Food and Waterborne Parasitology 28 (2022) e00180

Contents lists available at ScienceDirect

Food and Waterborne Parasitology

journal homepage: www.elsevier.com/locate/fawpar

Potential therapeutic effect of platelet-rich plasma and albendazole on the muscular phase of experimental *Trichinella spiralis* infection

Fatma M.A. Eissa [a], Ahmed H.A. Eassa [a], Rabab S. Zalat [b], Mohamed S. Negm [c], Marwa A. Elmallawany [a,*]

[a] Department of Medical Parasitology, Faculty of Medicine, Cairo University, Egypt
[b] Department of Medical Parasitology, Theadore Bilharz Research Institute, Egypt
[c] Department of Pathology, Faculty of Medicine, Cairo University, Egypt

ARTICLE INFO

Keywords:
T. spiralis
PRP
Albendazole
Histopathology
Drug therapy

ABSTRACT

Trichinellosis is a food-borne parasitic infection causing muscle damage. This study aimed to detect the potential therapeutic effect of platelet-rich plasma (PRP) alone or in combination with albendazole (ALB) on the muscular phase of experimental *Trichinella* infection in rats. The study was conducted on 70 rats divided into four main groups: healthy non-infected non-treated rats, non-infected rats treated with PRP, infected untreated rats (seven rats in each group), and an infected group of 49 rats. The infected group was further subdivided based on the drug therapy received. The effects of drug therapy were evaluated using parasitological and histopathological analyses.

The percent reduction in the number of *Trichinella spiralis* larvae per gram of muscle in the PRP-treated groups (one, two, and three doses) was 43.1%, 78.8%, and 86.1%, respectively. Groups treated with combined therapy of ALB & PRP (one, two, and three doses) showed overall reduction percentages of 87.7%, 90.9% and 95.2%, respectively. In contrast, the ALB-treated group showed a 69.4% reduction. All results of the abovementioned groups were statistically significant compared to the control-infected non-treated group.

The findings of the histopathological analysis were consistent with the parasitological results. Groups receiving combined therapy showed the most significant improvement in terms of the degree of inflammation and fibrosis. It can be concluded that PRP has a modulatory effect on the pathology caused by *T. spiralis larvae* in the muscular phase of trichinellosis.

To our knowledge, this is the first study to investigate the effect of PRP on the muscular phase of *T. spiralis* infection.

1. Introduction

Trichinella spiralis is a zoonotic nematode that causes *trichinellosis*. It is a worldwide food-borne parasitic disease caused by infection with nematode worms of the genus *Trichinella*. These parasites are a public health hazard with a particular concern over food safety. A large number of human cases occur annually by eating raw or undercooked pork or its products containing infective *Trichinella* larvae

* Corresponding author.
E-mail addresses: fatma.mamdouh@cu.edu.eg (F.M.A. Eissa), mamallawany@cu.edu.eg (M.A. Elmallawany).

https://doi.org/10.1016/j.fawpar.2022.e00180
Received 28 April 2022; Received in revised form 3 September 2022; Accepted 9 September 2022
Available online 14 September 2022

(Han et al., 2019; Xu et al., 2021; Basso et al., 2022).

The course of human infection with *T. spiralis* consists of three phases: an intestinal phase, a migratory phase, and a muscular phase. The parasite's entry into muscle cells causes inflammation in the host's muscle. These muscle larvae become encapsulated and are the primary cause of morbidity (Fabre et al., 2009; Bruschi and Chiumiento, 2011; Bruschi and Dupouy-camet, 2014).

The goal of treating trichinellosis is to alleviate muscular damage. Albendazole (ALB) and mebendazole are the main anti-helminthic drugs used to treat trichinellosis but low water solubility limits drug absorption. Accordingly, alternative safe and effective drugs are required (Caner et al., 2008; Gottestein et al., 2009; Nada et al., 2018). Several studies have reported the effective use of platelet-rich plasma (PRP) injections for various tendinopathies and muscle injuries. PRP is an autologous blood-derived product with elevated platelet concentrations compared to baseline as well as platelet-related growth factors (Gupta et al., 2012; Seow et al., 2021).

Several in vitro and in vivo studies have recently supported the use of PRP to treat muscle injury. PRP not only contains various cytokines and growth factors that may be beneficial to the healing of injured muscle but also plays a role in responding to pathogens through mechanisms including pattern recognition, early trafficking to sites of infection, direct antimicrobial functions, and potentiation of innate and adaptive immune mechanisms (Tsai et al., 2018; Yeaman, 2019). To our knowledge, the effect of PRP on trichinellosis, particularly in the muscular phase, has not yet been determined. Further studies are needed to assess the potential therapeutic effect of PRP as an alternative or adjuvant therapy.

2. Material & methods

2.1. Ethical considerations

The study protocol was approved by the ethical committee of Kasr Al-Ainy Faculty of Medicine and the Institutional Animal Care and Use Committee (IACUC), Cairo University. All experiments were carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines and approved by the institution for animal ethics concerning care for animals and safe disposal of their wastes at Theodor Bilharz Research Institute (TBRI).

2.2. Experimental animals

The current study was carried out on 70 laboratory-bred albino rats (*Rattus norvegicus*), 12–16 weeks old and weighing 150–200 g. The animals were purchased from the European Country Farms in Egypt and were housed in TBRI throughout the study on a standard diet containing 24% protein, 4% fat, and approximately 4–5% fibre and water at a temperature of 24 °C.

2.3. T. spiralis strain

The *T. spiralis* strain (Istituto Superiore di Sanità code: ISS6158) used in this study was granted by the Department of Medical Parasitology, Tanta Faculty of Medicine. Rats tested by stool examination, to exclude intestinal parasitic infection, were used to propagate *T. spiralis* at the Department of Medical Parasitology, TBRI. The infected rats were killed five weeks postinoculation and the skeletal muscle was removed, chopped and processed by the standard pepsin-HCl artificial digestion method to recover *T. spiralis*

