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EFFICACY OF MURINE PLATELET RICH-PLASMA VERSUS SPIRAMYCIN IN TREATMENT OF CHRONIC TOXOPLASMOSIS INFECTED MICE

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Abstract

Apicomplexan parasite, *Toxoplasma gondii*, is an obligate intracellular parasite, infecting and replicating within nucleated cells in many homoeothermic organisms, mammals and birds with the possibility of causing serious health problems. Recent studies indicated several side effects to the current drugs like Pyrimethamine® & Sulfadiazine®. This work evaluated murine platelet rich plasma (PRP) efficacy versus Spiramycin® treating toxoplasmosis infected mice parasitological, biochemical and histopathological studies. Fifty five immunocompetent male mice were divided into 5 groups of 11 mice each. G1: non-infected control, G2: infected non-treated control, G3: infected and treated with spiramycin, G4: infected and treated with murine PRP, and G5: infected and treated with combined spiramycin and PRP.

Spiramycin and PRP treated mice gave the highest reduction in *Toxoplasma* tissue cysts number, lowest level of serum malondialdehyde (MDA) and marked pathological improvement.

Key words: Mice, *Toxoplasma gondii*, Platelet rich plasma, Spiramycin, Malondialdehyde.

Introduction

Toxoplasma gondii that causes toxoplasmosis is a protozoan parasite distributed globally infects man, animals, birds, and the soil *T. gondii* infection leads to severe pathological impacts in the immunodeficient patients and the congenital fetus (Al-Malki (2021). Molan *et al.* (2020) reported that many environmental and human factors affect the *T. gondii* seroprevalence rates among various countries and continents. They added that monitoring the source and transmission assist public health authorities to clarify the risk factors involved, as well as focus on implementing optimal state-specific health policies target its transmission control. The transmission occurred from the ingestion cysts or oocysts dropped from cats in contaminated raw or under-cooked food or water, and congenital transmission (Hill and Dubey, 2002). Besides, the transmission widely varied among populations, mainly based on the food habits and culture, as by consumption unpasteurized milk (Tenter *et al,* 2000), nosocomial by organ transplantation (Barsoum, 2006), contaminated blood transfusion or by needlestick injury (Abdel-Motagaly *et al,* 2017), and congenital from mother to child (Saleh *et al,* 2016). Generally speaking, toxoplasmasis and/or antibodies against *Toxoplasma gondii* were reported worldwide with the highest rates in areas of the world that have hot, humid climates and lower altitudes, because oocysts survive better in these types of environments (CDC, 2018).

Toxoplasmosis is usually asymptomatic, but severe manifestations can occur in some patients, specially immunosuppressed ones (McLeod *et al,* 2020) as cervical lymphadenopathy, ocular toxoplasmosis or TORCH infections (Morsy *et al,* 2022) and CNS disorders that develop chronic inflammation in latent infection with neurobehavioral problems (Etewa *et al,* 2021).

During pregnancy, *T. gondii* can infect the fetus via placenta causing abortion or congenital abnormalities (Arefkhah *et al,* 2019), with affection of about 190,000 individuals worldwide annually (Saad *et al,* 2020). Pathogenesis is related to induction of oxidative stress and lipid peroxidation that cause overproduction of Reactive Oxygen free Radicals (ROR) with reduction of endogenous antioxidants (Motavalli *et al,* 2018) that makes Malondialdehyde (MDA); a biomarker of lipid peroxidation and oxida-

tive stress, increases in chronic acquired toxoplasmosis (Al-Kuraishy *et al,* 2019) and becomes a good indicative method for toxoplasmosis and assessment of its treatment (Kiran *et al,* 2019).

The anti-*Toxoplasma* drugs are effective during the acute phase caused by tachyzoites. Once they underwent into bradyzoites, tissue cysts developed causing chronic infection with the mainly ineffective treatment (Lang *et al,* 2018), however, there were trials to treat chronic toxoplasmosis (Radke *et al,* 2022). Various pharmacological agents were available such as Spiramycin® and Pyrimethamine® alone or combined with Sulfadiazine® (Remington *et al,* 2006), but these regimens have more or less side effects as myelotoxicity, which required discontinuation (Konstantinovic *et al,* 2019).

