tissue pieces.
5. The filtrate was discarded in the centrifuge at 3000 rpm for five minutes and two times after pouring the slurry and Phosphate buffer saline (PBS) buffer solution was added to the precipitate.
6. The mixture was discarded a third time and the precipitate was resuspended in 4-5 ml of physiological solution to which 1000 international units of penicillin and 100 mg of streptomycin were added to prevent bacterial contamination and put the suspension in the refrigerator at 4ºC until use.
7. 10 microliters of this suspension were taken and spread on a glass slide and left to dry in the air, then fixed in 70% methyl alcohol and left to dry and stained with Giemsa color to investigate the number of histological cysts of the parasite and to diagnose its possible stages [8].

Determining the injection dose:

For injection into the required mice, 100 tissue bags were counted and a dose of 1.0 ml of the previously prepared suspension mixture was prepared from the placentas on the basis of weight for each mouse to be injected into the chelation, using a wine medical syringe with a G21 gauge needle[9]. After the infection occurred, all mice were subjected to continuous monitoring, and upon noticing any signs of disease or the death of one of them, they were immediately dissected for the purpose of obtaining the brain, heart, liver and spleen and imprinting them on a glass slide, then staining them with Giemsa and examining them under the microscope to diagnose the parasite.

The live mice were left infected with chronic toxoplasmosis and the formation of tissue cysts in their tissues, which are required for the later stages of work [10].

Study of the effect of some drugs in the experimental treatment of toxoplasmosis in mice:

There are many drugs that have often been used in the treatment of acute toxoplasmosis after the efficacy of a number of them was evaluated in laboratory animals. Samples of these drugs were selected to evaluate their efficacy in the treatment of congenital toxoplasmosis. The following are the drugs used under study:

Clindamycin HCl:

The drug is in tablet form, of Jordanian origin, with a concentration of 150 mg. It was given at a dose of 100 and 200 mg/kg of body weight once daily.

4. Malarone (MAL):

The drug is in tablet form, of English origin, with a concentration of 350 mg. It was given at a dose of 100 and 200 mg/kg of body weight once daily.

Congenitally infected female mice:

As reported in [11], 40 female mice were placed with 20 male mice and left during the night hours for the purpose of insemination. For 10 days, pregnant females were infected with 20 tissue sacs by injection into the peritoneum , then the females were isolated until the births. The first-generation mice were subjected to tests to diagnose the parasite. After the births reached three weeks, the males were separated from the females and divided into groups. When they reached 4 weeks, treatment was started for a period of two months:

1 Negative control: No infection is developed in it and without drug administration, numbering 10 mice.

2- Positive control: The infection is created congenitally and without medication, and the number of 10 mice is 10 mice.

Congenitally infected female mice dosed with drugs for two months at the rate of (10) mice for each treatment

* Infected mice dosed with clindamycin at a dose of 200 mg/kg

*Infected rats dosed with malaron at a dose of 200 mg/kg

*Infected mice were given clindamycin and malarone at a dose of 100 mg/kg each.

*Infected mice were given clindamycin and malarone at a dose of 200 mg/kg each.

Histological stains:

These colors are used to color the components of the textiles. The colors included the following:

1. Hematoxylin-delafield and eosin staining

Delafield's and Eosin Stain (H&E):

Attended according to [12].

2.Mallory's triple stain (TS):

Use this stain to check for textile components that are dyed different colors in this stain. This colorant consists of three solutions and it was prepared and colored according to [12].

3. Histochemical stains:

It is used to investigate the main tissue components (carbohydrates, proteins and fats) in the brain and eye tissues under the current study and to investigate a number of special pathological variables, which included the following colors:

Toluidine Blue stain (TB):

It is a metachromatic stain (that is, the body tissues transform this dye into a color other than the original color of the blue one) and it is used to color fat and connective tissue, and to detect amyloidosis, and it is present and colored as stated in [12].

Periodic Acid Schiff stain (PAS)

This stain is used to detect the presence of carbohydrates in the tissues and is prepared and colored as stated in [13].

Congo red stain

For the detection of amyloides, it appears in red or pink color and is present and colored by it, as stated in [12].

Alcian Blue stain (AB) pH 2.5%:

This stain is used to detect the presence of carbohydrates and sulfur mucopolysaccharides and is prepared and colored as stated in [13].

Von kossa stain(VK):

Stain used to detect calcium salts deposits and appear black. Attend and color it as stated in [13].

Pearl`s Stain (PS):

A stain used to detect hemosiderin resulting from the decomposition of red blood cells and appear blue when stained with this stain. Attend and color it as stated in [13].

Feulgen stain(F):

To detect deoxyribonucleic acid (DNA) and RNA, and the occurrence of programmed cell death, prepare and color it as stated in [12].

Results and discussion

Isolation of the parasite from the placenta of aborted women:

In this study, the human placenta was the main source and the only tissue from which the parasite was extracted. The placenta achieved a 100% infection rate in adult mice injected with placenta suspension and the infection progressed to the chronic phase within two months.

Effect of Malarone and Clindamycin in Adult Mice Congenitally Affected by Chronic Toxoplasmosis:

Table (4) shows the effect of malaron and clindamycin in mice congenitally infected with chronic toxoplasmosis, as the number of dead mice was 8 in the positive control group, and it did not occur Fatalities when treated with malarone and clindamycin at a single dose of 200 and 100 and 200 mg/kg each.

A significant difference appeared in the rate of tissue cysts in the brain of mice that were killed after fo ur months from both groups (control and treatment). The use of the two mentioned drugs results in a reduction in the number of tissue cysts in the brains of mice treated with them.

The lowest rate of brain tissue cysts was 5.55 bags/brain when Malarone and clindamycin treatment at a dose of 200 mg/kg each, while the highest average number of brain tissue cysts was 272.22 bags/brain for the positive control group. On the other hand, the use of these two drugs was accompanied by a significant improvement in the brains and eyes of mice, according to histological observations.

The prolonged treatment with these two drugs in mice, in addition to its reduction in the number of deaths, led to a complete cure of chronic toxoplasmosis in these mice, as the bio-evaluation value reached 100% when treated with malarone and clindamycin at a dose of 200 mg/kg each.

Table 1. Effect of Malarone and Clindamycin in Mice Congenitally infected by Toxoplasmosis.

group	Dosage: mg/kg/d	(Duration of infection + duration of treatment + monitoring) 2+4 months			
		N. dead mice	% survival	Av. N. tissue cysts in the brain / cyst / mouse	% vital assessment (cure)
MAL	200	0	100	b16.66	80
CLD	200	5	50	a 294.44	10
MAL+CLD	100	0	100	b16.66	80
MAL+CLD	200	0	100	b5.55	100
negative control		0	100	0 c	100
positive control		9	10	a 327.77	0

Means with similar letters are vertically significant according to Duncan's test at a probability level of 0.05

Histopathological

No pathological histological changes were seen in the examined tissue sections from the organs removed from the healthy (uninfected) mice, as the cells and tissues of these mice appeared normal compared to the infected mice figure(1),(2),(3)and(4).

