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Muscular Myogenin Expression in Probiotics and Ivermectin Treated Trichinellosis Murine Model

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Abstract

Trichinellosis is a worldwide food-borne parasitic disease caused by eating raw or undercooked meat containing the infective larvae of *Trichinella* nematodes. Our study is concerned with assessing the prophylactic effect of probiotics on skeletal muscle degeneration in *T. spiralis* experimentally. Fifty mice were divided into five groups (ten mice each) classified as normal control group, infected control group, probiotic treated group, Ivermectin treated group and combined probiotic and Ivermectin treated group. Assessment of effectiveness of different treating agents used was done by parasitological, histopathological, and molecular assessments. The parasitological assessment was achieved through detection and counting of *T.spiralis* adult in intestinal fluid samples and encapsulated larvae in tongue and diaphragm muscles. Also, histopathological assessments were carried on tongue and diaphragm muscles and molecular assessments was done though myogenin expression. The results showed that the group treated with probiotic combined with Ivermectin had the best results in all parameters (parasitological, histopathological and molecular) followed by the group which was treated with Ivermectin and finally the probiotic treated group. The combined group showed the lowest *T.spiralis* count both adult (percentage reduction 67.4%) and larvae (percentage reduction 88.1%) , also showed the best improvement of the histopathological results in the form of increased reduction in the number of deposited larvae and the intensity of the inflammatory infiltrate as well as destruction of the surrounding capsule . And in correspond to the gene expression; the combined treated group had the best effect on reduction of myogenin gene expression with percentage reductions (80%).

Keywords: Trichinella spiralis, Myogenin, Gene expression, Probiotics and Ivermectin.

1. Introduction

Trichinella spiralis (*T. spiralis*) a zoonotic parasite of nematode type and having cosmopolitan distribution Rossi [1] with wide range of hosts as mammals, birds and reptiles Kongkaew [2]. In Egypt, it was reported both in man Abdel-Hafeez [3] and in slaughtered pigs in Cairo Abattoirs Nassef [4]. Infection is acquired by consuming improperly cooked meat containing *T. spiralis* larvae. In the

stomach the released larvae bury themselves in the small intestinal lining till the adult stage is developed Steel [5]. Then invade the enterocytes of the small intestine, where they mature to male and female adult worms then produce newborn larvae that invade the muscle cells and mature to the infecting stages to complete their life cycle Muñoz [6].

Because of the unique intracellular localization of *Trichinella* in the skeletal muscle Bruschi [7]. The invasion can damage the muscle cells, either directly or indirectly Bruschi [7] leading to serious muscle pain and other complications up to death Nassef [4].

Diagnosis of *Trichinella spiralis* done mainly by detecting larvae in muscle samples or by testing for serum anti-*Trichinella* antibodies or antibodies in the meat juice Abdel-Hafeez [3].

Myogenic regulatory factors (MRFs) include many genes as (MyoD, MyoD, myogenin, Myf5, Myf6 and MRF4) Wu [8], encoding basic proteins and determine muscle cell differentiation both in vitro and in vivo of which myogenin gene is the only MRF expressed in all skeletal muscle cell lines Soumillion [9].

Ren [10] previously identified the differentially expressed genes in *T. spiralis* using real time PCR, that helped better understanding host parasite interaction Ren [10].

So, studying expressed genes involved in nurse cell formation will be helpful in diagnosing, assessment of therapy and subsequently controlling trichinellosis.

Ivermectin and its related compounds are known to be the most essential anthelmintics Basyoni [11].

None of the used anti *Trichinella* drugs are considered fully effective against the encysted or newborn larvae of *T. spiralis*, because of their low bioavailability, moreover some of them are contraindicated both in pregnancy and in children less than 3 years. Thus, new effective anti-trichinellosis drugs are needed to control this important zoonotic disease Yadav [12].

Probiotic actions include production of anti-microbial molecules and modulation of the immune system Goudarzi [13] as it has a broad spectrum of activity Rondanelli [14]. Probiotics serve as supplement to the host microflora that provide protection against various enteric pathogens and known also to demonstrate promising results like improved gut barrier function adding to their unique ability to compete with pathogenic microbiota for adhesion to the gut and improve their colonization Kerry [15].

In parasitological field, probiotics were effective against *Cryptosporidium parvum, Giardia duodenalis, Ascaris suum, Schistosoma mansoni, Toxocara canis, Trichinella spiralis, Babesia microti* and *Trypanosoma cruzi* Obendorf [52].

Although probiotics are still in pipeline and requires further studies several forms of probiotics are available commercially and are in use in large amount Kerry [15].

This study aimed to assess the probiotics prophylactic effect on skeletal muscle degeneration induced by *T. spiralis* in experimentally using parasitological, histopathological and gene expression studies coding for myogenin.

2. Materials and Methods

2.1 Animals and parasite infection:

The present study was carried out on Parasitology Department Faculty of Medicine Zagazig University and Parasitology Department Faculty of Medicine Al Azhar University Egypt from august 2019 to august 2020. Fifty laboratory bred Swiss albino mice nearly 6

weeks old, weighing 25-30 gm each, were used in this study.

2.2 Ethical consideration:

The study was approved by the Research Ethics Committee, Faculty of Medicine, Al-Azhar University. All procedures related to animal experimentation met the International Guiding Principles for Biomedical Research Involving Animals as issued by the International Organizations of Medical Sciences and as approved by ethics committee of Zagazig university.