Spiramycin is a 16-member-ring macrolide antibiotic produced by *Streptomyces ambofaciens* (Chew *et al,* 2012) and has several side effects as drug resistance and toxicity. Moreover, without human vaccine and emergence of atypical *T. gondii* strains, control became more complicated (Montazeri *et al,* 2018). The Platelet Rich Plasma (PRP) is known to have evident anti-microbial, antioxidant and anti-inflammatory effects (Costa *et al,* 2021).

There are increasing evidences that platelets have an important role in the body's defense against different pathogens as well as anti-parasitic immunity (Li *et al,* 2020). This immunological role is mainly due to the receptors they carry and their storage granules that have a wide variety of immunologically active substance (Weyrich and Zimmerman 2004) like cytokines and interleukins (Nurden, 2011). As part of the innate immune system, platelets could recognize pathogens from all major classes of microorganisms. These interactions resulted in platelet activation and secretion of a broad range of peptides to form a first-line defense against infection. Activated platelets also, could release chemokines and express ligands to activate leukocytes to trigger both the innate and adaptive immune responses (Portier and Campbell, 2021). Also, platelets kill several microbial pathogens (Xu *et al,* 2018) as they are plentiful source of anti-microbial molecules (Tang *et al,* 2002) which activate other immune cells including promotion of leukocyte infiltration and neutrophil extracellular trap formation (Clark *et al,* 2007). PRP therapies are suitable treatment with clinical benefits and better improvement of some human diseases (Xuan *et al,* 2020).

The study aimed to evaluate therapeutic effect of murine Platelet Rich Plasma (PRP), taken from rats, as a new therapeutic agent against toxoplasmosis compared to spiramycin on experimental chronically Swiss Albino mice.

Materials and Methods

T. gondii: A-virulent strain (ME49) was provided by Theodor Bilharz Research Institute (TBRI). Maintenance of the strain was performed by serial injection of Swiss albino mice every 8 weeks intraperitoneally with 0.1ml of brain suspension of previously infected mice contained about $1x10^2$ cysts/ml.

Experimental design: Fifty five laboratory-bred male Swiss albino mice were included in this study. They were divided into five groups of 11 mice each. The mice were 10-weeks old and each weighing about 20-25gm. All mice were supplied with standard pellet food and water and were placed in cages of well ventilation (El-Fakhry *et al,* 1998). Mice of different study groups were maintained under controlled temperature of (25±2°C) and lighting (12h light/12h dark cycle). Stool examination was performed to exclude any parasitic infections (Garcia and Bruckner, 1977). After (45dpi), one mouse from each group was sacrificed to prove infection before the treatment as follows: GI: Non-infected control. GII: Infected untreated control. GIII: Infected, spiramycin treated (Rovamycin, Sanofiaventis, Egypt), was given at a dose of 200 mg/kg/day (Saleh *et al,* 2021), given at a fixed time daily for 10 days. GIV: Infected mice, PRP treated intraperitoneally at a dose of (0.5ml/kg), two days/

week for 4 weeks (Bausset *et al,* 2012). GV: Infected and received combined spiramycin and murine PRP, treated with half dose of spiramycin (100mg/kg/day) by oral route for ten days and half dose of PRP (0.25ml/kg) by intraperitoneal injection, two days a week for 4 weeks.

Platelet-Rich Plasma preparation: A double-spin method was used to obtain PRP from the male albino rats which were used as blood donors. Briefly, rats were anesthetized with ether and sacrificed then, blood was collected on (sodium citrate) anticoagulant solution (9 parts of blood: 1 part of sodium citrate). Hemocytometer was used to count platelets in 100µl of the anticoagulated blood. Basal platelet count was assayed about (600,000-900,000)/ul, the remaining blood was centrifuged at 1000rpm for15min at room temperature to separate plasma, which was collected and centrifuged at 3000rpm for 10min. to get PRP where erythrocytes were present at the bottom, white blood cells in the middle and plasma fluid at the top. After centrifugation, two-thirds of plasma fluid at the top was separated the platelet poor plasma (PPP) and the last third of plasma or platelet rich plasma (PRP). Platelets were counted and number was up to 1800000/ul. Final concentration of platelets obtained in PRP as approximately 3 times in the whole blood (Soliman *et al,* 2019).

Spiramycin tablets (Sigma-Aldrich) were purchased as powder. Calculation of the active ingredient in each tablet was done according to its weight. Each tablet has 1gm of spiramycin. 6 weeks post-infection, treatment was administered daily at a fixed hour for 10 consecutive days in a dose of (200mg/kg/day) to spiramycin group and 100mg/kg/day to the combined GVI. Dose per mouse was calculated and dissolved in 20ml water for oral administration by oral gavage (Saleh *et al,* 2021). Mice were then anaesthetized and sacrificed. Sacrificing mice of GIII was at (55dpi) while mice of GIV& GV were sacrificed at (90dpi), for parasitological, biochemical and histopathological pictures.