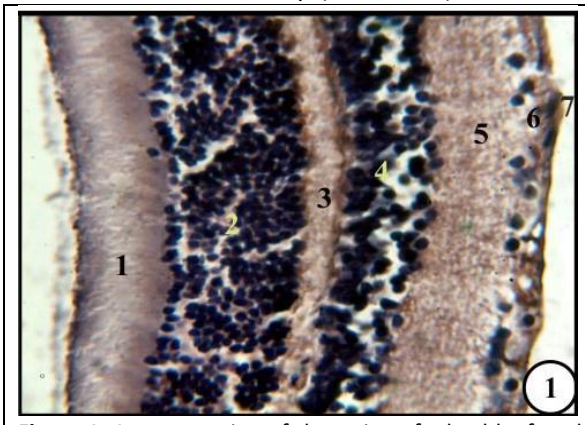

Figure 1. A cross section of the retina of a healthy female mouse showing the photoreceptor layer (1), outer nuclear layer (2), outer plexiform layer (3), inner nuclear layer (4), inner plexiform layer (5), and layer of cells Streptococcus (6) nerve fiber layer (7) (H&E.400x).

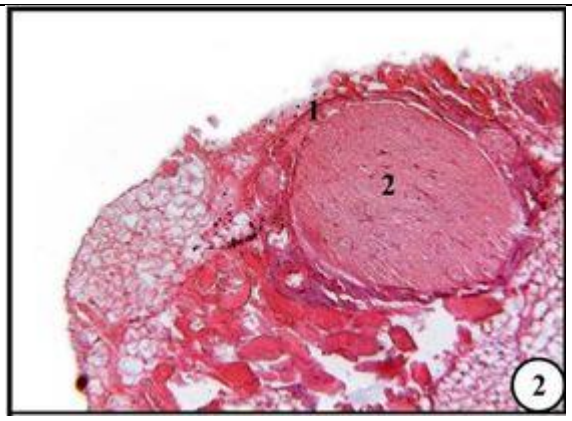

Figure 2. A cross section of the optic nerve of a healthy female mouse showing the nerve sheath (1) and nerve fibers (2) (H&E. 100x).

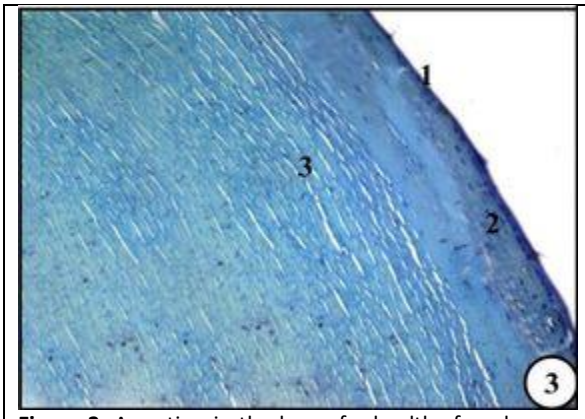

Figure 3. A section in the lens of a healthy female mouse showing the capsule (1), lens epithelium (2), lenticular fibers (3) (TB . 100x).

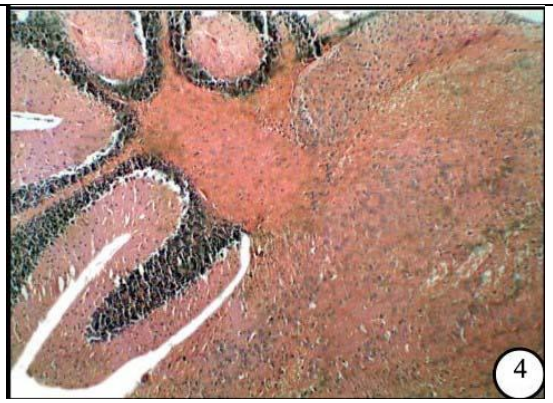

Figure 4. A cross section of the brain and cerebellum of an intact female mouse (H&E . 100x).

Females four months after congenital infection:

The pathological changes were irregularities in the entire retina and involved several folds with complete destruction of the photoreceptors and Bruch's membrane. Hyperplasia appears in both the nuclear layer and ganglion cells, thickening and reduction in the solid and choroidal components, with chorioretinitis, infiltration of inflammatory cells in the vitreous fluid and cataract formation. These lesions were in the retina and choroid towards the peripheral region (Figure 5).

In another eye, there was a wide break in the retina with its separation from the choroid and the occurrence of an inflammatory exudate in the vitreous fluid with reduction and condensation of the solid and choroidal components, and the occurrence of a central cataract, which led to its irregularity (Figure 6).

In another eye, several folds appeared in the retina and a complete destruction of the photoreceptor cells with their nuclei and the outer plexiform layer, leaving only the inner nuclear layer, the inner plexiform layer, ganglion cells and nerve fibers, and there appears to be hyperplasia in all of these layers, which led to the elongation of the retina and its inclusion in several folds. On the other hand, the solid and choroidal components have thickened with the presence of inflammatory cells in the choroidal area. Exudate was observed with amyloids debris in the vitreous fluid as well as inflammatory cells, which indicates inflammation of the vitreous fluid. (Figure 7).

Histopathological changes in the brains of adult female mice congenitally infected:

Histological sections revealed significant changes, as vascular congestion was observed in the cerebral cortex with constriction of blood vessels (Figure: 8). Extensive brain necrosis, fatty changes and the appearance of amyloidosis (photo 9) were noted. Extensive necrosis and fibrosis of the cerebral cortex were also noted (Figure 10).

The effect of treatment with malarone and clindamycin at a dose of 100 mg/kg synergistically in the eyes and brains of adult female mice with congenitally toxoplasmosis:

When these two drugs were used for a period of two months, less effects were observed, and the lesions were reduced, which consisted of condensation of the photoreceptor cell area and the presence of some necrosis in the inner nuclear layer, ganglion cell layers and nerve fibers (Figure: 11). As for the brain, some necrosis appeared and some blood vessels narrowed (Figure 12).