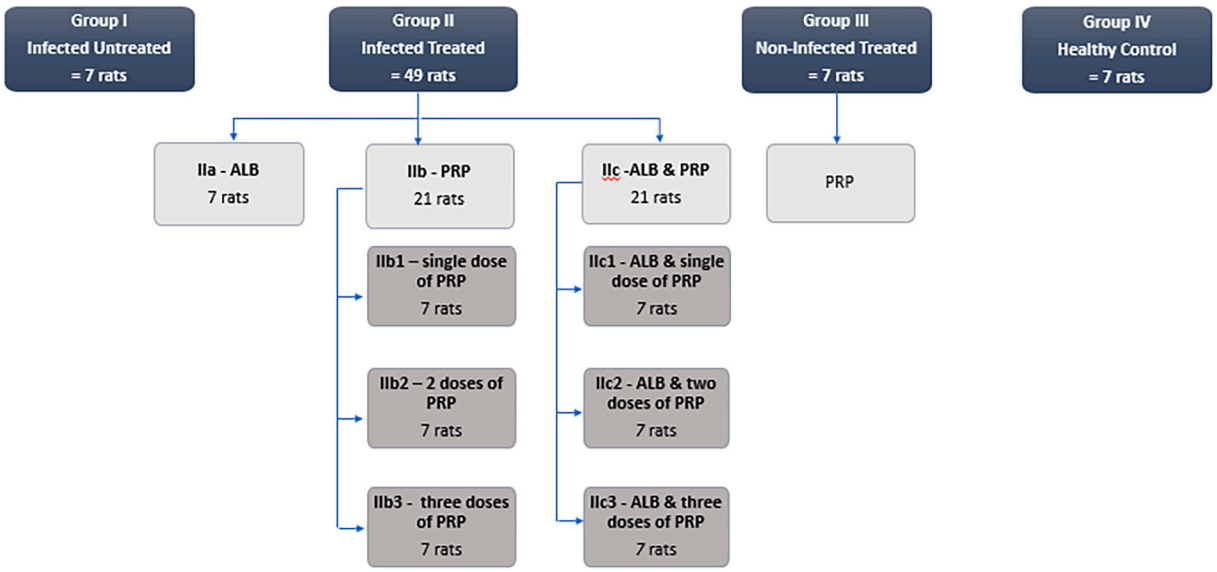

Fig. 1. Experimental design.

muscle larvae (Kapel et al., 2005; Shalaby et al., 2010). The larvae were sieved and washed by repeated sedimentation in distilled water and then counted in a cell counting chamber.

2.4. Experimental design

The sample size was calculated using the G*Power program (University of Düsseldorf, Düsseldorf, Germany), and information reported by Shalaby et al., 2010 justified the use of five rats in each group or subgroup. The 70 albino rats were divided into four main groups. Group I: infected untreated (positive control) rats; Group II: infected treated; Group III: non-infected treated rats; Group IV: non-infected non-treated (negative control) rats (Fig. 1). Each rat in Groups I and II was orally inoculated with 500 *T. spiralis* larvae (Soliman et al., 2011).

2.5. Administration of therapeutic agents

Therapeutic agents were administered 35 days post-inoculation during the chronic phase of the infection, according to Fahmy and Diab (2021). The time of inoculation and therapeutic agent administration are shown in Table 1.

Five weeks post-inoculation, ALB (Bendex) treatment was started. ALB (Bendex) 200 mg tablets manufactured and provided by Sigma-Egypt were used. Tablets were crushed, the powder was weighed and dissolved in distilled water, and the rats were intubated into the oesophagus at 50 mg/kg body weight for five consecutive days (Chung et al., 2001).

PRP was prepared using autologous rat plasma to minimize risk of cross-reaction or immune reactivity (Delos et al., 2014). From each rat, 3.14 mL of blood was transferred into anticoagulated blood sampling tubes with an equal volume of sterile saline injected as a fluid replacement (Messora et al., 2011). PRP was prepared according to Salarinia et al. (2017). The sediment (PRP) volume was adjusted to 0.5 mL with 10% calcium chloride solution and injected intramuscularly into the outer aspect of the right thigh in the same rat (Rtail et al., 2020). PRP was administered immediately after preparation instead of storage and delayed usage to avoid any effects that may result from prolonged storage or freeze/thaw cycles (Delos et al., 2014). PRP was freshly prepared following the above-mentioned procedure for the second and third doses of PRP that were administered to groups IIb2, IIb3, IIc2 and IIc3. Elapsed time between the first and second dose and between the second and third dose was two weeks.

2.6. Euthanasia

All rats were euthanized using intraperitoneal anaesthesia. Rats received anaesthetic–anticoagulant solution (500 mg/kg thiopental and 100 units/mL heparin) by intraperitoneal injection (Liang et al., 1987). Rats in Group IIa were sacrificed 45 days post-inoculation (Shalaby et al., 2010). Two weeks after PRP injection, rats in groups IIb, IIc, and III were sacrificed according to Contreras-Muñoz et al. (2017). Rats in Groups I and IV were sacrificed at the end of the experiment as shown in Table 1.

Samples of the diaphragm muscles, thoracic muscles, and thigh muscles were removed and samples from all groups were histopathologically examined (Shalaby et al., 2010). Additional samples of these three muscles were examined to detect and count *T. spiralis* larvae (Basyoni and El-Sabaa, 2013; Muñoz-Carrillo et al., 2016).

Table 1
The time of inoculation, therapeutic agent administration and euthanasia of all groups.

Groups	Time of inoculation	Time of therapeutic agent administration	Time of euthanasia
Infected untreated (group I)	All rats from all infected group were infected at the same time	Not treated	At the end of the experiment (69 days post infection)
Infected treated with ALB (group IIa)	All rats from all infected group were infected at the same time	Five weeks post infection	5 days post treatment (45 days post infection)
Infected treated with single dose of PRP (group IIb1)	All rats from all infected group were infected at the same time	Five weeks post infection	2 weeks post PRP treatment (49 days post infection).
Infected treated with two doses of PRP (group IIb2)	All rats from all infected group were infected at the same time	Five weeks post infection	2 weeks post second PRP dose (59 days post infection).
Infected treated with three doses of PRP (group IIb3)	All rats from at infected group were infected at the same time	Five weeks post infection	2 weeks post third PRP dose treatment (69 days post infection).
Infected treated with ALB and single dose of PRP (group IIc1)	All rats from all infected group were infected at the same time	Five weeks post infection	2 weeks post treatment (49 days post infection).
Infected treated with ALB and two doses of PRP (group IIc2)	All rats from all infected group were infected at the same time	Five weeks post infection	2 weeks post treatment (59 days post infection).
Infected treated with ALB and three doses of PRP (group IIc3)	All rats from all infected group were infected at the same time	Five weeks post infection	2 weeks post treatment (69 days post infection).
Non-infected treated with PRR (group III)	Not infected	At beginning of the experiment	2 weeks post treatment (49 days post infection).
Healthy control group (group IV)	Not infected	Not treated	At the end of the experiment (69 days post infection).

2.7. Drug effects evaluation

2.7.1. Counting the larvae in muscles

The excised muscle was weighed and digested by pepsin-HCl artificial digestion to release larvae, which were then counted using a stereomicroscope at 40× magnification. The larval load was calculated as the total number of larvae per gram of muscle and compared between groups to determine the percentage of reduction in larval load in the treated group as described by García et al. (2013). The pepsin-HCl artificial digestion method was the method of choice instead of ISO 7218:2007, as it is the standard in validation studies (Wojtkowiak-Giera et al., 2012).

2.7.2. Histopathological examination

Excised diaphragm muscles, thoracic muscles, and thigh muscles from each rat in all groups were fixed and processed by standard histological methods. Sections were stained with haematoxylin and eosin (H&E) to assess inflammatory infiltration by the number of white blood cells and picrosirius red to evaluate the collagen content by the area of new collagen fibres (Rtail et al., 2020). Inflammatory cellular infiltration was split into 5 grades: no inflammation, minimal (1% to 15% increase in inflammatory cellular infiltration), mild (16% to 35% increase in inflammatory cellular infiltration), moderate (36% to 65% increase in inflammatory cellular infiltration) and severe (66% to 100% increase in inflammatory cellular infiltration) compared to the control group. Additionally, fibrosis was split into 5 grades: no fibrosis, minimal (1% to 15% increase in fibrosis), mild (16% to 35% increase in fibrosis), moderate (36% to 65% increase in fibrosis) and severe (66% to 100% increase in fibrosis) compared to the control group.

