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STUDY ON THERAPEUTIC EFFICACY OF LACTOFERRIN LOADED ON SILVER-NANOPARTICLES AGAINST TRICHINELLA SPIRALIS PARENTERAL PHASE

By
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Abstract

Globally trichinellosis (*Trichinella* species) is a risky parasitic zoonosis Anthelminthic such as oral albendazole® and mebendazole® commonly used to treatment are poorly water soluble with unfavo- rable bioavailability. This study evaluated the therapeutic efficacy of lactoferrin loaded on silver nanoparticles on *Trichinella spiralis* infection in experimental infected mice by parasitological and histopathological parameters. Mice were classified into 8 groups each of 5 mice. G1: Non infected non treated (normal), G2: Infected non treated (infected control), G3: In-fected and albendazole, treated, G4: Infected and lactoferrin treated, G5: Infected and silver nanoparticles treated, G6: Infected and lactoferrin loaded on silver nanoparticles treated, G7: Infected and albendazole & lactoferrin, & G8: Infected and albendazole combined with lactoferrin loaded on silver nanoparticles treated.

The results showed a significant decrease in all treated groups, markedly in *T. spiralis* larval muscles treated with combination of albendazole and lactoferrin loaded on silver nanoparticles (92.39%), with muscles histopathological changes.

Keywords: *Trichinella spiralis*, albendazole, lactoferrin, silver nanoparticles, histopathology.

Introduction

Trichinella spiralis is the smallest viviparous nematode parasite, occurring in rodents, pigs, bears, hyenas and humans, causing trichinosis of global distributions (Zarlenga *et al,* 2020). Zoonotic trichinosis was not commonly encountered in Egypt, but domestic infection was reported in pigs, cats, and wild carnivores (Morsy *et al,* 2022). Man acquired infection by ingestion of undercooked meat containing encysted larvae from infected animals, mainly pigs (Pradhan and Karanth, 2023). The released larvae burrow beneath the small intestine epithelium, moult four times to reach male and female stages, return to gut lumen to mate, and the generate larvae disseminated via circulatory system to striated muscle (CDC, 2020). The muscle cells undergo morphological changes in form of nurse cell complex or capsule (Wu *et al,* 2008), within 17-21 days they develop to infective form (Atia *et al,* 2023). Most of cases are subclinical, gastrointestinal distress may be observed early in the first week (Ribicich *et al,* 2007). Symptoms include generalized fever, abdominal pain, diarrhea, nausea, vomiting, myalgias, or severe like myocarditis and encephalitis (Abdel Fadil *et al,* 2018). Also, *Trichinella* larvae cause inflammatory responses in the lungs, and central nervous system (Elmehy *et al,* 2021). Both Albendazole® and Mebendazole® are used to treat applications, but with much side effects (Khallaf *et al,* 2018).

Lactoferrin Lf, an iron-containing milk protein, is called a miracle molecule has many characters, such as immunomodulatory, anti-inflammatory, anti-parasitic, anticancer, and DNA-regulatory effects (Kowalczyk *et al,* 2022). Antiparasitic activity of Lf protein can bind and transport iron molecules which are crucial for parasite to enhance its pathogenicity and longevity (Anand, 2023). *Toxoplasma, Entamoeba, and Giardia* were eliminated by Lf (Zarzosa-Moreno *et al,* 2020). Besides, nanoparticles can delivery systems for medications or vaccinations to increase the therapeutic efficacy (Nassef *et al,* 2018).

The study aimed to assess the therapeutic efficacy of lactoferrin loaded on silver nano

particles on the parentral phase of *Trichinella spiralis* in experimental infected mice by parasitological and histopathological parameters.

Materials and Methods

The study was done at Biological Unit of Theodor Bilharz Research Institute (TBRI), Giza, and the Department of Parasiology, Faculty of Medicine, Benha University, between February 2022 and November 2023.

Parasite: A *Trichinella spiralis* strain was isolated from infected albino mice raised in the laboratory of TBRI.

