Journal of the Egyptian Society of Parasitology, Vol. 53, No.3, December 2023
J. Egypt. Soc. Parasitol. (JESP), 53(3), 2023: 559 – 568
Online: 2090-2549

EFFECTS OF INTRAMUSCULAR INJECTION OF PLATELET-RICH PLASMA ON LARVAL BURDEN OF *TRICHINELLA SPIRALIS* IN EXPERIMENTALLY INFECTED MICE

By
FATMA H. SHALAAN[1*], TAHANY A. A. AL-ATTAR[1], SHEREEN F. MAHMOUD[2], HUDA IBRAHIM ABD-ELHAFIZ[3], YOUSRA A. AL-GHALBAN[4] AND MONA F. FAHEEM[1,5***]**

[1]Department of Medical Parasitology, [2]Department of Pathology, [3]Department of Clinical Pharmacology, and [4]Department of Public Health and Community Medicine, Faculty of Medicine, Menoufia University, Menoufia and [5]Department of Basic Medical Sciences, Faculty of Medicine, King Salman International University, South Sinai Governorate, Egypt

(Correspondence: *dr.fatma.shaalan@gmail.com,ORCID:https://orcid.org/0000-000 2-2920-3018,**tahany.alattar.12@med.menofia.edu.eg, ORCID: https://orcid.org/000 0-0003-1758-5036mona.fahem.12@med.menofia.edu.eg ORCID: https://orcid.org/ 0000-0001-5713-9167)

Abstract

Trichinellosis (= trichinosis) is widespread food-borne zoonotic parasitosis caused by the nematode *Trichinella spiralis* triggering skeletal muscle injury. This study identified the therapeutic effects of platelet-rich plasma (PRP) as a safe choice in muscular phase of mice experimental infected with *T. spiralis*. Ninety male mice were divided into three main groups, GI: Negative control, GII: Positive control and GIII: *Trichinella* infected PRP treated. All mice were sacrificed on 14th day post infection (initiation of muscle cell invasion), on 21st day (early stage of nurse cell formation), on 28th day (formation of most nurse cells was completed), and on 35th day PI (convalescent period). The parasitological examination showed a significant reduction in the number of larvae detected in the infected PRP-treated ones 35th PI. Histopathological and immunohistochemical of vascular endothelial growth factor, caspase 3 analyses coincided with parasitological findings, on 35th day PI as well. There was a significant improvement in the in-flammatory cells with fewer muscle fibers degradation, indicating that PRP injection in trichinellosis muscular phase improved inflammatory reaction and reduce *T. spiralis* larval burden.

Keywords: Trichinellosis, Platelet-rich plasma, Vascular endothelial growth factor, Caspase 3.

Introduction

Trichinellosis is a worldwide food-borne disease caused by *Trichinella* species mainly *spiralis*, man can be infected by eating raw or undercooked meat from wild game, such as bear, or pork products containing encysted larvae (Eissa *et al,* 2022), but occasional infection was acquired by ingestion of reptile meat, such as lizards and turtles (Lo *et al*, 2009). In Egypt, trichinellosis (trichinosis) was occasionally reported in man, but only the domestic *T. spiralis* infection was reported in pigs, cats, rodents, and wild carnivores (Morsy *et al,* 2022). In human`s stomach, larvae were released by the host digestive processes, then enter the small intestine and burrow into the lamina propria of the villi in jejunum and ileum where they undergo four molts to reach the adult stage. Females are 2.2 mm in length; males 1.2 mm live in the small bowel is about four weeks. After 1 week, females release larvae that migrate to striated muscl-es where they encyst (Farid *et al,* 2019). *T. spiralis* heavy infection can cause gastroent-eritis resembling botulism (El-Bahnasawy *et al,* 2014). Nausea, diarrhea, vomiting, fatig-ue, fever, and abdominal discomfort are the first symptoms, also headaches, fevers, chil- ls, cough; face and eyes swelling, aching jo-ints and muscle pains, itchy skin, diarrhea, or constipation may follow the first sympto- ms (Gottstein *et al,* 2009).

The hosts' immune systems dramatically react to this parasitic disease. *Trichinella* infection results in a mixed Th1/Th2 im-

mune response with a Th2 immune response dominating during the intestinal period (Liao *et al,* 2018). This immune profile continues throughout the muscle phase along with an increase in regulatory T-cells (El-Aswad *et al,* 2020). Vascular endothelial growth factor (VEGF) is an angiogenesis factor that has crucial effects during inflammatory reaction (Shibuya, 2011). Moreover, VEGF is needed for healthy endothelium and myofibril tissues (Tang *et al,* 2004).

Benzimidazole derivatives, such as albendazole and mebendazole are used widely to treat human trichinellosis (Dea-Ayuela *et al,* 2015). Due to their poor water solubility and poor bioavailability, these medications were ineffective against the encapsulated larvae located along the striated muscles (Solana *et al,* 2009). Thus, new anti-*Trichinella* treatment must be developed (Yadav and Temjenmongla, 2012). Numerous medicinal plants and herbs have undergone experimental testing against trichinellosis, including leaf extracts of *Lasia spinosa* and *Artemisia absinthium* (Garcia *et al,* 2015).

Platelet-rich plasma (PRP) is a mixture composed of a high percentage of platelets in a little plasma contains a lot of growth factors capable to stimulate angiogenesis and differentiation of fibroblasts, like platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) insulin-like growth factor (IGF) and platelet-derived angiogenic factor (PDAF) (El-Kholy *et al,* 2021).

