Journal of the Egyptian Society of Parasitology, Vol. 53, No.2, August 2023
J. Egypt. Soc. Parasitol. (JESP), 53(2), 2023: 369 – 380
Online: 2090-2549

AN EXPERIMENTAL STUDY OF PROTECTIVE AND THERAPEUTIC EFFECTS OF PROBIOTICS ON INFECTION BY *TOXOPLASMA GONDII*

By
SALWA OSHIBA[1*], GEHAN SADEK[1,2], DINA ALLAM[3], ASMAA ELLAMEY[1], and TAHANY AL-ATTAR[1]
Department of Parasitology[1] and Department of Pathology[3], Faculty of Medicine, Menoufia University[1,2,3], and King Salman International University[2], Shebin ElKom, Menoufia Governorate[1,2,3] & South Sinai governorate[2], Egypt.
([*]Correspondence: sal_131977@yahoo.com)

Abstract

One of the most significant opportunistic parasites causing serious infections in humans is *Toxoplasma gondii* (*T. gondii*). Lack of maximally effective therapeutic drugs for chronic toxoplasmosis necessitates the search for a reliable and safe toxoplasmosis therapy. This study evaluated, compared, and contrasts the curative and preventative effects of probiotics on mice infected with the ME49 strain of *T. gondii*, as well as to contrast these effects with those of spiramycin. Probiotic effects were assessed using research in parasitology, histopathology, immunology, and immunohistochemistry. The results showed that probiotics were more helpful when administered as a preventative measure than when provided as a treatment for toxoplasmosis. *Toxoplasma gondii* cyst counts in the brain were successfully decreased by probiotics. The inflammation in the heart, eyes, and brain also lessened as a result. Besides, it led to a rise in interleukin-10 (IL-10) and a fall in Interferon-gamma (IFN-γ) serum levels. In the brain, heart, and eyes, it also reduced the expression of cluster of differentiation 3 (CD3) and Caspase3. It was less effective than spiramycin, nevertheless, as a therapy.

Keywords *Toxoplasma*, Probiotics, Experimental, Prophylaxis, Treatment.

Introduction

The commonest parasite zoonotic illness, toxoplasmosis, which is caused by *Toxoplasma gondii*, affects practically all warm-blooded animals, including humans. Around two billion individuals were affected globally (Mose *et al*, 2019). A persistent *T. gondii* infection affects around 30% of the world's population (Moncada *et al*, 2012). Humans who contract disease develop long-life seropositivity (Assimakopoulos *et al*, 2015). Consuming raw or undercooked meat that contains living tissue cysts and drinking or eating food that has been infected with *T. gondii* oocysts are the two major ways that people get infections (Dubey, 2008). The placenta also allows for vertical transmission (Robert-Gangneux and Darde, 2012).

Human populations have been reported to contain a wide range of *T. gondii* antibodies, ranged from 10% to 80%, according to studies conducted over the past three decades (Tenter *et al*, 2000). *T. gondii* infections are mostly asymptomatic in immunocompetent hosts, but 10-20% of individuals may experience symptoms. It can present with flu-like symptoms such as lymphadenopathy, myalgia, and fever. Some individuals have myocarditis, poly-myositis, lymphadenitis, or chorioretinitis (Martinot *et al*, 2020). The parasite can lead to serious and even fatal consequences in immunocompromised people, including encephalitis and serious ocular problems (Karimi *et al*, 2020).

T. gondii alters several neural signaling systems and causes a number of alterations in host neurons during chronic toxoplasmosis. Furthermore, parasites within neurons have been linked to direct neuronal death and atrophy (Etewa *et al*, 2021). When *T. gondii* is transmitted transplacentally during pregnancy, it can cause severe issues that can end in stillbirth, abortion, or congenital defects (Rostami *et al*, 2018). IFN-γ is produced by CD4 and CD8 T cells. It is a proinflammatory cytokine that prevents pathogens by causing inflammation. In contrast, IL-10 acts as an anti-inflammatory cytokine and restricts

the action of pro-inflammatory cytokines (Memon *et al*, 2022). Family of cysteinyl aspartate-specific proteases is known as caspases that control apoptosis. In brain cells, caspase-3 was identified as a key apoptotic mediator (Porter and Jänicke, 1999). The characteristic T cell surface protein complex known as CD3 is made up of four polypeptide chains: delta (δ), epsilon (ε), zeta (ζ), and gamma (γ). All T-cell stages and mature T-cells express CD3 at high levels. This protein's specificity made it crucial for determining the efficacy of an immune response to any stimuli and for identifying healthy and diseased T cells in tissues via immunohistochemistry labeling (Yahia *et al*, 2022).

World Health Organization defines probiotics as "Live organisms that, when administered in sufficient amounts, cause a health benefit to the host." They were initially prim arily used to manage bacterial and viral infections, but they are also employed to manage parasite infections (Travers *et al*, 2011). Probiotics are one of the dietary supplements most often utilized worldwide (Abid and Koh, 2019). The production of antibiotic substances and competition with pathogens for food sources and cell adhesion sites are two aspects of probiotics' mode of action. Probiotics also alter the metabolism of micrroorganisms, enhance the performance of digestive enzymes, and strengthen host defenses, which raise antibody production and macrophage activity (El-Sawah *et al*, 2020).

The present study aimed to evaluate, compare, and contrasts the curative and preventative effects of probiotics on mice infected with the ME49 strain of *T. gondii*, as well as to contrast these effects with those of spiramycin

Materials and Methods

At the Theodor Bilharz Research Institute (TBRI), in Giza, all experiments were conducted. Mice were maintained on a conventional commercial pelleted food in an air-conditioned room at 20 to 22°C under standard living circumstances at the animal house of TBRI.

Toxoplasma gondii strain (ME49) was obtained from Theodor Bilharz Research Institute (TBRI), Giza. Dissection and saline homogenization of *T. gondii*-infected mice brain were performed. A single drop of brain homogenate was examined under a light microscope to count the cysts in the cerebral tissue. A dosage adjustment was made to infective dose, which was changed to 10 cysts/ mouse administered orally by blunt feeding needle (Aboukamar *et al*, 2022).

Animals and study design: The study was performed on 70 Swiss Albino male mice, 6-8 weeks old, weighing 20-25 grams. They were divided into seven groups of 10 mice each. GI: Negative control group (non-infected, non-treated), each mouse received 0.2 ml of physiological saline solution. GII: Positive control group (infected non-treated), each mouse received 10 *T. gondii* cysts. GIII: Probiotic-prophylactic infected group: Mice received probiotics as prophylaxis, and then were infected by *T. gondii* cysts; each mouse received 0.1g/kg of probiotics daily for 21 days before infection and continued for 7 days post infection. GIV: Probiotic-treated infected group: Mice were infected, and probiotics treated, each mouse received 0.1g/kg of probiotics daily for 28 days after infection started from the first day of infection (Langarodi *et al*, 2012). GV: Spiramycin-treated infected group: Mice were infected, and treated with spiramycin, each mouse received 400mg/kg/ day for one week started one week before scarification. GVI: Probiotics-treated non-infected group, each mouse received 0.1g/ kg of probiotics daily for 28 days started at same time as the probiotic-treated infected group. GVII: Spiramycin-treated non-infected mice, each mouse received-spiramycin-400mg/kg/day for one week started one week before scarification, which was done at day 60 post-infection.