The animals were obtained and bred in the Animal House, Faculty of medicine, Zagazig University and housed and maintained in an institution responsible for animal ethics in accordance with the national and institutional guidelines. Mice were divided into five groups (10 each) and classified as normal control group, infected control group, probiotic treated group, Ivermectin treated group and finally combined probiotic and Ivermectin treated group.

The used *T. spiralis* isolate was obtained from infected albino mice in Parasitology Department Laboratory, Faculty of Medicine, Zagazig University. Maintained by consecutive passages in mice. Before the start of the experiment all mice were left for 3 days to adapt.Then each mouse was infected *per os* with 400 *T. spiralis* larvae/mouse on day 7 of treatment with probiotics Buckova [16].

2.3 Treating agents:

Probiotics: Lactobacilli (L.plantarun) was purchased and administered orally daily for seven days at a dose of 10*9 CFU/ml in 100 µl before mice infection Costamagna [17].
Ivermectin: Iverzine tablets were administered orally at a dosage of 4 µg /mouse/day at 0-, 5-, 15-, and 35-days post infection (PI) Soliman [18] and Basyoni [11].

2.4 Experimental design:

This study includes five groups of mice, 10 mice each as follows:GI (control normal group), GII (control infected group): Infected untreated, GIII (probiotic group): Treated with probiotics then infected with 400 *T. spiralis* larvae/mouse on day 7 of probiotics treatment, GIV (Ivermectin group): Infected then treated with Ivermectin and GV (combined group): Infected then treated with ivermectin and probiotics

On the 7th day pi one mouse from each group was sacrificed for detection of adult T. spiralis count in intestinal wash and on the 40th dpi, the rest of mice were sacrificed to show and count the larvae of the parasite Basyoni [11]. Subsequently, tissue samples were obtained (diaphragm, tongue, triceps, and biceps brachialis, and quadriceps femoralis muscles) which were removed for trichinoscopy, a small part of muscles was preserved in -80 until the primer was imported and another in formalin 10% for histopathology while the remaining major part of the muscles was digested for estimating the total larval count.

2.5 *T. spiralis* adult count:

The intestine was cut into pieces of nearly 1 cm each, incubated at 37 °C in 100 ml of Hanks balanced salt solution (HBSS) for 2 hours to allow the worms to migrate out of the tissue. The intestine was washed several times and centrifuged at 1500 rpm for 10 min. The sediment reconstituted in 3–5 drops of HBSS to be examined at a magnification of 10× to count the adult worms [19].

2.6 *T. spiralis* larval count:

The major parts of muscles were cut into pieces, digested in 1% concentrated HCl ,1% of (1/10.000) pepsin and 200 ml distilled water then incubated at 37 °C for

two hours under continuous agitation using a stirrer. The digested muscles were passed through a 50-mesh/in. sieved to remove the coarse particles. Then larvae were collected on a 200-mesh/in., sieved, twice washed with distilled water and suspended in 200 ml of tap water in a flask. The sedimented larvae were counted using a McMaster counting chamber Denham [20].

2.7 Histopathological study:

Parts of the tongue muscle and diaphragm from all groups were fixed in 10% formalin for 48 h, washed in water for 12 h, dehydrated in alcohol, cleared in xylene and embedded in paraffin blocks, then sectioned at 5 μm by microtome and stained with hematoxylin and eosin according to Drury [21]. The number of larvae per low power field was demonstrated and the intensity of the inflammatory reaction surrounding the capsule was evaluated.

2.8 Gene Expression study:

Using Myogenin gene which expresses muscle degenerative changes occurring during muscular phase of trichinellosis by the following steps:

2.8.1 Extraction of total RNA from muscle of mice:

Total RNA from homogenize tissue samples was isolated according to the manufacturer`s instruction using easy-spinTM [DNA free] Total RNA Extraction Kit, intron biotechnology, south korea from muscle of mice using Thermo-scientific RNA Mini Kit, this procedure yields an essentially pure fraction of total RNA after only a single round of purification without organic extractions or precipitations Peirson [22].

2.8.2 Reverse transcription PCR (RT-PCR):

Reverse transcription was performed according to the manufacturer`s instruction using (Maxime RT-PCR PreMixKit, intron biotechnology, south Korea) for cDNA synthesis.

2.8.3 Gene expression using real time quantitative PCR):

Quantitative RT–PCR was performed according to the manufacturer`s instructions using topreal™ qpcr 2x premix (SYBR Green with low rox), enzynomics, korea) Relative quantitation of myogenin gene expression was carried out by determining the amounts of targets and of an endogenous reference (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) gene from the standard curve.

The DTlite 5S1 REAL-TIME Thermal cycler 5 channel,48×0.2ml System (DNAtechnology co,Moscow, Russia) detection System was used to detect the PCR product obtained by binding of SYBR Green to dsDNA.To amplify specific mRNAs, the following primers were used: CW-myogenin OR: 50-AGG AGG CGC TGT GGG AGT T; CW-myogenin OF: 50-GGG CCC CTG GAA GAA AAG (Oustanina et al., 2004) And those for GAPDH were 5'-CTCATGACCACAGTCCATGC-3' and 5'-TTCAGCTCTGGGATGACCTT-3' Khositharattanakool [53]. CT indicates the number of PCR cycles required for the fluorescence signal to exceed the detection threshold value. The difference in CT values for two genes was used to calculate delta CT [ΔCT = CT (target gene) − CT (internal reference gene)]. A higher ΔCT indicates lower gene expression. We calculated the fold difference [fd = 2^ (− ΔΔCT)]. ΔΔCT = ΔCT (a target) − ΔCT (a reference) and have presented the relative gene expression of the above genes.