This study aimed to evaluate the efficacy of Platelet-Rich Plasma (PRP) on muscular larvae of *T. spiralis* experimentally infected male mice by parasitological, histopathological, and immunohistochemical parameters to determine its therapeutic impact.

Material and Methods

Ethical considerations: All the procedures were approved by Faculty of Medicine, Menoufia University Ethical Committee's Guidelines (IRB number 5/ 2023PARA 20) as well as the National and International Valid Guidelines of Theodore Bilharz Research Institute (TBRI). These agreed with the Helsinki Declaration Guidelines (2008).

Animals and infection: Clean laboratory bred 90 Swiss Albino male mice (7-8 weeks old & 18-20g weight) parasitic-free were used. Mice were cages and allowed normal diet and tap water. *Trichinella spiralis* larvae were obtained from infected pork from Cairo Governoment- al Slaughtered House, and kept alive in TBRI animal house by repeated passages in mice. Infective larvae were extracted from mice on 35th day post-infection (dpi). The mice were dissected out infected muscles were divided into minute pieces and digested in a 6% pepsin-HCl solution overnight at 37°C with continuous stirring. The recovered larvae were washed several times with sterile PBS and given orally as 200 larvae per mouse (Li *et al,* 2010).

Platelet-rich Plasma (PRP): Mice were anesthetized and blood was aseptically drawn from mouse's retro-orbital veins into centrifuged contained PBS (3.8%). Blood was centrifuged at 1000 rpm for 15min at 20°C, and plasma was centrifuged at 3000rpm for 10min. at 20°C to obtain the platelets were dissolved in PBS, pooled, and incubated at room temperature for 30min, on a rotating platform to eliminate platelet agglomerates. Platelets were counted by Sysmex KX-21 (Japan), as platelet 600×10^3/µl (Xie *et al,* 2013). PRP was freshly prepared each time and used within an hour and injected intramuscular injection a dose of 200µl for each mouse twice a week from the first day of infection (Kwon *et al,* 2012).

Experimental groups: Ninety mice were divided into three groups. G1: 10 mice uninfected control or negative control, GII: 40 mice *T. spiralis* infected, but not treated (Positive control). GIII: 40 mice *T. spiralis* infected and treated with PRP. Both GII &GIII were sacrificed at the same intervals as 10 mice in each time. GIIa & GIIIa were sacrificed on day 14th PI, GIIb & GIIIb were sacrificed on day 21st PI, GIIc & GIIIc were sacrificed on day 28th PI, and GIId & GIIId

were sacrificed on day 35th PI. At each time, mice of scarification muscles were dissected out from diaphragm, tongue, triceps, biceps brachialis, and quadriceps femoris. A portion of each was then fixed with 10% neutral buffered formaldehyde for histopathological and immunohistochemical analyses. Another portion was subjected to artificial digestion in order to measure the number of larvae.

Histopathological examination: Formalin-fixed muscles were processed paraffin-embedding, sectioned 4μm thick, stained with hematoxylin and eosin (H&E), and microscopically examined.

Immunohistochemical to detect vascular endothelial growth factor (VEGF) and caspase-3: After the manufacturer's instructions, immunohistochemical staining using avidin-biotin immune-peroxidase complex technique was used to examine both VEGF expression and caspase-3 in muscle tissues. Paraffin blocks' portions were mounted on clean slides and left overnight. By heating to 100°C, endogenous peroxidase activity was inhibited. Slides were placed in citrate buffer (pH 6.0) for antigen recovery. The primary polyclonal rabbit anti-mouse antibody for VEGF (Santa Cruz Biotechnology, Santa Cruz, CA, as 1:400) or primary polyclonal rabbit anti-mouse antibody for caspase-3 (Lab Vision, Goteborg, Sweden, as 1:200) was applied to slides and incubated overnight at 4°C. Anti-mouse biotinylated secondary antibody was applied to slides. Horseradish peroxidase solution complexes with avidin-biotin were added. The counterstaining with Meyer's hematoxylin, 3, 3' diaminobenzidinetetrahydrochloride (DAB) chromogen was placed onto the slides. After preparing negative control slides using the same procedure without the primary antibodies, all slides were microscopically examined

Immunohistochemical to evaluate VEGF and caspase-3**:** Both were semi-quantitatively evaluated using the immunohistochemistry score (IHS) by examining ten high-power fields (x400) of each slide. Cells with cytoplasmic brown staining for both proteins were considered positive. Combining the quantity score (positively stained cells %) with staining intensity score produced the average IHS. The quantity score varied from 0 to 4 (zero= no immunostaining; 1= 1-10% of cells positively stained; 2= 11-50% positively stained; 3= 51-80% positively stained; and 4 = >81% positively stained). From 0 to 3, staining intensity was graded (where 0= negative, 1= mild, 2= moderate, and 3= strong). The IHS ranged from 0 to 12 was calculated by multiplying quantity score by staining intensity score. IHS of 9-12= strong immunoreactivity (+3), 5-8=moderate (+2), 1-4=mild (+1), & 0= negative

Statistical analysis: Data were collected, tabulated, and analyzed using an IBM compatible personal computer with Statistical Package for the Social Science (SPSS) version 26. Qualitative data were expressed as number (N) and percentage (%). Chi- square test examined the significance for qualitative variables. Quantitative data were expressed as mean ($\bar{x}$) and standard deviation (SD). Student’s t- test was utilized to compare between two groups having quantitative parametric variables. Probability of error P value > 0.05 was not significant, P ≤0.05 was significant, and P ≤0.001 was highly significant.