2.8. Statistical analysis of data

Data were coded and analysed with the statistical analysis software package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were expressed using the mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were made using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables, while nonparametric Kruskal–Wallis test and Mann–Whitney test were used for nonnormally distributed quantitative variables. P values <0.05 were considered statistically significant (Chan, 2003).

3. Results

3.1. Parasitological assessment of treatment groups

3.1.1. The effect of ALB, PRP, and combined ALB/PRP treatments compared to the infected untreated group

No larvae were detected in the non-infected treated control groups in the thigh, thoracic muscles, or diaphragm that were taken after euthanasia and artificially digested. The mean numbers of larvae per gram of muscle in the infected untreated group and all the infected treated groups are shown in Table 2.

As depicted in Table 2, the maximum reduction in the number of larvae was detected in the infected treated group with ALB & three doses of PRP, which was found to be statistically significant. In addition to the infected untreated group, the infected group treated with ALB alone had the highest mean numbers, with a statistically significant difference. It was also noted that the mean numbers of larvae in the infected untreated group were significantly different compared to all infected treated groups. The only exception was with results of diaphragm muscles of the group infected and treated with a single dose of PRP, which had no statistical significance.

In all groups, the mean number of larvae per gram of diaphragm muscle was higher than that in thoracic muscles. The lowest mean numbers of larvae were detected in thigh muscles, with no statistically significant difference compared to the diaphragm and thoracic muscles.

Table 2
Comparison between the infected untreated group and infected treated groups regarding the number of larvae per gram of muscles (LPG) expressed as the mean ± SD.

		Mean number of larvae (± SD) per gram		
		Thigh muscles	Thoracic muscles	Diaphragm
Group I	Infected untreated group	210 ± 25.5	338 ± 54.04	945 ± 33.17
Group IIa	Infected treated with ALB	52.86 ± 19.86*	115.96 ± 15.65 *	306.62 ± 82.56 *
Group IIb1	Infected treated with single dose of PRP	23.01 ± 14.8*	218.82 ± 23.92 *	897.5 ± 162.85
Group IIb2	Infected treated with two doses of PRP	9.76 ± 5.37*	29.74 ± 8.4*	473.06 ± 106.45*
Group IIb3	Infected treated with three doses of PRP	5.5 ± 3.71*	22.44 ± 2.78 *	307.1 ± 92.97 *
Group IIc1	Infected treated with ALB and single dose of PRP	10.27 ± 7.09*	43.95 ± 39.52*	180.31 ± 13.78*
Group IIc2	Infected treated with ALB and two doses of PRP	3.68 ± 2.18*	23.17 ± 9.73*	177.18 ± 37.7*
Group IIc3	Infected treated with ALB and three doses of PRP	2.38 ± 2.3*	12.04 ± 2.75*	91.8 ± 27.33*

p-value < 0.05.
* Statistically significant compared to corresponding value in infected untreated group (Group I).

3.1.2. Reduction in the average number of larvae in muscle samples in the infected treated group, according to the treatment regimen

The percentages of reduction in the mean numbers of larvae among the infected treated group compared to the infected untreated group were calculated and are presented in Table 3 and Fig. 2.

The results revealed that the highest reduction percentages were achieved by combined treatment with ALB & three doses of PRP, as reduction percentages were 98.9% in thigh muscles, 96.4% in thoracic muscles, and 90.3% in muscles of the diaphragm. The reduction percentages in thigh muscles, thoracic muscles, and diaphragm in the ALB and two doses of PRP-infected treated groups were 98.3%, 93.2% & 81.3% in thigh muscles thoracic muscles, and diaphragm, respectively. In contrast, the lowest reduction percentages were in the PRP-infected group's single dose, 89% in thigh muscles, 35.3% in thoracic muscles, and 5% in the diaphragm.

All the above-mentioned differences were statistically significant compared to the control infected group.

3.2. Histopathological assessment of therapeutic effect

In the non-infected non-treated control group (Group IV), histopathological examination of muscle sections revealed normal muscle appearance with regular muscle bundles and peripherally located bland nuclei separated by thin fibrous stroma. In all examined muscles, the histopathology of Group I (the infected untreated rats) revealed moderate inflammatory cellular infiltration with severe fibrosis (Fig. 3). In contrast, no pathological changes were detected in Group III (the non-infected rats treated with PRP), as the muscle sections demonstrated regular bundling with peripherally located bland nuclei that were separated by thin fibrous stroma.

Examination of muscle sections from the infected group treated with ALB (Group IIa) demonstrated moderate inflammatory cellular infiltration and moderate to severe fibrosis with granuloma formation (Fig. 4). Examination of muscle sections from the groups infected with PRP demonstrated mild inflammatory cellular infiltration and moderate fibrosis with granuloma formation in Group IIb1 (infected rats treated with a single dose of PRP), showed mild inflammatory cellular infiltration with mild fibrosis in Group IIb1 (infected rats treated with a single dose of PRP) and mild inflammatory cellular infiltration with minimal fibrosis in Group IIb3 (infected rats treated with three doses of PRP) (Fig. 5).

In contrast, examination of muscle sections from groups that received combined drug therapy of ALB and PRP showed mild inflammatory cellular infiltration with mild fibrosis with granuloma formation in Group IIc1 (infected treated with ALB and a single dose of PRP) and no inflammatory cellular infiltration with minimal fibrosis in both Group IIc2 (infected treated with ALB and two doses of PRP) and Group IIc3 (infected treated with ALB and three doses of PRP) (Fig. 6).

4. Discussion

In experimentally infected rats, two therapeutic agents (ALB and PRP) were tested on the muscular phase of trichinellosis. The effects of these two agents were studied separately and in combination. The number of rats in each group and subgroup increased to seven instead of five rats, as recommended by Shalaby et al. (2010), to account for potential losses during breeding. The therapeutic effect of each treatment model was assessed and included parasitological assessment via mean numbers of larvae per gram of thigh muscles, thoracic muscles, and diaphragm muscles and histopathological assessment for inflammatory infiltration and fibrosis in muscle biopsies from the same muscles.

Regarding the parasitological assessment, the highest larval load was observed in the diaphragm in all infected rats, which aligns with the findings of Kapel et al. (2005), who stated that the highest load of larvae was in the tongue, followed by the diaphragm, and then other striated muscles. They illustrated that predilection sites were determined mainly by host species and the age and level of infection.

All treatment models demonstrated a significant decrease in the mean numbers of *T. spiralis larvae* compared to the infected untreated group but none achieved complete eradication of larvae.

In this study, ALB treatment at a dose of 50 mg/kg/day for five consecutive days showed a 74.83% reduction in the number of larvae in thigh muscles, 65.69% reduction in the number of larvae in thoracic muscles, and 67.55% reduction in the number of larvae in the diaphragm, an overall average reduction of 69.36%, compared to the infected untreated group, with statistically significant differences.

Table 3
Percentages of reduction of larvae in the muscles of all infected treated groups, compared to the infected untreated group.