2.8.4 Statistical analysis:

Expression of the quantitative values of all measured parameters was done, as mean ± standard deviation (SD). Then the

collected data were analyzed by ANOVA test determine the significance of differences between all groups using Statistical Package for Social Sciences (SPSS), version 14.0. The difference was considered statistically significant when $P < 0.05$, highly significant when $P < 0.01$ or unsignificant when $P > 0.05$. Drug efficacy or percentage of reduction (R %) was also calculated using this equation (R %) = 100× (mean number in controls minus mean number in treated mice) / mean number in controls.

3. Results

3.1 Parasitological results:

Count of T. spiralis Adults in small intestine:

In contrast to the control infected untreated group ($p < 0.001$), the mean number of worms counted in the small intestine was significantly reduced in the combined treated group (21.75), followed by the Ivermectin treated group (44) and finally the probiotic treated group (53) with a percentage reduction (67.4%, 34.08% and 20.6%) respectively (Table. 1).

T. spiralis larval count in the muscle:

In contrast to the control infected untreated group ($p= 0.000$), the mean number of larvae in the muscle of the combined treated group (1050) was significantly reduced, followed by the Ivermectin treated group (1800), and finally the probiotic treated group (5450) with a percentage of reduction (88.1%, 79.6% and 38.2 %) respectively (Table. 2).

Table (1): Mean T. spiralis worm count and their percent reductions in the small intestine of different treated groups compared to the infected control group.

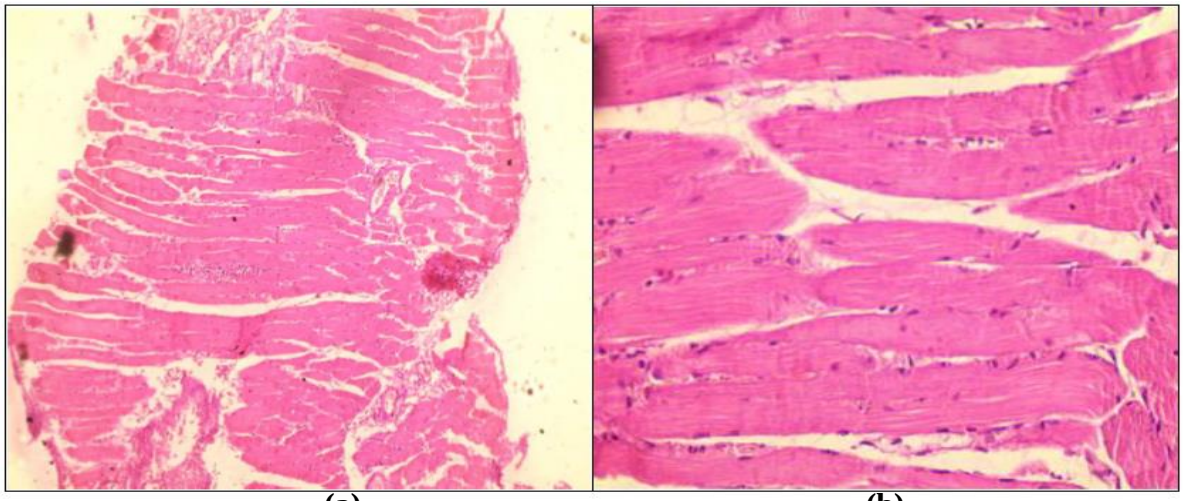
	GII Infected control	**GIII Probiotic**	**GIV Ivermectin**	**GV Combined**
Mean ± SD	66.75 ± 3.77	53 ± 2.94	44 ± 4.32	21.75 ± 6.23
R %	--	20.6%	34.08%	67.4%
F	70.96			
P-Value	0.000*			
Post hoc	--	P1 (GII& GIII) = 0.006*	P2 (GII&GIV) = 0.000*	P3 (GII& GV) = 0.000*

Table (2): Mean T. spiralis larval count and their percent reductions in the muscle of different treated groups compared to the infected control group.