Isolation and inoculum preparation: Five weeks post-infection (PI), mice were sacrificed and dissected out for muscles, which were digested in a solution of 1% pepsin and 1% HCl in 200ml of distilled water. By an electric stirrer, the mixture was incubated at 37°C with continuous agitation for 2 hours. To eliminate coarse particles, the digested product was sieved via a 50-mesh/inch sieve. After collecting encysted larvae recovered via a 200-mesh/inch filter, washed twice with tap water and then suspended in a conical flask containing 150ml of tap water. The sediment larvae were tallied using a binocular microscope and three samples, each of which was 20µm in diameter, obtained from the sediment and placed on a slide. The supernatant fluid was thereafter discarded. 300 larvae per mouse were introduced orally into mice.

Drugs: 1-Albendazole suspension was obained from the Sigma Pharmaceutical Co., Egypt, and given at a dose of 50mg/kg. 2-Lactoferrin was prepared at TBRI, Giza, in a dose of 50mg/kg. Preparation was done using chromatography based on hydrophobic interactions with milk acid. 3- Silver nanoparticles were prepared at TBRI, Giza in a dose of 50mg/kg, prepared by using chemical reduction (Solomon *et al,* 2007).

Experimental design: Forty laboratory bred adult Swiss Albino mice weighed 20-30gm were classified into 8 groups of 5 mice each: G1: Neither infected nor treated (negative control), G2: Infected non-treated (positive or infected control), G3: Infected and treated with albendazole in a dose of 50 mg/kg, G4: Infected and treated with lactoferrin in a dose of 50mg/ kg, G5: Infected and treated with silver nanoparticles in a dose of 50mg/kg, G6: Infected and treated with lactoferrin loaded on silver nanoparticles in a dose of 50mg/kg, G7: Infected and treated with albendazole in a dose of 50mg/kg in combination with lactoferrin in a dose of 50mg/kg, and G8: Infected and treated with albendazole in a dose of 50mg/kg in combination with lactoferrin loaded on silver nanoparticles in a dose of 50mg/kg. Treatment started on the 31st day post-infection for five successive days. Mice were sacrificed on 35^{th} day PI to determine the efficacy on *T. spiralis* larvae (Nada *et al,* 2018).

Drugs' efficacy was assessed: I- Parasitological by counting of *T. spiralis* larvae on the 35^{th} day post-infection. Larval counting was done by taking three samples, each was formed of 20 microns from the sediment, then spreading it on the slide and counted by using binocular light microscope x40 power.

Histopathological: Muscle specimens were obtained from parts of the thigh muscles. Following fixation in 10% formalin, specimens were dried, cleaned, and embedded in paraffin blocks. Five-micron-thick paraffin se ctions were stained with hematoxylin and eosin, and microscopically examined.

Ethical consideration: The study was approval by Faculty of Medicine's Research Ethical Committee of Benha University (No. MD 5-2-2022).

Statistical analysis: Data were collected, computerized and analyzed by SPSS version 20 (Statistical Package for Social Sciences). Descriptive data measures were mean and standard deviation. ANOVA test evaluated between means of more than two grou- ps. $P < 0.05$ was significant***,** and $P < 0.01$ was very significant******. Reduction rate (R) was calculated from treated and positive control as follows: $R= [(A-B)/A] \times 100\%$, where A= mean number larvae recovered from positive control & B= mean number recovered from

treated mice (Feldman *et al*, 2003).

Results

Parasitological a significant *T. spiralis* larval count reduction was in treated mice as to G2 (P2<0.001**). G8 gave lowest (680.0± 192.35) compared to G2 (8940.0±1021.27). G5 gave highest (6000.0±254.95) as compared to G2 (8940.0±1021.27). Highest reduction count (92.39%) was in G8, followed by G7 (87.7%), G3 (79.64%), G6 (64.65%), G4 (44.52%) and least was in G5 (32.89%).

Histopathological, G1 showed normal muscular architecture; G2 showed encysted larvae and intact capsules encompassed by intense inflammatory cellular infiltrate (pericapsular plasma-lymphocytic inflammatory cellular infiltration). G3 showed encysted larvae (some with degenerated capsules) surrounded by intact capsules, macrophages and lymphocytes constitutes a moderate inflammatory cellular infiltration. G4 showed a decline in larval count accompanied by capsule degradation and a slight infiltration of inflammatory cells and muscular sections of silver nanoparticles treated mice, G5 showed fragmentation, vacuolation and capsular degeneration of most larvae accompanied by an infiltration of inflammatory cells, predominantly macrophages and lymphocytes. G6 showed a decline in quantity and deterioration of larvae with a mild inflammation.