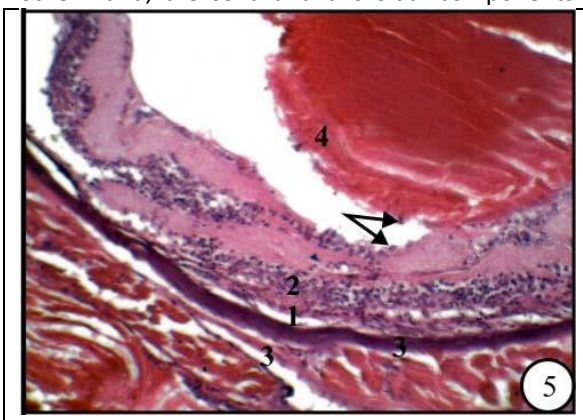

Figure 5. cross-section of an adult mouse eye after the congenital injury shows irregularity in the entire retina

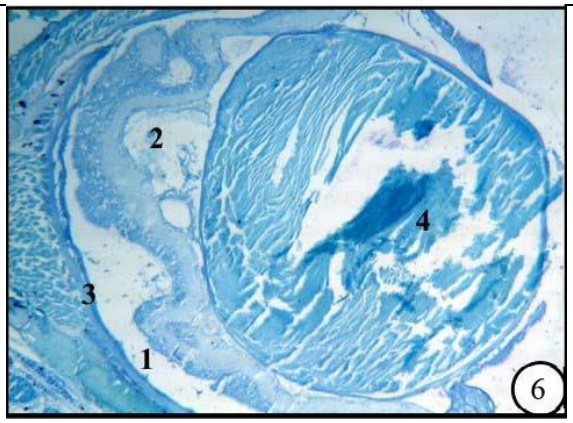

Figure 6. cross-section of the eye of an adult mouse after the

and inclination of several folds with complete destruction of the photoreceptor cells and Brooch's membrane (1) and hyperplasia appears in both the nuclear layer and ganglion cells (2) and appeared Condensation and reduction in solid and placental components(3) With inflammation of the choroid and retina, infiltration of inflammatory cells into the vitreous fluid (arrows) and cataracts (4) (H&E. 100x).

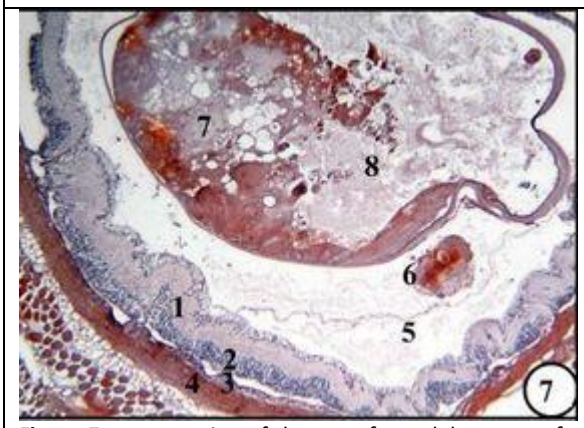

Figure 7. cross-section of the eye of an adult mouse after the congenital injury shows an area near the exit of the optic nerve in which a scar connecting the retina to the choroid (arrows) (CO.100x).

congenital injury shows an area near the exit of the optic nerve in which a scar connecting the retina to the choroid (arrows) (H&E. 100x).

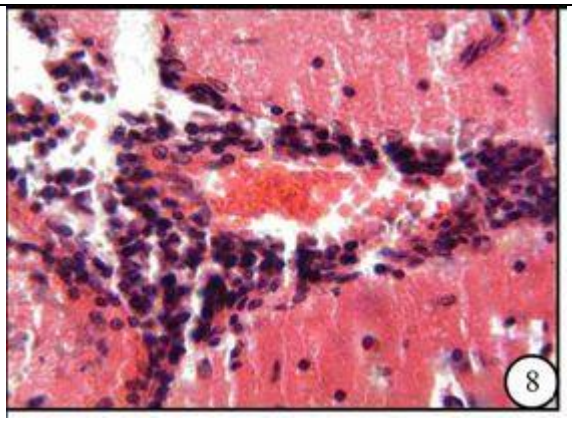

Figure 8. A cross section of the brain of an adult mouse after the congenital injury. Congestion of blood vessels in the cerebral cortex with condensation of blood vessels (H&E. x400).

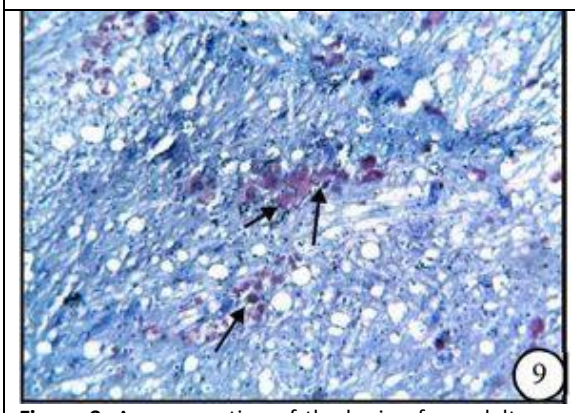

Figure 9. A cross section of the brain of an adult mouse four months after congenital infected noted amyloidosis (arrows) (TB. 100x).

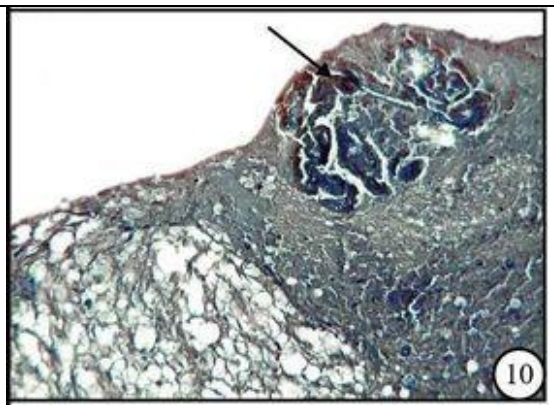

Figure 10. A cross section of the brain of an adult mouse after congenital infected. Note the fibrosis (arrow) (TS. 100x).

The effect of treatment with malarone and clindamycin at a dose of 200 mg/kg synergistically in the eyes and brains of adult female mice congenitally infected with chronic toxoplasmosis:

A noticeable improvement was observed during treatment for two months with these two drugs, as the retina appeared normal with some necrosis in the two layers of ganglion cells and disintegration in the inner and outer nuclear layers (Figure13). As for the optic nerve, it also appeared normal, except for a slight increase in glial cells in the form of rows (Figure 14). As for the lens, it looked normal except for a slight necrosis in its lens fibers (Figure 15). The brain appeared normal and free of tissue cysts (Figure 16).