	GII Infected control	**GIII Probiotic**	**GIV Ivermectin**	**GV Combined**
Mean ± SD	8825 ± 1164.4	5450 ± 680.68	1800 ± 529.15	1050 ± 129
R %	--	38.2%	79.6%	88.1%
F	97.321			
P Value	0.000*			
Post hoc	--	P1 (GII& GIII) 0.000*	P2 (GII&GIV) 0.000*	P3 (GII& GV) 0.000*

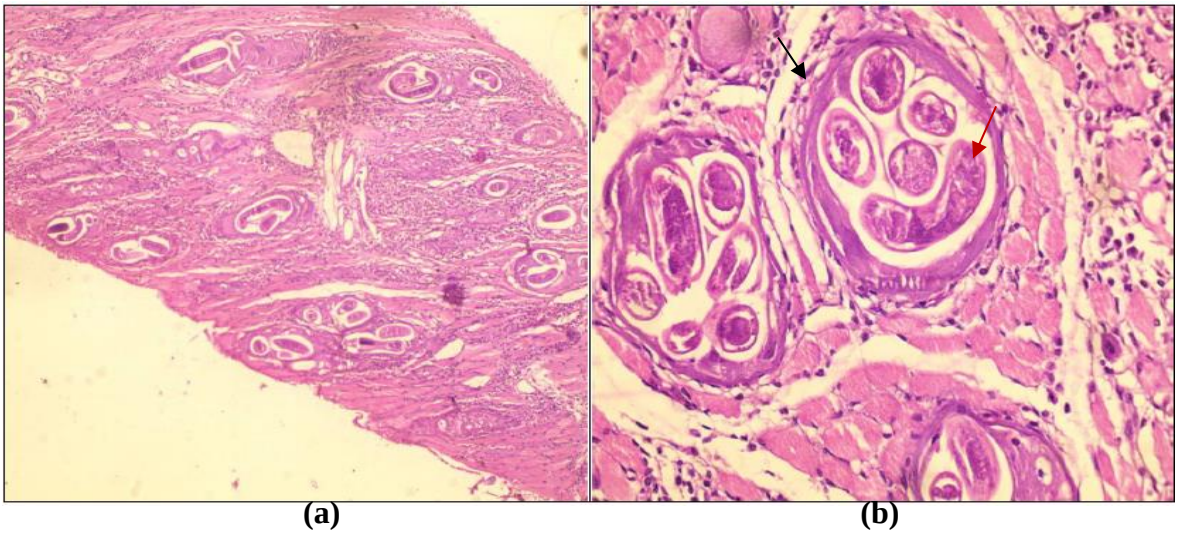
3.2 Histopathological:

Multiple encysted larvae with heavy inflammatory infiltrates around their capsule and some areas of degenerated muscle fibers were found in sections taken from the tongue and diaphragm of the infected control group (GII). A collagenous capsule encased each larva, as well as massive inflammatory cellular infiltrations (Fig.2). In contrast to the infected control group, treated groups showed varying degrees of reduction in the number of deposited larvae as well as the intensity of the inflammatory infiltrate, with higher significance in the combined treated group (GV) in the form of decreased encysted larvae count as well as destruction of the surrounding capsule (Fig.5) followed by the ivermectin treated group (GIV) (Fig.4) and the least results were observed in the probiotic treated group (GIII) (Fig.3).



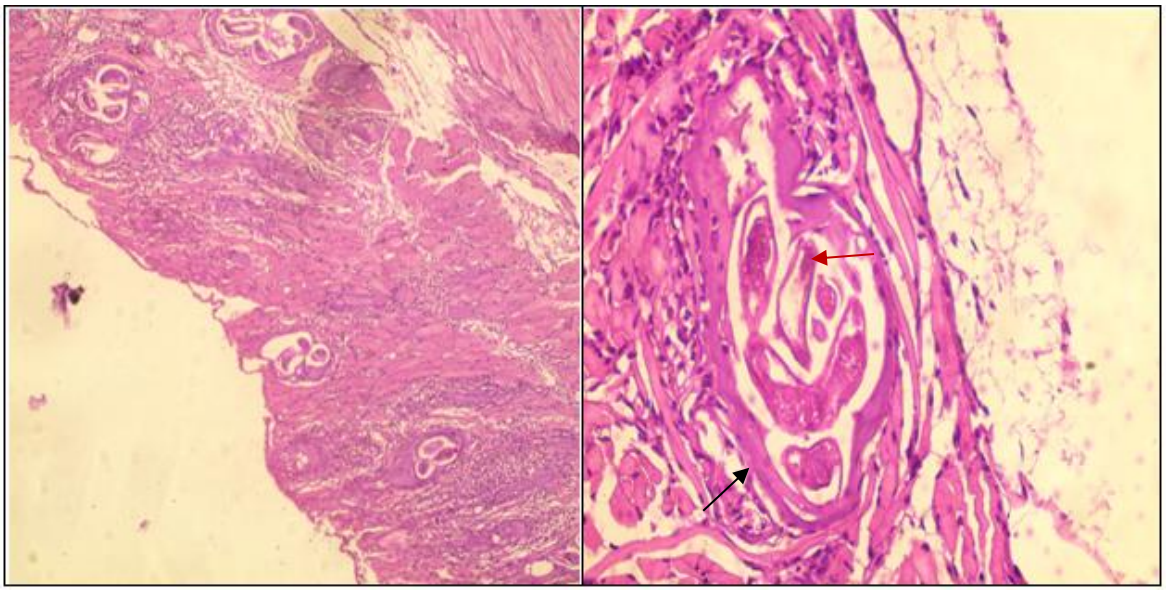
(a) **(b)**

Figure (1): H&E-stained cut sections in the diaphragm of normal group of mice showing no observed histopathological changes in muscular sections (Fig. 1.a 100x) and (Fig. 1.b 400x).