Parasitological study: It was performed at TBRI. Brain tissues of sacrificed mice from each group were dissected and homogenized in a tube containing saline. For the purpose of counting cysts, a drop of brain homogen-

ate was applied to a slide, coated with a slide cover, and inspected under a light microscope.

Histopathological study (Drury and Wallington, 1980): At 60 days after infection (dpi), mice were sacrificed. Each mouse had its heart, brain, and eyes removed, and then preserved in 10% formalin. They were dehydrated by ethanol in ascending concentrations: 70% for 30 minutes, 80% for 40 minutes, 95% for an hour, and ultimately 100% for three different changes of one hour each. Xylene was used to wash the samples twice. The melted paraffin wax included dehydrated tissues. Tissue samples were sliced using a microtome, placed on clean glass microscope slides, and stained with hematoxylin and eosin (H & E) after cooling.

Immunohistochemical study (Etewa *et al*, 2021): To extract CD3 and Caspase3 staining, the brain, eyes, and heart (4mm-thick) were placed in a 10mmol/l citrate buffer solution with a pH of 6.0 and cooked at 80°C for 30 minutes in microwave. After washing in water, 3.0% H_2O_2 in methanol was administered for 20 minutes to halt endogenous peroxidase activity. Slides were treated with regular goat serum for 20 minutes at room temperature to prevent non-specific staining. Anti-Caspase3 and rabbit polyclonal anti-CD3 (1:100) were added for an hour at room temperature. Diaminobenzidine, streptavidin horseradish peroxidase-conjugated tertiary antibody, and biotinylated secondary antibody were used. Sections were then stained with Mayer's hematoxylin as a counterstain. Using a light microscope at x40, positive cells for Caspase 3 and CD3 had brownish cytoplasmic staining that was semi-quantitatively scored. Staining intensity was graded as follows: 0= no cells, 1= few cells (low densities), 2= many cells, with high densities.

Quantitative estimation of interleukin 10 (IL-10) & interferon gamma (INF-γ) by sandwich ELISA in serum samples (Meira *et al*, 2014): Blood samples estimated the levels of IL-10 & IFN-γ in sera of all mice by ELISA before their scarification at day 60 post-in-fection. An antibody that is specific to mouse IL-10 and IFN-γ has been pre-coated on the micro-ELISA plate. Wells of micro-ELISA plate were filled with standards, samples, and an exclusive antibody and then adding biotinylated detection antibodies specific for mouse IL-10, IFN-γ, and Avidin-Horseradish Peroxidase (HRP) conjugate to each microplate well. During washing, free components were taken out. To each well, the substrate solution was added. Only wells contained mouse IL-10, IFN-γ, biotinylated detection antibody, and Avidin-HRP conjugate would exhibit blue coloring. Enzyme-substrate process was halted, and color changed to yellow, by introducing a stop solution. A wavelength of 450nm + 2nm was used to spectrophotometrically estimate optical density (OD). Relationship between tOD value and mouse IL-10 and IFN-γ levels was linear. Concentration of mouse IL-10 and IFN-γ in samples was calculated by comparing the samples' OD to the standard curve.

Statistical analysis: Statistical Package for Social Sciences (SPSS) version 26 (SPSS Inc. Released 2018) was used on an IBM-compatible personal computer to be tabulated and analyzed. Armonk, NY: IBM Corp., IBM SPSS Statistics for Windows, version 26.0. For the relationship between qualitative variables, chi-square test was used.One-way ANOVA test compared more than two groups using quantitative variables regularly distributed. For post hoc analysis, least significant difference test was used. Probability of P-value: non-significant difference if $P > 0.05$, significant difference if $P < 0.05$, highly significant difference if $P < 0.001$

Ethics Statement: The protocol was approved by the Institutional Ethical Committee, Faculty of Medicine, Menoufia University (IRB: 3/2022 PARA 20), which went with the Standard Declaration of Helsinki (2016).

Results

Parasitological results: there was a discernible drop in number of *T. gondii* cysts, with

a highly significant difference between them (P 0.001). Lowest mean number of cysts was in spiramycin-treated infected (59.4±6.3) with a reduction of 70.35%, probiotic-prophylactic infected (81.4±4.7) with 59.3% reduction and then probiotic-treated infected (104.4±9.7) with 47.5% reduction.

Immunological results: IFN-γ showed that the highest value was in GII (25.62±1.03), which decreased with treatment. The highest reduction was in GV (8.97±0.44), followed by GIII (12.78±1.06), and then GIV (18.69± 0.89), with highly statistically significant (P < 0.001) between all groups except between GI & GVI (P5=0.197), GI & GVII (P6= 0.755), and GVI & GVII (P21= 0.326). Values of serum IL-10 increased with treatment. The highest value was in GV (137.84±1.76), followed by GIII (97.53±3.5), and then GIV (79.12±1.8), with highly statistically significant (P < 0.001) between all groups except between GI & GVI (P5= 0.942), GI & GVII (P6= 0.756), and GVI & GVII (P21= 0.544).

Histopathological results; Brain positive control (GII) was 100% strong inflammation with the greatest levels of brain edema, hemorrhage, and inflammation. With therapy, these abnormal findings in the brain tissues improved. The spiramycin-treated (GV) showed the greatest reduction in brain inflammation (80% mild, 20% moderate), followed by probiotic-infected (GIII) with a prophylactic infection (70% moderate, 20% mild, 10% severe), and the probiotic-treated infected (G IV) with 60% severe, 40% moderate. The most significant reduction in brain hemorrhage was in GV (90% mild, 10% moderate), and then in GIII (70% moderate, 30% mild), and lastly in GIV (40% severe, 60% moderate). Brain edema was improved in GV (90% mild, 10% moderate), GIII (50% moderate, 50% mild), and G IV (30% severe, 50% moderate, 20% mild).

Heart showed that GII had 100% strength in terms of cardiac inflammation, hemorrhage, and edema and edema. With therapy, the abnormal cardiac tissues were improved. most (80% mild, 20% moderate), followed by GIII (70% moderate, 10% mild, 20% severe), and lastly in GIV (70% severe, 30% moderate). Heart hemorrhage was improved in GV (80% mild, 20% moderate), followed by GIII (90% moderate, 10% mild), and lastly in GIV (60% severe, 40% moderate). Also, GV had the greatest reduction in cardiac edema (100% mild), followed by GIII (50% moderate, 50% mild), and GIV (40% severe, 60% moderate).

