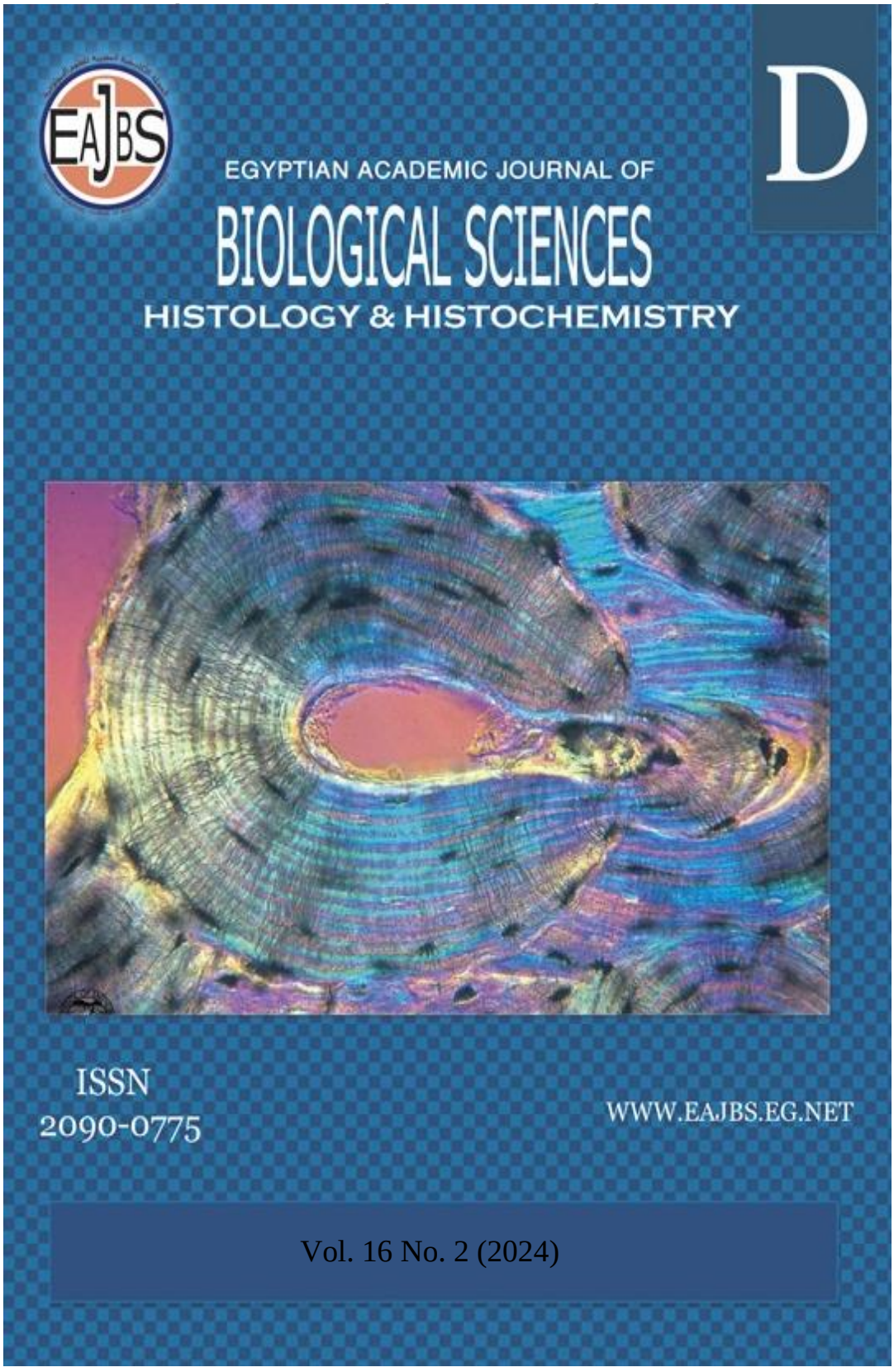

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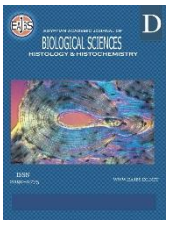