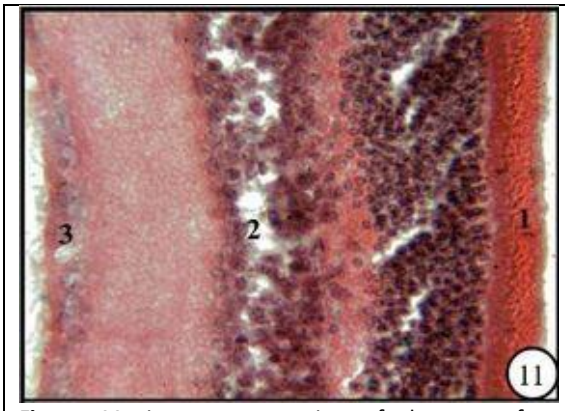

Figure 11. is a cross section of the eye of a congenitally infected adult mouse treated with malarone and clindamycin at a concentration of 100 mg/kg synergistically. Note the condensation of the photoreceptor cells (1) and the presence of some necrosis in the inner nuclear layer (2) and the two layers of ganglion cells and nerve fibers (3) (H&E.x400).

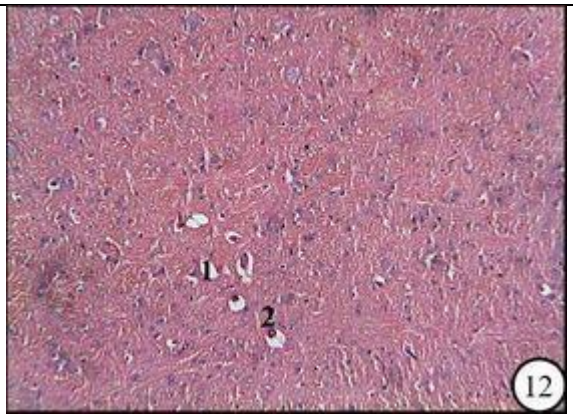

Figure 12. is a cross section of the brain of a congenitally infected adult mouse treated with malarone and clindamycin at a concentration of 100 mg/kg synergistically. Note the necrosis (1) and stenosis of some blood vessels in it (2) (H&E. 100x).

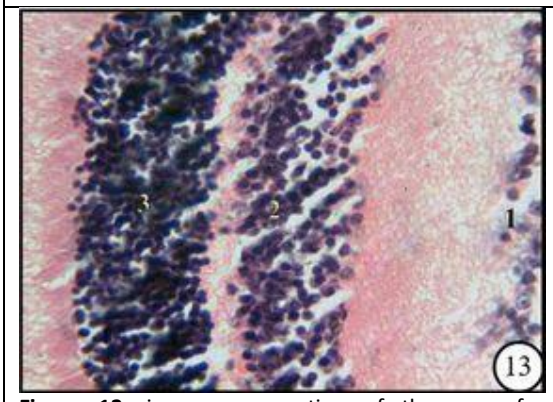

Figure 13. is a cross section of the eye of a congenitally infected adult mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. The retina appears normal with some necrosis in the ganglion cell layers (1) and disintegration in the inner and outer nuclear layers (2 and 3) (H&E. x400).

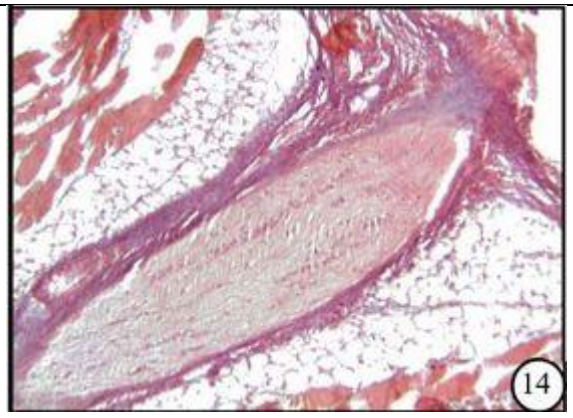

Figure 14. is a cross section of the optic nerve of a congenitally infected adult mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. The nerve appears normal with rows of glia (H&E. 100x).

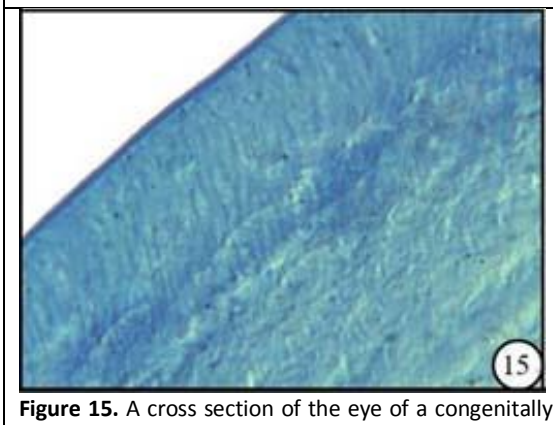

Figure 15. A cross section of the eye of a congenitally infected adult mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. Note the lens is normal except for a slight necrosis in some of its fibers (TB. 100x).

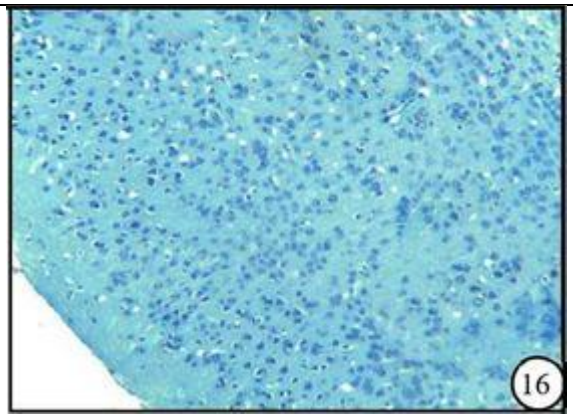

Figure 16. is a cross section of the brain of a congenitally infected adult mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. The brain appears quite normal (TB. 100x).

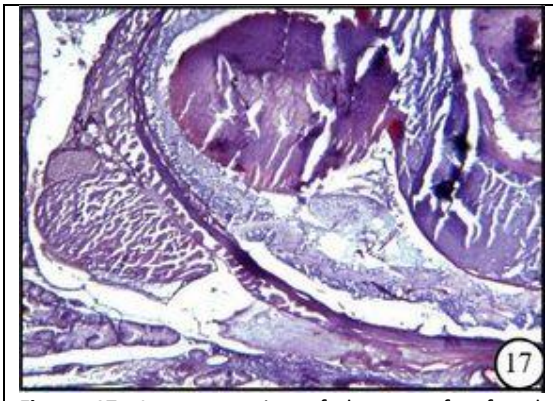

Figure 17. A cross section of the eye of a female mouse congenitally infected showing the strong positive lens and scleral affinity of these females whose eyes were white macroscopically (PAS . x40).

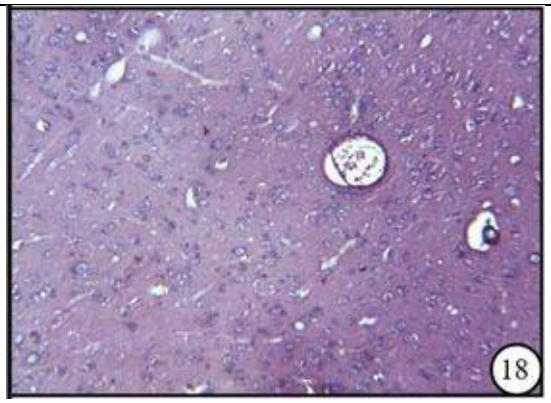

Figure 18. A cross section of the brain of a congenitally infected female mouse showing moderate positive affinity. Note necrosis, vessel stenosis and edema (PAS.100x).

