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Effect of Platelet Rich Plasma versus Conventional Therapy on Early and Late ME 49 *Toxoplasma Gondii* Infection in Immunocompromised Mice

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Abstract

TOXOPLASMOSIS is a highly prevalent worldwide opportunistic parasitic infection caused by *Toxoplasma gondii*. It is associated with various morbidities in immunocompetent patients while in immunosuppressed patients, it can be fatal. Available drugs cannot eradicate the infection completely and induce several side effects. Thus, the present work aimed to evaluate the possible therapeutic effects of platelet rich plasma (PRP) alone or in combination with pyrimethamine and sulfadiazine (PYR/SDZ) in treatment of early and late toxoplasmosis. The research was carried out on 60 mice, divided into two experiments (A: early and B: late toxoplasmosis), 30 mice each. In each experiment, the mice were divided into 5 subgroups; I: non-infected, non-treated, II: infected, non-treated, III: infected and treated by PYR/SDZ, IV: infected and treated by PRP alone and V: infected and treated by PRP+ PYR/SDZ. All mice were assessed parasitologically (tissue cyst count), histopathologically (brain sections) and immunologically (IFN- γ level). The best results were recorded in PRP+ PYR/SDZ group with 90.06% and 89.08% reduction percentages in tissue cyst count in early and late stages respectively, resolving all the inflammatory changes and the highest increase of IFN- γ level among all groups. PRP alone yielded 61.58% and 47.16% reduction percentages in early and late stages respectively, with great improvement of the histopathological findings and high elevation of IFN- γ level compared to 81.64% and 60.46% reduction percentages in mice treated with PYR/SDZ alone in early and late stages respectively, with improvement of the histopathological changes and the lowest increase of IFN- γ level. It can be concluded that PRP combined with PYR/SDZ can be a good candidate for treatment of acute and chronic toxoplasmosis.

Keywords: Early and late toxoplasmosis, PRP, PYR/SDZ, Brain tissue cyst, IFN- γ.

Introduction

Toxoplasmosis is a worldwide disease caused by an obligatory intracellular coccidian, *Toxoplasma gondii* (*T. gondii*), that especially targets reticuloendothelial cells, but can infect all human cell types [1] Approximately one-third of the world's population has been estimated to have chronic *T. gondii* infection [2]

There are two clinical phases associated with toxoplasmosis in humans: (I) acute, where the extremely replicative tachyzoites propagate all over the human body, and (II) chronic, where tissue cysts containing bradyzoites form, primarily in the nervous system and skeletal muscle (it may persist in the host's body for life) [3, 4].

The infection in immunocompetent individuals typically has a mild or asymptomatic acute phase which usually does not require treatment [5, 6]. Medication to immunocompetent patients should be given only if they have serious and long-lasting manifestations, disseminated infection, eye involvement or infection acquired in laboratory [7, 8]. In immunocompromised patients, toxoplasmosis is considered as a serious opportunistic infection that is mostly caused by latent cysts reactivation in chronic infection patients. In HIV patients, despite the use of highly active antiretroviral therapy (HAART), toxoplasmosis remains to be the most common cause of neurological opportunistic infection in Europe, with toxoplasmic encephalitis as the most common clinical manifestation [9].

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Unfortunately, in non-HIV patients, the prognosis is worse and the infection is more likely to disseminate than be confined to the central nervous system. It is particularly life-threatening in bone marrow or hematopoietic stem cell transplant patients, as mortality rate ranges from 38% to 67% in spite of treatment [10].

Pyrimethamine-sulfadiazine (PYR/SDZ) combination is the most effective treatment currently available for toxoplasmosis. Also, trimethoprim combined with sulfamethoxazole as well as spiramycin are the other main choices for the clinical treatment of toxoplasmosis [11]. However, these therapies have serious side effects that may lead to stoppage of the treatment regimens. Also, these drugs are only effective in eradication of tachyzoites in early infection, but they lack efficacy to eliminate *T. gondii* tissue cysts to cure chronic infection which can also reactivate in immunocompromised hosts, leading to a potentially fatal outcome. Besides, many of currently used drugs cannot cross the blood brain barrier to treat CNS disease caused by *T. gondii* [12].

T. gondii seropositivity has been correlated with neurological and mental disorders, as well as cognitive impairment [13]. Moreover, chronic toxoplasmosis has been associated with different neuropsychiatric disorders including memory loss, hyperactivity disorder, attention deficit, bipolarism and schizophrenia [14, 15]. Therefore, there is a critical need for safe and effective therapies that have the efficacy to treat early and late toxoplasmosis especially in immunocompromised patients [16].

