Prophylactic and therapeutic efficacy of berberine on chronic toxoplasmosis in diabetic or hypertensive mice

Original Article

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ABSTRACT

Background: Possible association between toxoplasmosis, and chronic diseases, *e.g.*, hypertension (HTN) and diabetes mellitus (DM) are not uncommon. Therefore, searching for a new, safe, alternative and efficient natural product to treat toxoplasmosis in patients complaining of hypertension or diabetes is critically needed.
Objective: To investigate the potential prophylactic and therapeutic efficacy of berberine on chronic toxoplasmosis in diabetic, and hypertensive mice compared to Spiramycin©.
Material and Methods: A total number of 77 mice were divided into normal (n.=7), hypertensive (n.=35) and diabetic (n.=35) mice. Hypertension and type 1 diabetes were induced by 8% NaCl, and 2% Alloxan monohydrate, respectively. Both hypertensive, and diabetic mice were subdivided into five equal subgroups: control, berberine-prophylactic, Spiramycin©-treated, berberine-treated, and combined treatment (berberine and Spiramycin©). Berberine efficacy was assessed using parasitological and histopathological parameters.
Results: Parasitological assessment showed that all treated subgroups showed a statistically significant reduction in the median tissue cysts count compared to infected control subgroups. Combined (berberine and Spiramycin©) showed the highest reduction rates of cysts count in both hypertensive and diabetic subgroups. For the prophylactic potential efficacy, berberine showed the least reduction rate in tissue cyst count in both hypertensive and diabetic subgroups. Histopathological assessment demonstrated moderate to marked improvement of brain, spleen and kidney inflammatory changes in all treated subgroups, the highest was recorded in the combined treated subgroups.
Conclusion: Berberine is a promising adjuvant in treating toxoplasmosis associated with chronic diseases including diabetes mellitus and hypertension.

Keywords: berberine; diabetes; hypertension; Spiramycin©; toxoplasmosis.

Received: 16 July, 2024; **Accepted:** 30 August, 2024.

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Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 17, No. 2, August, 2024.**

INTRODUCTION

Toxoplasmosis is one of the most significant issues in public health affecting up to 74.5% worldwide with variable clinical presentations depending on the host's immunological status[1]. Remarkably, in immunocompromised patients, chronic toxoplasmosis may reactivate, resulting in a potentially lethal condition. The probable correlation between toxoplasmosis and chronic disorders such as DM and HTN is up challenging and still not understood. Due to a decreased host immune system in DM, the current hypothesis suggests that diabetic patients are more vulnerable to opportunistic infections including toxoplasmosis[2,3].

The possible role of *T. gondii* in DM induction may be due to inflammatory destruction of β pancreatic cells, with subsequent inability to produce sufficient insulin. Acute and chronic pancreatitis, as well as diabetes, could develop as a result of this sequelae[4]. Besides, people with cardiovascular diseases and HTN are more liable to develop toxoplasmosis. In fact, toxoplasmosis may affect hemodynamic control or increase dopamine which is known to increase the blood pressure. Therefore, toxoplasmosis becomes a risk factor for developing HTN in some individuals[5].

There is continuous challenge in the treatment of toxoplasmosis since bradyzoites are unaffected by the commonly used drugs, and the blood-brain barrier further complicates the management of brain tissue cysts. Several therapeutic regimens are used for management of toxoplasmosis, but pyrimethamine/sulfadiazine combination is the

 DOI: 10.21608/puj.2024.304987.1257

current gold standard[6]. Unfortunately, there are many drawbacks regarding their restricted activity against *T. gondii* tissue cysts, reported resistance, and serious unpleasant side effects[7].

Currently, herbal plant extracts are widely introduced as a potential source of treatment for several diseases, not only for their role in promoting the immune system but also due to their low cost, easy accessibility, and fewer side effects[8]. Berberine is an alkaloid extracted from various plants and is used medically as herbal supplement. It contains a collection of bioactive compounds with analgesic, anti-diabetic, anti-obesity, anti-hyperlipidaemic, anti-hypertensive, antimicrobial actions[9] and for anti-memory impairment[10]. As an anti-parasitic drug, berberine showed potential or promising therapeutic efficacy on *Plasmodium* spp.[11], *Leishmania* spp.[12], *G. lamblia*[13], *T. vaginalis*[14], *E. histolytica*[15], and *T. gondii*[10]. Moreover, berberine showed potential prophylactic effect against several medical diseases including atrial fibrillation[16], DM[17], diabetic nephropathy[18], and colorectal carcinoma[19].