Histochemical changes in the eyes and brains of adult female mice with congenital toxoplasmosis:

Periodic Acid-Schiff (PAS):

The results of using this stain showed that the components of the retina and the brain were negative, except for the lens capsule and the brain envelope, which were positive in female mice of the control group.

Histochemical changes of eyes and brains of adult female mice congenitally infected toxoplasmosis :

In these affected females, the lens, retina, and sclera were all strongly positive for PAS (Figure 17). The optic nerve was also positive, and the cornea was positive as well. As for the brain of these newborns, it was also positive, he noticed necrosis, narrowing of the vessel and edema (Figure 18).

Histochemical changes of the effect of malarone and clindamycin treatment at a dose of 200 mg/kg synergistically in eyes and brains of adult female mice with congenital toxoplasmosis:

The affinity for the stain was negative in the retina, except for the photoreceptor cells, which were positive when treated with malarone and clindamycin at a dose of 200 mg/kg synergistically for two months (Figure 19). As for the cornea, its stamens were positive (Figure 20). The optic nerve was positively covered and part of the surrounding tissue (Figure 21). As for the brain, the affinity was negative, and a slight condensation appeared in the periphery of the brain (Figure 22).

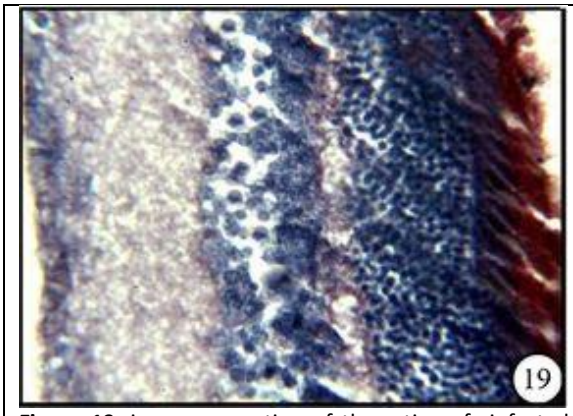

Figure 19. is a cross-section of the retina of infected female mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. Shows intense positive affinity for photoreceptor cells and moderate positive affinity for the outer and inner plexiform layers (PAS. 400x).

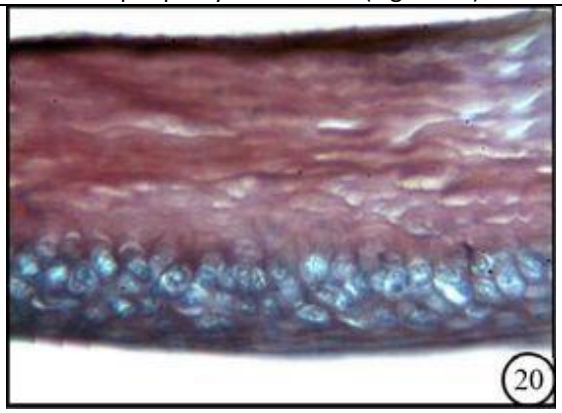

Figure 20. is a cross-section of the cornea of infected female mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. Note the positive affinity of its wedges (PAS. x400).

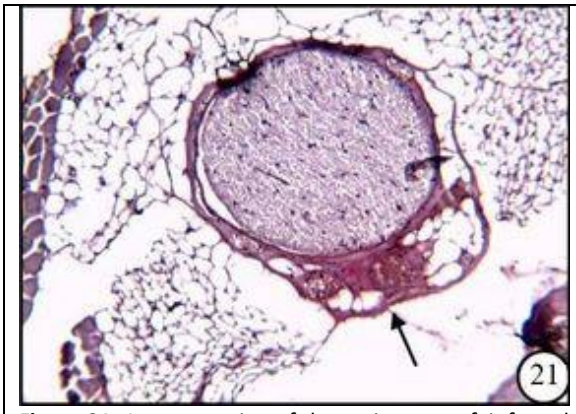

Figure 21. A cross section of the optic nerve of infected female mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. Note the intense positive affinity for the optic nerve sheath (arrow) (PAS. 100x).

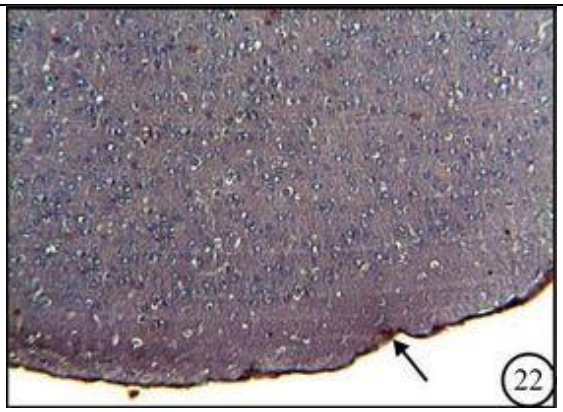

Figure 22. is a cross section of the brain of a female mouse infected and treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. Shows weak positive brain affinity (arrow) (PAS. 100x).

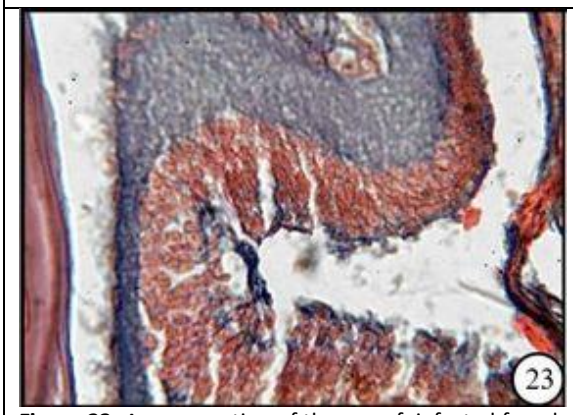

Figure 23. A cross section of the eye of infected female mouse showing moderate positive affinity for part of the retina (AB. x400).

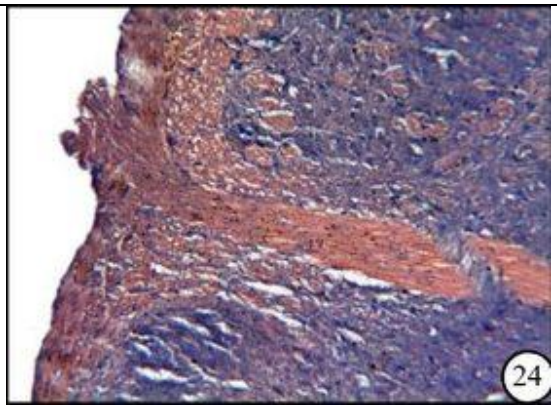

Figure 24. A cross section of the brain of a female mouse infected showing strong positive affinity in some parts (AB. 100x).