G8 was better treated than G7, showed larval vacuolation and degeneration with mild macrophages and lymphocytes invasion.

Details were in tables (1) and figures (1, 2, 3, 4, 5, 6, 7, 8, 9, &10).

Table 1: Comparison between different groups regarding *Trichinella spiralis* larval count (n=5).

Groups	Mean ±SD	R	P2
G1: Negative control	0.0 ±0.0	NO	
G2: Positive (infected) control	8940.0 ±1021.27	0%	
G3: Albendazole	1820.0 ±432.43	79.64%	<0.001**
G4: Lactoferrin	4960.0 ±384.71	44.52%	<0.001**
G5: Silver nanoparticles	6000.0 ±254.95	32.89%	<0.001**
G6: Lactoferrin loaded on silver nanoparticles	3160.0 ±357.77	64.65%	<0.001**
G7: Albendazole and Lactoferrin	1100.0 ±158.11	87.7%	<0.001**
G8: Albendazole and Lactoferrin loaded on silver nanoparticles	680.0 ±192.35	92.39%	<0.001**
ANOVA	233.23		
P value	<0.001**		

P = significance between different groups, P2= significance between G2 & other treated mice, **highly significant (P <0.01).

Discussion

The present study showed that *T. spiralis* larval count in mice treated with albendazole showed a reduction rate of (79.64%). This result more or less agreed with Nassef *et al.* (2019), who found reduction rate of (71.3%) *T. spiralis* larvae treated with albendazole and also, with Eissa *et al.* (2022), who reported a reduction rate of (69.36%). However, result disagreed with El-Melegy *et al.* (2019, they reported a reduction rate of (40.18%) with mebendazole treated *T. spiralis* larvae.

In the present study, *T. spiralis* larvae in mice treated with lactoferrin was (44.52%). This agreed with Ordaz-Pichardo *et al.* (2012), who found that bovine lactoferrin reduced amoebic liver abscess in hamster model, intra-gastric treated (2.5mg/100g) for 8-days duration didn't have clinical manifestations and significant abscess diminishing.

The present study showed that *T. spiralis* larval count in silver nano-particles mice treated gave a reduction rate of 32.89%. This agreed with El-Melegy *et al.* (2019), they reported a reduction rate of (38.46%) in silver nanoparticles treated mice. Also, result agreed with Abd-Elrahman *et al.* (2021), they reported that larvae in muscle subjected to sub-lethal dosage of silver nanoparticles were significant destroyed.

In the present study, *T. spiralis* larvae treated with lactoferrin loaded on silver nanoparticles showed (64.65%) reduction rate, which was higher than lactoferrin treated mice. This agreed with Godot *et al.* (2000), who found that nanoparticles had greater tissue penetration power into alveolar hydatid cysts, leading to more destruction of the ger-

minal layer. Also, it agreed with Anand *et al.* (2015), they reported that nanoformulation increased the therapeutic potential of lactoferrin and anti-toxoplasmal activity was significantly of its proliferation in many organs and prolonged infected mice survival to 25 post infections. Also, Anand *et al.* (2016) reported that lactoferrin nanocapsules inhibited *Plasmodium berghei* parasite than native protein, and after 35 days post-infection, when parasitemia dropped to 4-5%, with significant infected mice survival.

The present study showed that *T. spiralis* larvae treated by utilizing a blend of albendazole and lactoferrin gave a high reduction rate of 87.7%, which was greater than mice treated with albendazole alone. This more or less agreed with Salama *et al.* (2021), who reported a reduction rate of 90.6% with albendazole, and agreed with Fahmy and Diab (2021) who found a reduction rate of *T. spiralis* larvae of 86.2% with combination albendazole-mefloquine mice.

In the present study, *T. spiralis* larvae treated by utilizing a blend of albendazole and lactoferrin loaded on silver nanoparticles gave highest reduction rate of 92.39% due to the synergistic action of combined treatment and nanoparticles. This agreed with Nassef *et al.* (2019) who found that the highest reduction rate of 97.3% in larval count in mice treated by nanoparticles loaded with full dosage of albendazole. Moreover, El-Melegy *et al.* (2019) reported that mice treated with mebendazole loaded on silver nanoparticles gave a reduction rate of 92.25%.