Parasitological: Brain cysts quantification: brains of sacrificed mice were crushed individually in a mortar, and 5ml of normal saline were added to get brain emulsion homogenates (Djurkovic-Djakovic *et al,* 2002). Total number of cysts per mouse brain was determined by adding 20ul drop of brain homogenate onto microscopic slides and counted under light microscopy. This count was multiplied by 25 to obtain tissue cysts/1ml of brain suspension. Then, mean cysts number in each group was calculated.

Biochemical: Malondialdehyde levels in samples were measured by using thiobarbituric acid reaction method of (Bahrami *et al,* 2016): Estimation of MDA using colorimetric method (Kit of Bio diagnostic, Cat. No. MD. 25 29, Egypt) was used to estimate serum MDA levels, a working solution contained 15% trichloroacetic acid, 0.375% thiobarbituric acid and 0.25 N hydrochloric acid was prepared. For each sample, 250μl serum and 500μl working solution were mixed and placed in boiling water for 10min. After cooling, they were centrifuged at 3000rpm for 10min. Then, 200μl of each supernatant was transferred to microplates and the optical density of samples was measured at 535nm. MDA values of were expressed as nmol/mL.

Histopathological: Samples of brain, liver and kidney tissues of all groups were collected, fixed in 10% formalin, dehydrated in ascending grades of ethanol and embedded in paraffin for further processing, serial sections of 5µm thick stained with H & E stain (Drury and Wallington 1980), and light microscopical examined.

Statistical analysis: Data were analyzed by using Statistical Package for the Social Sciences SPSS version 22 (Armonk, 2013). Quantitative data were the mean, range and standard deviation. ANOVA F-test calculated the difference between quantitative variables among different groups (Chan 2003).

Ethical considerations: The study was approved (ZU-IACUC/3/F/143/2020), by Ethical Committee of Zagazig University which went with the guidelines of Helsinki (2000).

Results

There was high significant difference (P = 0.000) in mean of brain cysts in GIII, GIV & GV compared to GII. There were significant differences (P =0.000) in reduction among groups compared to GII with the highest reduction in brain cyst viability was in mice treated with combined spiramycin and PRP, GIII & GIV with rate of 17.9%, 30.8% & 64% respectively. The least reduction was in GIV treated with PRP alone as compared to GIII & GV. There was high significant difference (P =0.000) in mean number of brain-tissue cysts among groups. A high significant difference (P =0.000) in mean level of MDA in GIII, GIV & GV in relation to GII with highest reduction of MDA level was in spiramycin & PRP mice then spiramycin alone, with least reduction in MDA level by PRP alone. Mean MDA level among groups to one another showed high significant difference (P =0.000) in all except GIII versus GIV without significant difference.

Brain in GI showed normal picture, but in GII showed multiple mature tissue cysts, neuronal degeneration and marked lymphocytic inflammatory cellular infiltrate. GIII showed tissue cyst burden, less than in GII with moderate lymphocytic inflammatory cellular infiltrate, and GIV showed mild reduction in infection burden but markedly reduced inflammatory infiltrate compared to GII. GV showed the best reduction in burden cysts without inflammatory infiltrate than using spiramycin or RRP alone.

Liver in G1 showed normal picture, but in GII showed several pathological changes due to toxic effects in disturbance of the hepatic architecture, distortion of liver cell plates with variable hepatocyte ballooning degeneration. Hepatic lobules reveal intense lymphocytic inflammatory cellular infiltrate with dilatation of the central vein and blood sinusoids and presence of tissue cyst. Regarding spiramycin treated GIII, hepatic sections showed moderate periportal lymphocytic inflammatory cellular infiltrate, congestion and dilatation of blood sinusoids with some vaculation, less than GII, & GIV showed similar changes with noticeable reduction in hepatic lobular inflammation and normal sized central vein and sinusoids without improvement of ballooning degeneration, but some pathological changes were improved in GV that showed mild lymphocytic inflammatory cellular infiltrate with normal sized central vein and sinusoids.