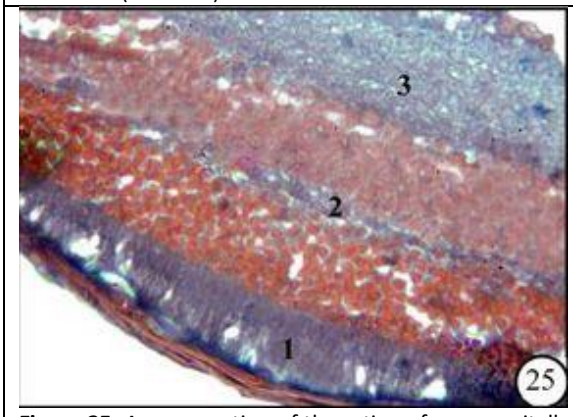

Figure 25. A cross section of the retina of a congenitally infected female mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. Shows the highly positive photoreceptor layer (1) and the moderately affinity plexiform layers (2) and (3) (AB, x400).

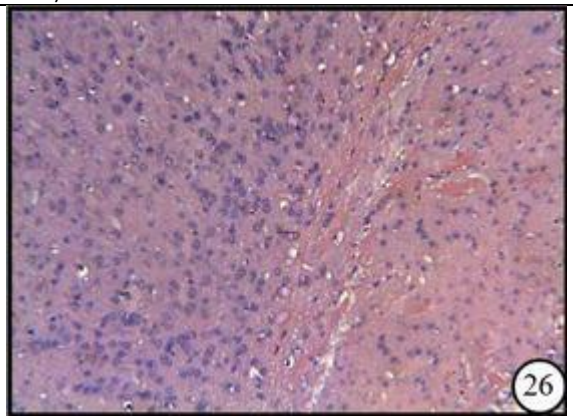

Figure 26. a cross section of the brain of a congenitally infected female mouse treated with malarone and clindamycin at a concentration of 200 mg/kg synergistically. Shows moderate positive affinity (AB. 100x).

Histochemical changes of eyes and brains of adult female mice congenitally infected with toxoplasmosis:

The results of for the congenitally infected females, the retina was moderately positive, and the lens was negative (Figure 23). As for the brain, it showed a very positive color tone (Figure 24).

Histochemical changes of the effect of malarone and clindamycin treatment at a dose of 200 mg/kg synergistically in eyes and brains of adult female mice with chronic toxoplasmosis:

As for the congenitally infected females who were treated with malarone and clindamycin for two months at a dose of 200 mg/kg synergistically, the photoreceptor cell layer and the inner and outer plexiform medial affinity layers appeared (Figure 25). The brain was affinity negative (Figure 26).

Von Kossa stain:

Histochemical and histochemical changes of eyes and brains of congenitally infected mice with toxoplasmosis and treatment:

The mice showed calcifications in the retina and lens (Figure 27). As well as simple calcifications in the atrophied nerve (Figure 28). And calcification appeared in the brain (Figure 29).

Perl`s stain:

Histochemical pathological changes of eyes and brains of adult female mice infected with chronic toxoplasmosis after four months and congenitally infected:

The results of staining with this color showed the appearance of hemosiderin resulting from the decomposition of red blood cells in the retina of congenitally infected mice with (Figure 30). As for the brain, hemoglobin appeared in a number of parts of the brain of infected adult mice (Figure 31). Hemosiderin was not seen in the previous drug-treated groups.

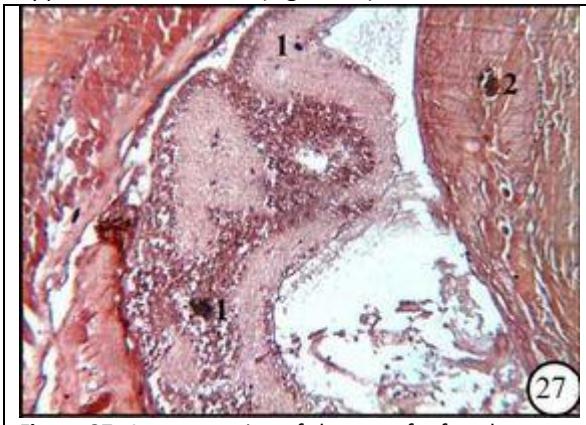

Figure 27. A cross section of the eye of a female mouse congenitally infected showing some calcifications in the retina (1) and lens (2) (VK. 100x).

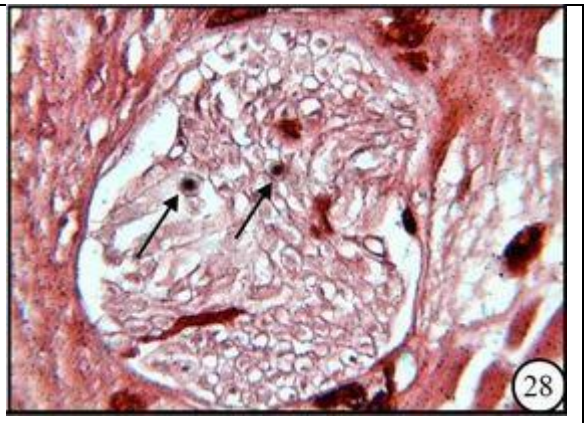

Figure 28. A cross-section of the optic nerve of a female mouse congenitally infected showing some minor calcifications in the atrophic nerve (arrows) (VK. x400).

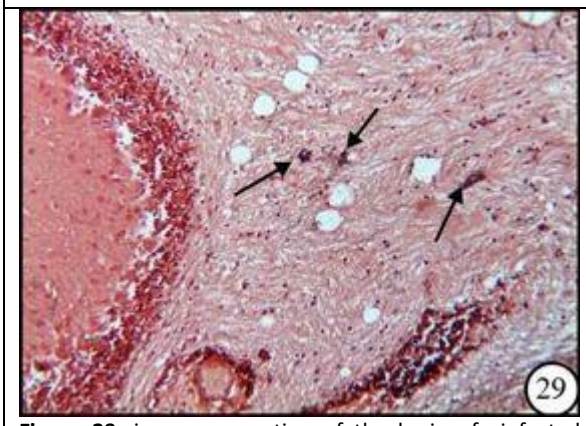

Figure 29. is a cross section of the brain of infected female mouse showing some calcifications (arrows) (VK. 100x).