Thus, the present study aimed to evaluate the potential effect of berberine extract as a prophylactic and therapeutic agent for the treatment of toxoplasmosis among mice with hypertension or diabetes compared to standard treatment by Spiramycin© for future development of a new, safe, and effective compound used for treating the disease.

MATERIAL AND METHODS

This experimental case-control study was conducted at the Medical Parasitology Department of Theodor Bilharz Research Institute (TBRI) during the period from January to April 2022

Study design: Two groups of mice (hypertensive and diabetics) infected with *T. gondii* were divided into subgroups treated with berberine, Spiramycin©, combined therapy in addition to prophylactic berberine subgroups. Parameters used for evaluating berberine efficacy include parasitological and histopathological assessment.

Experimental animals: A total of 77 laboratory-bred Swiss albino male mice, aged 6-8 w, and weighing an average of 20-25 g were provided from TBRI, Egypt. During the study, mice were kept in plastic cages at suitable temperature and humidity and fed standard diet containing 4% fat, 4-5% fiber, 24% protein, and water *ad-libitum*.

***T. gondii* strain:** The ME49 chronic strain was obtained from TBRI where tissue cysts from brains of infected mice were isolated and used for induction of chronic infection in experimental mice. Briefly, brain tissues were collected, homogenized, and stained with Giemsa stain. Under oil immersion lens, *T. gondii* tissue cysts were counted in 10 different fields, and the average number in 20 µl was calculated[20].

Drugs preparation and dose adjustment: Spiramycin© (Sigma Aldrich Inc, USA) was purchased, and dissolved in PBS (pH 7.2). Mice were orally inoculated using esophageal tube at a dose of 200 mg/kg/d for 14 consecutive d[21]. The drug was given either singly or in combination with berberine herbal plant to *Toxoplasma* infected hypertensive, and diabetic mice. Berberine is extracted from the Indian barberry root, and was obtained from Sigma Aldrich Inc, USA. It was dissolved in PBS (pH 7.2), and a dose of 20 mg/kg/d was orally inoculated using an esophageal tube for 14 consecutive d. This regimen was given for treatment, and prophylaxis after and before infection with Me49 *T. gondii* strain, respectively[22].

Induction of chronic diseases: Hypertension was induced by oral administration of high salt (8% NaCl for 7 consecutive d) to mice subgroups. The blood pressure (BP) level was checked on experimental day 14 using tail cuff technique (LSI Lectica, LE5001 device). The BP level was recorded three times per week till end of study. Mice were considered hypertensive when systolic BP is more than 140 and diastolic BP is higher than 90[23]. Type 1 DM (T1DM) was induced by oral administration of single dose (40 mg/kg) of 2% Alloxan monohydrate (ALDRICH chemistry, South Africa) diluted in 0.9% saline and given to mice subgroups for 5 consecutive d. The blood glucose level was measured on experimental day 14 from a tail vein blood sample using a glucometer (Auto-coding premium, INFOPIA device) to check for induced hyperglycemia. Blood glucose level was estimated 3 times per week till end of study, and levels more than 180 mg /dl represented diabetes[24].

Mice infection: Each mouse was orally infected by 200 µl of PBS containing 1000±200 tissue cysts isolated from *Toxoplasma*-infected mice[25].

Study groups: Mice were classified into 2 main groups: control (GA) and treated (GB) groups. Control group (n.=21) was subdivided into: A1; non-infected non-treated mice (negative control), A2; *Toxoplasma*-infected hypertensive non treated mice (positive control 1), and A3; *Toxoplasma*-infected diabetic non treated mice (positive control 2). While the treated group (n.=56) was subdivided into: B1; hypertensive mice treated by berberine before *Toxoplasma* infection (prophylactic subgroup), B2; *Toxoplasma*-infected hypertensive mice, treated by Spiramycin© (control drug), B3; *Toxoplasma*-infected hypertensive mice, treated by berberine, B4; *Toxoplasma*-infected hypertensive mice treated by combined berberine and Spiramycin©, B5; diabetic mice treated by berberine before *Toxoplasma* infection (prophylactic subgroup),

B6; *Toxoplasma*-infected diabetic mice treated by Spiramycin© (control drug), B7; *Toxoplasma*-infected diabetic mice treated by berberine, B8; *Toxoplasma*-infected diabetic mice treated by combined berberine and Spiramycin©.