Kidney in GI showed normal architecture, but in GII showed tissue cysts with marked lymphocytic inflammatory cellular infiltrate. GIII showed moderate lymphocytic inflammatory cellular infiltrate with tissue cyst compared to GII. GIV showed mild lymphocytic inflammatory cellular infiltrate without pathological lesions, and GV showed recovery of most pathological changes with mild inflammation and restoration of normal renal structures.

Details were given in tables (1, 2, 3 & 4) and figures (1, 2 & 3)

Table 1: Comparison between mean brain cyst counts among treated groups:

Groups	Mean ± SD	Reduction %	F	P
GII	91± 6.4	-	614.8 .000**	.000*
GIII	30.8±3.9	66%		
GIV	64± 3.4	29.6%		
GV	17.9± 1.8	80.3%		

F: F test (ANOVA) *Significant, p< (.0001) **Highly significant, p (0.000)

Table 2: LSD Comparing brain cyst counts among treated groups:

Groups	P
GII versus GIII	.000*
GII versus GIV	.000*
GII versus GV	.000*
GIII versus GIV	.000*
GIII versus GV	.000*
GIV versus GV	.000*

LSD: Least significant difference, *Significant (p<.0001), **Highly significant (p<0.000)

Table 3: Comparison between mean serum levels of MD (Malondialedehyde) among treated mice

Groups	Mean ± SD (nmol/ml)	F	P
GII	57.7±4.9	78.5	.000**
GIII	31.5±9.6		
GIV	32.2±2.2		
GV	21.3± 1.2		

F: F test (ANOVA), *Significant, p< (.0001) **Highly significant, p (0.000)

Table 4: Least significant difference of serum levels of MD among treated mice

Groups	P
GII versus GIII	.000**
GII versus GIV	.000**
GII versus GV	.000**
GIII versus GIV	.475
GIII versus GV	.000**
GIV versus GV	.000**

Highly significant, p (0.000), NS: Non significant, P (0.475), **LSD: Least significant difference

Discussion

No doubt, chronic toxoplasmosis is a life-long infection characterized by cysts formation as an immune evasion mechanism and resulted in brain lesions (Etewa *et al,* 2019). Tissue cysts develop resistance against most of the available anti-*Toxoplasma* drugs due to its' hard cyst walls and low bradyzoites metabolism (Montazeri *et al,* 2018). But, the combination of sulfadiazine and pyrimethamine represented the main chemotherapy of toxoplasmosis (Saleh *et al,* 2014). However, this combination was not so effective against the *T. gondii* cysts except with long duration of treatment up to 21 days (Silva *et al,* 2019). Although, Peters *et al.* (2007) in USA reported no clinical association between sulfadoxine/pyrimethamine use and kernicterus was reported despite the extensive use of both and related compounds to treat maternal malaria and congenital toxoplasmosis in near-term pregnant women and newborns. Stoner *et al.* (2017) in Zambia, as malaria prevalence was low, national guidelines continue to recommend that all pregnant women receive sulfadoxine-pyrimethamine (SP) for malaria prophylaxis monthly at every scheduled antenatal care visit after 16 weeks of gestation. HIV-positive women must receive co-trimoxazole prophylaxis for HIV and not SP, but many still received SP. Nevertheless, Keyhani *et al.* (2020) reported a need for agents of natural products or biosynthetic agents as an alternative drug with higher toxoplasmosis efficacy with neglected side-effects.

In the present study, spiramycin showed significant difference (P <0.001) in mean *T. gondii* cysts number in brain sections of all groups, as the cyst count indicated infection density in tissues (El-Temsahy *et al,* 2002).

In the present study, spiramycin induced a moderate reduction in brain cysts number when compared to the infected untreated control group. This agreed with Omar *et al.* (2021) who showed that spiramycin produced significant reduction in brain tissue cyst count as compared with infected control mice. This agreed with Dunay *et al.* (2018) reported that spiramycin was effective mainly against acute toxoplasmosis with a high concentration in the placenta. Also, Nasr *et al.* (2020) who reported the effectiveness of spiramycin in chronic murine infections was ability to reduce brain cyst numbers. Allam *et al.* (2021) analyzed the efficacy of spiramycin in comparison to infected control mice, reported a significant reduction in parasite count in infected mice treated with spiramycin, but without complete parasite eradication.