Platelet rich plasma (PRP) is a high-platelet concentration plasma; three to five times higher than normal blood level [17]. Besides its high level of platelets, PRP has a huge concentration of cytokines, chemokines and growth factors which released in the affected areas. These factors promote migration, proliferation and differentiation of undifferentiated vital cells to the affected site to initiate their recovery [18, 19]. Moreover**,** PRP has many properties as hemostatic, anti-inflammatory and immunological functions [20]. Besides, PRP has proven to potentiate both innate and adaptive immune response [21].

According to recent studies, PRP is believed to possess antimicrobial characteristics that would render it as an appropriate biological therapy, especially when used in combination with the conventional therapy [22]. Also, it had been used in trials to treat different parasitological infections and it had shown beneficial effects including *Schistosoma mansoni* [23, 24] C*ryptosoridium parvum* [25] and *Trichinella spiralis* [21]. Thus, the aim of the present research was to evaluate the possible therapeutic efficacy of PRP alone or in combination with the conventional drugs (PYR/SDZ) in treatment of acute and chronic stages of toxoplasmosis in immunocompromised mice.

Material and Methods

Experimental animals

The present study was carried out on 60 laboratory-bred male Swiss albino mice, 6 – 8 weeks old, 25-30 gm each at the time of the experiment. Two male albino rats 12–16 weeks old, 250-300 gm each. They were used as blood donors for PRP preparation throughout the experiment. Two laboratory-bred male Swiss albino mice were used as a source of infection to the sixty mice in the experiment. They were previously infected with *T. gondii* ME49 strain 8 weeks prior to our experiment. The animals were housed in TBRI animal house during the period from April to June 2023, and were well nourished with standard feeding regimens and tap water, kept in specialized cages at 24 ºC temperature and 45-55% humidity.

Immunosuppression

All sixty mice received immunosuppressive drug; dexamethasone sodium phosphate (Dexazone). Dexazone (0.25 µg/g/day) started 14 days before infecting mice and continued throughout the experiment. It was administrated orally using esophageal tube [26].

Induction of infection

Brains of two infected mice (8 weeks post-infection) were isolated, homogenized with saline using tissue homogenizer and assessed for cysts count in 20 µl using oil immersion lens (x100) microscopically. After that, the obtained suspension was adjusted to reach a concentration of 100 cysts/0.2 ml. This dose was administrated to each mouse orally using oesophageal tube [27].

Drug preparation

Each drug (PYR and SDZ) was obtained in powder form, transformed into suspension using 1% DMSO and administrated orally for 10 days using oesophageal tube. The dose administrated was 12.5 mg /kg /day for PYR, and 200 mg /kg /day for SDZ [28, 29].

Platelet rich plasma

Two ml blood were collected from rat retro-orbital sinus using capillary tube into a sodium citrate-anticoagulant tube and was centrifuged at 1000 rpm for 15 min (1st spin). The top layer was separated as plasma containing platelets and then centrifuged at 3000 rpm for 10 min (2nd spin). Afterwards, the lower third of plasma was collected as PRP and dissolved in PBS (1:1) (Fig. 1) [24] PRP (0.5 ml/kg) [25] was injected using insulin syringe intravenously into lateral tail vein of each mouse once per week for 3 weeks [30].

Experimental design

Sixty immunosuppressed male albino mice were employed in this research. They were then divided into two main experiments (A and B), 30 in each. Each experiment was further subdivided into 5 groups (each 6 mice) as follows; Group I: non-infected non-treated (negative control group), Group II: infected non- treated (positive control group), Group III: infected and treated with PYR+SDZ combination, Group IV: infected and treated with PRP and Group V: infected and treated with PYR+SDZ and PRP combination.

The two experiments were designed to study the efficacy of tested therapeutics on early and late toxoplasmosis respectively. In early infection (Experiment A), the treatment was administrated from day 5 post-infection and mice were sacrificed at day 35 post-infection, while in late infection (Experiment B), the treatment was administrated from 35 post-infection and mice were sacrificed one week after the end of treatment.

Therapeutics efficacy evaluation

Mice received intraperitoneal injection of 500 mg / kg thiopental and 100 units / ml heparin as anesthetic anticoagulant solution [21], then euthanized by decapitation [26]. The efficacy of therapeutics was estimated according to parasitological, histopathological and immunological parameters.