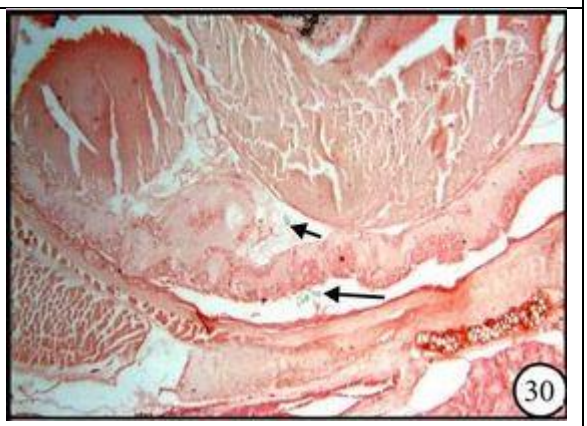

Figure 30. A cross section of the eye of a female mouse congenitally affected showing some hemosiderin in the retina and vitreous (arrow) (PS. x40).

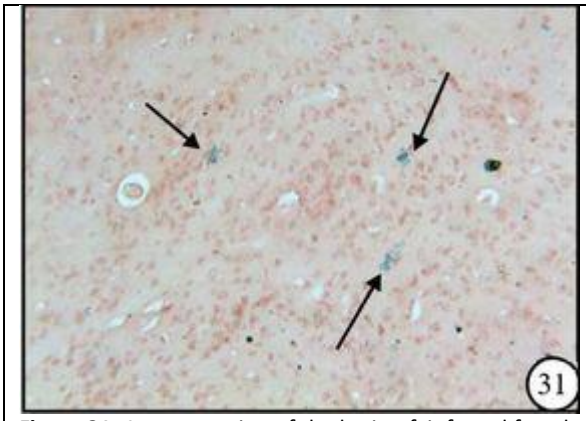

Figure 31. A cross section of the brain of infected female mouse showing some hemosiderin (arrows) (PS 100x).

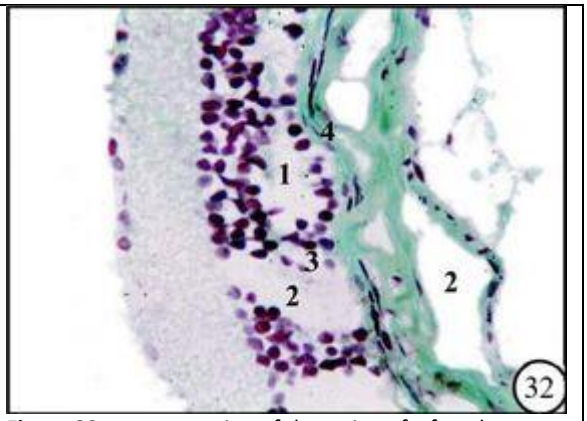

Figure 32. a cross-section of the retina of a female mouse infected with chronic toxoplasmosis , shows a Flexenrontersteiner flower (1), edema (2), programmed cell death of some cells of the inner nuclear layer (3), and the destruction of photoreceptor cells and their nuclei (4) (F. x400).

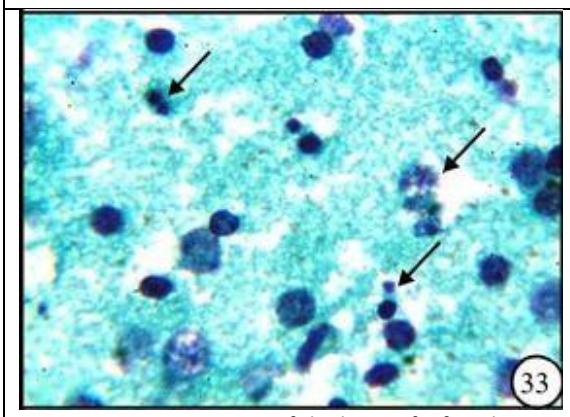

Figure 33. a cross section of the brain of a female mouse infected with chronic toxoplasmosis showing programmed cell death of some cells in the brain (arrows) (F. x 1000).

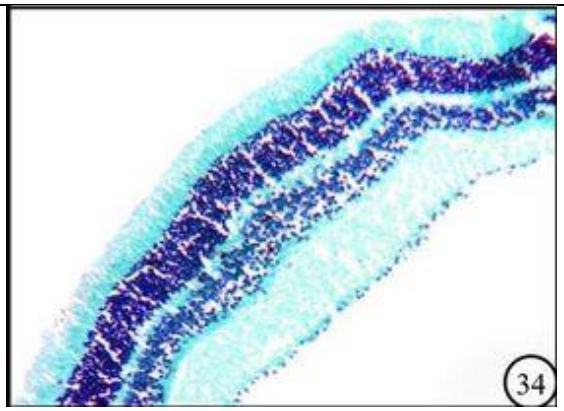

Figure 34. is a cross section of the retina of a female mouse infected with chronic toxoplasmosis treated with malarone and clindamycin at a concentration of 200 mg/kg. The retina appears normally (F. 100x).

Feulgen stain :

In the congenitally infected females, after four months, the stained showed the phenomenon of Flexenrontersteiner flower with the appearance of edema in the retina as well as the programmed cell death of the inner nuclear cell layer, and it clearly showed a complete destruction of the photoreceptor cells and their nuclei (Figure 32).

As for the brain of these females, a clear programmed death of glial and neuronal cells appeared in this stain (Figure 33). This stain also showed that the retina appeared somewhat normal to a large extent in mice treated with malarone and clindamycin at a dose of 200 mg/kg (Figure 34), and the cornea appeared somewhat normal. The brain appeared to be largely normal, and the phenomenon of programmed cell death did not appear and was reduced or stopped when using these two drugs.

Discussion

Parasite isolation:

We can conclude from the results we obtained that the Toxoplasma parasite can be isolated from the placentas of aborted women, and the isolated parasite infection can be induced in mice , and the placenta can be considered a source that reflects the condition of congenital infection .Numerous experiments and research on the placenta confirmed the importance of its role and effectiveness in protecting the fetus from injuries that occur in the mother[14].

Malaron was chosen for its unique characteristics among the wide spectrum drugs against parasitic infections. It consists of two drugs, the first is atovaquan and the second is proquanil hydrochloride, which is used in the treatment of malaria and has achieved excellent results in this field. This drug interferes with two different pathways, the first of which includes the

biosynthesis of pyrimidines necessary for the replication of DNA and RAN. Malaria parasite Disrupts Deoxythymidylate Synthesis [15].

Atovaquan is characterized by a high lipid solubility, penetration into brain tissue, and a high presence in the cerebrospinal fluid. It has a high effect on Toxoplasma parasite in the glass and in the body of the living organism and has a high ability to affect the slow-reproducing whin and the histocystic stage[16].