Generally, Platelet rich plasma (PRP) is an autologous blood derivative rich in active growth factors (GFs), some of which are hepatocyte growth factor (HGF); insulin-like growth factor-I (IGFI) and epidermal growth factor (Shoeib *et al,* 2018). PRP powerful therapeutic potentials is due to ability of del-

ivering great variety of biologically active GFs to injury site as being simple, effective, safety and ability (Frechette *et al*, 2005). Also, it was related to powerful healing and an up-growing role in regenerative medicine (Alves and Grimalt, 2018).

In the present study, mice treated by murine RRP was the least one in reducing tissue cysts with reduction in inflammatory infiltrate compared to other treated mice. But, the present mice treated with PRP and spiramycin gave highly significant reduction of number of brain cysts as compared to infected control with mild inflammatory cellular infiltrate. This can be explained that combinations of PRP with other drug gave higher efficacy. Meshkini *et al.* (2021) found that alum-PRP mixture combined with the excretory- secretory antigens vaccine (ESA), significantly enhanced the vaccine potency and showed that ESA vaccine when applied with alum-PRP efficiently stimulated the development of cellular immune responses to *T. gondii* by increasing IFN-γ & IL-5 production. This agreed with El-Aswad *et al.* (2018) who used PRP alone in treatment of schistosomiasis and found that PRP did not show any significant decrease in mean worm number as compared to infected group without effect on total worm burden or egg numbers and concluded that PRP neither have anti-helminthic nor anti-fecundity actions on *S. mansoni*.

In the present study, in non-infected, non-treated mean MDA level in serum of infected mice was (10.8±0.16). But, in infected non-treated mean serum level of MDA reached was (57.7±4.9). This agreed with Karaman *et al.* (2008) who reported that reflected the oxidative stress induced by *T. gondii* infection increased the MDA level and decreased glutathione (GSH) concentration in serum of *T. gondii* seropositive patients. Also, Al-Kuraishy *et al.* (2020) reported that MDA levels were significantly higher in asymptomatic *Toxoplasma* seropositive patients compared to healthy ones. This was explained by Zhang *et al.* (2018) who reported that after tissue damage, lipid peroxidation occurred in huge quantities, with MDA being the most prominent process, led to cell form disruption, inflammation, and necrosis.

In the present study, in spiramycin treated mice the mean MDA level was (32.2±2.2) with statistically significant difference in relation to the infected mice (57.7±4.9). PRP treatment alone or in combination with spiramycin resulted in a highly significant reduction in serum MDA level (27.8±1.8) and (21.3±1.2) respectively. Keshk and Zahranb (2019) reported that reduction in lipid peroxidation was attributed by PRP to suppress the oxidative stress. They also explained the beneficial effects of PRP in different experimental models of acute and chronic renal injury. This agreed with Hegab *et al.* (2019) who found that PRP on renal oxidative stress markers of a rat model of Adriamycin induced chronic kidney disease with a significant decrease in MDA levels.

In the present study, there was histopathological improvement of treated brain, liver and kidney sections versus infected non treated control ones. This agreed with Saleh *et al.* (2021) who found severe inflammatory reaction with many *Toxoplasma* cysts in the cerebral tissue and neuronal degeneration. Also, liver sections of infected untreated mice showed hepatic ballooning degeneration and necrosis, which agreed with Silva *et al.* (2013). Infected liver sections showed diffuse inflammation in form of perivascular cuffing of lymphocytic infiltrate with vascular dilation and congestion. This agreed with Wang *et al.* (2019), and Omar *et al.* (2021) reported that pathological changes were due to *T. gondii* toxic effect on liver tissue.

In the present study, spiramycin treated mice showed moderate reduction of tissue cysts with moderate inflammatory cell infiltrate. This agreed with Etewa *et al.* (2018) who reported that brain tissues in RH strain infected mice treated with spiramycin gave a moderate reduction in pathological changes.

In the present study, the PRP treated mice

showed the least pathological improvement with mild inflammatory infiltrate. This agreed with El-Aswad *et al.* (2018) who found that PRP alone didn't reduce mean hepatic granuloma number, but disagreed with Salem *et al.* (2018) who reported that rat PRP markedly improved dimethylnitrosamine induced liver fibrosis by signifcant decreased liver hydroxyproline content.

In the present study, combination between PRP and spiramycin caused a highly significant improvement of hepatic degeneration and inflammatory infiltrations compared to infected mice. This agreed with Soliman *et al.* (2019) who reported that PRP has anti-inflammatory effects. The anti-inflammatory effect of PRP may be attributed to its capability to increase the intracellular expression of anti-inflammatory mediators (IL-4, IL-10, & IL-13) and to decrease IL-1 β -mediated catabolic effects (Moussa *et al,* 2017).