Histopathology of the eyes in GII showed the greatest level of ocular inflammation, hemorrhage, and edema (100% strong), improved by treatment. The significant reduction in ocular inflammation was in GV (80% mild, 20% moderate), followed by GIII (70% moderate, 10% mild, 20% severe), and then GIV (70% severe, 20% moderate, 10% strong). Ocular hemorrhage was improved in GV (90% mild, 10% moderate), next in GIII (60% moderate, 20% mild, 20% severe), and then in GIV (80% severe, 20% moderate). Ocular edema was improved the most in GV (90% mild, 10% moderate), in GIII (50% moderate, 40% mild, 10% severe), and then in GIV (50% severe, 50% moderate).

Immunohistochemical results: Positive control showed greatest expression of CD3 in brain tissues (100% score 2), therapy reduced CD3 expression. In prophylactic probiotic infected, 80% scored 1, and 20% scored 2. Expression in GIV received probiotic treatment was 60% for score 1 and 40% for score 2. In spiramycin-treated, 100% scored 1 with highly significant (P <0.001) among groups. Also, the highest expression of caspase3 in brain tissues was in GII 100% for score 2. Caspase3 expression decreased with treatment. In GIV, expression was 70% for score 1 & 30 % for score 2. In GIII, it was 90% for score 1 & 10% for score 2. In GV, it was 100% for score 1, with highly significant (P <0.001) between groups. CD3 expression in cardiac tissues was greatest in GII (100% for score 2). With therapy, Heart inflammation in GV improved CD3 expression was reduced to 70% for score 1 and 30% for score 2 in GIV while 90% for score

1 &10% for score 2 in GIII. It was 100% in GV for score 1, with statistically highly significant way (P <0.001) in all groups. Also, GII (100% for score 2) gave greatest expression of caspase3 in cardiac tissues. Therapy, expression of caspase 3 was reduced to 60% for score 1 & 40% for score 2 in GIV, 80% for score 1 and 20% for score 2 in GIII. It was 100% in GV for score 1. All groups differed in a highly significant way (P <0.001). CD3 was expressed most highly in ocular tissues in GII (100% for score 2). With therapy, CD3 expression was reduced to 70% for score 1 and 30% for score 2 in GIV. It was 80% for score 1 and 20% for score 2 in GIII. It was 100% in GV for score 1. All groups differed with highly significant (P < 0.001). Also, GII (100% score 2) gave highest expression of caspase3 in ocular tissues. With therapy, expression of caspase 3 was reduced to 80% for score 1 & 20% for score 2 in GIV. 90% for score 1 & 10% for score 2 in GIII, and 100% in GV for score 1, with highly significant (P<0.001) in all groups.

Details were given in tables (1, 2 & 3) and figures (1, 2, 3, 4, 5, 6, 7 & 8).

Table 1: Comparison of mean number of *T. gondii* cysts in brain tissue at day 60 post-infection of groups.

Groups & Number of mice	Cyst count	GII reduction%	F test	P value	Post Hoc test
GI (Negative control) n=10	0		726.353	<0.001**	P1<0.001**
GII (Positive control) n=10	199±7.3				P2<0.001**
GIII (Infected + probiotic prophylaxis) n=10	81.4±4.7	59.3%			P3<0.001**
Group IV (Infected + probiotic treatment) n=10	104.4±9.7	47.5%			P4<0.001**
GV (Infected+ spiramycin treatment) n=10	59.4±6.3	70.35%			P5=1
GVI (Probiotic treated) n=10	0	0			P6=1
GVII (Spiramycin treated) n=10	0	0			P7<0.001**
					P8<0.001**
					P9<0.001**
					P10<0.001**
					P11<0.001**
					P12<0.001**
					P13<0.001**
					P14<0.001**
					P15<0.001**
					P16<0.001**
					P17<0.001**
					P18<0.001**
					P19<0.001**
					P20<0.001**
					P21=1

P1: Comparison between GI & GII, P2: between GI & GIII, P3: between GI & GIV, P4: between GI & GV, P5 between GI & GVI, P6: between GI & GVII, P7: between GII & GIII, P8: between GII & GIV, P9: between GII &GV, P10: between GII & GVI, P11: between GII & GVII, P12: between GIII & GIV, P13: between GIII & GV, P14: between GIII & GVI, P15: between GIII & GVII, P16: between GIV & GV, P17: between GIV & GVI, P18: between GIV & GVII, P19: between GV & GVI, P20: between GV & GVII, P21: between GVI & GVII

Table 2: Comparison of the serum levels of IFN-γ in all groups at day 60 post-infection.

groups & Number of mice	IFN-γ range	F test	P value	Post Hoc test
GI (Negative control)	6.07±0.66	848.128	<0.001**	P1<0.001**
GII (Positive control)	25.62±1.03			P2<0.001**
GIII (Infected + probiotic prophylaxis)	12.78±1.06			P3<0.001**
GIV (Infected +probiotic treatment)	18.69±0.89			P4<0.001**
GV (Infected +spiramycin treatment)	8.97±0.44			P5=0.197
GVI (Probiotic treated)	6.57±0.91			P6=0.755
GVII (Spiramycin treated)	6.19±0.84			P7<0.001**
				P8<0.001**
				P9<0.001**
				P10<0.001**
				P11<0.001**
				P12<0.001**
				P13<0.001**
				P14<0.001**
				P15<0.001**
				P16<0.001**
				P17<0.001**
				P18<0.001**
				P19<0.001**
				P20<0.001**
				P21=0.326

Comparison between groups (from P1:P21) as in Table (1).

Table 3: Comparison of the serum levels of IL10 in groups at day 60 post-infection.

Groups & Number of mice	IL 10 range	F test	P-value	Post Hoc test
GI (Negative control)	9.6±0.8	138.869	<0.001**	P1<0.001**
GII (Positive control)	59.2±3.4			P2<0.001**
GIII (Infected + probiotic prophylaxis)	97.53±3.5			P3<0.001**
GIV (Infected +probiotic treatment)	79.12±1.8			P4<0.001**
GV (Infected +spiramycin treatment)	137.84±1.76			P5=0.942
GVI (Probiotic treated)	9.54±0.73			P6=0.756
GVII (Spiramycin treated)	9.31±0.92			P7<0.001**
				P8<0.001**
				P9<0.001**
				P10<0.001**
				P11<0.001**
				P12<0.001**
				P13<0.001**
				P14<0.001**
				P15<0.001**
				P16<0.001**
				P17<0.001**
				P18<0.001**
				P19<0.001**
				P20<0.001**
				P21=.544

Comparison between groups (from P1:P21) as in Table (1).