Results

No larvae were detected in any muscle of GI mice taken after scarification. But, there were mean numbers of larvae per gram of muscle in subgroups of GII sub-groups and all GIII, with reduction percentages in the mean numbers of the larvae when compared to the corresponding mice sub-groups sacrificed at the same times. Highly significant (p= <0.001) reduction was in infected PRP-treated SG on 35th day PI.

GI muscular histology was normal. At 14th dpi, skeletal muscle tissues of both GII & GIII were more or less normal. On day 2st pi, GII showed significant muscle damage in vicinity of numerous *T. spiralis* larvae, but GIII showed the less larval number and the

muscles showed fewer signs of damage. On day 28th pi, GII showed an increasing number of larvae formation, and most nurse cells was completely development, with muscle regeneration and the majority of muscle fibers were normal, but GIII showed lower larvae numbers with dense lymphocytic infiltrate and better muscle regenerations. On the day35th pi, GII showed same results as in 28th dpi, but GIII showed few larval number with marked inflammatory reactions and nearly completed muscle regeneration with high significant differences (P<0.001) between both GII & GIII.

Immunohistochemical staining showed negative VEGF expression on day 14th pi in GII & GIII and skeletal muscles of GII & GIII at 21st dpi mildly stained (+1) with VEGF. On day 28th pi, GII still ex-pressed VEGF at a mild (+1) level, but GIII showed strong (+3) expression of VEGF. The same pattern of protein expression continued on day 35th pi in GII and GIII still showed strong (+3) VEGF expression. Regarding the caspase-3 protein expression, at 14th dpi, both GII & GIII didn't show any evidence of caspase-3 protein. Brown cytoplasmic expression of caspase-3 was mild (+1) in GII, but moderate caspase-3 expression (+2) in GIII on day 21th pi.

Enzyme was in cytoplasm of surrounding infiltrating inflammatory cells and in nurse cells. GII on day 28th pi showed mild (+1) caspase-3 cytoplasmic expression in cellar cytoplasm, but GIII showed strong expression (+3). On day 35th pi, GII showed weak (+1) expression of caspase-3, but GIII showed strong expression (+3) of the same protein.

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

Table 1: Comparison between GII & GIII as to larval number per gram of muscles.

Positive control (GII)	No larvae	PRP treated (GIII)	No larvae	Reduction	Significance	P value
14th	0±0	14th	0±0	-		p= --
21st	25.1±2.76	21st	18.5±3.3	26.29 %	*t* test = 4.84	<0.001
28th	67.4±3.13	28th	32.7±1.8	51.45%	*t* test = 29.9	<0.001
35th	98.2±2.9	35th	26.1±2.07	73.42%	*t* test = 45.4	<0.001

Table 2: Immunohistochemical expression of VEGF between GII & GIII.

Day PI	Expression degree	Positive control (GII)		PRP treated (GIII)		Significance test	P value
		No	%	No	%		
14	- Negative	10	100	10	100	X^2=----	
	- Mild	0	0	0	0		P=---
	- Moderate	0	0	0	0		
	- Severe	0	0	0	0		
21	- Negative	1	10	0	0	X^2=1.05	
	- Mild	9	90	10	100		> 0.05
	- Moderate	0	0	0	0		
	- Severe	0	0	0	0		
28	- Negative	0	0	0	0	X^2=16.4	
	- Mild	9	90	1	10		<0.001
	- Moderate	1	10	0	0		
	- Severe	0	0	9	90		
35	- Negative	0	0	0	0	X^2=20	
	- Mild	10	100	0	0		<0.001
	- Moderate	0	0	0	0		
	- Severe	0	0	10	100		

Table 3: Immunohistochemical expression of Caspase 3 between GII & GIII.

Day PI	Expression degree	Positive control (GII)		PRP treated (GIII)		Significance test	P value
		No	%	No	%		
14	- Negative	10	100	10	100		
	- Mild	0	0	0	0	X^2=----	P=---
	- Moderate	0	0	0	0		
	- Severe	0	0	0	0		
21	- Negative	1	10	0	0	X^2=20	
	- Mild	9	90	0	0		<0.001
	- Moderate	0	0	9	90		
	- Severe	0	0	1	10		
28	- Negative	0	0	0	0	X^2=20	
	- Mild	10	100	0	0		<0.001
	- Moderate	0	0	1	10		
	- Severe	0	0	9	90		
35	- Negative	0	0	0	0	X^2=20	
	- Mild	9	90	0	0		<0.001
	- Moderate	1	10	0	0		
	- Severe	0	0	10	100		

Discussion

Generally speaking, *Trichinella spiralis* induced myopathy is an inflammatory myopathy that was difficult to treat unless parasitosis was combated in its early intestinal phase before reaching the muscles (Abou Rayia *et al,* 2023). Meanwhile, myopathies are a heterogeneous group of disorders primarily affecting the skeletal muscle structure, metabolism or channel function and interfering in daily life activities (Nagy and Veerapaneni, 2023). Nevertheless, PRP becomes a popular biological tool in regenerative medicine due to its strong healing characteristics as well as its ability to improve tissue fibrosis (El-Sharouny *et al.* 2019). PRP's use in regenerative medicine has rapidly expanded to include a wide range of clinical disorders such as ulcers, bone diseases, and tissue damage (Scully *et al,* 2018).