		Percentage of reduction (%) in			
		Thigh muscles	Thoracic muscles	Diaphragm	Average
Group IIa	Infected treated with ALB	74.83*	65.69*	67.55*	69.36 *
Group IIb1	Infected treated with single dose of PRP	89.04*	35.26*	5.027	43.1 *
Group IIb2	Infected treated with two doses of PRP	95.35*	91.2*	49.94*	78.83 *
Group IIb3	Infected treated with three doses of PRP	97.38*	93.36*	67.5*	86.08 *
Group IIc1	Infected treated with ALB and single dose of PRP	95.11*	87*	80.92*	87.68 *
Group IIc2	Infected treated with ALB and two doses of PRP	98.25*	93.15*	81.25*	90.88 *
Group IIc3	Infected treated with ALB and three doses of PRP	98.87*	96.44*	90.29*	95.2 *

p-value<0.05.

* Statistically significant compared to corresponding value in infected untreated group (Group I).

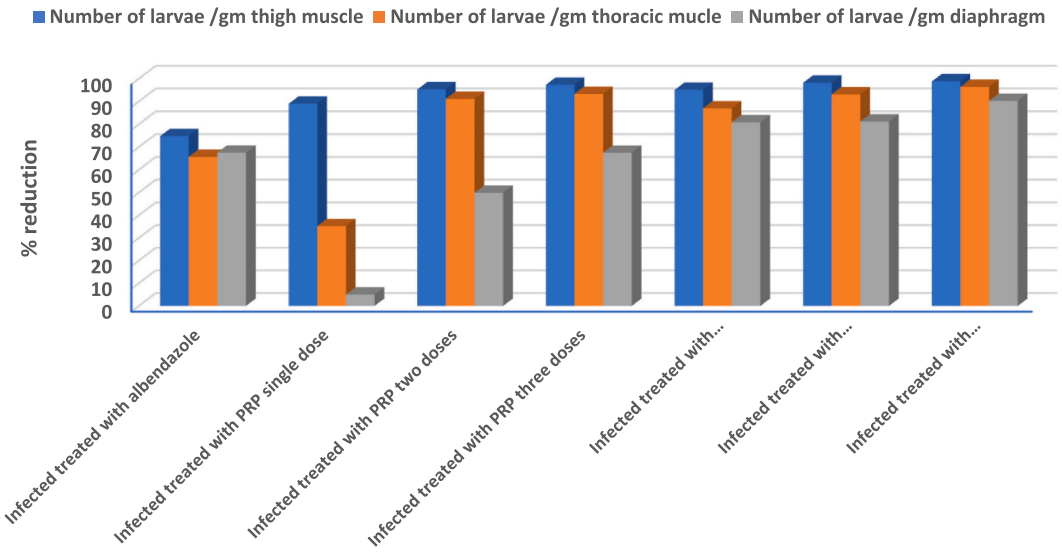

Fig. 2. Percentages of larval reduction expressed as the mean number of larvae per gram of muscle in all infected treated groups.

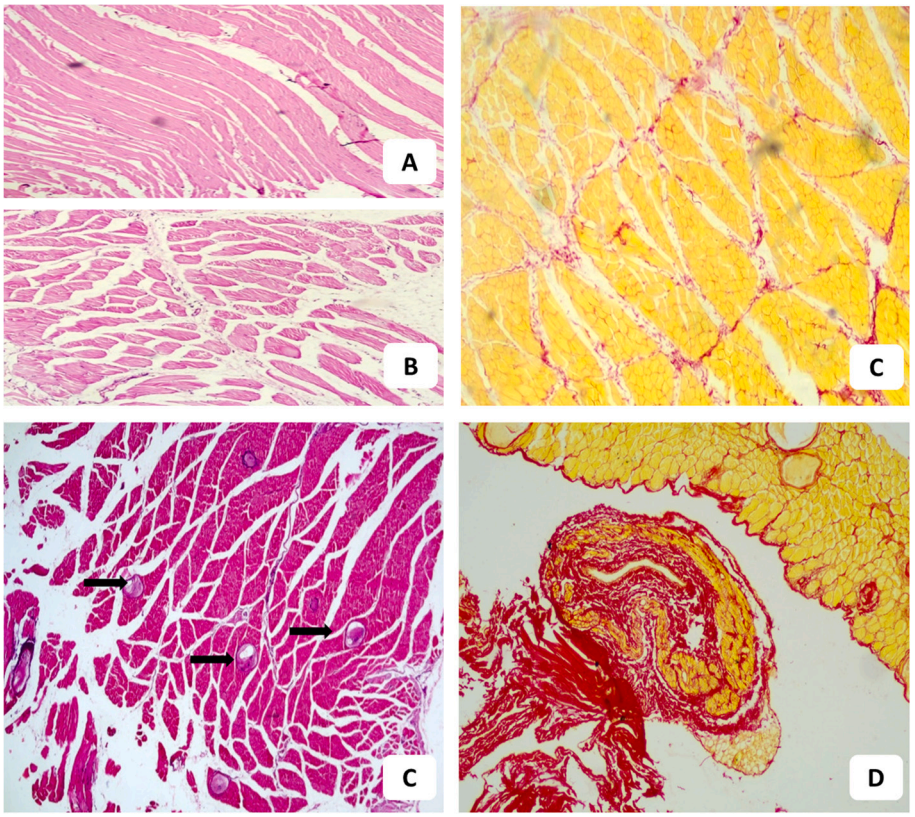

Fig. 3. Skeletal muscle sections from the healthy control group and infected untreated group.
(A (Longitudinal section) & B (Transverse section): H&E, 100×) (C: Picrosirius, 100×) of skeletal muscle section from the healthy control group showing regular muscle bundles with peripherally located bland nuclei separated by thin fibrous stroma.
(C &D): Skeletal muscle section from the infected untreated group showing multiple *T. spiralis* larvae (arrow) associated with moderate inflammatory cellular infiltration (C: H&E, 200×) and severe fibrosis (Red color) (D: Picrosirius, 100×).

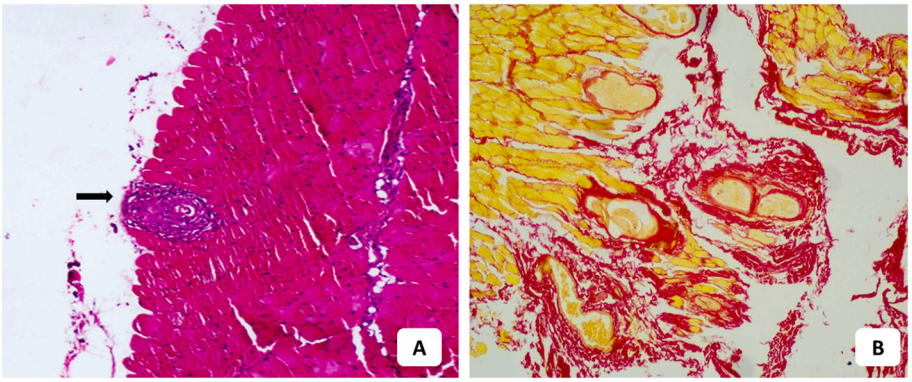

Fig. 4. Skeletal muscle section from the infected group that was treated with ALB.
(A: H&E, 100×) showing moderated inflammatory cellular with granuloma formation (arrow) associated with moderate to severe fibrosis (Red color) (B: Picro-sirius, 200×).