Discussion

Toxoplasma gondii is one of the most pathogenic opportunistic parasites with a variety of intermediate hosts, particularly in immunocompromised individuals, infection can result in severe, sometimes fatal brain and ocular disorders (Zhai *et al*, 2020).

In the present study, the therapy caused a significantly lower mean number of *T. gondii* cysts in brain tissue. The group that received spiramycin treatment had the fewest cysts in their brain tissue, followed by group received probiotics as a preventative measure, and then group that received probiotic treatment. This agreed with Memon *et al*. (2022) in China, who reported that coccidia-infected hens that were administered diclazuril, probiotic, and probiotic combined with diclazuril exhibited a considerable decrease in oocyst shedding in feces. Whether taken with or without anti-coccidial drugs, probiotic supplementation increases immunity and lessens the consequences of coccidia infection. Also, the present study agreed with another study, which claimed that koumiss, a food high in probiotics, may reduce number of brain cysts in mice with chronic *T. gondii* infection (Yan *et al*, 2022). Besides, the present results concurred with El-Sawah *et al*, (2020), who claimed that probiotics were more advantageous when given as a prevent ative measure than when given as a cure for an illness. Moreover, the study results were consistent with Shaaban *et al*, (2021), they suggested that probiotic prophylactic and therapeutic groups significantly reduced the *Giardia* cysts number shed in feces

When it comes to immunological outcomes, the mean value of IFN-γ dropped with therapy whereas the mean value of IL10 rose. The group infected and treated with spiramycin had the greatest drop in IFN-γ, followed by the probiotic-prophylactic infected group and the probiotic-treated infected. The group that got probiotics as a therapy had the lowest value of IL10, followed by mice received probiotics as a prophylaxis, and group received spiramycin treatment. This agreed with Yan *et al.* (2022), who reported that administration of koumiss (rich in probiotics) resulted in a decrease in IFN-γ levels and an increase in IL-10 levels, showing that koumiss may reduce proinflammatory factors and increase anti-inflammatory factors. But, Guitard *et al*. (2006) didn't observe a significant effect of probiotics administration on mucosal cytokines (IFN-γ and IL10) expression in *C. parvum*-infected neonatal

rats compared with uninfected control rats

In the current study, there was a significantly lower level of inflammation, hemorrhage, and edema in all treatment groups compared to the positive control group when it came to the histological examination of the brain, heart, and eye of the examined groups. The spiramycin-treated group showed the greatest improvement, followed by the probiotic-infected group as a precaution, and finally the probiotic-treated infected group.

The present findings were corroborated by a certain study, which reported that various pathological abnormalities in the brain improved following spiramycin treatment and that brain gliosis was significantly decreased (Omar *et al*, 2021).

Therapeutic treatment of probiotics to *Giardia*-infected mice improved the pathological mucosal alterations, restored normal mucosal architecture, and reduced inflammation (Sanad *et al*, 2020). The present results also agreed with Memon *et al*. (2022), who found that probiotic therapy improved the cecal lesions in coccidiosis-infected mice. Besides, in agreement with the present results, probiotic supplementation helped in restorating normal mucosal architecture with increased villi and crypts ratio and decreased inflammatory reaction in small intestine of *Giardia*-infected mice (Shukla *et al*, 2013). Probiotics when given to *C. parvum*, newborn infected rats didn't appreciably alter rats' small intestinal damage (Guitard *et al*, 2006).

In the present immunohistochemistry study, the positive control group showed greatest levels of CD3 expression in their tissues (brain, heart, & eye). With therapy, CD3 expression was reduced. The spiramycin-treated group showed the greatest decline, which was subsequently followed by probiotic infection as a preventative measure and infection treated with probiotics. This agreed with those who found that probiotic therapy lowered CD3 expression in intestinal mouse tissues infected with *Cryptosporidium* (Gaber *et al*, 2022). Besides, the positive control mice with chronic toxoplasmosis showed a considerable increase in CD3 expression by activated astrocytes, indicating increased quantity and function of astrocytes (Etewa *et al*, 2021).

In the present study, positive control group had the greatest levels of Caspase3 expression in the tissues (brain, heart, & eye). With therapy, the expression of caspase 3 was reduced. The spiramycin-treated group saw the greatest decline, which was subsequently followed by probiotic infection as a preventative measure and infection treated with probiotics. This result agreed with El-Khadragy *et al*. (2019), who reported that probiotic therapy reduced the amount of the apoptotic marker caspase-3 in the hepatic tissues of mice infected with *S. mansoni*. Besides, the intestinal tissues of giardiasis-infected mice showed that the expression of caspase-3 apoptotic activity was elevated in positive control group and decreased with probiotic therapy (Shaaban *et al*, 2021)

Conclusion

Probiotics have greater protective than curative benefits on experimental toxoplasmosis. This was evidenced by the drop in the number of cysts in the brain and a decline in the pathological alterations in the tissues of brain, heart, and eyes.

They reduced serum levels of IFN-γ, while simultaneously increasing serum levels of IL-10. Besides, they decreased the immunohistochemistry expression of CD3 and Caspase3 in the tissues.

Acknowledgments

The authors would like to thank the manager of Theodore Bilharz Research Institute Animal House for his kind assistance during the current study on experimental mice.

Authors' contribution: AE, TA, SO & GS; Planning and experimental design. AE, TA, SO, & GS: Mice infection, parasitological and immunological studies. AE, & DA: Histopathological and immunohistochemical studies. AE, TA, DA, SO, & GS: Statistical analysis, interpretation, and paper writing. TA, DA, SO, & GS: Critical revision of the manuscript. All authors read and approved

the final manuscript.

Authors' declarations: Authors reported that they neither have a conflict of interest nor received any funds for public, commercial, or not-for-profit sectors.

References

Abid, MB, Koh, CJ, 2019: Probiotics in health and disease: Fooling mother nature? Infection 47, 6: 911-7.

Aboukamar, WA, Elhenawy, AA, Elmehankar, MS, Elzoheiry, MA, El-Gamal, R, *et al,* 2022: Activity of isoflavone biochanin A in chronic experimental toxoplasmosis: impact on inflammation. Parasitol. Res. 121, 8:2405-14.

Assimakopoulos, SF, Stamouli, V, Dimitropoulou, D, Spiliopoulou, A, Panos, G, *et al*, 2015: *Toxoplasma gondii* meningoencephalitis without cerebral MRI findings in a patient with ulcerative colitis under immunosuppressive treatment. Infection 43:589-93.

Drury, RAB, Wallington, EA, 1980: Carleton's Histological Technique; Oxford, New York, Toronto, Oxford University Press, 5th edition.

Dubey, JP, 2008: The history of *Toxoplasma gondii*–the first 100 years. J. Eukaryot Microbiol. 55:46775.

El-Khadragy, MF, Al-Olayan, EM, Elmallah, MI, Alharbi, AM, Yehia, HM, *et al,* 2019: Probiotics and yogurt modulate oxidative stress and fibrosis in livers of *Schistosoma mansoni*-infected mice. BMC Complement. Alter. Med. 19, 1:1-13.