In the present study, PRP decreased the number of *Trichinella* larvae, particularly on day 35th pi as compared to positive control. Eissa *et al.* (2022) found that a single PRP decreased in mean number of *T. spiralis* larvae/gram of thigh muscles, thoracic muscles, and diaphragm, with an average reduction of 43.1%. This decrease jumped to 78.83% when multiple doses were used. This agreed with Shalaby et al. (2010), who reported histological improvement of inflammation surrounding *Trichinella* infected muscle cells, Also, both *Attia et al.* (2015) and Eid *et al.* (2020) reported massive inflammatory cellular infiltration surrounding *Trichinella* larvae in muscles compared to positive control. Li and Ko (2001) explained the diverse histopathology findings with distinct cellular responses caused by the use of different strains of mice during the *T. spiralis* muscle phase. Meanwhile, El-Aswad *et al.* (2018) who reported that PRP reduced hepatic fibrosis in mice infected with *Schistosoma mansoni.*

In the present study, the PRP significantly improved the histopathological changes in small intestinal villi. This agreed with Eissa *et al.* (2022) in Egypt, who found that PRP markedly decreased inflammation and fibrosis in skeletal muscles in Trichinellosis. Chellini *et al.* (2019) reported that the anti-inflammatory and antifibrotic effect of PRP was due to release of various growth factors upon platelet activation, acting as anti-inflammatory agents by blocking the production of monocyte chemotactic protein-1 (MCP-10 & decreasing fibrosis by suppressing collagen production. Meanwhile, the treating efficacy of PRP more or less agreed with El-Kholy *et al.* (2021), who dealt with murine cryptosporidiosis treated infection with PRP in conjunction with Nitazoxanide. They found that the pathologic and inflammatory consequences of cryptosporidiosis on the small intesti-

nal villi were recovered as well as liver and portal tracts in immunocompromised hosts restored liver architecture with reduced portal tract pathology.

Besides, Chazaud *et al.* (2003) found that VEGF is a potent chemo-attractant for macrophages Fujimaki *et al.* (2016) reported that VEGF plays an important role in muscular remodeling, angiogenesis, and inflammatory response and can initiate an inflammatory response and release growth factors such as IGF-1 and anabolic hormones (Tonkin *et al,* 2015). At this moment the situation is reversed as the macrophage converts from the pro-inflammatory to anti-inflammatory that aided in reduction of inflammation and activation of satellite cells, as well as the regeneration of myofibrils (Arnold *et al,* 2007).

In the present study, PRP-treatment showed strong (+3) VEGF expression causing a significant improvement in the inflammatory response, collagen deposition, fibrotic tissue, and other growth factors for collagen production. Brentnallet *et al.* (2013) reported that the most significant executioner of caspase was caspase-3 triggered by the intrinsic and extrinsic mechanisms. Thus, this result went with Pakshir and Hinz (2018) in Canada, who reported that PRP prevented the transition of fibroblasts into myofibroblasts. Chellini *et al.* (2018) added that the main drivers of tissue scarring were by activation of both VEGF-A and VEGF-A receptor-1-mediated inhibiting the TGF-β1/Smad3 signaling path way. Again, El-Aswad *et al.* (2020) reported that the trichinosis produces apoptosis rather than necrosis in infected muscle cells, and that the muscle damage was caused by the invading larvae and inflammatory cells accumulating increasing levels of reactive oxygen species and other damaging free radicals

The present study showed that the PRP-treated mice on day 35th pi significantly up-regulated apoptotic factor caspase 3 and modulated programmed cell infection death. This was clear by marked expression in muscle around the *Trichinella* larvae and decreased their count. This result agreed with Borrione *et al.* (2014), who suggested that PRP into the skeletal muscles of rats or mice exposed to various injurious traumas showed muscular healing by inflammatory response modulation and increased macrophage in the injury site stimulating myogenic response. Besides, El-Aswad *et al.* (2018) reported that PRP triggered apoptosis of activated hepatocyte stellate cells in murine schistosomiasis by elevating both caspase-3 expression, and matrix metalloproteinase-9 initiated their apoptosis.

Conclusion

The intramuscular PRP administration showed a significant decrease in mean number of larvae in muscles, as a promising therapeutic effect on *T. spiralis* larvae.

Authors' declarations: They declared that they neither have any conflict of interest nor received any funds with companies.

Authors' contributions: Shalaan, FH, Alattar, TAA, and Faheem, MF, did the all practical part, and followed up outcome data parasitological, histopathological and immunohistochemical studies. Abd-Elhafiz, HI, prepared PRP, decided dose and wrote methodology. Mahmoud, SF, examined muscle sections and interpreted histopathological and immunohistochemical results. Al-Ghalban, YA, Faheem, MF, Shalaan, FH, Al-attar, T AA, and Abd-Elhafiz, HI, did statistical analysis. Shalaan, FH, wrote the manuscript.

All authors critically revised and approved the final manuscript version for publication.

Acknowledgments

Thanks are due to Dr. Bahaa Eldeen W. El-Aswad, Professor of Parasitology, Faculty of Medicine, Menoufia University for his kind input.