i) Parasitological evaluation

After scarification, one half of brain tissue was homogenized with 1 ml saline by tissue homogenizer. Twenty microns of the obtained suspension were examined either directly or as methanol fixed film stained with Giemsa (to determine tissue cysts count in brain of each mouse) [31]. Mean cysts count in each group was assessed and percentage of reduction of cysts in each single group was calculated via the following equation [32]:

$$\frac{Mean\ number\ of\ tissue\ cysts\ \ recovered\ from\ positive\ control\ group - Mean\ number\ of\ \ tissue\ cysts\ recovered\ from\ treated\ group}{Mean\ number\ of\ tissue\ cysts\ recovered\ from\ positive\ control\ group} x100$$

ii) Histopathological examination

The second half of the brain of each mouse was collected in formalin 7%, dehydrated in rising series of ethyl alcohol, cleared in xylol and embedded in paraffin. Sections (5-micron thick each) were then cut using microtome, processed for staining with H&E stain [31]. First criteria of inflammation were assessed including neutrophil infiltrate, mononuclear (lymphocytes and plasma cell) infiltrate, astrogliosis and the extent of brain edema. The results were used to determine the intensity of inflammation for each group, classified as four grades: no inflammatory reaction (grade 0), mild inflammatory reaction (grade 1), moderate inflammatory reaction (grade 2) and severe inflammatory reaction (grade 3) according to [33].

iii) Immunological assessment

Interferon gamma is the absolute requirement for controlling toxoplasmosis infection either in the acute phase or in case of reactivation of latent infection [34]. It was measured in the sera of all mice of each group using "Mice IFN-γ Sandwich ELISA kit" according to kits manufacturer's instructions. Blood used was collected just prior to scarification from retro-orbital sinus [35]. The mean level of IFN-γ for each group was calculated, also the percentage of increase of IFN-γ level in each treated group, in comparison to group II (infected, non-treated), was calculated using the following equation:

$$\frac{Mean\ level\ of\ IFN\ \gamma\ in\ each\ treated\ group - Mean\ level\ of\ IFN\ \gamma\ in\ positive\ control\ group}{Mean\ \ level\ of\ IFN\ \gamma\ in\ positive\ control\ group} x100$$

Statistical analysis

The statistical package for the social sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA) was used to code and enter the data. For quantitative variables, the mean and standard deviation were used to summarize the data; and for categorical variables, the frequencies (number of samples) and relative frequencies (percentages) were used. When comparing two groups, the unpaired t test was used for comparisons, and when comparing more than two groups, the analysis of variance (ANOVA) with multiple comparisons post hoc test was used (Chan, 2003 a). Chi square ($\chi 2$) test was used for comparing categorical data (Chan, 2003 b). P-values less than 0.05 were considered statistically significant.

Results

Parasitological assessment

Using light microscope (x100), tissue cysts appeared by direct examination as shiny spherical bodies with a wide range of size, well defined cyst wall and bradyzoites within them were seen as granular appearance (Fig. 2). In Giemsa-stained films, the cysts could be seen as well circumscribed spherical bodies, deeply stained with bradyzoites inside as granules using fine adjustment (Fig. 3).

All treated groups, in both early and late toxoplasmosis experiments, revealed statistically significant percentages of reduction compared to positive control group (Fig. 4). The best therapeutic efficacy was recorded with combination therapy (PYR/SDZ + PRP) with (90.06%) and (89.08%) reduction percentages of brain tissue cysts in early and late stages respectively, followed by group III (PYR/SDZ) and lastly group IV (PRP) (Tables 1 and 2).

In all treated groups, treatment of early toxoplasmosis revealed a better efficacy in reduction of brain cysts count than in late infection.

Histopathological assessment

All treated groups in both early and late toxoplasmosis infections revealed some degree of resolving the inflammatory signs that were found in infected, non-treated group (Fig. 5 and 6).

In group V**,** using the combination therapy of PRP with conventional treatment had the best results, with resolution of all the pathological abnormalities that occurred in the brain. Treatment induced the tissue to return to its normal histology again in both early and late stages of infection.

On early treatment with PRP alone, it resolved some of the pathological changes that occurred in the brain in early toxoplasmosis and only mild inflammation had remained. However, PRP induced complete resolution of inflammatory changes in late toxoplasmosis.

Group III (infected and treated with conventional therapy PYR/SDZ) histopathological examination revealed mild inflammation in brain tissue in both early and late toxoplasmosis infection (Table 3).

Immunological Assessment

All treated groups in early and late stages of toxoplasmosis revealed an increase in IFN-γ level in serum; being higher in early stage but with no significant difference than the late stage. The highest increase in IFN-γ level in both early and late toxoplasmosis infection was recorded with combination therapy (PYR/SDZ + PRP). It was more than tripled in both experiments with statistically significant difference compared to positive control level with **(**201.65 %) and (230.97%) increase percentages in early and late experiments respectively. This was followed by PRP therapy alone with statistically significant increase compared to positive control group in both early and late infection. PYR/SDZ induced the least increase in IFN-γ level (statistically significant only in late but not early infection compared to positive control group) (Table 4&5).