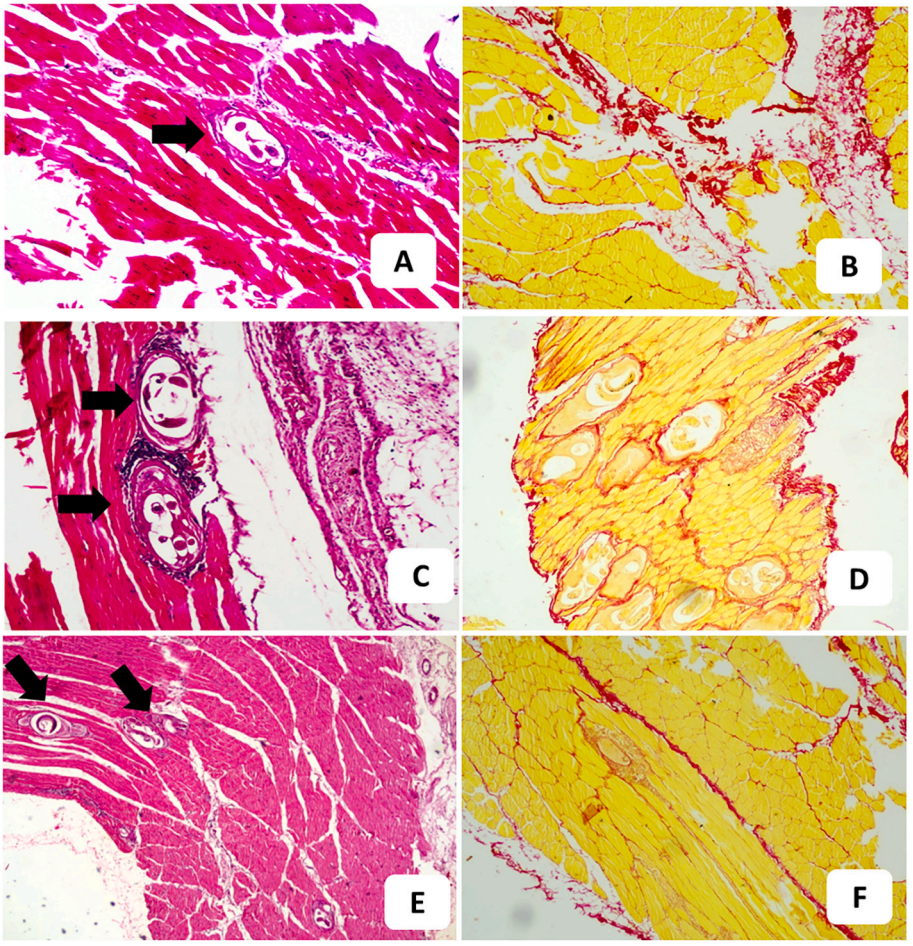

Fig. 5. Skeletal muscle section from the infected group that was treated with PRP.
(A&B): Skeletal muscle section from the infected group that was treated with a single dose of PRP showing a *T. spiralis* larva (arrow) associated with mild inflammatory cellular infiltration (A: H&E, 200×) and moderate fibrosis (Red color) (B: Picrosirius, 200×).
(C&D): Skeletal muscle section from the infected group that was treated with two doses of PRP showing two *T. spiralis* larvae (arrows) associated with mild inflammatory cellular infiltration (C: H&E, 200×) with mild fibrosis (Red color) (D: Picrosirius, 200×). (E &F): Skeletal muscle section from the infected group that was treated with three doses of PRP showed two *T. spiralis* larvae (arrows) associated with mild inflammatory cellular infiltration (E: H&E, 100×) and minimal fibrosis (Red color) (F: Picrosirius, 100×).

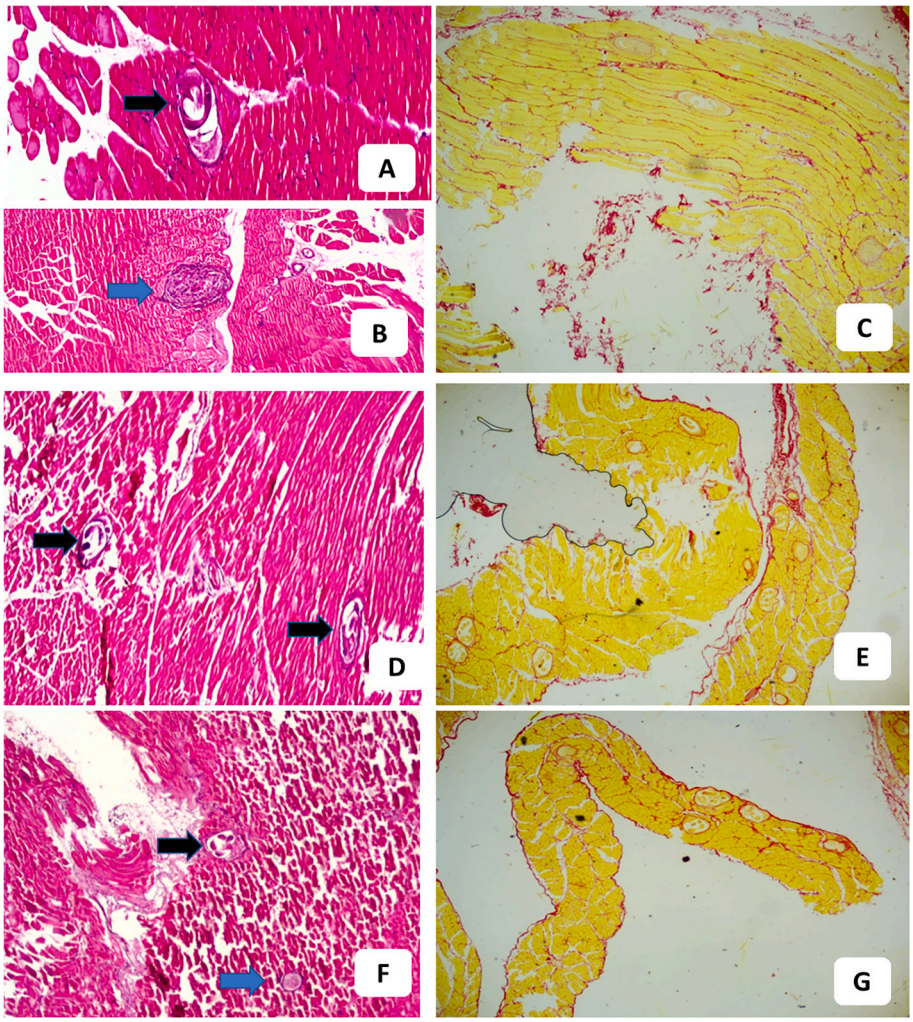

Fig. 6. Skeletal muscle section from the infected group that was treated with combined therapy ALB and PRP.
A, B& C: Skeletal muscle section from the infected group that was treated with ALB and a single dose of PRP showing one viable (black arrow) and one degenerated (blue arrow) *T. spiralis* larvae surrounded with granuloma associated with mild inflammatory cellular infiltration (A: H&E, 200× & B: H&E, 100×) and mild fibrosis (Red color) (C: Picrosirius, 100×).
D&E: Skeletal muscle section from the infected group that was treated with ALB and two doses of PRP showing multiple *T. spiralis* larvae (arrows) with no inflammatory cellular infiltration (D: H&E, 100×) and associated with minimal fibrosis (Red color) (E: Picrosirius, 100×).
F&G: Skeletal muscle section from the infected group that was treated with ALB and three doses of PRP showing one viable (black arrow) and one degenerated (blue arrow) *T. spiralis* larvae with no inflammatory cellular infiltration (F: H&E, 100×) and associated with minimal fibrosis (Red color) (G: Picrosirius, 100×).