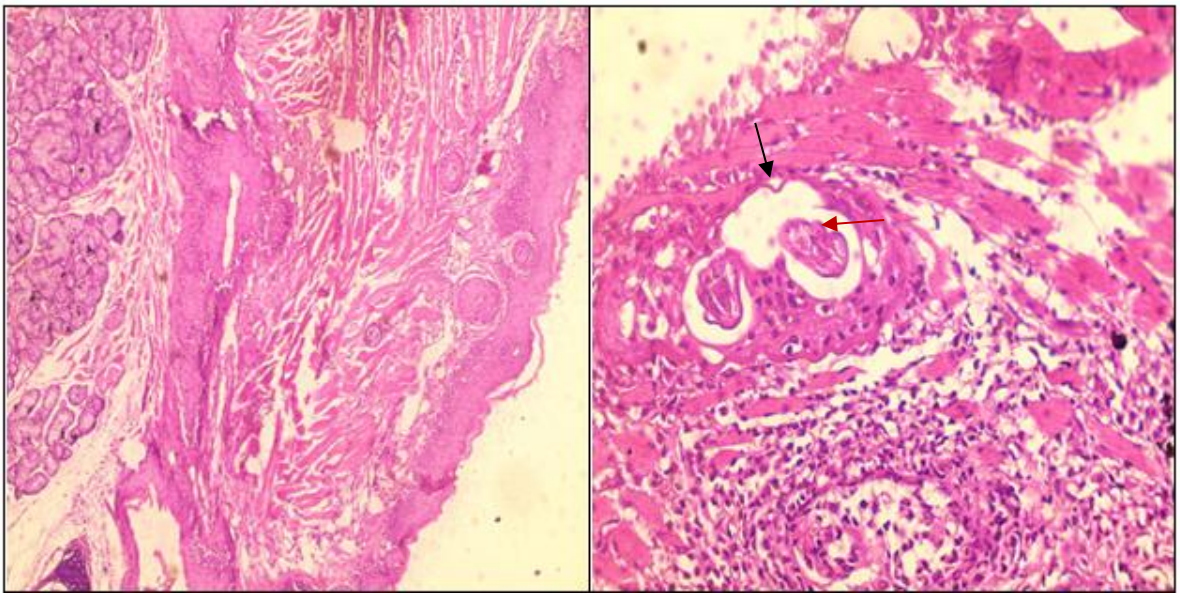
(a) **(b)**

Figure (2): H&E stained cut section of diaphragm muscles of the infected, non-treated group revealed the presence of a massive number of encysted *T. spiralis* larvae surrounded by a thick intact capsule and a massive number of chronic inflammatory cells infiltrating muscle bundles and surrounding the encysted larvae (fig.2a 100x) and cut section of tongue muscles of infected, non-treated group stained with H&E revealed *T. spiralis* larvae surrounded by a thick intact capsule (black arrow) and a massive number of chronic inflammatory cells (blue arrow) infiltrating muscle bundles and surrounding the complete larvae (red arrow) (fig.2b 400x).



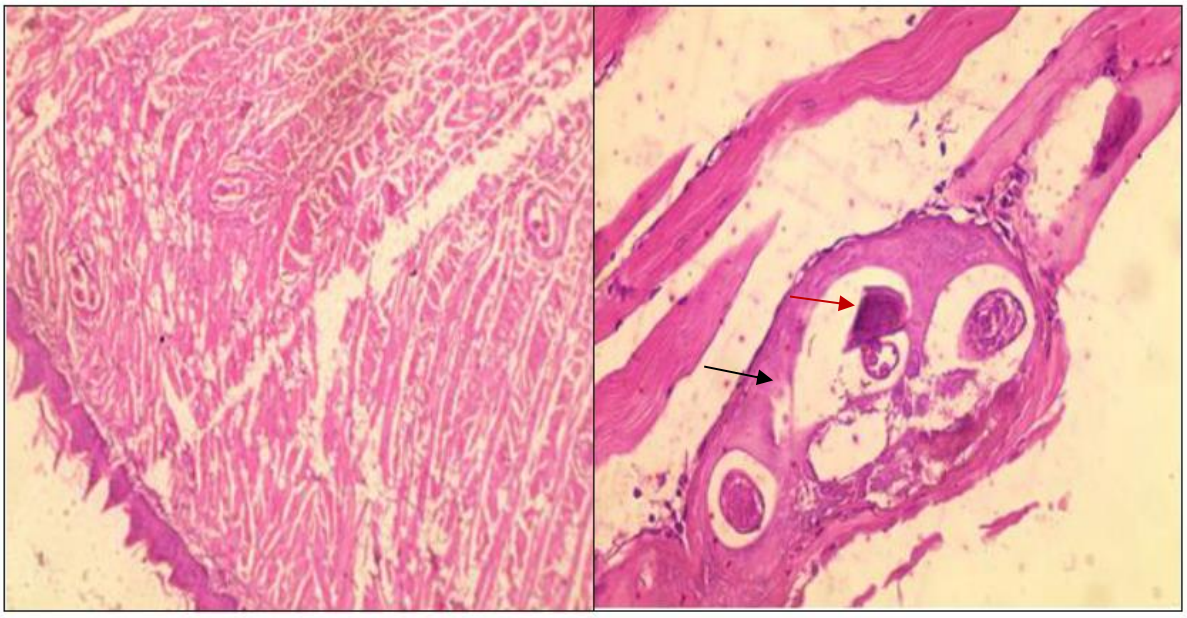
(a) **(b)**

Figure (3): H&E stained cut section in the diaphragm and tongue of infected mice treated with *L.plantarum* revealed slight decrease in the number of *T.spiralis* . with a fewer number of encysted larvae than the infected, non-treated group with heavy inflammatory cellular infiltration surrounding them (fig.3a 100x) and H&E cut section in the diaphragm and tongue of infected mice treated with *L.plantarum* revealed the capsule in most of the larvae appeared thick and complete (black arrow). It also showed degeneration of the larvae (red arrow) in the form of fragmentation and invasion by inflammatory cellular infiltrate (blue arrow) (fig.3b 400x).