Discussion

Toxoplasma gondii is one of the best adapted *Toxoplasma gondii* is one of the best adapted parasites It can stay in its hosts for a long duration of time, possibly a lifetime [36]. The basic treatment for toxoplasmosis is PYR/SDZ. However, tissue cysts are resistant against most of the currently used anti-*Toxoplasma* medications and all drugs are usually accompanied by adverse effects and toxicity that increase with the long duration of treatment [11- 37]

Platelet rich plasma contains 3–5 times plasma baseline platelet concentration [38]. Platelets can recognize pathogens and initiate immune response against them. This results in platelets activation and secretion of mediators that help in activation, multiplication, differentiation and movement of the target cells to the site of infection and forming a primary-line of defense against infection. Also, activated platelets could release chemokines to activate leukocytes infiltration and neutrophil extracellular trap formation [39- 40- 24]. Additionally, PRP has been shown to have great anti-inflammatory, anti-microbial, and anti-oxidant properties [41].

In the present work, concerning the use of single PRP therapy, mice showed 61.58% and 47.16 % percentages of reduction of brain tissue cysts in early and late infection respectively. These results agree with [42] study, which showed 29.8% reduction of brain tissue cysts in chronic toxoplasmosis (although not in a percentage as high as in the current work). Furthermore, the present study results agree with the reported PRP positive impact on other parasitic infections such as [21-24- 25]. On the contrary, [23] found statistically insignificant reduction in *S. mansoni* worm burden on using PRP alone.

On the other hand, in the current work, the use of PYR/SDZ alone, showed a major decrease in numbers of tissue cysts whether in early or late stages of infection with 81.64 and 60.46 % percentages of reduction respectively (higher than PRP, but lower than combination therapy). Similarly, [1] study revealed 94.5% and 52.1% percentages of reduction in brain tissue cysts in the acute and chronic stages of infection respectively. Moreover, [44] yielded 98.12% and 70.14 % percentage of reduction of brain tissue cysts in the acute and chronic stages respectively. On the contrary, [29] unexpectedly got better results in treatment of the chronic stage than the acute stage (reduction rates 76.7% and 51.5% respectively). Moreover, [44] used lower doses of PYR/SDZ for a longer duration and found dramatic decrease in brain cyst burden in case of chronic infection. On the other hand, an in vivo study in treatment of chronic toxoplasmosis, only yielded 39.29 % reduction percentage [45].

In the present work, the best improvement in parasitological assessment was in mice of group V (PYR/SDZ+PRP) in both early and late infection with 90.06% and 89.08 % reduction percentages respectively. Likewise. [22], reported that PRP exhibits a synergistic efficacy with antibiotics, and this special benefit offers a promising result in the treatment of bacteria that are resistant to antibiotics. In agreement, [42] stated that administration of PRP in combination with spiramycin resulted in statistically significant reduction of Toxoplasma brain tissue cysts by 80.3% in treatment of chronic infection. Similarly, different trials have been performed and confirmed the synergistic effect of PRP in combination with several conventional drugs

used in the treatment of other parasites like PRP + praziquantel in treatment of *S. mansoni* [23] and PRP + albendazole in treatment of *Trichinella spiralis* [21].

Histopathological examination of brain sections of mice in group III (PYR/SDZ) in both early and late stages showed reduction of inflammatory response (from grade 2 in infected non-treated group to only grade 1 "mild inflammatory reaction"), but lacked restoration of tissue to normal architecture like when using PRP. Similar results were recorded by [1- 28- 29- 44]. On the other hand. [46], revealed no improvement of the inflammatory response after treatment. They showed cerebral hyper cellularity, diffuse reactive gliosis and diffuse inflammatory reaction. Also, [43] observed only a slight reduction of the inflammatory response in brain tissues in mice acutely infected with *T. gondii* and no improvement at all in chronically infected mice after PYR/SDZ treatment.

Histopathological examination of mice brain sections in group IV (single PRP) in early stage showed a mild inflammatory reaction (grade 1), while in late stage, it revealed recession of all inflammatory reactions with normal brain tissue appearance (grade 0). Furthermore, mice in group V (PYR/SDZ + PRP), in both early and late stages, showed normal brain tissue with no trace of the inflammatory reaction or edema (grade 0). This anti-inflammatory effect of PRP may be attributed to release of platelets growth factors that block monocyte chemotactic protein-1 (MCP-1) production as reported by [47- 48]. Moreover, PRP has the ability to modulate the immune response by different mechanisms that end in inhibiting inflammatory process and promoting tissue repair [49]. Similarly, [42] reported marked reduction in inflammatory cells in brain tissue of *T. gondii* infected mice treated with PRP alone and a nearly complete restoration of normal brain tissue in the group treated with PRP combined with spiramycin. Similarly, many authors confirmed the promising anti-inflammatory effects of PRP either when used singly or when combined with the conventional medications in treatment of different parasitic infections [24- 25].