Mice sacrifice: Animal scarification was performed after 45 days in control subgroups (A1, A2, A3), 47 days in diabetic subgroups (B5 - B8) and 60 days post infection (DPI) in subgroups (B1- B4). Mice were intraperitoneally injected with 500 mg/kg thiopental and 100 unit/ml heparin (anesthetic– anticoagulant solution)[26].

Assessment of drug efficacy

Parasitological assessment[27]: Tissue cysts were counted in the isolated mice brains using oil immersion lens (×100). The average tissue cysts number in ten different fields of each mouse's brain, and the mean number of the 7 mice were calculated to evaluate the reduction in their numbers. The decrease in *Toxoplasma* cysts' count in treated group compared to infected untreated control group was expressed as percent reduction (PR) and calculated by the formula: PR=(cyst count in untreated group–cyst count in the treated group)/cyst count in untreated group) X 100.

Histopathological assessment: Brain, spleen and kidney tissues were excised from each mouse to assess the histopathological and inflammatory changes and for detection of tissue cyst. Briefly, collected tissue samples were preserved in formol-water 7%, dehydrated in ascending series of ethyl alcohol one hour each. Samples were xylene-cleared, paraffin-embedded, microtome-cut into 5 µm thick sections, and hematoxylin and eosin (H&E)-stained[28]. Examination of tissue sections was performed using light microscope and digital slide scanner (Leica APERIO LV1 microscopy).

Statistical analysis: Data coding, entering and analysis was conducted using the statistical package for the Social Sciences (SPSS; IBM Corp., Armonk, NY, USA) version 25 for Microsoft Windows 10. Quantitative data were analyzed by calculating minimum, maximum, median, and interquartile range (IQR). For independent samples, Kruskal Wallis test followed by pairwise comparison analysis was used. A significant difference was considered at $P \leq 0.05$.

Ethical consideration: The protocol of the present study was approved by Institutional Animal Care and Use Committee, Beni-Suef University (BSU- IACUC) with approval number (021-140). Mice feeding, housing, and handling was done following the recommendations of "Guide for the care and use of laboratory animals". Besides, a committee concerned with animal ethics at TBRI approved the study.

RESULTS

Parasitological assessment: Unstained brain tissue cysts appeared round to oval in shape with thin cyst wall and average size of 5-50 µm. Whereas, Giemsa-stained tissue cysts were purple stained containing from a few to hundreds of bradyzoites with terminal nucleus (Fig. 1).

In the infected hypertensive positive control subgroup (A2), the median number of *Toxoplasma* tissue cysts were 1600 with a decrease in number in other hypertensive infected treated subgroups (B1-B4). The highest reduction rate (59.2% and 51.7%) in median cyst number was recorded in the infected hypertensive subgroup treated with combined berberine and Spiramycin© therapy (B4) and berberine (B3) respectively with significant statistical differences compared to control subgroup (A2) ($P \leq 0.05$). The least reduction rate of *Toxoplasma* tissue cyst (34.1%) was recorded in the prophylactic subgroup (B1) with non-statistical difference with positive control subgroup (A2) (Table 1) and (Fig. 2). Although the reading of

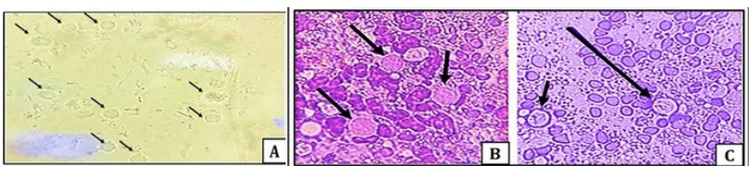

Fig. 1: Unstained *Toxoplasma* tissue cysts (arrows) in mice brain (**A,** ×100); Giemsa-stained cysts in mice brain showing bradyzoites with terminal nucleus (arrows) (**B, C,** × 400).

Table 1. Median count and reduction% of brain tissue cysts in infected hypertensive mice groups.

Subgroups	Tissue cyst count			IQR	Reduction %	Statistical analysis
	Median	Minimum	Maximum			*P* value
A2	1600	1440	2040	300	--	
B1	1052	958	1228	220	34.1	
B2	940[a]	800	1200	270	40.6	**0.001***
B3	797[a]	600	1050	271	51.7	
B4	665[a,b]	400	880	270	59.2	

Hypertensive subgroups; A2: Positive control; **B1:** Prophylactic; **B2:** Spiramycin©; **B3:** Berberine; **B4:** Combined therapy; **IQR:** Interquartile range; Statistical analysis was carried out using the Kruskal Wallis test followed by pairwise comparison analysis; **[a]:** Significantly ($P \leq 0.05$) different from controls (A2); **[b]:** Significantly ($P \leq 0.05$) different from hypertensive mice (B1); ***:** Significant ($P \leq 0.05$).

systolic and diastolic blood pressure decreased in berberine treated subgroups (B3 and B4), it showed non statistically significant difference compared to non-treated hypertensive control mice (Table 2).