Reference

Abou Rayia, DM, Izzularab, BM, Harras, S, Ghafar, MTA, Azzam, AR, *et al,* 2023: Stem cell biotherapy: A new remedy for *Trichinella spiralis*-induced inflammatory myopathy. Parasitol. Int. 102773. https://doi.org/10.1016/j.par-in t . 2023.102773

Arnold, L, Henry, A, Poron, F, Baba-Amer, Y, Van Rooijen, N, *et al,* 2007: Inflammatory

monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J. Exp. Med 204, 5:1057-69.

Attia, RA, Mahmoud, AE, Farrag, HMM, Makboul, R, Mohamed, ME, *et al,* 2015: Effect of myrrh and thyme on *Trichinella spiralis* enteral and parenteral phases with inducible nitric oxide expression in mice. Mem.do Inst. Oswaldo Cruz 110:1035-41.

Borrione, P, Grasso, L, Chierto, E, Geuna, S, Racca, S, *et al,* 2014: Experimental model for the study of the effects of platelet-rich plasma on the early phases of muscle healing. Blood Transfus. 12, 1: S221-8.

Brentnall, M, Rodriguez-Menocal, L, De Guevara, RL, Cepero, E, Boise, LH, 2013: Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol. 14, 32. https://doi.org/10.1186/1471-2121-14-32

Chazaud, B, Sonnet, C, Lafuste, P, Bassez, G, Rimaniol, AC, *et al,* 2003: Satellite cells attract monocytes and use macrophages as a support to escape apoptosis and enhance muscle growth. J. Cell Biol. 163, 5:1133-43.

Chellini, F, Tani, A, Vallone, L, Nosi, D, Pavan, P, *et al,* 2018: Platelet-Rich plasma prevents in vitro transforming growth factor-β1-induced fibroblast to myofibroblast transition: Involvement of vascular endothelial growth factor (VEGF)-A/VEGF receptor-1-mediated signaling. Cells 7, 9:142. https://doi.org/10.3390/ cells 7090142

Chellini, F, Tani, A, Zecchi-Orlandini, S, Sassoli, C, 2019: Influence of platelet-rich and platelet-poor plasma on endogenous mechanisms of skeletal muscle repair/regeneration. Int. J. Mol. Sci. 20, 3:683. https://doi.org/10.3390/ijms2003 0683

Dea-Ayuela, MA, Sadkowska-Todys, M, Wroblewski, J, Torrado-Durán, JJ, Golab, E, *et al,* 2015: Immunoproteomic analysis of *Trichinella spiralis* larval crude antigens recognized by sera from patients with trichinellosis after treatment with albendazole. Trop. Biomed. 32, 4: 613-24.

Eid, RK, Ashour, DS, Essa, EA, El Maghraby, GM, Arafa, MF, 2020: Chitosan coated nanostru- ctured lipid carriers for enhanced in vivo efficacy of albendazole against *Trichinella spiralis.* Carbohydrate Polymers 232:115826.

Eissa, FMA, Eassa, AHA, Zalat, RS, Negm, MS, Elmallawany, MA, 2022: Potential therapeutic effect of platelet-rich plasma and albendazole on the muscular phase of experimental *Trichinella spiralis* infection. Food Waterborne Parasitol. 28 e00180 https://doi.org/10.1016/j. fawpar. 2022. e00180

El-Aswad, BEW, Ammar, AI, Mahmoud, SF, Soliman, SS, Abd El-Atty, AF, 2020: Immunohistochemical evaluation of interleukin-23 and cyclooxygenase-2 in the muscles of mice infected with *Tri-chinella spiralis.* Trop. Biomed. 37, 1:75-88.

El-Aswad, BEW, El-Refai, SA, Mahmoud, SF, Helwa, MA, 2018: Human platelet-rich plasma alleviates liver fibrosis in murine *Schistosomiasis mansoni.* Med. J. Cairo Univ. 86, 7: 3807-23.

El-Bahnasawy, MM, Aly, NZ, Abdel-Fattah, MA, Morsy, TA, 2014: Botulism as a food poisoning: What is it? JESP 44, 1:211-20.

El-Kholy, WA, Elgohary, SA, El Kholy, AA, El-Ashkar, AM, 2021: The efficacy of platelet rich pl-asma as adjuvant therapy in the treatment of cryptosporidiosis in experimentally infected immuno- suppressed rats. PUJ 14, 2:162-70

El-Sharkawy, H, Kantarci, A, Deady, J, Hasturk, H, Liu, H, *et al,* 2007: Platelet-rich plasma: Growth factors and pro-and anti-inflammatory properties. J. Periodontol. 78, 4:661-9.

El-Sharouny, SH, Rizk, AA, EE, Rashed, LA, Sayed, WM, & Elmoneam, MDAA, 2019: Analysis of the therapeutic role of platelet-rich plasma against cisplatin-induced hepatotoxicity in rats: Controversy between oxidative and apoptotic markers. Eur. J. Anat. 23, 3:201-13.

Farid, AS, Fath, EM, Mido, S, Nonaka, N, Horii, Y, 2019: Hepatoprotective immune response during *Trichinella spiralis* infection in mice. J. Vet. Med. Sci. 81, 2:169-76.

Fujimaki, S, Machida, M, Wakabayashi, T, Asashima, M, Takemasa, T, *et al,* 2016: Functional overload enhances satellite cell properties in skeletal muscle. Research Article | Open Access 2016 | Article ID 7619418 | https://doi.org/ 10.1155/2016/7619418

García-Rodríguez, JJ, Andrés, MF, Ibañez-Escribano, A, Julio, LF, Burillo, J, *et al,* 2015: Selective nematocidal effects of essential oils from two cultivated *Artemisia absinthium* popu-

lations. Z. Naturforsch. C. J. Biosci. 70, 9/10: 275-80.