Biological Effects of *Toxoplasma gondii* Parasite:1: Ultrastructural Cytological Changes of Hepatocytes Nuclei of The *Toxoplasma gondii* Me49-Strain-Infected Albino Male Mice

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ABSTRACT

Aims: the infection with *Toxoplasma gondii* parasite causes some dangerous diseases_such as the disease toxoplasmosis in the human in the world. Therefore, the present work done to investigate some ultrastructure changes in the nuclei of cells of the liver in *Toxoplasma gondii*-infected male mice for both 3 months and 5 months. **Results:** The density of all chromatin types (prefrail, granular and associated nucleolus chromatin and extended chromatin) of nucleus in *Toxoplasma gondii* infected-mice for both 3 months and 5 months increased versus there control mice and it markedly increased in infected-mice for 5 months versus infected mice 3 months. the number of nucleoli in hepatocytes nucleus markedly increased in control 5 months versus control 3 months and not changed in *Toxoplasma gondii* infected-mice with time. **Conclusion:** *Toxoplasma gondii* Me49 strain-infection has stimulatory effect on the density chromatin distribution in normal hepatocytes nucleus and no effect on the number of nucleoli in hepatocytes nucleus but the age of animal increased the number of nucleoli in normal hepatocytes nucleus.

INTRODUCTION

Toxoplasma gondii is an intra-cellular protozoan parasite that infects many animals as well as humans with worldwide distribution. The disease is characterized by tachyzoite proliferation in the acute stage, followed by a chronic stage of latent cysts within the central nervous system (Skariah *et al.*,2010). *Toxoplasma gondii* is an obligate intracellular protozoan parasite that infects most species of warm-blooded animals, including humans, and causes the disease toxoplasmosis. *Toxoplasma*. *gondii* is a master manipulator, capable of completely rewiring the key signaling pathways of its various hosts (Naor *et. al.*, 2018). The clinical manifestations are affected by the strain of the parasite and immunity of the host. The estimated prevalence of latent toxoplasmosis among immunocompromised individuals is 35.9% (Wang *et, al.*, 2017). *Toxoplasma*. *gondii* depends on secreted effectors to subvert diverse host cellular functions promoting growth during the acute stages of infection (tachyzoite) and support differentiation into a persistent stage (bradyzoite) in host neuronal and muscle cells (Cygan etmml, 2020).

Hence, some of the effectors are secreted only during the tachyzoite stages and disappear from the host nucleus upon parasites differentiation into its latent form, while others continue to persist in bradyzoite infected cells (Mayoral *et. al.,* 2020). In immunocompetent individuals, the disease is usually asymptomatic in 60% of cases. However, it can have serious complications on the fetus, neonates, and in immunocompromised individuals (Robert *et. al.,* 2012). The cysts remain dormant in the brain of infected humans and can reactivate in immunocompromised patients resulting in acute toxoplasmic encephalitis which may be fatal (Chew *et.al.,* 2012). There are few or no published preclinical articles concerned with biological effects of *Toxoplasma gondii,* therefore we designated the present work; a fine structure changes in nuclei of the hepatocytes of *Toxoplasma gondii* infected male mic as a part of big research project of the preclinical studies of the biological effects of this parasite on the male mice; cytological, histochemical and pathological studies.

MATERIALS AND METHODS

Animals:

A total number of 40 adult male Swiss albino mice weighed 18 ± 2 gm and aged 90 ± 2 days were obtained from Theodor Bilharz research Institute, Cairo, Egypt. male mice were subjected to the same experimental conditions of an artificial light – dark cycle (12 h-12h), temperature (23±2 c), and humidity (37-40 %). They were supplied standard food and water ad libitum. The experiments of this research were conducted in the Autoradiographic lab. in faculty of science, south valley university, under supervision of Dr. Abdelbaset Aref Mohamed Aref. The experiments were conducted in the lab. achieve stability of environmental conditions, the separation between treated animals and control ones, and IACUC goals as in (Aref, *et.al.,* 2021a).