(a) **(b)**

Figure (4): H&E-stained cut section in the diaphragm and tongue of infected mice treated with ivermectin showed a moderate degree of larval deposition. (fig.4a 100x) and Cut section in the diaphragm and tongue of infected mice treated with ivermectin stained with H&E showed fragmentation of the larvae (red arrow) and invasion by inflammatory cellular infiltrate (blue arrow), Capsules around most of the larvae showed thinning in some areas (black arrow) (fig.4b 400x).



(a) **(b)**

Figure (5): H&E stained cut section in the tongue of infected mice treated with probiotic and ivermectin showed marked reduction in number of *T.spiralis* (fig.5a 100x) and cut section in the diaphragm of infected mice treated with probiotic and ivermectin stained with H&E, showed homogenised larvae (red arrow), vacuolation and splitting of the capsule (black arrow) into thin layers with diffuse inflammatory cellular infiltration (blue arrow) surrounding and invading the capsule (fig.5b 400x).

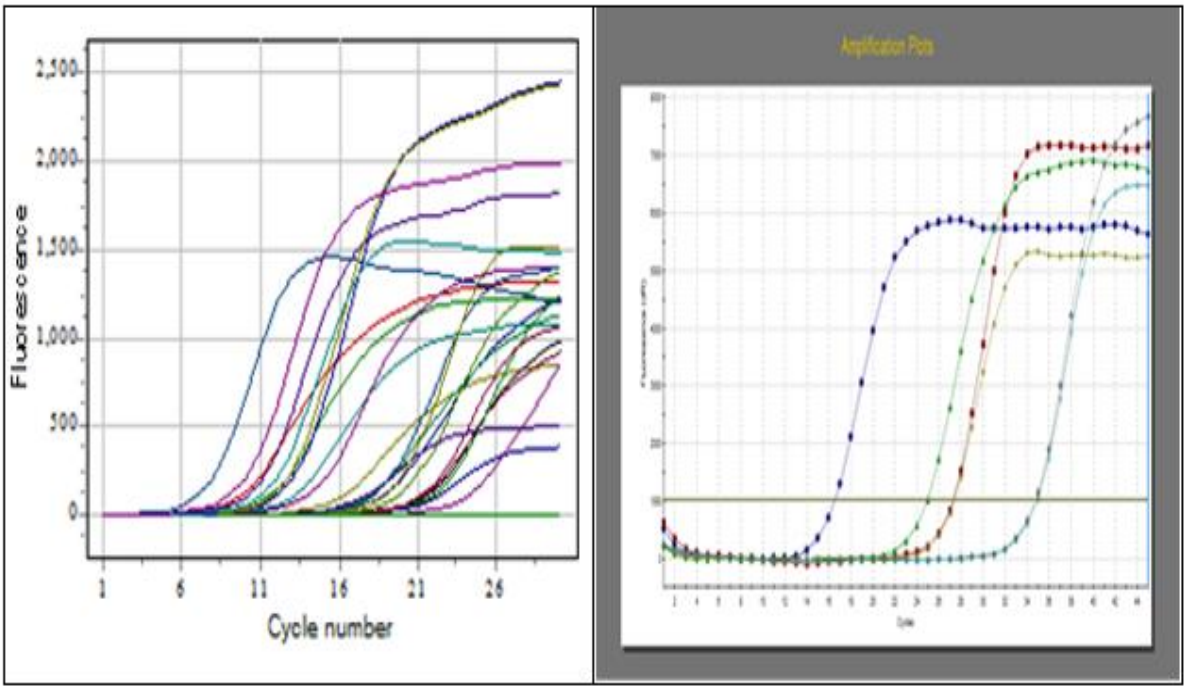


Figure (6): Showing myogenin gene amplification curves with variable crossing point values.

3.3 Gene expression results:

On day 35 PI, real-time PCR was used to detect myogenin gene mRNA expression during T. spiralis nurse cell formation in muscle tissue. The infected non-treated group had a mean number of Myogenin mRNA in muscle tissue of 2.714 ±1.77, while the other groups had 1.83 in Probiotic treated mice, 0.7225 in Ivermectin treated mice, and the lowest mean (0.542) in the combined treatment group with percentage reductions (32.5%, 37.4% and 80%) respectively and a statistically significant reduction compared to the infected control group P value = 0.04* (Table. 3).

Table (3): Mean T. spiralis myogenin expression in the muscles of different treated groups compared to the infected control group.