In the current work, serum samples from mice in group III (PYR/SDZ) showed elevation in IFN- γ level by 24.21% and 41.76% compared to positive control group in early and late stages of infection respectively (statistically significant difference in comparison to negative control groups). These results indicate that treatment had improved mice ability to produce IFN-γ. This could be attributed to the destruction of infected cells and parasites by the drugs, therefore antigens were released and caused activation of T cells to produce cytokines including IFN- γ [50]. These present results were compatible to [43- 46- 50- 51], as they all stated rise of IFN- γ levels in PYR/SDZ treated groups. On the other hand, [1] stated a decrease in IFN-γ levels in mice serum samples in both acute and chronic toxoplasmosis after treatment with PYR/SDZ combination. Furthermore, a decrease in the IFN-γ levels in mice serum samples chronically infected with *T. gondii* and treated with PYR/SDZ combination was recorded by [44- 52].

In the present work, mice in group IV (PRP) showed a great rise in IFN-γ levels compared to infected non-treated group with 132.65% and 152.56 % increase percentages in early and late stages, respectively. Nevertheless, the major rise in IFN-γ levels was observed in group V (PYR/SDZ + PRP) in both early and late stages with increase percentages of 201.65 and 230.97 %, respectively in comparison to positive control group, indicating the great efficacy of combined therapy in stimulating the immune system to get rid of infection. Being a recent topic of research until now, there are no available studies that correlate the relation between PRP and IFN- γ levels in experimentally infected mice with any parasite. However, the current work results coincide with the immunomodulatory functions attributed to PRP administration. It has a major role in IFN- γ production that in turn promotes nitric oxide (NO) production, which may have an impact on parasite control due to its direct anti-parasitic and immunoregulatory properties [1- 53].

Conclusion

All the used therapeutics yielded better efficacy in reduction of brain tissue cyst count in the early toxoplasmosis compared to late infection.

Combined therapy groups (PYR/SDZ + PRP) in both early and late toxoplasmosis, yielded the best results, in which there was the highest percentage of brain tissue cysts reduction compared to usage of either therapeutic alone, resolving of all inflammatory signs from brain tissue and with the highest elevation to IFN- γ serum levels. Thus, adding PRP to the conventional therapy can be employed as a promising anti-*Toxoplasma* agent for both acute and chronic infections

Recommendation

Trials on conjugation of PRP with other new safe agents such as nanoparticles in treatment of toxoplasmosis are recommended to get novel treatment options with less side effects.

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This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The protocol of this study was approved by the ethical committees of Kasr Al-Ainy School of Medicine as well as Cairo University Institutional Animal Care and Use Committee (CU-IACUC) (number: CU III F 46 22).

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Author's contributions

"Authors contribute equally in this work".

TABLE 1. Comparison of Experiment A (early treatment) tissue cyst count, percentage of reduction and P value obtained with the different studied therapeutics:

Groups	Tissue Cysts (Mean ±SD)	Percentage of reduction	P value
Group A.I	0±0		
Group A.II	5900±433.59		
Group A.III	1083.33±204.12	81.64 %	* <0.001 $ <0.001
Group A.IV	2266.67±326.6	61.58 %	* <0.001
Group A.V	586.67±74.48	90.06 %	* <0.001 # 0.03 $ <0.001

*: p value compared to group II. #: p value compared to group III.
$: p value compared to group IV.
Note: P value ≤ 0.05 is considered statistically significant difference.

TABLE 2. Comparison of Experiment B (late treatment) tissue cyst count, percentage of reduction and P value obtained with the different studied therapeutics:

Groups	Tissue cysts (Mean ±SD)	Percentage of reduction	P value
Group B.I	0±0		
Group B.II	7266.67±776.32		
Group B.III	2873.33±174.2	60.46 %	* <0.001 $ 0.001
Group B.IV	3840±134.46	47.16 %	* <0.001
Group B.V	793.33±58.88	89.08 %	* <0.001 # <0.001 $ <0.001

*: P value compared to group II. #: P value compared to group III.
$: P value compared to group IV.
Note: P value ≤ 0.05 is considered statistically significant difference.

TABLE 3. Comparison between the intensity of inflammation within brain tissue of different groups in both early and late experiments:

Groups	Intensity of Inflammation (grades)	
	Early Infection	**Late Infection**
Group I	0	0
Group II	2	2
Group III	1	1
Group IV	1	0
Group V	0	0

Group I: non-infected non-treated, Group II: infected non- treated, Group III: infected and treated with PYR/SDZ, Group IV: infected and treated with PRP and Group V: infected and treated with PYR/SDZ+PRP. Grades of inflammation from 0-2 where (0): no inflammation; (1): mild inflammation and (2): moderate inflammation.