Similarly, McCracken (1978) demonstrated that ALB resulted in a 67% reduction when used at a dose of 50 mg/kg/day for five days as treatment for *T. spiralis* infection in mice. Several studies have investigated the efficacy of albendazole treatment. Reduction percentages in the number of *T. spiralis* larvae ranged from 28% to 98% (Bany et al., 1992; Lopez-Garcia et al., 1997; Nassef et al., 2019).

Diversity in ALB treatment protocols, including dosage and period of administration, resulted in diversity in reduction percentages of larvae count. In addition, the efficacy of ALB in treating trichinellosis is strictly related to the administration time. Indeed, it is more effective in the early stages of infection when the worms are still present in the intestinal mucosa or newborn larvae are migrating from intestinal vessels to muscles. Its relatively weaker effect on the extraintestinal phase encountered in this study could be due to its low gastrointestinal absorption and systemic bioavailability, as well as poor tissue levels (Kazura, 2014; Cohen et al., 2017; Long et al., 2017; Priotti et al., 2017).

In the current work, PRP revealed that a single dose of 0.5 mL/kg resulted in 89.04%, 35.26% and 5.027% reductions in the mean numbers of larvae per gram of thigh muscles, thoracic muscles, and diaphragm, respectively, with an average reduction of 43.1%. This reduction percentage increased to 95.35%, 91.2% and 49.94% using double doses on the same muscles, respectively, with an average reduction of 78.83%. However, compared to the infected untreated group, the use of three doses showed high percentages of reduction: 97.38%, 93.36% and 67.5%, with an average of 86.08%, with a statistically significant difference.

These promising PRP treatment results revealed that a single dose of PRP has less effect than ALB therapy. Nonetheless, double doses and three doses have a more significant effect than ALB alone. The use of ALB and three doses of PRP was the most effective therapeutic combination in the current study, with the highest percentage of larval muscle reduction. To our knowledge, this study may be the first to study the effect of PRP on the muscular phase of *T. spiralis* infection.

This promising anti-*Trichinella* effect appears consistent with findings that PRP has antimicrobial properties, particularly when combined with conventional therapy (Andia and Abate, 2013; Zhang et al., 2019). When activated, PRP produces several antimicrobial proteins. These bioactive molecules are responsible for PRP's ability to kill pathogens by interacting with the outer cell membrane and increasing its permeability, thereby influencing protein synthesis. Additionally, these molecules may also target intracellular proteins that affect DNA synthesis or inhibit the activity of enzymes (Yeaman and Bayer, 1999; Tang et al., 2002; Ho et al., 2016; Bechinger and Gorr, 2017; Zhang et al., 2019).

Based on the current study findings, PRP has a synergistic effect with ALB. Similarly, Zhang et al. (2019) stated that PRP displays a synergistic effect with antibiotics, and this unique advantage provides a promising outcome in treating antibiotic-resistant bacteria.

It is important to note that in the current work, all muscles receiving local PRP injection (thigh muscles) demonstrated the highest reduction percentage in both PRP-treated groups and groups of combined therapy. PRP administration as a therapy in Group III (noninfected rats treated with PRP) did not result in any complications. Similarly, Grassi et al. (2018) did not detect any complications of IM injection of PRP aside from discomfort at the injection site, haematoma or hyperesthesia, or pain.

Regarding the histopathological assessment in the present study, examination of muscle sections from non-infected rats showed regular muscle bundles with peripherally located nuclei separated by thin fibrous stroma. Nevertheless, examining muscle sections from infected untreated rats showed moderate inflammatory cellular infiltration with severe fibrosis and heavy invasion with *T. spiralis* encysted larvae.

Similar results were reported by Shalaby et al. (2010), who discovered that muscle sections from infected untreated rats revealed a focus of inflammation surrounding the infected muscle cells. Furthermore, Attia et al. (2015) and Eid et al. (2020) reported that muscle sections from infected untreated mice showed massive inflammatory cellular infiltration around larvae. Li and Ko (2001) explained the variable histopathological findings with different cellular responses due to using different strains of mice during the muscular phase of *T. spiralis.* This finding may explain the diversity in the degree of inflammatory infiltration between the current study and other studies.

In the current work, ALB treatment relatively improved the pathological findings compared to the infected untreated group, as an examination of muscle sections from ALB-treated rats showed moderate inflammatory cellular infiltration and moderate to severe fibrosis with granuloma formation. Likewise, Attia et al. (2015) and Eid et al. (2020) stated that muscles from ALB-treated mice showed massive inflammatory infiltration. Similarly, Nassef et al. (2019) reported that ALB-treated mice showed inflammatory cellular infiltrate surrounding the larvae, which is consistent with the findings of Salama et al. (2022). In contrast, the muscle sections from mice treated with ALB and prednisolone showed mild to moderate inflammatory infiltration around larvae.

Conversely, Shalaby et al. (2010) previously stated that ALB-treated rats showed only a few local inflammatory infiltrations in the examined muscle sections compared to the infected untreated group in their experimental study. Likewise, Nada et al. (2018) reported that muscle sections from mice treated with ALB showed mild inflammatory infiltrate around larvae in their study.

Similarly, Huang et al. (2020) illustrated that inflammatory cellular infiltration around the larvae in ALB-treated mice was significantly reduced, in addition to the pathological damage of muscle cells compared to the infected untreated control group.

Concerning PRP therapy, the current study results revealed that PRP significantly improved the degree of inflammation and reduced fibrosis. Examination of muscle sections from rats receiving a single dose of PRP showed mild inflammatory cellular infiltration and moderate fibrosis. The muscle sections from rats receiving two doses of PRP showed mild inflammatory cellular infiltration with mild fibrosis. In contrast, the muscle sections of rats receiving three doses of PRP showed mild inflammatory cellular infiltration with minimal fibrosis. This anti-inflammatory and antifibrotic effect of PRP is probably due to the release of various growth factors upon platelet activation, acting as anti-inflammatory agents by blocking the production of monocyte chemotactic protein-1 (MCP-1) and decreasing fibrosis by suppressing collagen production, as reported by El-Sharkawy et al. (2007) and Kodama et al. (2010).

Regarding the combined therapy, the results of this study demonstrated that the use of ALB combined with PRP resulted in substantial improvement in inflammation and fibrosis, which was established in the muscle sections of rats receiving a single dose of PRP and ALB showing mild inflammatory cellular infiltration with mild fibrosis. Furthermore, muscle sections from rats receiving ALB with two or three doses of PRP demonstrated no inflammatory cellular infiltration with minimal fibrosis.