In the diabetic control group (A3), the median tissue cyst count was 1570. The reduction rate of *T. gondii* tissue cysts in the group treated with berberine (B7) and combined berberine and Spiramycin© therapy (B8) was 57.1% and 60% respectively, even higher than infected diabetic mice treated with Spiramycin© (B6) which was 56% (Fig. 3). The difference was significantly decreased in all treated groups ($P \leq 0.05$) compared to control group (A3). The median number of *Toxoplasma* tissue cysts in the prophylactic group (B5) were 970 with 40.6% reduction which was not statistically different from control group (Table 3, and Fig. 4). Random blood sugar level in berberine treated groups (B7 & B8) was lower than diabetic control group (A3) with a non-statistically significant difference ($P=0.193$) (Table 4).

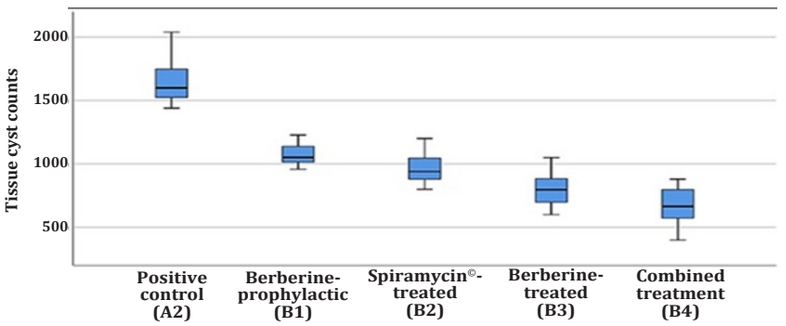

Fig. 2. Comparison between median tissue cysts counts in experimentally *Toxoplasma*-infected hypertensive mice groups.

Table 2. Blood pressure level in experimentally infected hypertensive mice group.

Subgroups	Blood pressure level (mmHg)			IQR	Statistical analysis
	Median	Minimum	Maximum		*P* value
A2	140/90	135/85	142/95	5/5	
B1	138/89	135 /80	140/90	5/5	
B2	138/90	135/85	145/95	5/10	*P*1=0.869
B3	135/85	130/80	140/90	5/4	*P*2= 0.217
B4	133/85	130/80	140/95	5/10	

Hypertensive subgroups; A2: Positive control; **B1:** Prophylactic; **B2:** Spiramycin©; **B3:** Berberine; **B4:** Combined therapy; **IQR:** Interquartile range; Statistical analysis was carried out using the Kruskal Wallis test followed by pairwise comparison analysis; ***P*1:** For systolic blood pressure; ***P*2:** For diastolic blood pressure.

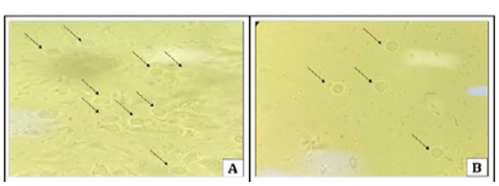

Fig. 3: Difference in number of *Toxoplasma* tissue cysts (arrows) among diabetic control group **(A)** and combined berberine and Spiramycin© treated group **(B)** ×1000.

Table 3. Median count and % reduction of *Toxoplasma* brain tissue cysts in infected diabetic mice groups.

Subgroups	Tissue cyst count			IQR	Reduction %	Statistical analysis
	Median	Minimum	Maximum			*P* value
A3	1570	1420	2025	270	--	
B5	970	850	1120	160	40.6	
B6	703[a]	600	1000	283	56.0	**0.001***
B7	70[a]	580	850	200	57.1	
B8	652[a]	400	815	255	60.0	

Diabetic subgroups; A3: Positive control; **B5:** Prophylactic; **B6:** Spiramycin©; **B7:** Berberine; **B8:** Combined therapy; **IQR:** Interquartile range; Statistical analysis was carried out using the Kruskal Wallis test followed by pairwise comparison analysis; **[a]:** Significantly ($P \leq 0.05$) different from controls (A3); ***:** Significant ($P \leq 0.05$).