Gottstein, B, Pozio, E, Nöckler, K, 2009: Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin. Microbiol. Rev. 22, 1:127-45.

Kwon, DR, Park, GY, Lee, SU, 2012: The effects of intra-articular platelet rich plasma injection according to the severity of collagenase induced knee osteoarthritis in a rabbit model. Ann. Rehab. Med. 36, 4:458-465.

Li, C, Ko, R, 2001: Inflammatory response during the muscle phase of *Trichinella spiralis* and *T. pseudospiralis* infections. Parasitol. Res. 87: 708-14.

Li, F, Cui, J, Wang, ZQ, Jiang, P, 2010: Sensitivity and optimization of artificial digestion in the inspection of meat for *Trichinella spiralis*. Foodborne Path. Dis. 7, 8:87985.

Liao, C, Cheng, X, Liu, M, Wang, X, Boireau, P, 2018: *Trichinella spiralis* and tumors: Cause, coincidence or treatment? Anticancer agents. Med. Chem. 18, 8:1091-9.

Lo, YC, Hung, CC, Lai, CS, Wu, Z, Nagano, I, *et al,* 2009: Human trichinosis after consumption of soft-shelled turtles, Taiwan. Emerg. Infect. Dis. 15, 12:2056-8

Morsy, TA Sallam, TA, Hawam, SM, 2022: Trichinosis (trichinellosis) in man and animals with reference to Egypt: An overview JESP 52, 3:431-42.

Nagy, H, Veerapaneni, KD, 2023: Myopathy. In: StatPearls [Internet]. Treasure Island (FL): Stat-Pearls Publishing.

Pakshir, P, Hinz, B, 2018: The big five in fibrosis: Macrophages, myofibroblasts, matrix, mechanics, and miscommunication. Matrix Biol. 68/ 69:81-93.

Scully, D, Naseem, KM, Matsakas, A, 2018: Platelet biology in regenerative medicine of skeletal muscle. Acta Physiol. (Oxf) 223(3):e13071. doi: 10.1111/apha.13071

Shalaby, MA, Moghazy, FM, Shalaby, HA, Nasr, SM, 2010: Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. Parasitol. Res. 107:17-25.

Shibuya, M, 2011: Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and proangiogenic therapies. Genes Canc. 2, 12: 1097-105.

Solana, H, Scarcella, S, Virkel, G, Ceriani, C, Rodríguez, J, et al, **2009:** Albendazole enantiomeric metabolism and binding to cytosolic proteins in the liver fluke *Fasciola hepatica*. Vet. Res. Commun. 33, 2:163-73.

Tang, K, Breen, EC, Gerber, HP, Ferrara, N M, Wagner, PD, 2004: Capillary regression in vascular endothelial growth factor-deficient skeletal muscle. Physiol. Genomics 18, 1:63-9.

Tonkin, J, Temmerman, L, Sampson, RD, Gallego-Colon, E, Barberi, L, *et al,* 2015: Monocyte/macrophage-derived IGF-1 orchestrates murine skeletal muscle regeneration and modulates autocrine polarization. Mol. Therap. 23, 7:1189-200.

Xie, X, Zhao, S, Wu, H, Xie, G, Huangfu, X, He, Y, Zhao, J, 2013: Platelet-rich plasma enhances autograft revascularization and reinnervation in a dog model of anterior cruciate ligament reconstruction. J. Surg. Res. 3, 1: 214-22.