IACUC Approval of Project Number:

IACUC-SVU-EYGPT SVU/ F S 3/2022 – P. N.	0	0	3 (1)

Cysts Parasite Infection:

Cysts of avirulent Me49 *Toxoplasma gondii* strain was obtained from infected mice, Medical Parasitology Department, Faculty of Medicine, Zagazig University, Egypt. 1 Mice infected with cysts of avirulent ME49 *Toxoplasma. gondii* strain was sacrificed; whole mice brain was homogenized with 2 ml of saline (0·85% NaCl) and cysts of parasite were counted microscopically in 50µl of the homogenate, and the count was multiplied by 20 to obtain the number of tissue cysts per 1 ml of brain homogenate. If cysts were not found, another 50µl was examined in the same manner of (Dubey *et al.,* 2012 and Barrios *et al.,*2021). Infection with cyst of avirulent strain of *Toxoplasma. gondii* (Me49) was initiated by oral administration of 10 cysts in 0.1 ml of diluted brain suspension using a 19-gauge gavage needle.

Experimental Design:

For Study the biological effects and long-term infection of Toxoplasma. gondii Me49 on brain of male albino mice, intervals 3 and 5 post infection. Forty male Swiss albino mice were divided into 4 groups, each containing 10 males as following:

Group (C_3): control mice were sacrificed after 3 months.

Group (T_3): *Toxoplasma. gondii* infected mice which were sacrificed after 3 months post infection.

Group (C_5): control mice were sacrificed after 5 months.

Group (T_5): *Toxoplasma. gondii* infected mice which were sacrificed after 5 months post infection.

Electron Microscopic Examination (Fine structure of the cell):

Electron Microscopic Preparation:

After dissection of experimental

animals, tissues of liver were prepared into a small tissue block (1mm3). Then they were fixed quickly in a primary fixative of Karnovsky solution (2% Paraformaldehyde/2.5% Glutaraldehyde which then buffered with a 0.2M Cacodylate Buffer) overnight at 4 degrees. Then washed by 0.1M Cacodylate Buffer before Post Fixative of 2% aqueous OsO4/0.2M Cacodylate Buffer 1:1 solution for 2 hours in the refrigerator Then washed by 0.1M Cacodylate Buffer. Tissues were then dehydrated through a graded series of ethanol then pass through Propylene Oxide 15 min twice finally put in 1:1 PO/Epon resin (Epon 812 recipe) overnight, change out to fresh Epon812 for 1-3 hours (Reynolds, 1963 and Coggeshall, 1967). Polyethylene capsules are placed in a holder and a drop of fresh Epon812 is placed in the capsules and the specimen is transferred to the appropriate capsule. The blocks are cured for 48 hours in a 60 C^{o} oven. Then cut with an ultra-microtome 1 micron or thick sections are taken to find the area of interest then set to 70 nm section thickness. Final step is Stain grids in 8% uranyl acetate for 2 hrs. followed by Stain grids in lead citrate for 5 min. After drying the sections, they were examined by transmission electron microscope at EM Unit, south valley University, Egypt.

RESULTS

The Chromatin Distribution in Hepatocytes Nucleus:

In control mice for 3 months, the density chromatin distribution in hepatocytes nucleus was normal (Fig. 1a). In *Toxoplasma gondii* Me49- strain-infected mice for 3 months, the density of all chromatin types (prefrail, granular and associated nucleolus chromatin and extended chromatin) in nucleus was more slightly than those of control mice (Fig.1 a and b).

In control mice for 5 months, the density chromatin distribution in hepatocytes nucleus was more markedly increased than that of control mice for 3 months (Fig.1 a and c). In *Toxoplasma gondii* Me49-strain-infected mice for 5 months, the density of all chromatin types (prefrail, granular and associated nucleolus chromatin and extended chromatin) in nucleus was markedly increased than that of control mice for 5 months (Fig.1 c and d).

The density of all chromatin types (prefrail, granular and associated nucleolus chromatin and extended chromatin) in nucleus of *Toxoplasma gondii*-infected mice for both 3 months and 5 months increased versus that control mice and it more increased in infected-mice for 5 months versus infected mice for 3 months.

Toxoplasma gondii Me49-strain-infection has stimulatory effect on density chromatin distribution in normal hepatocytes nucleus.

The Number of Nucleoli in Hepatocytes Nucleus:

In control mice for 3 months, the number of nucleoli in hepatocytes nucleus was normal. In *Toxoplasma gondii* Me49 strain infected mice for 3 months, the number of nucleoli in hepatocytes nucleus not change than that of control mice (Fig.1 a and b).