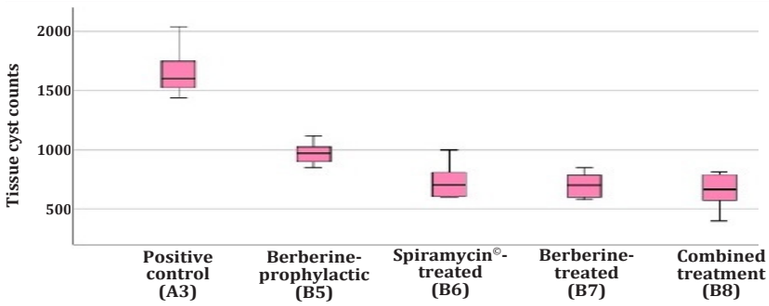

Fig. 4. Comparison between *Toxoplasma* median cysts' count in experimentally infected diabetic mice groups.

Table 4. Random blood sugar in experimentally infected diabetic groups.

Subgroups	Rndom blood sugar (mg/dl)			IQR	Statistical analysis
	Median	Minimum	Maximum		*P* value
A3	182	166	204	21	
B5	170	150	195	16	
B6	173	162	190	21	0.193
B7	164	148	180	28	
B8	164	149	183	29	

Diabetic subgroups; A3: Positive control; **B5:** Prophylactic; **B6:** Spiramycin©; **B7:** Berberine; **B8:** Combined therapy; **IQR:** Interquartile range; Statistical analysis was carried out using the Kruskal Wallis test followed by pairwise comparison analysis.

Histopathological assessment

- **Brain sections:** Negative control subgroup (A1) showed normal brain tissue, normal six layers of cerebral cortex, normal neurons and neuroglial cells. The brain section from infected control subgroups A2 (hypertensive) and A3 (diabetic) showed diffuse degeneration of neural cells, marked neutrophil vacuolation, dilated congested blood vessels with perivascular space, chronic inflammatory infiltrate associated with presence of tissue cysts of *T. gondii* (Figs. 5 A-C).

The brain tissue from infected hypertensive group treated by Spiramycin© (B2) and berberine (B3) showed few scattered degenerated neural cells, congestion and minimal neutrophil vacuolation with scattered mononuclear cell infiltrate in absence of *T. gondii* tissue cysts. Whereas in the subgroup treated by combined berberine and Spiramycin© (B4), the brain tissue showed almost normal neurons with scattered mild mononuclear cell infiltrate in absence of *T. gondii* tissue cysts (Figs. 5 D-F).