El-Sawah, AA, Aboelhadid, SM, El-Nahas, EN, Helal, HE, Korany, AM, *et al,* 2020: Efficacy of probiotic *Enterococcus faecium* in combination with diclazuril against coccidiosis in experimentally infected broilers. J. Appl. Microbiol. 129, 4:1020-8.

Etewa, S, Sarhan, M, Moawad, H, Mohammad, S, Samir, M, *et al,* 2021: Behavior and neuro-psychiatric changes in experimental chronic toxoplasmosis: Histopathological and immunohistochemical studies. PUJ 14, 2:183-92.

Gaber, M, Galal, LAA, Farrag, HMM, Badary, DM, Alkhalil, SS *et al,* 2022: The effects of com-mercially available *Syzygium aromaticum, Anethum graveolens, Lactobacillus acidophilus* LB, and zinc as alternatives therapy in experimental mice challenged with *Cryptosporidium parvum*. Infect. Drug Resist. 15:171-82.

Guitard, J, Menotti, J, Desveaux, A, Alimardani, P, Porcher, R, *et al,* 2006: Experimental study of the effects of probiotics on *Cryptosporidium parvum* infection in neonatal rats. Parasitol. Res. 9, 5: 522-7.

Karimi, M, Tabaei, SJS, Ranjbar, MM, Fathi, F, Jalili, A, *et al,* 2020: Construction of a synthetic gene encoding the Multi-Epitope of *Toxoplasma gondii* and demonstration of the relevant recombinant protein production: A vaccine candidate. Galen Med. J. 9:1708-15.

Langarodi NM, Mohammadi M and Mehr MRA, 2012: Effect of Protexin (probiotic) and formic acid on performance of broilers. Animal Product. Res. 1, 3:9-15.

Martinot, M, Greigert, V, Farnarier, C, Dardé, ML, Piperoglou, C, *et al,* 2020: Spinal cord toxo-plasmosis in a young immunocompetent patient. Infection 48:299-302.

Meira, CS, Pereira-Chioccola, VL, Vidal, JE, de Mattos, CCB, Motoie, G *et al,* 2014: Cere bral and ocular toxoplasmosis related with IFN-γ, TNF-α, and IL-10 levels. Front. Microbiol. 5:492-501.

Memon, FU, Leghari, IH, Rajput, N, Gadahi, JA, Sahito, JZA, 2022: Immunomodulatory and ameliorative effects of probiotic in combination with diclazuril on broilers under coccidia infection. J. Appl. Microbiol.132, 4:3181-8.

Moncada, PA, Montoya, JG, 2012: Toxoplasmosis in the fetus and newborn: an update on prevalence, diagnosis and treatment. Exp. Rev. Anti-Infect. Ther. 10:815-28.

Mose, JM, Kamau, DM, Kagira, JM, Maina, N, Ngotho, M, *et al,* 2019: Organ pathology and associated IFN-γ & IL-10 variations in mice infected with *Toxoplasma gondii* isolate from Kenya. Parasit. Parasit. Dis. 10:1-20.

Omar, M, Abaza, BE, Mousa, E, Ibrahim, SM, Rashed, HE, *et al,* 2021: Effect of spiramycin vers- us aminoguanidine and their combined use in experimental toxoplasmosis. J. Parasit. Dis. 45, 4:1014-25.

Porter, AG, Jänicke, RU, 1999: Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 6, 2:99-104

Robert-Gangneux, A, Dardé, ML, 2012: Epidemiology of and diagnostic strategies for toxoplasma-osis. Clin. Microbiol. Rev. 25, 2:264-96.

Rostami, A, Karanis, P, Fallahi, S, 2018: Advances in serological, imaging techniques and molecu- lar diagnosis of *Toxoplasma gondii* infection. Infection 46:303-15.

Sanad, MM, Aboel Maaty, DAE, Abd Elaal NF, Al-Ghandour AMF, Farag AI *et al*, 2020:

Ther-apeutic efficacy of probiotics against experimental giardiasis: Histopathological, histochemical and ul-trastructural changes. JESP 50, 1:21-32.

Shaaban, Y, Hassan, Z, Hussein, R, Hassan, A, Salama, D, 2021: Evaluation of the role of combin-ed prebiotic and probiotic supplements as prophylactic and therapeutic agents against experimental gi-ardiasis. PUJ 14, 2:193-203.

Shukla, G, Kaur, H, Sharma, L, 2013: Comparative therapeutic effect of probiotic *Lactobacillus casei* alone and in conjunction with antiprotozoal drugs in murine giardiasis. Parasitol. Res. 112, 6: 2143-9.

Tenter, AM, Heckeroth, AR, Weiss, LM, 2000: *Toxoplasma gondii*: From animals to humans. Int. J. Parasitol. 30:1217-58.

Travers MA, Florent I, Kohl L, Grellier P, 2011: Probiotics for the control of parasites: An overview. J. Parasitol. Res. 20:1-12.

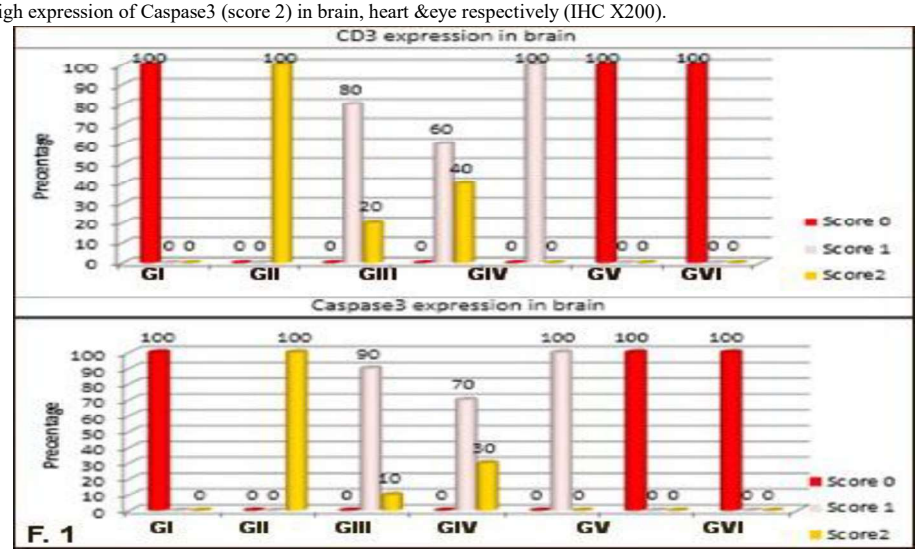
Yahia, SH, Etewa, SE, Saleh, NS, Mohammad, SM, Aboulfotouh NI *et al,* 2022: Histopathologi- cal, immunohistochemical and biochemical studies of murine hepatosplenic tissues affected by chronic toxoplasmosis. J. Parasitol. Res, 2022: 1-17.