The results of using the drug Malaron at a dose of 200 mg / kg, in this study, showed that no deaths occurred until the end of the observation period, which is 6 months, which made the survival rate 100%. Other drugs, the number of brain tissue cysts in female mice was significantly reduced compared to the control group, while the rate of biological evaluation and recovery was high (80%).

This is due to the high susceptibility of the drug, which is included in the synthesis of Malarone, to the cerebral tissue sacs through the effect on the slow-growing and immature alveoli as well [17], as well as the effect of the second compound proquanyl hydrochloride, which is included in the drug's synthesis, and its high effect in inhibiting The enzyme dihydrofolate reductase, which is, it is similar in its effect to the drug permethamine in terms of its effect on the action of the same enzyme [18]. Also, these results converged with what was found by [19] when they used atovaquan to treat chronic infection with 20 tissue cysts of *Toxoplasma gondii* of ME49 typeII strain type II and treatment with this drug At a dose of 100 mg/kg for two weeks, it reduced the number of cerebral tissue cysts significantly.

Clindamycin was selected for its antiparasitic properties [20], and this drug targets the process of protein synthesis in the cytoplasm and apicoplast. Acute at a dose of 400 mg/kg when given with food. [21,22] indicated that injecting mice with 200 rapidly multiplying RH strains and treating them with clindamycin at a dose of 400 mg/kg for 10 days led to an increase in survival to 40%, but with the infection remaining. [19] indicated that the drug gave protection from death by 60-86% at a dose of 400 mg/kg when injected with 104 rapidly multiplying ovaries into the intercostal cavity.[23] indicated that the use of this drug for the treatment of acute ocular toxoplasmosis at a dose of 12.5 mg/kg for 21 days did not improve and did not reduce the death rate.

When using malaron in combination with clindamycin at a dose of 200 mg/kg, they showed a significant effect on survival rates that reached 100%, no deaths, reduction in the number of tissue cysts and an increase in cure rates to 100%. This may be due to the synergistic effect of the two drugs together, as many The sources indicated that many drugs increase their effectiveness in eradicating parasitic infections, for example, the traditional combined treatment of pyrmethamine with sulfadiazine.[24]

Histopathological changes to the brain and eyes of congenitally infected females after four months:

The irregularity of the entire retina and its folding are several folds, with complete destruction of the photoreceptors and Bruch's membrane, hyperplasia of both the nuclear layer and ganglion cells, thickening and reduction of the sclera and choroid components, and cataract formation. These lesions are due to the direct effect of the parasite during the growth and development process of the visual system, as between[25] that infection during pregnancy leads to cataracts, increased pressure in the eye and around the eye, strabismus, optic neuritis, and retinal necrosis. Sometimes hypoplasia or hyperplasia occurs, and this may result from a chromosomal abnormality [26].

And the reduction that appeared in another eye represented by the optic papilla and optic nerve fibrosis in the area of choroidal retinitis and atrophy of the optic nerve as a result of the reduction of the fibers in the area of fibrosis with its loosening, and the detachment of the retina as well and the almost complete destruction of the lens. It may result from a loss and lack of neural connections. These results are similar to what [27]indicated that congenital toxoplasmosis in human fetuses results in changes and inflammation of the optic nerve and the optic papilla and their atrophy, and the occurrence and edema of the optic papilla.

As for the congenitally infected female mice treated with previous drugs, the effects were less severe compared to the control treatment. When malarone and clindamycin were used at a dose of 100 and 200 mg / kg, their positive effect was very clear in the eyes and brains of these females, and this is probably due to the synergistic effect of the two drugs together as well as an increase The dose, which causes a reduction of the parasite and tissue cysts, and thus tissue lesions and the resulting complications. These results are consistent with what [28,29] indicated that the lesions in the eyes of children with congenital toxoplasmosis are highly visible and that a number of lesions can appear during the life of the child, and that the focal scarred foci can become active, and therefore it must be treated with a group of drugs for a long time.

[30] indicated that the synergistic treatment with pyrimethamine and sulfadiazine for congenital

toxoplasmosis was eight times more effective than using each drug separately.

Histochemical changes in the eyes and brains of adult and congenitally infected female mice:

When using the PAS stain , the strong positive affinity indicates the extent of damage and destruction in the cells and components of the eye as a result of multiple lesions and parasite activity, or perhaps as a result of the increase in glycoproteins produced by the ciliary body that feed the lens and cornea [31,32] and that the accumulation of These substances as a result of cell breakdown or discharge outside the eye may increase the intensity of this reaction.

As for the dye Alician blue at the pH 2.5, the positive results for some parts of the eye and the optic nerve indicate an increase in the presence of sulfur mucopolysaccharides of a weak acidic nature, which may be due to the effect of the multiplication of the parasite and the presence of tissue cysts within the various components of the eye, as the intensity of the interaction increases with the severity of the presence The parasite, which is used by it as food for metabolic activities.

The results of the Von Kossa stain for infected adult mice showed that after four months, calcifications appeared in the plexiform layer of the retina, and minor calcifications appeared in the optic nerve. Calcifications also appeared in the brain of infected adult mice. Also, calcifications appeared in the retina and lens of mice , and this result is consistent with what [33] indicated about the occurrence of calcifications in the retina of congenitally infected mice, as well as the occurrence of calcification in the brain, and this result is consistent with what [34].

As for the Perls stain, it showed the appearance of hemosiderin resulting from the decomposition of red blood cells in some parts of the brain of infected mice and in the retina of the eye. Deep and superficial, capillary thickening, retinal detachment haemorrhage, focal necrosis, exudate, and altered retinal structure. And the excess of hemosiderin causes damage to the organ as a result of generating free oxygen radicals, which leads to a failure in the organ's performance of its function, and this condition is called hemochromatosis.[35,36]

When using treatment with malarone and clindamycin at a dose of 200 mg/kg synergistically, it led to a change in the response of the eyes and brains to most of the colors from very positive to positive or negative as a result of controlling the parasite's proliferation and eliminating most of the tissue cysts in the brains and eyes as well as the various parts of the body.

Conclusions

From the results obtained in the current study, which included a number of experiments, the following can be concluded:

Isolation of *Toxoplasma gondii* from the placentas of aborted women can be considered as a useful and good diagnostic method as well as its use to confirm the serodiagnosis if infection occurred in mice with albinism.

Most of the chronic injuries in mice with albinism ended in visual or cerebral impairments, or both

synergistic treatment with malarone and clindamycin at a dose of 200 mg/kg resulted in a reduction in the number of cerebral tissue cysts and prevented fatalities in adult congenitally infected mice with complete recovery.

And a longer percentage of survival in infected and drug-treated mice compared to infected and untreated mice.

Acknowledgements

The authors are very grateful to the University of Mosul, the College of Education for the Pure Science, Department of Biology, which helped to improve the quality of this work.

Competing Interests

The authors should declare that there are no competing interests.

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