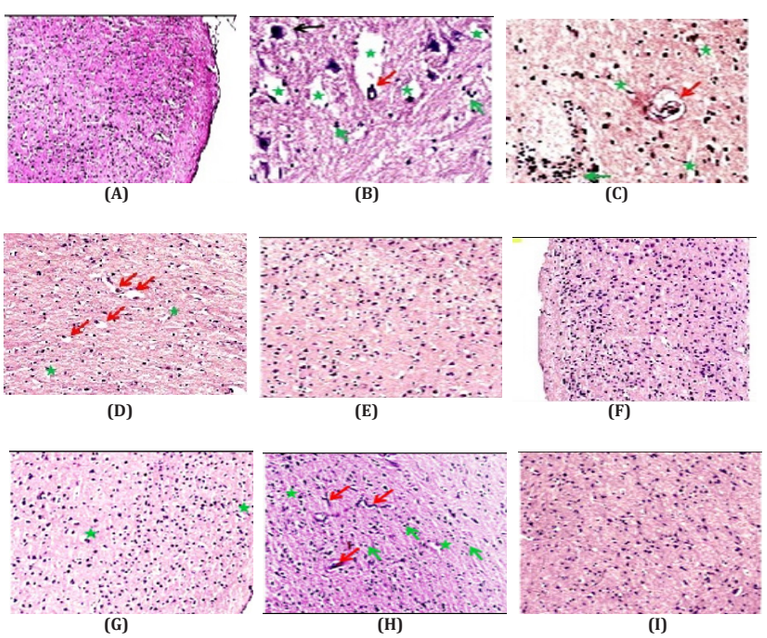

Fig. 5. Light microscopy of brain section from **(A)** negative control subgroup (H&E stain ×200); **(B)** diabetic control subgroup showing tissue cyst (black arrow), neutrophil vacuolation (green stars), dilated congested blood vessels (red arrow), and chronic mononuclear cell infiltrate (green arrows); **(C)** hypertensive control subgroup showing neutrophil vacuolation (green stars), dilated congested blood vessels (red arrow), and chronic mononuclear cell infiltrate (green arrow) (H&E stain ×400); **(D)** Spiramycin©-treated subgroup showing degenerated neural cells, congestion (red arrows), and neutrophil vacuolation (green stars); **(E)** berberine-treated subgroup showing degenerated neural cells, with minimal neutrophil vacuolation; **(F)** combined treated subgroup showing almost normal neurons (H&E stain ×200); **(G)** Spiramycin©-treated subgroup showing neutrophil edema (green stars); **(H)** berberine-treated subgroup, the brain tissue shows scattered degenerated neural cells (red arrows), neutrophil vacuolation (green stars), and mild mononuclear cell infiltrate (green arrows); **(I)** combined treated subgroup showing slightly normal neurons (H&E stain ×200).

The brain tissue from infected diabetic subgroups treated by Spiramycin© (B6) and berberine drug (B7) showed few scattered degenerated neural cells, congestion, minimal neutrophil edema with mild mononuclear cell infiltrate and absence of *T. gondii* tissue cysts, while the brain section from infected diabetic subgroup treated by combination of berberine and Spiramycin© (B8) showed slightly normal neurons with mild mononuclear cell infiltrate (Figs. 5 G-I).

- **Spleen sections:** Negative control subgroup (A1) showed normal splenic tissue consisting of white pulp formed of lymphoid follicles with central arterioles and red pulp containing splenic sinusoids. Spleen section from infected control subgroup A2 (hypertensive) and A3 (diabetic) showed marked disruption of splenic architecture with increase in megakaryocytes and multiple *T. gondii* tissue cysts associated with foci of hemorrhage (Figs. 6 A-C).

The spleen section from infected hypertensive subgroup treated by Spiramycin© (B2) showed moderate edema and foci of extravasated RBCs with absence of *T. gondii* tissue cysts. Whereas spleen section from infected hypertensive subgroup treated by berberine drug (B3) and the combined berberine and Spiramycin© (B4) showed almost normal splenic architecture with mild edema and mild congestion in absence of *T. gondii* tissue cysts (Figs. 6 D-F).

Spleen section from infected diabetic subgroup treated by Spiramycin© (B6) showed moderate congestion and foci of hemorrhage in absence of *T. gondii* tissue cysts. That from infected diabetic subgroup treated by berberine drug (B7) and the combination of berberine and Spiramycin© (B8) showed almost normal splenic architecture with scattered foci of hemorrhage in absence of *T. gondii* tissue cysts (Figs. 6 G-I).