	GII Infected control	**GIII Probiotic**	**GIV Ivermectin**	**GV Combined**
Mean ± SD	2.714 ± 1.77	1.83 ± 1.01	0.7225 ± 0.297	0.542 ± 0.258
R %	--	32.5%	37.4%	80%
F	3.8			
P-Value	0.04*			

4. Discussion

Trichinellosis is a severe food-borne parasitic zoonosis caused by a nematode of the genus Trichinella, with a wide host range and global distribution Godzik [23].

The most common symptom of trichinosis is diarrhea, but the infection can also cause fever, periorbital edema, and myositis. Depending on the number of larvae consumed, patients may develop serious disease such as myocarditis, pneumonia, or encephalitis Grove [24], Ibrahim [25]. Females enter the intestinal mucosa and deposit larvae, then travel through the circulatory system, disseminating throughout the organs until they reach the skeletal muscle fibers, partially destroying them and causing the toxic allergic reaction that is characteristic of Trichinellosis Costamagna [17].

After invasion, newly born larvae migrate into muscle cells, causing a remarkable series of cell physiological changes that convert the fully differentiated muscle cell into one that supports the larva's growth and development. Nurse cell formation is the name given to this process Patra [26]. Myogenic regulatory factors MyoD and myogenin (important for muscle myogenesis and regeneration) are over expressed in infected muscle tissues during *T. spiralis* infections, according to gene expression analysis, and the MyoD factor is highly expressed in the satellite cells of infected muscles Hafez [27].

Antihelminthics, mainly benzimidazole derivatives like albendazole and mebendazole, are commonly used in traditional therapy. However, the effectiveness of these benzimidazole derivatives is limited by the following factors: 1) low water solubility, 2) increasing antihelminthics resistance, 3) contraindication in children and pregnancy Buckova [16].

Because there has been no particularly effective treatment for the muscular phase of trichinellosis to date, the alternative treatment was used. Immunomodulators, which boost the immune system's non-specific response, are thus used as an

alternative to treat this and other parasite diseases Costamagna [17].

Probiotic bacteria El Temsahy [28], natural proteins Othman [29], and substances like myrrh, thyme, or artemisinin Abou Rayia [30] and Ibrahim [25] have all been shown to have promising anti-parasitic properties.

Probiotics have been used to control parasite infections in the last decade, primarily in intestinal diseases, but also in non-gut infections Travers [31].

Probiotics are live microorganisms that, when given in sufficient amounts, provide health benefits to the host (FAO/WHO, 2002). The prophylactic effect of probiotics versus the therapeutic effect of ivermectin on skeletal muscle degeneration in *T. spiralis* infected mice was demonstrated in this study using different methods of assessment including parasitological, histopathological and gene expression methods of assessment.

Regarding the number of intestinal worms in this study, probiotics was effective on the mean count of adult T.spiralis in samples of intestinal content that were (53 ± 2.94) in comparison to the infected non treated group (66.75 ± 3.77) with (20.6%) percentage of reduction which was statistically significant (P value<0.05) also probiotics was effective on the mean count of muscle larvae / mouse that was (5450 ± 680.68) in comparison to that of infected non treated group which was (8825 ± 1164.4) with (38.24%) percentage of reduction with statistically significant (P value<0.05) .

Our findings were in agreement with many authors Bautista [32], Costamagna [17], Martnez [33], 2011 and El Temsahy [28], Vasconi [34] explained the resistance to infection with probiotic treatment is due to the ability to prevent the development of infective larvae by removing adult worms from the small intestine, limiting the fertility of adult females, and destroying newborn larvae. Dvoroznakova [35] also stated that the host can elicit an immune response in response to parasite invasion by activating immune mechanisms at the intestinal level, where the parasite and gut microflora interact and modify each other as well as the host immune system. By strain specific adhesion, competition with pathogens, and other mechanisms, probiotic strains have the potential to inhibit or remove pathogens Azaïs [36], and Butel, [37].

However, the findings of this study differed from those of Dvoroznakova [35], in that the numbers of adult worms in mice treated with lactobacilli did not differ from those infected non treated mice. They explained their findings by saying that the variability may be due to the different lactobacilli strains used, as well as the different dosage and treatment schedule. In addition, our findings differed from those of Buckova [16] as there was no effect on worm burden during the intestinal phase of the infection and they suggested that it might be caused by the worse adhesive capacity of lactobacilli as they thought that the attachment of probiotics to the gut epithelium is an important determinant to achieve their beneficial effect on the host organism Buckova [16].

Our findings were supported by others Bautista [32], Costamagna [17], Martnez [33], El Temsahy [28]; Dvoroznakova [35] and Buckova [16].
Bacterial strains produce lactic and acetic acid, hydrogen peroxide, proteinaceous enterocins and bacteriocins. All of them are important in pathogen exclusion Šuskovic [38] and Laukova [39]. These bacterial substances may also have an impact on the larvae's vitality and contribute to their death, especially through hydrogen peroxide Buckova [16]. The reduced numbers of *T. spiralis* muscle larvae caused by bacterial strains could be linked to low female fecundity or the destruction of

newborn larva during migration to the host muscles Buckova [16].