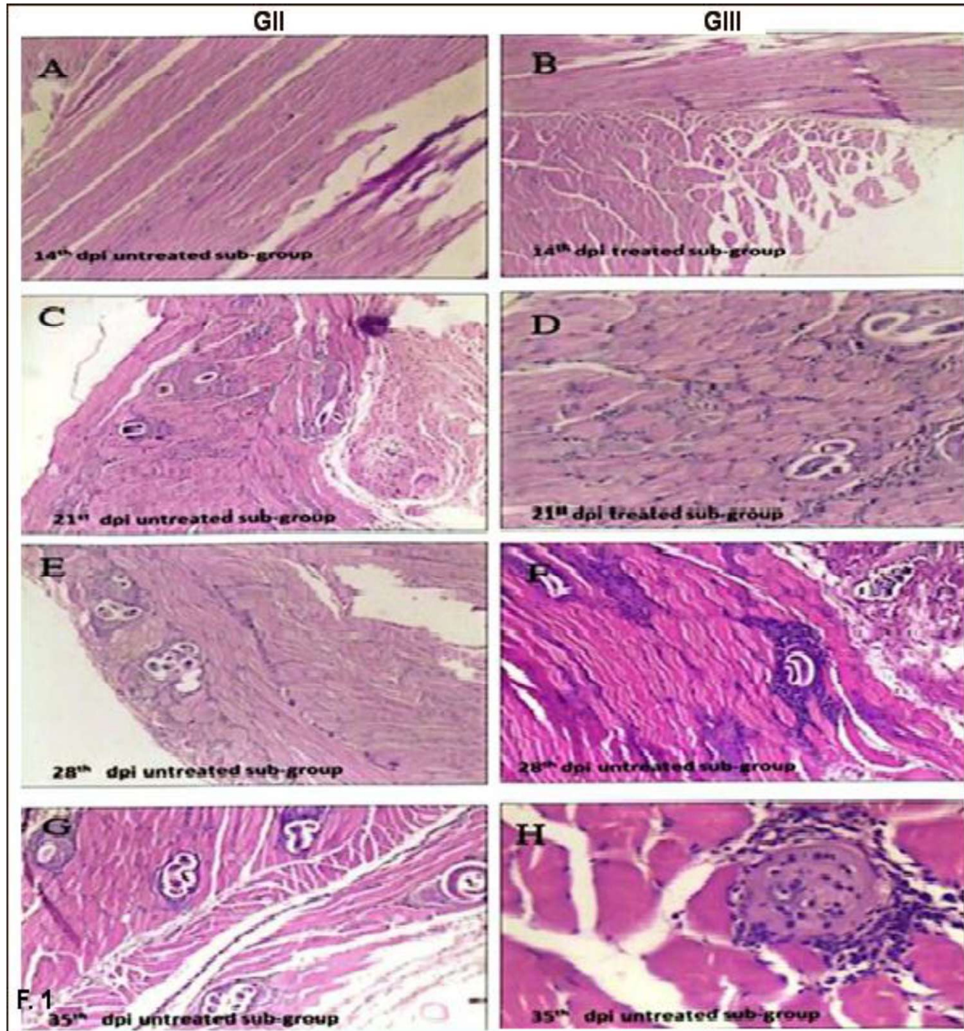
Yadav, AK, Temjenmongla, 2012: Efficacy of *Lasia spinosa* leaf extract in treating mice infected with *Trichinella spiralis*. Parasitol. Res. 110, 1:493-8.