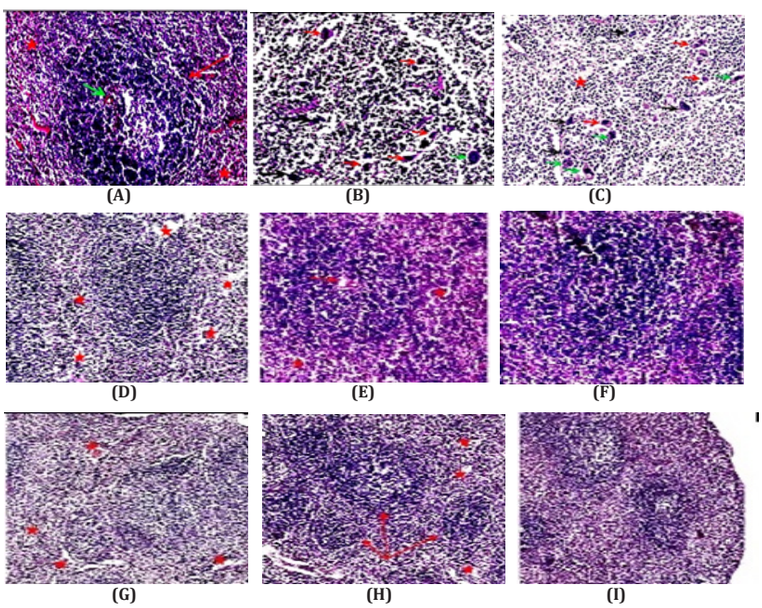

Fig. 6. Light microscopy of spleen section from **(A)** negative control subgroup showing white pulp (red arrows), central arterioles (green arrows), and red pulp (red stars); **(B)** hypertensive control subgroup showing increase megakaryocytes (black arrows), and multiple tissue cysts (green and red arrows); **(C)** diabetic control subgroup showing increase megakaryocytes (black arrows), multiple tissue cysts (green and red arrows), and foci of hemorrhage (red star) (×200); **(D)** hypertensive Spiramycin©-treated subgroup, showing edema (red star) (×100); **(E)** hypertensive berberine-treated subgroup showing edema (red stars), and congestion (red arrow) (× 200); **(F)** hypertensive combined treated subgroup showing almost normal splenic architecture (×200); **(G)** diabetic Spiramycin©-treated subgroup showing congestion and foci of hemorrhage (red stars) (×100); **(H)** diabetic berberine treated subgroup showing almost normal splenic architecture (red arrows), and foci of hemorrhage (red stars) (×100); **(I)** diabetic combined treated subgroup showing almost normal splenic architecture (×100).

- **Renal section:** Negative control subgroup (A1) showed normal renal tissue, normal glomeruli consisting of capillary tuft encircled by Bowman's capsule, distal convoluted tubules with large lumen and proximal convoluted tubules with round lumen and eosinophilic cytoplasm. While renal section from infected control A2 (hypertensive) and A3 (diabetic) subgroups showed marked capillaries glomerular tuft congestion with Bowman's capsule swelling and dilatation. The surrounding renal tubules showed diffuse severe acute tubular necrosis (Figs. 7 A-C).

Renal section from infected hypertensive subgroup treated by Spiramycin© (B2) and berberine drug (B3) showed moderate congested capillary glomerular tuft and few tubules showed degenerative cloudy swelling with minimal intraluminal hyaline cast in absence of interstitial inflammatory cells. Meanwhile, renal section from infected hypertensive subgroup treated with a combination of berberine and Spiramycin© (B4) showed almost normal renal glomeruli with minimal tubular degenerative changes (Figs. 7 D-F).

Renal section from infected diabetic subgroups treated by Spiramycin© (B6) and berberine drug (B7) showed moderate congested capillary glomerular tuft and few tubules showed degenerative hydropic changes, intraluminal epithelial cell cast with absence of interstitial inflammatory cells. While renal section from infected diabetic subgroup treated by combination of berberine and Spiramycin© (B8) showed almost normal renal glomeruli with absence of renal tubular degenerative and interstitial inflammatory changes (Figs. 7 G-I).

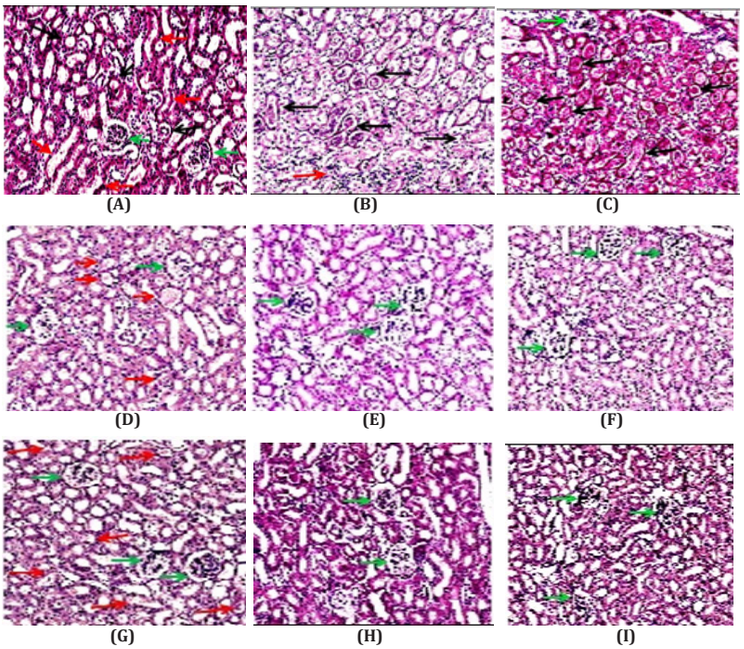

Fig. 7. Light microscopy of kidney section from **(A)** negative control subgroup showing normal glomeruli (green arrows), proximal convoluted tubules (black arrows), and distal convoluted tubules (red arrows); **(B)** hypertensive control subgroup showing tubular dilatation (black arrows), and chronic inflammatory cell infiltrate (red arrow); **(C)** diabetic control subgroup showing glomerular tuft congestion (black arrows), Bowman's capsule swelling and dilatation (green arrow) (H&E stain ×200); **(D)** hypertensive Spiramycin©-treated subgroup showing congested capillary glomerular tuft (green arrows), and degenerative cloudy swelling (red arrows); **(E)** hypertensive berberine treated subgroup showing congested capillary glomerular tuft (green arrows); **(F)** hypertensive combined treated subgroup showing almost normal renal glomeruli (green arrows); **(G)** diabetic Spiramycin©- treated subgroup showing congested capillary glomerular tuft (green arrows), and degenerative hydropic changes (red arrows); **(H)** diabetic berberine-treated subgroup showing congested capillary glomerular tuft (green arrows); **(I)** diabetic combined treated subgroup showing almost normal renal glomeruli (green arrows) (H&E stain ×200).