TABLE 4. Comparison of Experiment A (early treatment) mean serum levels of IFN-γ and observed percentages of increase in different treated groups:

Groups	IFN-γ level (Mean ±SD)	Percentage of increase	P value
Group A.I	30.08±9.21		
Group A.II	84.81±16.02		* 0.002
Group A.III	105.34±13.13	24.21 %	* <0.001 # 1.000
Group A.IV	197.31±29.9	132.65 %	* <0.001 # <0.001 $ <0.001
Group A.V	255.83±31.76	201.65 %	* <0.001 # <0.001 $ <0.001 @ 0.001

*: p value compared to group I. #: p value compared to group II.
$: p value compared to group III. **@**: p value compared to group IV.
Note: P value ≤ 0.05 is considered statistically significant difference.

TABLE 5. Comparison between mean serum levels of IFN-γ, their percentage of increase and P value in different groups of Experiment B (late infection):

Groups	IFN-γ level (Mean ±SD)	Percentage of increase	P value
Group B.I	27.23±10.06		
Group B.II	73.42±12.49		* <0.001
Group B.III	104.08±13.99	41.76 %	* <0.001 # 0.029
Group B.IV	185.43±23.02	152.56 %	* <0.001 # <0.001 $ <0.001
Group B.V	243±17.56	230.97 %	* <0.001 # <0.001 $ <0.001 @ <0.001

*: P value compared to group I. #: P value compared to group II.
$: P value compared to group III. **@**: P value compared to group IV.
Note: P value ≤ 0.05 is considered statistically significant difference.

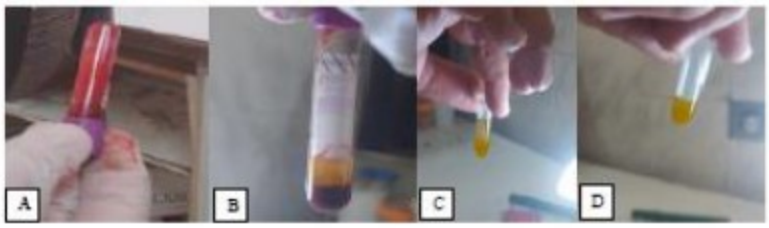

Fig. 1. PRP preparation, A: blood collection in anti-coagulant tube, B: separation of whole blood into upper plasma and lower RBCs after 1st spin, C: separation of plasma into upper PPP and lower PRP after 2nd spin and D: PRP.

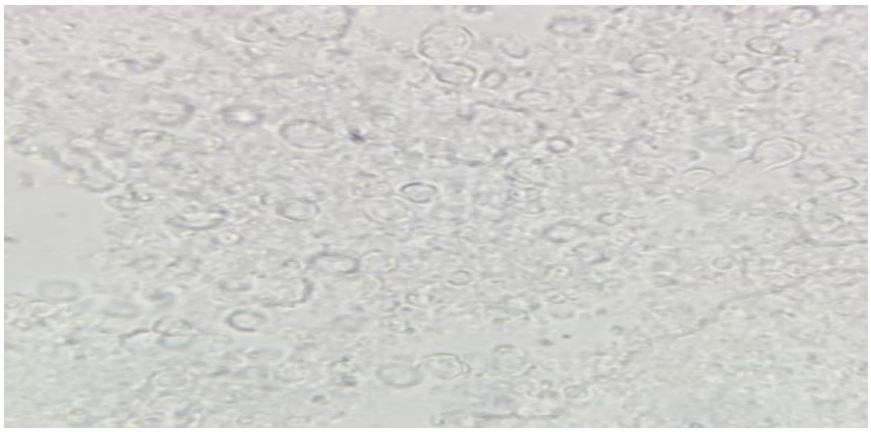

Fig. 2. *Toxoplasma gondii* tissue cyst in brain tissue homogenate without staining, appearing as shiny refractile spherical body, well defined outer cyst wall and granular inner structure (bradyzoites) (x100).