In control mice for 5 months, the number of nucleoli in hepatocytes nucleus markedly increased comparing with than that of control mice for 3 months (Plat 1. Fig. c). In *Toxoplasma gondii* Me49- strain-infected mice for 5 months, the number of nucleoli was less than that of control mice for 5 months (Fig.1 c and d).

The number of nucleoli in hepatocytes nucleus markedly increased in control 5 months versus that of control 3 months but not changed in *Toxoplasma gondii* infected-mice with time.

Toxoplasma gondii Me49 strain-infection has no effect on the number of nucleoli in hepatocytes nucleus but the age of animal increased the number of nucleoli in normal hepatocytes nucleus.

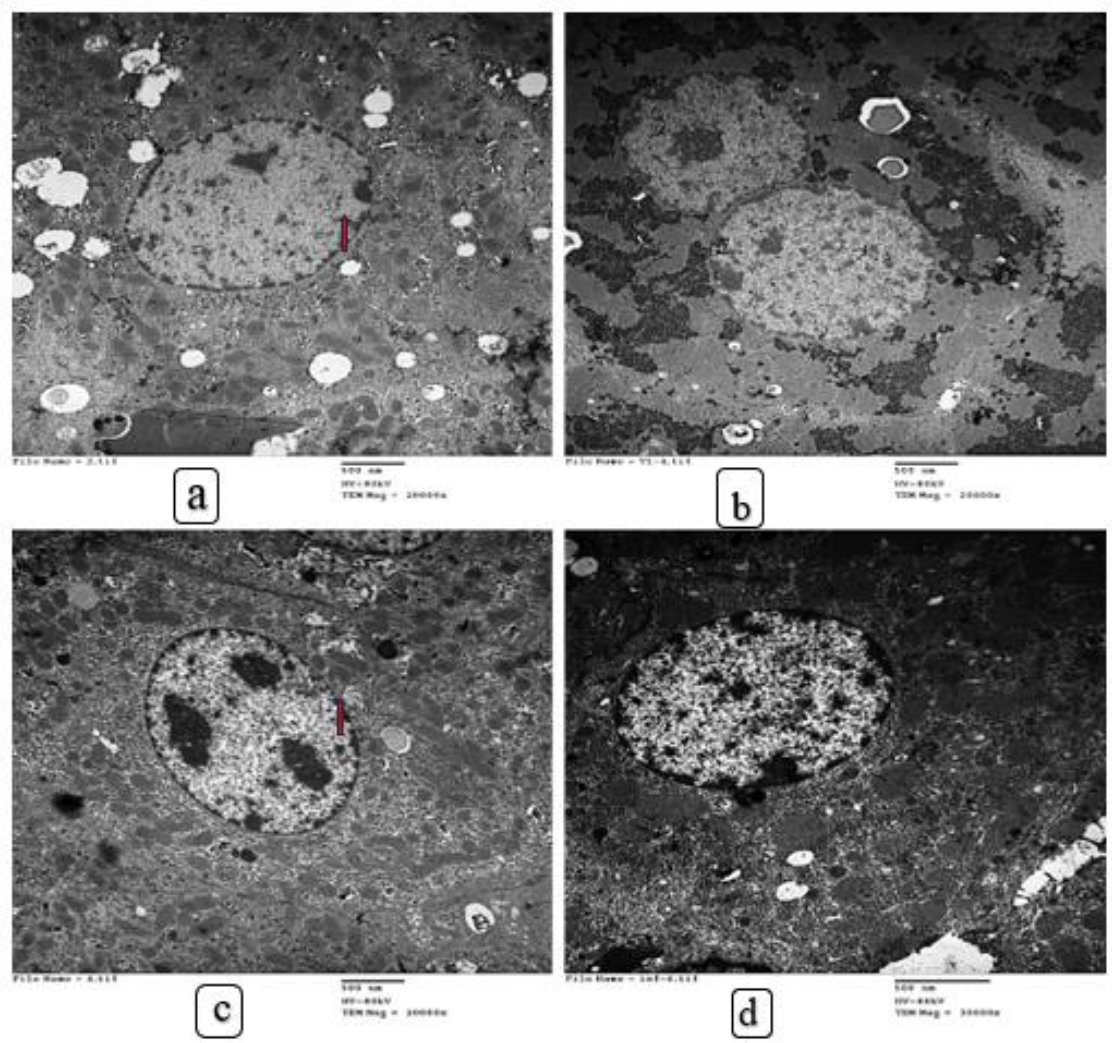

Fig. 1 (a, c, b and d): Electromicrographs showing density distribution of chromatin in hepatocytes nuclei in both control mice (a and c represent control mice for 3months and 5 months respectively) and *Toxoplasma gondii* Me49 strain infected mice (b and d represent infected mice for 3 months and 5 months respectively). The density of all chromatin types (prefrail, granular and associated nucleolus chromatin and extended chromatin) in nuclei of both infected mice for 3 months and 5 months increased versus control mice and it more increased in infected mice for 5 months versus infected mice 3 months. Also, the number of nucleoli increased in nucleus of control mice 5 months versus control mice 3 months and it decreased in nucleus of infected mice for 5 months versus infected mice for 5 months.

DISCUSSION

The infection with *Toxoplasma gondii* parasite causes some dangerous diseases in the world such as the disease toxoplasmosis (Yap *et. al.,* 2016 and Naor *et. al.,* 2018). There are few or no published preclinical articles concerned with biological effects of *Toxoplasma gondii*, therefore we designated the present work; a fine structure changes in nuclei of the hepatocytes of *Toxoplasma gondii* infected male mic as a part of big research project of the preclinical studies of the biological effects of this parasite on the male mice; cytological, histochemical and pathological studies

The present work done as preclinical research to investigate some ultrastructure of the cell; the density chromatin distribution and the number of nucleoli; in the hepatocyte's nucleus in *Toxoplasma gondii*-infected male mice for both 3 months and 5 months.

Our results showing the density of all chromatin types (prefrail, granular and associated nucleolus chromatin and extended chromatin) of hepatocytes nuclei in *Toxoplasma gondii* infected-