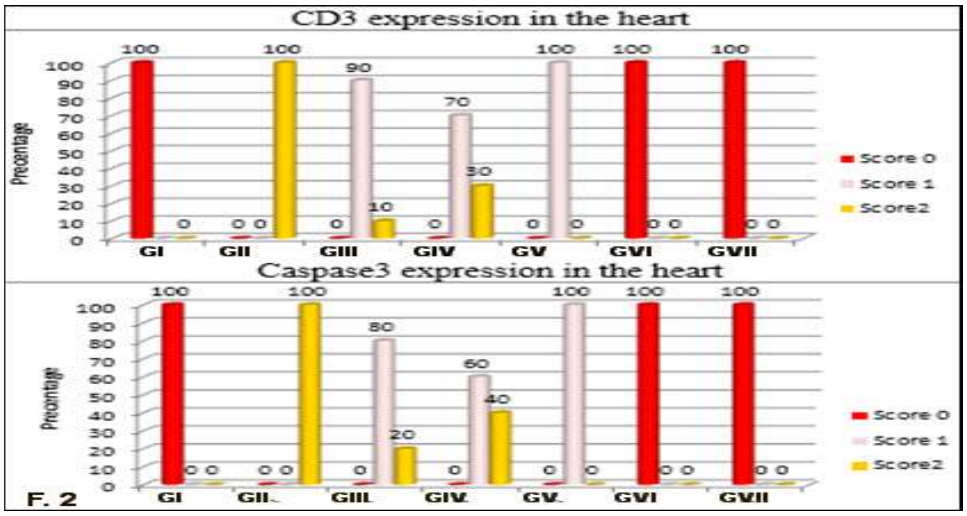
Yan, X, Sun, Y, Zhang, G, Han, W, Gao, J, *et al*, 2022: Study on the antagonistic effects of koumiss on *Toxoplasma gondii* infection in mice. Front. Nutr. 2260:1-11.

Zhai, B, He, JJ, Elsheikha, HM, Li, JX, Zhu, XQ *et al,* 2020: Transcriptional changes in *Toxoplasma gondii* in response to treatment with monensin. Parasit. Vectors 13, 1:1-11.

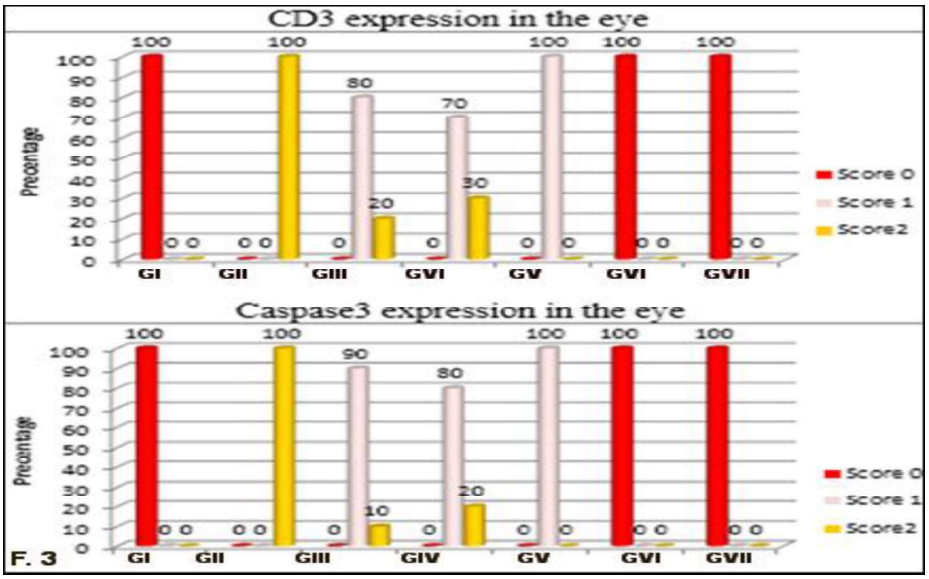
Explanation of figures

Fig. 1: Score and percentage of CD3 and Caspase3 expressions in brain tissues of groups at day 60 post-infection.
Fig. 2: Score and percentage of CD3 and Caspase3 expressions in heart tissues of groups at day 60 post-infection.
Fig. 3: Score and percentage of CD3 and Caspase3 expressions in eye tissues of groups at day 60 post-infection.
Fig. 4: Histopathological examination of brain tissue: a Normal brain section of G1 showed normal brain tissue, normal neurons, and no inflammation. (H& E stain x100). b. Brain tissue section of GII showed strong perivascular inflammatory cells (vasculitis) (red arrow) (H& E stain x200). c. Brain tissue section of GIII showed moderate inflammatory cells (lymphocytes) (yellow arrow) and moderate vasculitis (red arrow) (H&E X200). d. Brain section of GIV showed severe inflammatory infiltrate of mononuclear cells (lymphocytes) (yellow arrow) and mild vasculitis (red arrow) (H&E X200). e. Brain section of GV showed a mild inflammatory reaction, parenchymal mononuclear infiltrates (yellow arrow), some degenerative changes, and perivascular edema (red arrow) (H& E stain x200).
Fig. 5: Histopathological examination of heart tissues: a. Normal heart section G1showed normal heart tissue normal myocytes, and no inflammation. (H& E stain x100). b. Heart section of GII, showed strong interfibrillar hemorrhage (yellow arrow) (H& E stain x200).c. Heart section of GIII showed moderate inflammatory infiltrate mainly with lymphocytes (red arrow) and interfibrillar hemorrhage (yellow arrow) (H&E X200). d. Heart section of GIV showed severe inflammatory reaction (red arrow) and interfibrillar hemorrhage (yellow arrow) (H&EX200). e. Heart section of G showed mild inflammatory reaction (red arrow) and interfibrillar hemorrhage (yellow arrow) (H&EX100).
Fig. 6: Histopathological examination of eye tissues: a. Eye section of GI showed normal retina with no inflammation (H&EX400). b. Eye section of GII showed strong inflammation with loss of nuclei in outer nuclear layer (strong vacuolation of ONL) (H&EX400). c. Eye section of GIII showed moderate inflammation with loss of nuclei in outer nuclear layer (moderate vacuolation of ONL) (H&EX400). d. Eye section of GIV showed severe inflammation with loss of nuclei in outer nuclear layer (severe vacuolation of ONL) (H&EX400). e. Eye section of GV, showed mild inflammation with mild loss of nuclei in outer nuclear layer (mild vacuolation of ONL) (H&EX400).
Fig. 7: Immunohistochemical of CD3 in brain, heart, & eye: A, B, & C showed negative expression of CD3 (score 0) in brain, heart, & eye respectively, D, E, & F showed low expression of CD3 (score 1) in brain, heart, & eye respectively, G, H, & I showed high expression of CD3 (score 2) in brain, heart, & eye respectively (IHC X200).
Fig. 8: Immunohistochemical results of Caspase3 in brain, heart & eye: A, B, & C showed negative expression of Caspase3 (score 0) in the brain, heart, and eye respectively. D, E &F showed low expression of Caspase3 (score 1) in brain, heart, and eye respectively, G, H, & I showrf high expression of Caspase3 (score 2) in brain, heart &eye respectively (IHC X200).