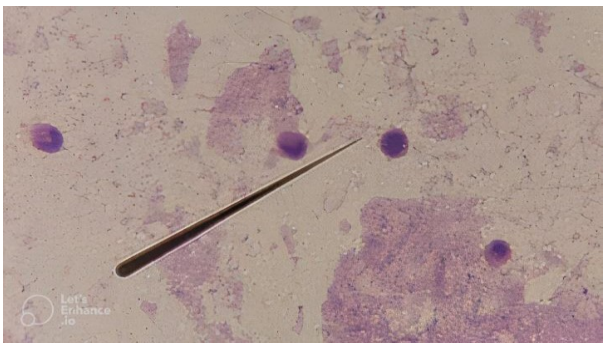

Fig. 3. *Toxoplasma gondii* tissue cyst in brain tissue homogenate after staining with Giemsa stain. It appeared as a spherical deeply stained granular body (x100)

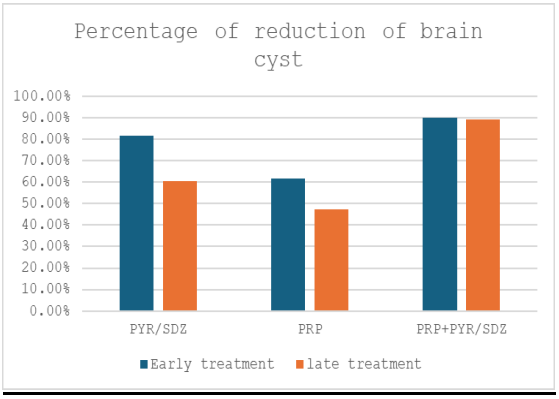

Fig. 4. A graph summarizing the efficacy of studied therapeutics on the percentage of reduction of Toxoplasma tissue cysts in different treated groups in both early and late stages of infection. The evident increase in efficacy of combined therapy especially on late stage of infection could be clearly observed compared to the use of single therapy.

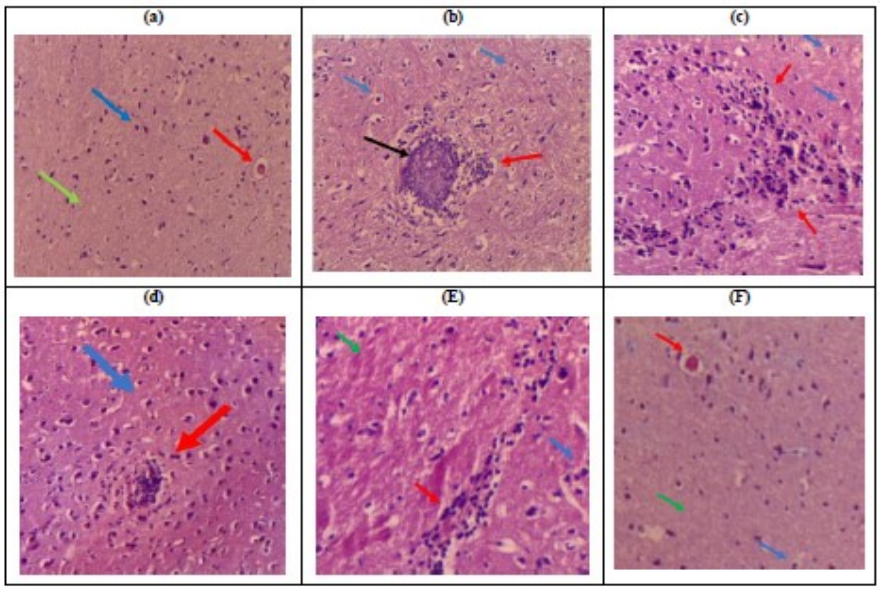

Fig. 5. Brain tissue sections from early stage (H&E stained) of A) group I showing normal brain tissue criteria with normal appearance of astrocytes (blue arrow) in a fibrillary background (green arrow) and normal vasculature with no perivascular inflammation (red arrow), B and C) group II showing Toxoplasma tissue cyst (black arrow) with heterogenous inner structure representing bradyzoites with moderate inflammatory cells aggregates (red arrow). Astrocytosis also appeared as enlarged nuclei of astrocytes and swollen cytoplasm around them (blue arrows), D) group III showing mild reactive hypertrophy and hypercellularity of astrocytes "astrocytosis" (blue arrow) and mild perivascular inflammation (red arrow), E) group IV showing astrocytosis (blue arrow), increased thickness of astrocytic foot processes making the fibrillary background more dense "gliosis" (green arrow) and mild perivascular inflammatory aggregates (red arrow) and F) group V showing restoration of the normal appearance of astrocytes (blue arrow), normal density of astrocyte foot processes "fibrillary background" (green arrow) and absence of perivascular inflammatory infiltrates (red arrow) with no visible signs of inflammation or edema within the brain tissue. (mag. A and F (x100), B, C, D and E (x200).