In addition, it is noteworthy that PRP alone was more effective than ALB alone. Muscle sections from PRP-treated groups showed minimal inflammatory cellular infiltration with minimal fibrosis, and those treated with ALB demonstrated moderate inflammatory infiltration and moderate to severe fibrosis.

However, to our knowledge, no previous studies have investigated the histopathological effects of PRP against *T. spiralis* infection. Nevertheless, El-Aswad et al. (2018) stated that PRP has an anti-fibrotic effect against *S. mansoni*-induced liver fibrosis in experimentally infected mice, associated with improving liver enzymes, particularly when combined with praziquantel. In addition, they recommended combining praziquantel and PRP as a therapy for treating *S. mansoni* liver fibrosis and confirmed its applicability in the case of human liver fibrosis caused by schistosomiasis *mansoni.*

Finally, the histopathological assessment results in the current study are consistent with the parasitological assessment results, as the groups treated with combined ALB and PRP demonstrated significant improvement in inflammation and fibrosis.

In conclusion, the present study revealed that PRP has a modulatory effect on the pathogenesis effects caused by *T. spiralis* larvae on the muscular phase of trichinellosis in experimentally infected rats, which was evident with two & three doses of PRP, proving it more effective than conventional ALB treatment. However, this was not observed with a single PRP dose. Various studies using more or higher doses of PRP are recommended to investigate its utmost effective treatment potential as a single therapy for the muscular phase

of trichinellosis.

Furthermore, when compared to using each PRP and ALB therapy separately, the combined PRP and ALB therapy resulted in the highest percentages of larval reduction with significant improvement in inflammation and fibrosis. Therefore, this combination may be a very promising treatment option, given their synergistic effect. Additional studies with different PRP and ALB doses are recommended to achieve the best treatment outcomes.

Finally, even though PRP was injected intramuscularly into the thigh muscles, there was a reduction in the mean number of larvae in the thoracic and diaphragm muscles, indicating a systemic effect of PRP therapy. Hence, studies investigating the potential therapeutic effect of PRP in the migratory phase of *T. spiralis* may be recommended.

The current study expands the therapeutic use of PRP alone and in combination with the standard chemotherapeutic agents currently used to treat *T. spiralis* infection, potentially as a treatment for other parasitic diseases, particularly those affecting the muscles.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank the Medical Parasitology Department, Faculty of Medicine, Tanta University, for granting the *T. spiralis* strain used in this study.

References

Andia, Abate, 2013. Platelet-rich plasma: underlying biology and clinical correlates. Regen. Med. 8 (5), 645–658. https://doi.org/10.2217/rme.13.59.

Attia, et al., 2015. Effect of myrrh and thyme on *Trichinella spiralis* enteral and parenteral phases with inducible nitric oxide expression in mice. Mem. Inst. Oswaldo Cruz 110 (8), 1035–1041. https://doi.org/10.1590/0074-02760150295.

Bany, et al., 1992. Efficacy of albendazole against *Trichinella pseudospiralis* and *Trichinella spiralis* in mice. Wiad. Parazytol. 38 (3&4), 143–146.

Basso, et al., 2022. Evaluation of the PrioCHECK™ Trichinella AAD kit to detect Trichinella spiralis, T. britovi, and T. pseudospiralis larvae in pork using the automated digestion method Trichomatic-35. Parasitol. Int. 86, 102449. https://doi.org/10.1016/j.parint.2021.102449, 2022 Feb. Epub 2021 Sep 2. PMID: 34481946.

Basyoni, El-Sabaa, 2013. Therapeutic potential of myrrh and ivermectin against experimental *Trichinella spiralis* infection in mice. Korean J. Parasitol. 51 (3), 297. https://doi.org/10.3347/kjp.2013.51.3.297.

Bechinger, Gorr, 2017. Antimicrobial peptides: mechanisms of action and resistance. J. Dent. Res. 96 (3), 254–260. https://doi.org/10.1177/0022034516679973.

Bruschi, Chiumiento, 2011. *Trichinella* inflammatory myopathy: host or parasite strategy? Parasit. Vectors 4 (1), 42. https://doi.org/10.1186/1756-3305-4-42.

Bruschi, Dupouy-camet, 2014. Trichinellosis. In: Bruschi, F. (Ed.), Helminth Infections and Their Impact on Global Public Health. Springer, pp. 229–273. https://doi.org/10.1093/cid/ciu891.

Caner, et al., 2008. Comparison of the effects of Artemisia vulgaris and Artemisia absinthium growing in western Anatolia against trichinellosis (*Trichinella spiralis)* in rats. Exp. Parasitol. 119 (1), 173–179. https://doi.org/10.1016/j.exppara.2008.01.012.

Chan, 2003. Biostatistics102: quantitative data – parametric & non-parametric tests. Singap. Med. J. 44 (8), 391–396.

Chung, et al., 2001. Efficacy of flubendazole and albendazole against *Trichinella spiralis* in mice. Parasite 8 (2001), 195–198. https://doi.org/10.1051/parasite/200108s2195.

Cohen, et al., 2017. Infectious Diseases, Fourth edition. Elseiver Health Sciences.

Contreras-Muñoz, et al., 2017. Postinjury exercise and platelet-rich plasma therapies improve skeletal muscle healing in rats but are not synergistic when combined. Am. J. Sports Med. 45 (9), 2131–2141. https://doi.org/10.1177/0363546517702864.

Delos, et al., 2014. The effect of platelet-rich plasma on muscle contusion healing in a rat model. Am. J. Sports Med. 42 (9), 2067–2074. https://doi.org/10.1177/0363546514540272.

Eid, et al., 2020. Chitosan coated nanostructured lipid carriers for enhanced in vivo efficacy of albendazole against *Trichinella spiralis*. Carbohydr. Polym. 232, 115826. https://doi.org/10.1016/j.carbpol.2019.115826.

El-Aswad, et al., 2018. Human platelet rich plasma alleviates liver fibrosis in murine *Schistosomiasis Mansoni*. Med. J. Cairo Univ. 86 (2018), 3807–3823. https://doi.org/10.21608/mjcu.2018.61850.

El-Sharkawy, et al., 2007. Platelet-rich plasma: growth factors and pro-and anti-inflammatory properties. J. Periodontol. 78 (4), 661–669. https://doi.org/10.1902/jop.2007.060302.

Fabre, et al., 2009. Immunity to *Trichinella spiralis* muscle infection. Vet. Parasitol. 159 (3&4), 245–248. https://doi.org/10.1016/j.vetpar.2008.10.051.

Fahmy, Diab, 2021. Therapeutic efficacy of albendazole and mefloquine alone or in combination against early and late stages of *Trichinella spiralis* infection in mice. Helminthologia 58 (2), 179–187, 8 Jun. 2021. https://doi.org/10.2478/helm-2021-0016.

García, et al., 2013. Novel albendazole formulations given during the intestinal phase of *Trichinella spiralis* infection reduce effectively parasitic muscle burden in mice. Parasitol. Int. 62 (6), 568–570. https://doi.org/10.1016/j.parint.2013.08.009.