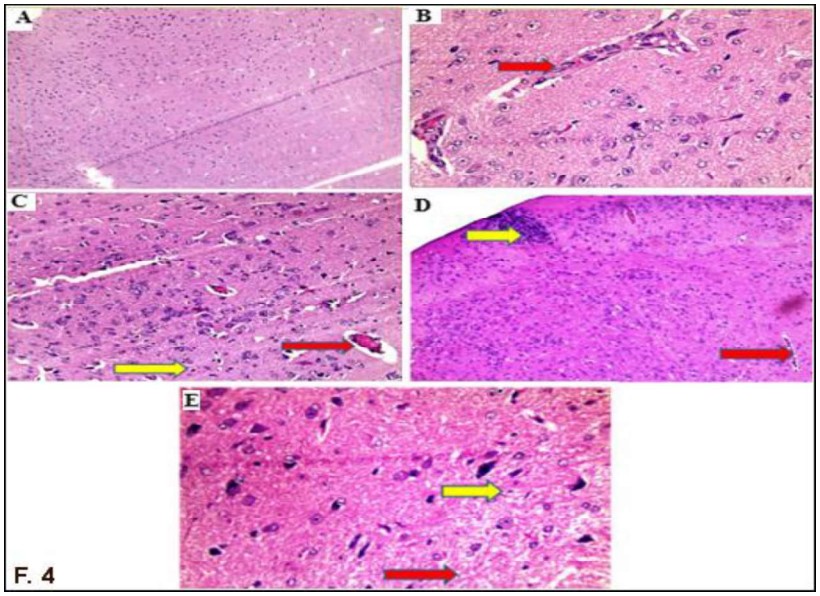



CD3 expression in the heart
Precentage
GI GII GIII GIV GV GVI GVII
Score 0
Score 1
Score2
Caspase3 expression in the heart
Precentage
GI GII GIII GIV GV GVI GVII
Score 0
Score 1
Score2

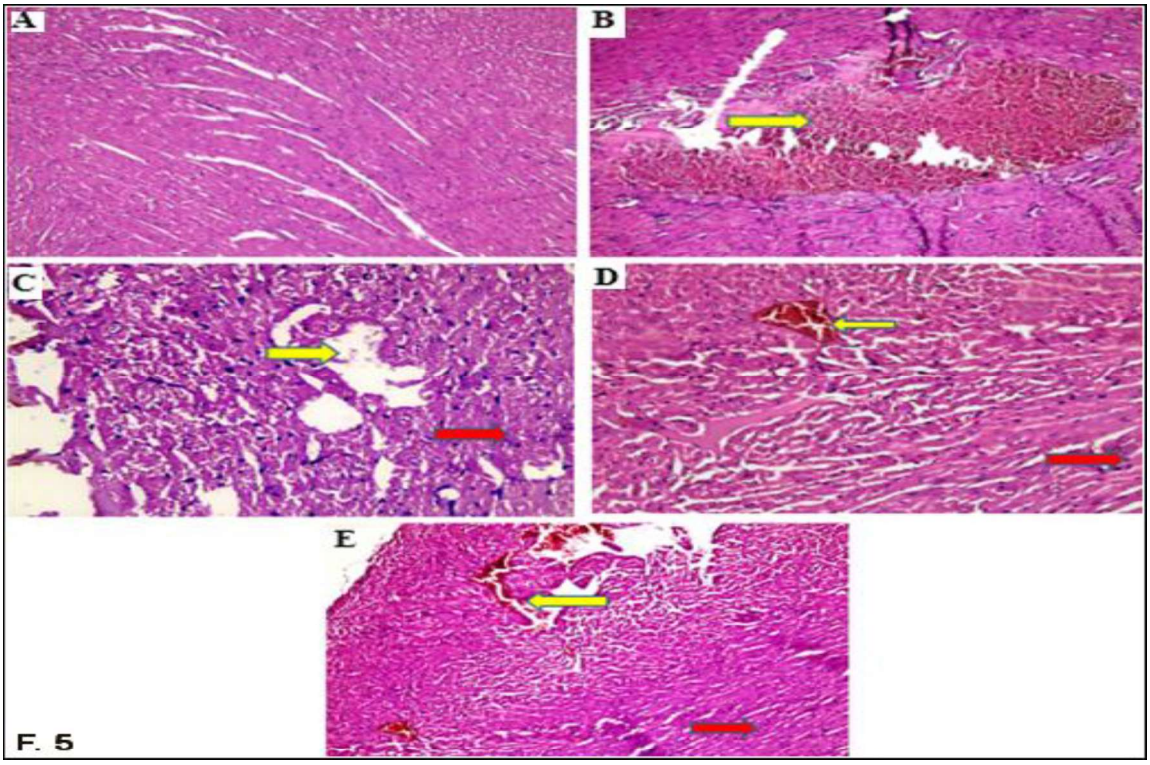
F. 2


CD3 expression in the eye
Precentage
GI GII GIII GVI GV GVI GVII
Score 0
Score 1
Score2
Caspase3 expression in the eye
Precentage
GI GII GIII GIV GV GVI GVII
Score 0
Score 1
Score2