DISCUSSION

During the last decade, several studies investigated the potential efficacy of different natural products in treating acute or chronic toxoplasmosis or both. The studies investigated propolis[29], berberine[30], curcumin nano emulsion[31,32], and probiotics, *e.g.*, *Lactobacilli acidophilus*[33]. The reduction rate of tissue cysts counts in the current study recorded in berberine treated-hypertensive and -diabetic mice (51.7% and 57.1%, respectively) may be explained by its broad anti-inflammatory effect[30]. Nevertheless, limited studies were concerned with the potential effect of berberine on chronic toxoplasmosis. Mahmoudvand *et al*.[26] showed that berberine significantly reduced tachyzoite load in acutely infected mice in comparison to the control group. However, the therapeutic impact of berberine on pathogens other than *T. gondii* was clarified by its ability to break down the cell membrane, interfere with DNA replication, modify the host immunological responses or probably inhibit the synthesis of toxins and other virulence factors[11,34]. In addition, it promotes oxidation by increasing reactive oxygen intermediates, and depleting thiols leading to apoptosis[12,35].

In the current study, combined treatment reduced the median tissue cyst count better than berberine monotherapy in hypertensive (59.2% versus 51.7%), and diabetic (60% versus 57.1%) mice which confirmed the synergistic effect of both treatments. This may be due to increased penetration of Spiramycin© into the blood brain barrier leading to more reduction in *T. gondii* tissue cysts[6]. This synergistic effect was previously recorded in liposomes/berberine, and berberine/Albendazole© combinations in decreasing the parasites burden compared to single treatment in visceral leishmaniasis[35], and trichinellosis[36], respectively.

In the present study, the least reduction rate of tissue cyst was recorded in the prophylactic berberine subgroup. Research on the prophylactic effects of berberine against chronic toxoplasmosis are scarce, and no *in vivo* studies focusing entirely on berberine for this purpose. However, several studies were recently conducted to investigate its potential prophylactic efficacy in clinical diseases. The investigators claimed that it exhibited promising results[16-19].

The reading of systolic and diastolic BP level was lower in berberine treated subgroups. However, its use for only two weeks at low dose might be insufficient to make statistically significant difference with non-treated mice. Notably, the hypotensive effect of berberine was confirmed when administered at high dose and for months[37]. On the other hand, random blood sugar level was lower in berberine-treated than diabetic control subgroup with no statistical significance. Berberine may be considered as regulator of blood glucose and its hypoglycemic effect may not be obvious in the current study due to the administration of low dose for short duration. Other studies[38-40] observed that combining berberine with hypoglycemic drugs improve their performance where it inhibits gluconeogenesis and decreases insulin resistance primarily by repairing the destructed or exhausted islets of Langerhan of the pancreas.

Our histopathological results revealed improvement of all the studied tissues in berberine-treated hypertensive and diabetic subgroups which confirms the anti-inflammatory effect of berberine. In concordance

with our results, other studies showed improvement in the histopathological changes in berberine-treated groups in brain tissue in acute toxoplasmosis[22], spleen tissue in visceral leishmaniasis[35], and renal tissues in schistosomiasis *mansoni*[41].

In conclusion, berberine extract proved promising therapeutic effect on chronic toxoplasmosis when associated with chronic diseases including T1DM, and hypertension. As prophylactic potential efficacy, further studies are recommended to discover the best berberine dosage, prophylactic duration, and potential adverse effects.

Authors' contributions: Zalat RS, Abd El Wahab WM, Farouk RR and Ali MI contributed to study design and performance of the parasitological studies. The pathological studies were carried out by Ahmed MM and Mousa AMA. The original draft was written by Ismail MAM, Ghieth MA, and Abdel Gawad SS. All authors contributed in the proposal of the study topic, data analysis, and revising the manuscript. They approved the authorship and accepted the manuscript final version before publication.

Conflict of interest: The authors declare that they have no conflict of interest.

Funding statement: No funding.

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