In the present study, PRP treated mice either alone or in combinations with dose of (0.5ml/kg), and (0.25ml/kg)) respectively, as regarding kidney tissue, showed restoration of normal structure which agreed with Salem *et al.* (2018) who denoted that PRP accelerated the recovery of renal function and repaired kidney structure after damage induced by cisplatin.

Conclusion

The murine PRP combined with spiramycin reduced the *Toxoplasma gondii* (ME49) strain tissue cysts and improved pathology in brain, liver & kidney tissues. Combinations showed a marvelous anti-oxidant effect to lower MDA level in serum of infected mice.

However, the PRP alone gave the minimal anti-*Toxoplasma* results and minimal ability to decrease brain cysts with little improvement of inflammatory infiltrate.

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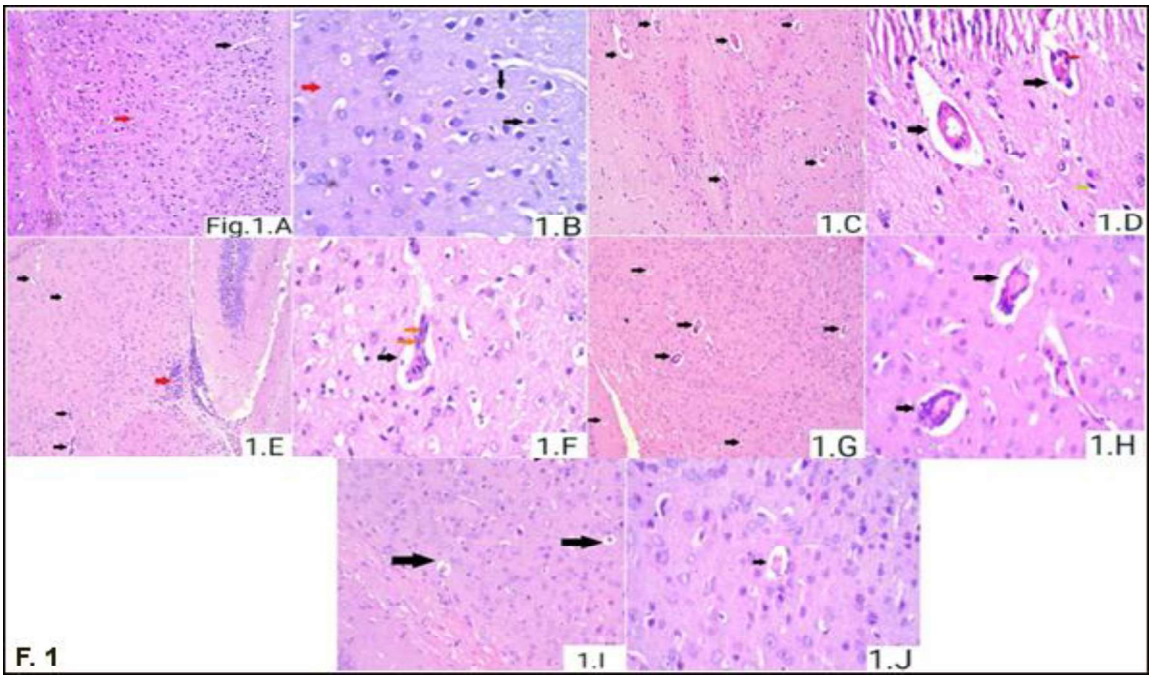
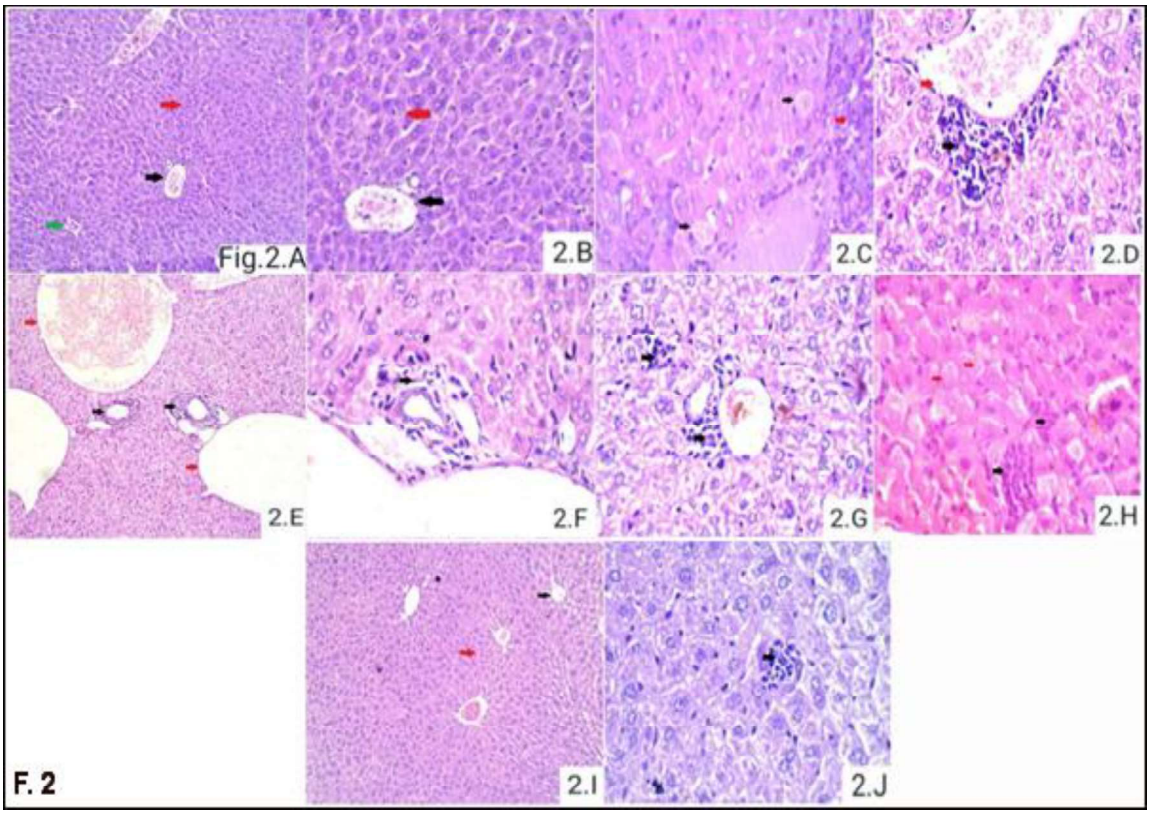
Explanation of figures