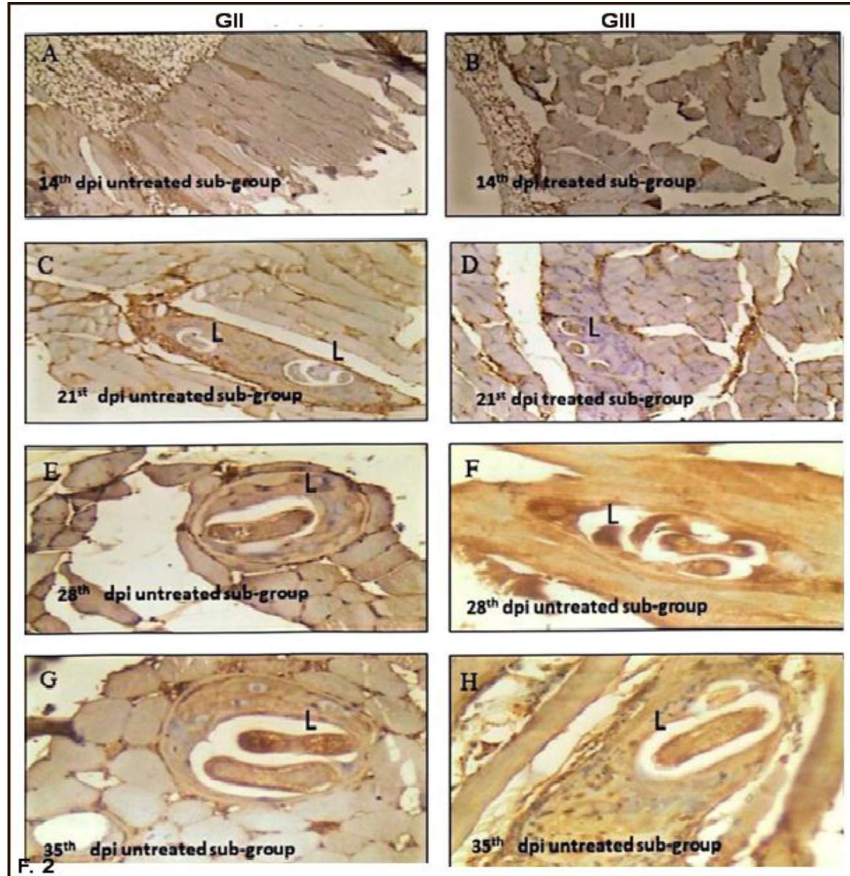
Explanation of figures

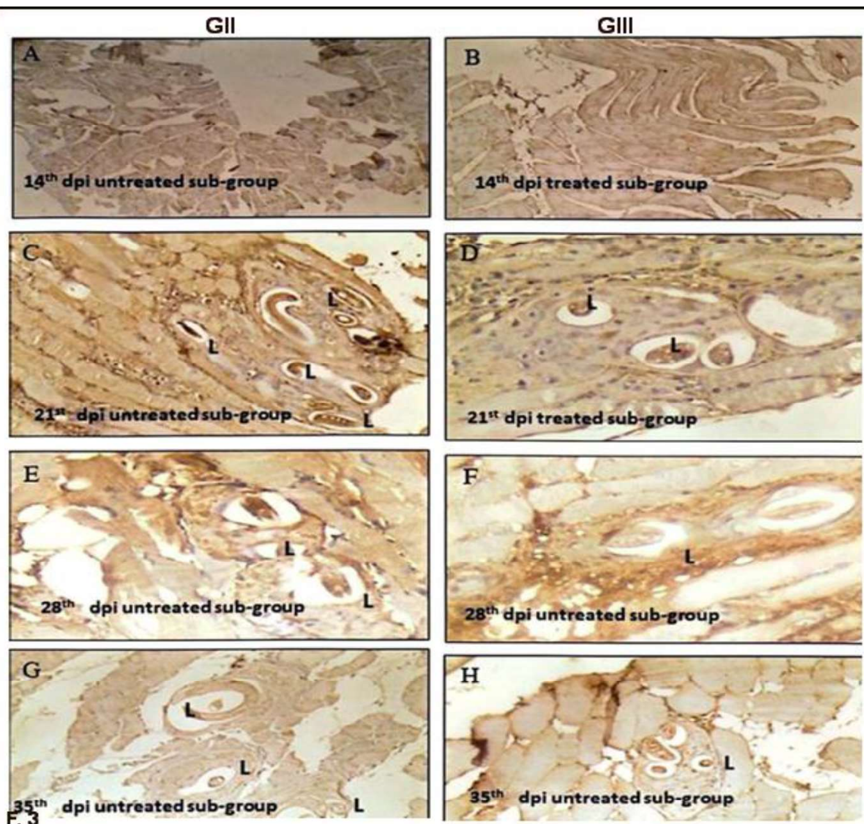
Fig. 1: Histopathology of muscles (H&E): A, B- showed transverse section in skeletal muscle tissue of GII & GIII at 14 dpi, with normal appearance of muscle fibers, average distribution of peripherally located, and flattened nuclei (x100). C- showed TS in skeletal muscle tissue of GII at 21 dpi, with larval deposition within muscle fibers surrounded by chronic inflammatory reaction mainly lymphocytes (x100). D- showed TS in skeletal muscle tissue of GIII at 21 dpi, with larval deposition within muscle fibers surrounded by chronic inflammatory reaction mainly lymphocytes (x200). E- showed TS in skeletal muscle tissue of GII at 28dpi, with larval deposition within muscle fibers and lymphocytic infiltration (x100). F- showed TS in skeletal muscle tissue of GIII at 28 dpi, with larval deposition within muscle fibers surrounded by dense lymphocytic infiltrate (x100). G- showed TS in skeletal muscle tissue of GII at 35 dpi, with larval deposition within muscle fibers with destructed larvae surrounded by marked chronic inflammatory reaction mainly lymphocytes (x100). H- showed TS in skeletal muscle tissue of GIII at 35 dpi, with destructing larva surrounded by marked chronic inflammatory reaction mainly lymphocytes (x400).

Fig. 2: Immunohistochemical expression of VEGF between GII & GIII: A, B- TS in skeletal muscle of GII &GIII at 14dpi showed negative VEGF immunostaining expression (x100). C- TS in skeletal muscle of GII at 21dpi showed mild cytoplasmic VEGF expression in muscle bundles with larval deposition (x200). D- TS in skeletal muscle of GIII at 21 dpi showed mild cytoplasmic VEGF immunohistochemical expression in muscle bundles with larval deposition (x200). E- TS in skeletal muscle of GII at 28dpi showed mild cytoplasmic VEGF expression in skeletal muscle bundles with larval deposition (x400). F-TS in skeletal muscle of GIII at 28dpi showed strong cytoplasmic VEGF expression in muscle bundles with larval deposition (x400). G- TS in skeletal muscle of GII at 35dpi showed mild cytoplasmic VEGF expression in muscle bundles with larval deposition (x400). H- TS in skeletal muscle of GIII at 35dpi showed strong VEGF cytoplasmic expression in muscle bundles with larval deposition (x400)

Fig. 3: Immunohistochemical expression of Caspase 3 between GII & GIII: A,B- TS in skeletal muscle of GII & GIII at 14 dpi showed negative caspase 3 stain expression, no larva (x100). C- TS in skeletal muscle of GII at 21dpi showed mild cytoplasmic caspase 3 immunohistochemical expression in muscle bundles with larval deposition (x200). D- TS in skeletal muscle of GIII at 21dpi showed moderate cytoplasmic brown caspase 3 immunohistochemical expression in muscle bundles with larval deposition (x400). E- TS in skeletal muscle of GII at 28dpi showed mild cytoplasmic caspase 3 stain immunohistochemical expression in muscle bundles with larval deposition (x400). F- TS in skeletal muscle of GIII at 28dpi showed strong cytoplasmic brown caspase 3 immunohistochemical expression in muscle bundles with larval deposition (x200). G- TS in skeletal muscle of GII at 35dpi showed mild cytoplasmic brown caspase 3 expression in muscle bundles with larval deposition (x200). H- TS in skeletal muscle of GIII at 35dpi showed strong cytoplasmic caspase 3 expression in muscle bundles with larval deposition (x200).




GII
GIII
A
14th dpi untreated sub-group
B
14th dpi treated sub-group
C
L
L
21st dpi untreated sub-group
D
L
21st dpi treated sub-group
E
L
28th dpi untreated sub-group
F
L
28th dpi untreated sub-group
G
L
35th dpi untreated sub-group
H
L
35th dpi untreated sub-group
F. 2


GII
GIII
A
14th dpi untreated sub-group
B
14th dpi treated sub-group
C
L
L
L
L
21st dpi untreated sub-group
D
L
21st dpi treated sub-group
E
L
L
28th dpi untreated sub-group
F
L
28th dpi untreated sub-group
G
L
L
L
35th dpi untreated sub-group
H
L
35th dpi untreated sub-group
F. 3