Gottestein, et al., 2009. Epiolemiology, diagnosis, treatment and control of trichinellosis. Clin. Microbiol. Rev. 22 (1), 127–145. https://doi.org/10.1128/CMR.00026-08.

Grassi, et al., 2018. Is platelet-rich plasma (PRP) effective in the treatment of acute muscle injuries? A systematic review and meta-analysis. Sports Med. 48 (4), 971–989. https://doi.org/10.1007/s40279-018-0860-1.

Gupta, et al., 2012. Evidence-based indications of platelet-rich plasma therapy. Expert. Rev. Hematol. 2021 https://doi.org/10.1080/17474086.2021.1860002.

Han, et al., 2019. Immunomodulatory effects of *Trichinella spiralis* excretory-secretory antigens on macrophages. Exp. Parasitol. 196, 68–72. https://doi.org/10.1016/j.exppara.2018.10.001.

Ho, et al., 2016. Systematic analysis of intracellular-targeting antimicrobial peptides, bactenecin 7, hybrid of pleurocidin and dermaseptin, proline-arginine-rich peptide, and lactoferricin B, by using Escherichia coli proteome microarrays. Mol. Cell. Proteomics 15 (6), 1837–1847. https://doi.org/10.1074/mcp.M115.054999.

Huang, et al., 2020. Sanguinarine has anthelmintic activity against the enteral and parenteral phases of *Trichinella* infection in experimentally infected mice. Acta Trop. 201 (2020), 105226. https://doi.org/10.1016/j.actatropica.2019.105226.

Kapel, et al., 2005. Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. Vet. Parasitol. 132 (1&2), 101–105. https://doi.org/10.1016/j.vetpar.2005.05.036.

Kazura, 2014. Tissue nematodes. In: Bennett, J.E., Dolin, R., Blaser, M.J. (Eds.), Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 8th edition. Elsevier Health Sciences, p. 3208.
Kodama, et al., 2010. Thrombocytopenia exacerbates cholestasis-induced liver fibrosis in mice. Gastroenterology 138 (7), 2487–2498. https://doi.org/10.1053/j.gastro.2010.02.054.
Li, Ko, 2001. Inflammatory response during the muscle phase of *Trichinella spiralis* and *T. pseudospiralis* infections. Parasitol. Res. 87 (9), 708–714. https://doi.org/10.1007/s004360100420.
Liang, et al., 1987. Proceeding of the First Sino- American Symposium, 1, pp. 34–48.
Long, et al., 2017. Principles and Practice of Pediatric Infectious Diseases E-Book. Elsevier Health Sciences. https://doi.org/10.1001/jama.1997.03550180093048.
Lopez-Garcia, et al., 1997. Albendazole versus ricobendazole (albendazole-sulphoxide) against enteral and parenteral stages of *Trichinella spiralis* in mice. Int. J. Parasitol. 27 (7), 781–785. https://doi.org/10.1016/s0020-7519(97)00052-0.
McCracken, 1978. Efficacy of mebendazole and albendazole against *Trichinella spiralis* in mice. J. Parasitol. 214–219. https://doi.org/10.2307/3279659.
Messora, et al., 2011. A standardized research protocol for platelet-rich plasma (PRP) preparation in rats. RSBO Revista Sul-Brasileira de Odontol. 8 (3), 299–304.
Muñoz-Carrillo, et al., 2016. Current aspects in trichinellosis. Parasit. Parasit. Dis. 65–87. https://doi.org/10.5772/intechopen.80372.
Nada, et al., 2018. Therapeutic effect of *Nigella Sativa* and ivermectin versus albendazole on experimental trichinellosis in mice. J. Egypt. Soc. Parasitol. 48 (1), 85–92. https://doi.org/10.21608/jesp.2018.77029.
Nassef, et al., 2019. Therapeutic efficacy of chitosan nanoparticles loaded with albendazole on parenteral phase of experimental trichinellosis. J. Egypt. Soc. Parasitol. 49 (2), 301–311. https://doi.org/10.21608/jesp.2019.68134.
Priotti, et al., 2017. Albendazole microcrystal formulations based on chitosan and cellulose derivatives: physicochemical characterization and *in vitro* parasiticidal activity in *Trichinella spiralis* adult worms. J. Am. Assoc. Pharm. Sci. 18 (4), 947–956. https://doi.org/10.1208/s12249-016-0659-z.
Rtail, et al., 2020. Improvement of skeletal muscle regeneration by platelet-rich plasma in rats with experimental chronic hyperglycemia. Biomed. Res. Int. https://doi.org/10.1155/2020/6980607.
Salama, et al., 2022. The curative effect of zingiber officinale and cinnamomum zeylanicum extracts on experimental *Trichinella spiralis*. J. Parasit. Dis. 46 (1), 24–36. https://doi.org/10.1007/s12639-021-01412-y (2022) Mar. Epub 2021 Jun 19. PMID: 35299906; PMCID: PMC8901936.
Salarinia, et al., 2017. Platelet rich plasma: effective treatment for repairing of spinal cord injury in rat. Acta Orthop. Traumatol. Turc. 51 (3), 254–257. https://doi.org/10.1016/j.aott.2017.02.009.
Seow, et al., 2021. Platelet-rich plasma injection for the treatment of hamstring injuries: a systematic review and meta-analysis with best-worst case analysis. Am. J. Sports Med. 49 (2), 529–537. https://doi.org/10.1177/0363546520916729.
Shalaby, et al., 2010. Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. Parasitol. Res. 107 (1), 17–25. https://doi.org/10.1007/s00436-010-1827-9.
Soliman, et al., 2011. Therapeutic efficacy of dormectin, ivermectin and levamisole against different stages of *Trichinella spiralis* in rats. Türkiye Parazitolojii Dergisi 35 (2), 86. https://doi.org/10.5152/tpd.2011.22.
Tang, et al., 2002. Antimicrobial peptides from human platelets. Infect. Immun. 70 (12), 6524–6533. https://doi.org/10.1128/IAI.70.12.6524-6533.2002.
Tsai, et al., 2018. Platelet-rich plasma releasate promotes regeneration and decreases inflammation and apoptosis of injured skeletal muscle. Am. J. Sports Med. 46 (8), 1980–1986. https://doi.org/10.1177/0363546518771076.
Wojtkowiak-Giera, et al., 2012. Trichinellosis in mice: effect of albendazole on the glutathione transferase in the intestines. Folia Parasitol. 59 (4), 311.
Xu, et al., 2021. The immune protection induced by a serine protease from the *Trichinella spiralis* adult against *Trichinella spiralis* infection in pigs. PLoS Negl. Trop. Dis. 15 (5), e0009408 https://doi.org/10.1371/journal.pntd.0009408.
Yeaman, 2019. The role of platelets in antimicrobial host defense. Platelets 523–546. https://doi.org/10.1016/B978-0-12-813456-6.00029-1.
Yeaman, Bayer, 1999. Antimicrobial peptides from platelets. Drug Resist. Updat. 2 (2), 116–126. https://doi.org/10.1054/drup.1999.0069.
Zhang, et al., 2019. Platelet-rich plasma for the treatment of tissue infection: preparation and clinical evaluation. Tissue Eng. B Rev. 25 (3), 225–236. https://doi.org/10.1089/ten.teb.2018.0309.