Fig. 1: Brain sections stained with (H&E): A- GI showed normal brain tissue with normal astrocytes (red arrow) and normal blood vessels (black arrow, X100). B- G1showed normal pyramidal shape of neurons (black arrow) with intact neuropil (dendrites and axons of neurocytes) (red arrow, X400). C- GII showed multiple mature *T. gondii* tissue cysts (black arrow, X100). D- GII showed multiple mature tissue cysts (black arrow) with intracystic bradyzoites (red arrow)(X400). E-GIII showed moderate number of mature tissue cysts (black arrow) with moderate lymphocytic inflammatory cellular infiltrate (red arrow) in relation to infected group (X100). F- GIII showed mature tissue cyst, (black arrow) with multiple bradyzoites (orange arrow, X400). G- GIV showed multiple mature tissue cysts (black arrow, X100). .H-GIV showed multiple mature tissue cysts (black arrow, X400). I- GV showed few distorted tissue cysts (black arrow) with reduction of mature tissue cysts(X100). J- GV showed few distorted tissue cysts (black arrow) with reduction of mature toxoplasma tissue cysts(X400).

Fig. 2: Liver sections stained with (H&E): A- GI showed normal hepatoportal area (black arrow), normal size central vein (green arrow) with normal size hepatocytes arranged in cords (red arrow, X100). B- G1showed normal hepatoportal area (black arrow) with normal size hepatocytes arranged in cords (red arrow, X400). C- GII showed distortion of hepatic architecture, severe lymphocytic inflammatory cellular infiltrate (black arrow), with ballooning degeneration (red arrow) (X100). D- GII showed severe lymphocytic inflammatory cellular infiltrate (black arrow) and dilatation of central vein (red arrow, X400). E- GIII showed moderate lymphocytic periportal inflammatory cellular infiltrate (black arrow) with dilatation of hepatic blood vessels (red arrow, X100). F-GIII showed moderate lymphocytic periportal inflammatory cellular infiltrate with hepatic degeneration (black arrow, X400). G- GIV showed mild lymphocytic periportal inflammatory cellular infiltrate (black arrow, X400). H- GIV showed focal lymphocytic inflammatory cellular infiltrates (black arrow) and ballooning degeneration (red arrow, X400). I- GV showed restoration of normal size of central vein (black arrow) with restoration of normal architecture of liver (red arrow, X100). .J- GV showed mild focal lymphocytic inflammatory cellular infiltrates (X400).