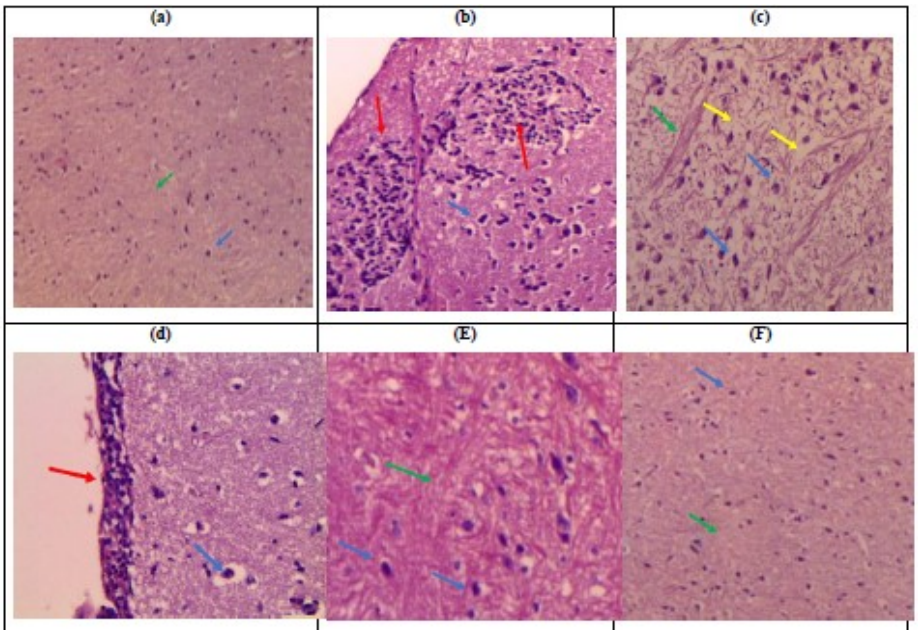

Fig. 6. Brain tissue sections from late stage (H&E stained) of A) group I showing normal brain tissue criteria with normal appearance of astrocytes (blue arrow) in a fibrillary background (green arrow), B, C and D) group II showing moderate inflammatory cells aggregates within brain tissue and meninges (D) (red arrow), and (blue arrows), increasing density of the astrocytic foot processes "gliosis" (green arrow) and moderate edema appeared as diffuse vacuolation of brain tissue (yellow arrows), E) group III showing mild reactive hypertrophy and hypercellularity of astrocytes "astrocytosis" (blue arrow) with increase in density of fibrillary background (green arrow) and F) group IV and V showing restoration of the normal appearance of astrocytes (blue arrow) and normal density of astrocyte foot processes "fibrillary background" (green arrow). (Mag. A and F (x100), B, C, D and E (x200).

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تأثير البلازما الغنية بالصفائح الدموية مقابل العلاج التقليدي في المرحلة المبكرة والمتأخرة لعدوى المقوسة الغوندية (سلالة إم إي 49) في الفئران المثبطة مناعيا

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الملخص

داء المقوسات هو عدوى طفيلية منتشرة بشكل كبير في جميع أنحاء العالم. يهدف العمل الحالي إلى تقييم التأثيرات العلاجية للبلازما الغنية بالصفائح الدموية بمفردها أو بالاشتراك مع البيريميثامين والسلفاديازين في علاج المرحلة المبكرة والمتأخرة لداء المقوسات في الفئران المثبطة مناعيا. تم تقسيم 60 فأرًا مضعف المناعة إلى تجربتين؛ التجربة "أ" للمرحلة المبكرة للعدوى والتجربة "ب" للمرحلة المتأخرة للعدوى. تم تقسيم كل من التجربتين إلى خمس مجموعات فرعية؛ (1): غير مصابة وغير معالجة، (2): مصابة وغير معالجة ، (3): مصابة وتعالج بالبيريمثامين/سلفاديازين ، (4) مصابة وتعالج بالبلازما الغنية بالصفائح الدموية بمفردها و (5): مصابة وتعالج بالبيريمثامين/سلفاديازين و البلازما الغنية بالصفائح الدموية معا. تم تسجيل أفضل النتائج في مجموعة البلازما الغنية بالصفائح الدموية االمجتمعة مع البيريميثامين والسلفاديازين مع نسب انخفاض 90.06% و89.08% في عدد الحويصلات النسيجية في المراحل المبكرة والمتأخرة على التوالي مقارنة بالمجموعة الغير معالجة، كما أدى إلى حل جميع الأعراض الالتهابية وأعلى زيادة في مستوى الانترفيرون جاما في جميع المجموعات الأخرى. ومن ثم، تم استنتاج أن البلازما الغنية بالصفائح الدموية المقترنة مع الأدوية التقليدية (بيريمثامين/سلفاديازين) يمكن ترشيحها علاجًا جيدًا للمراحل الحادة والمزمنة من داء المقوسات سواء من خلال الفحص الطفيلي أو الباثولوجي أو المناعي

الكلمات الدالة: المرحلة المبكرة والمتأخرة لعدوى المقوسات ، البلازما الغنية بالصفائح الدموية.