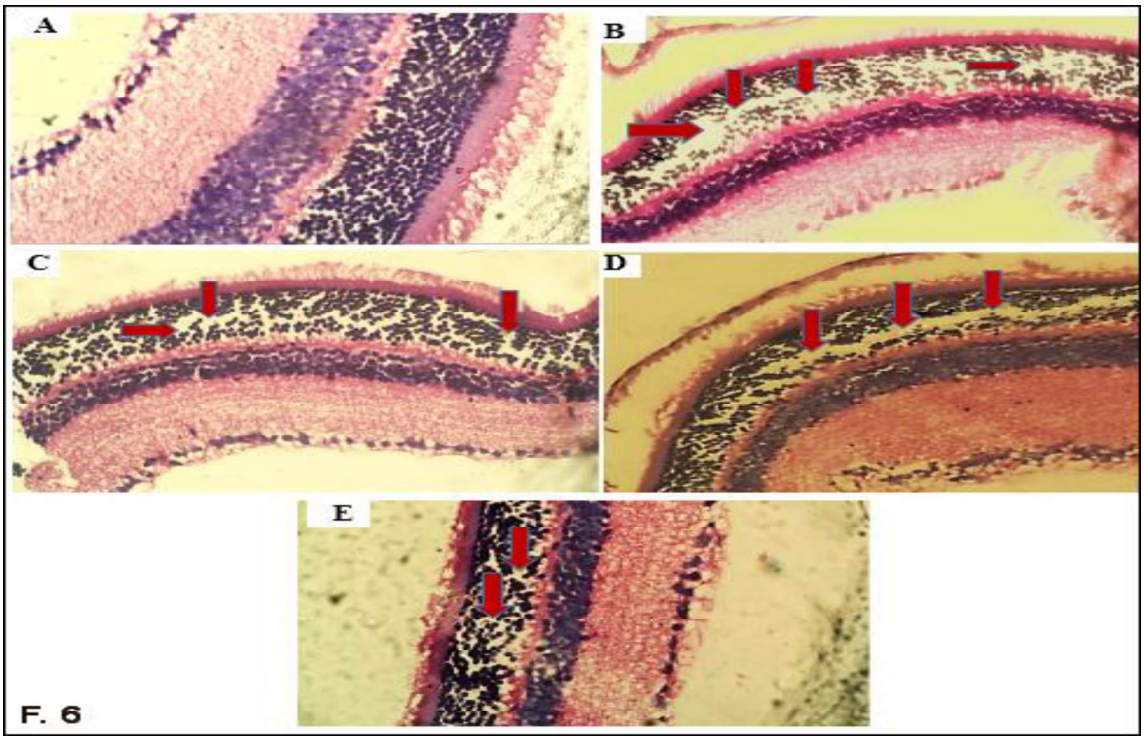
F. 3


A
B
C
D
E

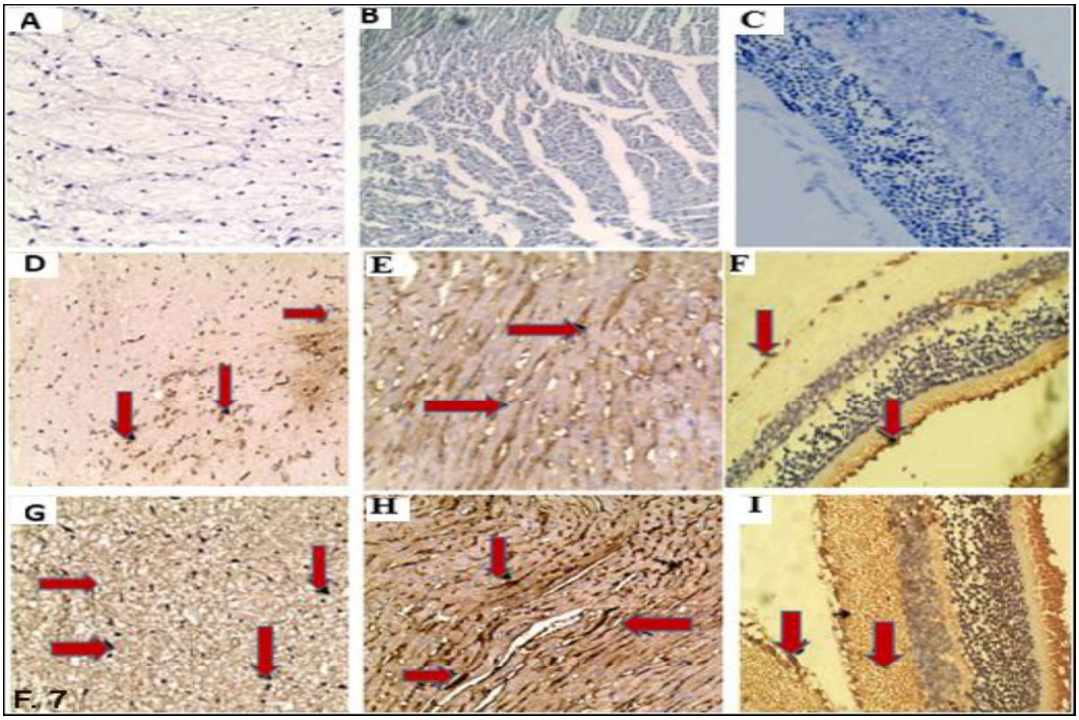
F. 4

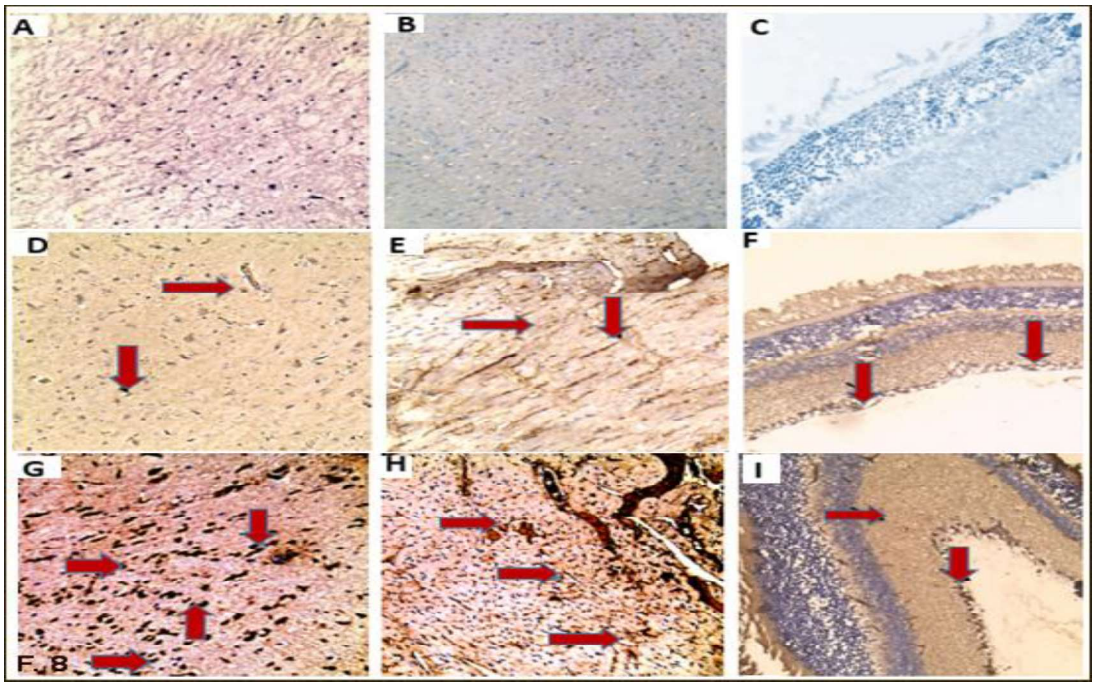


F. 5



F. 6


A
B
C
D
E
F
G
H
I
F. 7


A
B
C
D
E
F
G
H
I
F. 8