mice for both 3 months and 5 months increased versus those of control mice and it markedly increased in infected-mice for 5 months versus infected mice 3 months the number of nucleoli in hepatocytes nucleus markedly increased in control 5 months versus control 3 months and not change in *Toxoplasma gondii* infected-mice with time.

The Chromatin Distribution in Hepatocytes Nucleus:

The present data the density of all chromatin types in nucleus of *Toxoplasma gondii*-infected mice for both 3 months and 5 months increased versus that control mice and it more increased in infected-mice for 5 months versus infected mice for 3 months. *Toxoplasma gondii* Me49-strain-infection has stimulatory effect on density chromatin distribution in normal hepatocytes nucleus.

The Number of Nucleoli in Hepatocytes Nucleus:

Our results showing the number of nucleoli in hepatocytes nucleus markedly increased in control 5 months versus that of control 3 months but not changed in *Toxoplasma gondii* infected-mice with time. *Toxoplasma gondii* Me49 strain-infection has no effect on the number of nucleoli in hepatocytes nucleus but the age of animal increased the number of nucleoli in normal hepatocytes nucleus.

Conclusion and Recommendation:

Conclusion: *Toxoplasma gondii* Me49 strain-infection has stimulatory effect on the density chromatin distribution in normal hepatocytes nucleus and no effect on the number of nucleoli in hepatocytes nucleus but the age of animal increased the number of nucleoli in normal hepatocytes nucleus.

Declarations:

Ethics Approval: Ethical approval was obtained from the Ethical Committee of the Faculty of Science, South Valley University, Egypt. With the approval number (No. 004/06/24). Consent to participate from participants is not applicable as it is an animal experimental study.IACUC-SVU-Egypt approval of project number 3 (1).

Conflict of Interest: There is no conflict of interest among authors.

Author contribution: Each author took part in the design of the study, contributed to data collection, and participated in writing the manuscript. The manuscript is neither being published nor being considered for publication elsewhere until a decision is reached by this journal. The authors declared no conflict of interest.

Data availability statement: The collection of data developed and/or assessed throughout the present work is available through the corresponding author upon reasonable request.

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