The result of ivermectin treated group in this study showed great effectiveness against *T. spiralis* as the mean count of adult in intestinal content samples were (44 ± 4.32) in ivermectin treated group in comparison to the infected non treated group (66.75 ± 3.77) with statistically significant difference (P value<0.05) and (34.1%) percentage of reduction and the mean count of muscle larvae / mouse was (1800 ± 529.15) in comparison to that of infected non treated group which was (8825 ± 1164.4) with (79.6%) percentage of reduction that was statistically significant (P value<0.05) (Table. 2). Our results corresponding to adult worms were agreed with that of Freedman [40], Kamel [41], Omar [42], El-Azzouni [43] and Basyoni [11]. However, different results were reported by Song [44] who found ivermectin less effective against the adult phase.

Ivermectin's anthelmintic activity, according to Barton [45], can be attributed to modulating GABA-gated chloride channels, which are more accessible in nematodes than in vertebrates. According to El-Azzouni [43], ivermectin has a direct impact on adults, as demonstrated by topographic changes that lead to adult destruction, as well as a reduction in the number of larvae.

Regarding the drug effects on the muscle phase the results of this study were in agreement with that of El-Azzouni [43], Song [44] and Basyoni [11]. In the present study the best effect in parasitological assessment was observed in the group treated with both ivermectin and probiotic as the mean count of adult *T. spiralis* in intestinal content samples were (21.75± 6.23) in comparison to the infected non treated group (66.75 ± 3.77) with (67.4%) percentage of reduction which was statistically significant (P value<0.05) (Table. 1) and the mean count of muscle larvae / mouse was (1050 ± 129) in comparison to that of infected non treated group (8825 ± 1164.4) with (88.1%) percentage of reduction which was statistically significant (P value<0.05) (Table. 2).

This is the first study to evaluate the combined effect of both ivermectin and probiotic on intestinal and muscular phases in *T.spiralis* infection. The reported effect of both ivermectin and probiotic might be due to synergistic effect of both drugs on adult and larvae.

In terms of histopathological findings, our study demonstrate the histopathological effect of probiotic on mice tissue infected with *T.spiralis* and found that it has an effect less than both that of ivermectin and their combination as all treated groups showed a significant reduction in the mean number of muscular larvae when compared to the infected control group of mice. Ivermectin plus probiotic had the best effectiveness against muscular stages, followed by ivermectin alone, with probiotic having the lowest efficacy.

In the present study, large numbers of *T. spiralis* larvae were diffusely present in muscle sarcoplasm with a huge number of chronic inflammatory cells infiltrating muscle bundles and surrounding the encysted larvae with some degeneration in muscle fibers in the infected non-treated group. A collagenous capsule encased each one, as well as massive inflammatory cellular infiltrations. These results agreed with Attia [46] and Ibrahim [25].

On the other hand, Dyab [47], Basyoni [11] and Kamel [41] disagreed with our results because their histopathological examination showed that encysted larvae were replacing muscle fibers and that inflammation was minimal to absent, with only a few lymphocytes. When compared to infected control group, there was a significant reduction in the number of larvae and the intensity of the inflammatory

infiltrate in all of our treated groups. The larvae evidently showed degeneration and were replaced by amorphous material surrounded by areas of coagulative necrosis with inflammatory cells, and many of cysts showed marked degenerative changes.

El-Azzouni [43] reported that the ivermectin efficacy against encysted *T. spiralis* larvae was 73.5% when injected at week 6 PI. Different results were demonstrated by Soliman [18] who found that ivermectin, and levamisole were unsuccessful in reduction of larval counts in the diaphragms of infected rats when injected at day 35 PI. Basyoni [11] explained that this discrepancy could be due to the different medication schedules.

Regarding the results of molecular gene expression, our study demonstrated an increased expression of myogenin in infected control group that decreases among the treated groups with variable degrees according to the drug used. These findings were consistent with those of Hafez [27], who found that myogenic regulatory factors, MyoD and myogenin, are overexpressed in infected muscle tissues during *T. spiralis* infections, with vaccinated unchallenged mice group exhibited moderate positive expression and mild expression of myogenin in vaccinated challenged group.

Also, Shavlakadze [48] agreed with our results as they reported an increase of Myogenin, MyoD and myogenic regulatory factors (MRFs)2 in *T. spiralis* infected muscle and Bentzinger [49] suggested that the highly conserved MyoD, Myf5, myogenin, and MRF4 genes are collectively expressed in the skeletal muscle lineage and are therefore referred to as MRFs.

Wu [8] discovered that Myogenic regulatory factors (MyoD and myogenin) were overexpressed in infected muscle tissue of both *T. spiralis* and *T. pseudospiralis* infections, indicating that the invasion of newborn larvae cause de-differentiation of infected muscle cells, characterized by loss of muscle cell characteristics, changes in muscle gene expression, and up-regulated expression of cell differentiation related genes such as myogenin, in infected muscle tissues.

These results also agreed with Berghella [50] who stated that gene expression profiling is a sensitive and powerful tool to study the cellular differentiation process and found that Myog expression in the mature myofiber is eventually decreased Timmons [51].

This could explain our results of downregulated expression of myogenin in treated groups. The improvement with ivermectin and probiotic may be explained as these drugs selectively influence these molecular pathways and enable the mobilization of satellite cells in diseased muscle, thereby enhancing the tissue's own regenerative capacity Bentzinger [49].

5. Conclusion:

Probiotics have a minimal prophylactic effect against *T. spiralis* infection and cannot be used alone, but that when combined with the ivermectin drug in the treatment of *T. spiralis* infection in mice, the results were better than when the drugs were used independently.

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