Fig. 3: Kidney sections stained with (H&E): A- GI showed normal histological structure of renal glomeruli (black arrow) and normal renal tubules (red arrow, X100). B- G1showed normal histological structure of renal glomeruli (black arrow) and normal renal tubules (red arrow, X400). C- GII showed tissue cyst (black arrow, X400). D- GII showed marked lymphocytic inflammatory cellular infiltrate (black arrow, X400). E- GIII showed tissue cyst (black arrow, X400). F-GIII showed moderate lymphocytic inflammatory cellular infiltrate (black arrow, X400). G- GIV showed mild lymphocytic inflammatory cellular infiltrate (black arrow, X400). H- GIV showed restoration of normal structure of kidney tissue (X100). I- GV showed restoration of normal structure of kidney tissue (X400).





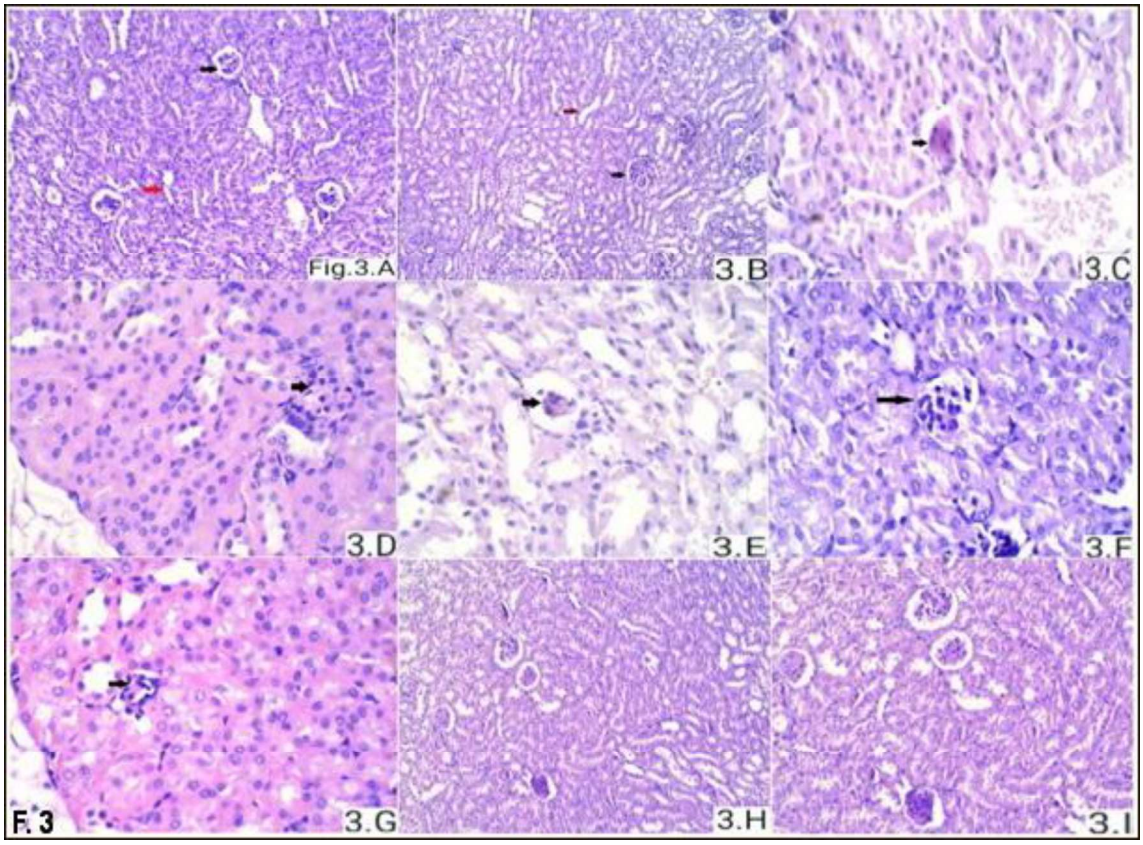


Fig.3.A
3.B
3.C
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