In the present study, muscles of positive control have many *Trichinella* encysted larvae with intact capsules encompassed by intense inflammatory cellular infiltrate (pericapsular plasma-lymphocytic inflammatory cellular infiltration). But, in albenazole treated mice *Trichinella* encysted larvae (some with degenerated capsules) contained within intact capsules and exhibiting a moderate infiltration of inflammatory cells, primarily lymphocytes and macrophages of mononuclear nature. Besides, Fahmy and Diab (2021) reported that albenazole treated infected mice showed larval deposition surrounded by mild muscle inflammation. Also, this agreed with Balaha *et al.* (2020) who found that *T. spiralis* infected mice treated with albendazole gave a reduced amount of encapsulated larvae encircled by a modest infiltration of inflammatory cells.

The present study showed that muscles of lactoferrin treated mice showed a decline in larval quantity accompanied with its capsule degradation and a minor inflammatory cellular infiltration. This was due to the fact that lactoferrin reduced proinflammatory cytokines such as TNF-α. Mice treated with silver nanoparticles showed capsular degeneration accompanied by an invasion of inflammatory cells, mainly macrophages and lymphocytes. This agreed with Nassef *et al.* (2019), who found that nanoparticles treated trichinellosis infected mice exhibited fragmentation of larvae and invasion by inflammatory cellular infiltrate. Also, it agreed with El-Melegy *et al.* (2019), who found that mice treated with silver nanoparticles caused larval capsule thinning, internal structures sever necrosis, less lymphocytic infiltration surrounding them, and a smaller necrosis area of muscle fibres. Besides, Hassan *et al.* (2021) showed that nanoparticles treated *T. spiralis* infected mice showed muscles histopathological inflammatory changes compared to positive control.

In the present study, muscles of lactoferrin loaded on silver nanoparticles treated mice showed decrease in larval population and degeneration accompanied with a mild inflammatory edema. This agreed with El-Melegy *et al.* (2019), who reported that muscles treated with mebendazole loaded on nanoparticles gave marked thinning and destruction of capsulated larvae, including internal structures and lysed, leaving nearly typical skeletal muscle fibers with striations and peripheral nuclei without lymphocytic infiltr ation. Formerly, Samarasinghe *et al.* (2014) reported the potential therapeutic utility of

bovine lactoferrin nanoformulateds in treating chronic inflammatory rheumatic illnesses and osteoarthritis. The present study showed that muscles of mice treated with albendazole and lactoferrin have larval vacuolation with capsular degeneration with mild invasion via inflammatory cells mainly macrophages and lymphocytes. This agreed with Fahmy and Diab (2021), who reported that mice given combined treatment of mefloquine and albenazole had significantly fewer encysted larvae, with degenerative changes in contents and entirely invaded with inflammatory cells. Besides, it agreed with Salama *et al.* (2022), who found that larvae treated with albendazole and prednisolone showed a mild to moderate inflammatory cellular infiltrate. Again, Balaha *et al.* (2020) found that Albendazole and resiniferatoxin-treated mice exhibited a limited quantity of *T. spiralis* larvae and mild inflammation; some muscles retained normal striation.

The present study showed that muscles of mice treated with albendazole and lactoferrin loaded on silver nanoparticles gave the least inflammation intensity, larval vacuolation and degeneration. Positive control gave significantly histopathological decease in the inflammatory cellular infiltration due to synergistic action of both drugs and nanoparticles. This agreed with Nassef *et al.* (2019), who reported rare inflammation intensity and deposition of *T. spiralis* larvae in mice treated with nanoparticles loaded on albendazole full dose. Also, Hassan *et al.* (2021) reported that mice given a combined treatment of Ivermectin® and Nitazoxanide® loaded on nanoparticles gave significant reduction in number of *T. spiralis* encysted larvae, inflammatory infiltrates and destruction due to the inflammatory cell invasion, and marked larval complete degeneration.

Conclusion

Muscle phase of trichinellosis was treated effectively with lactoferrin when coated on silver nanoparticles and albendazole as well.

This was documented by marked decrease in larval numbers and muscles improvement.

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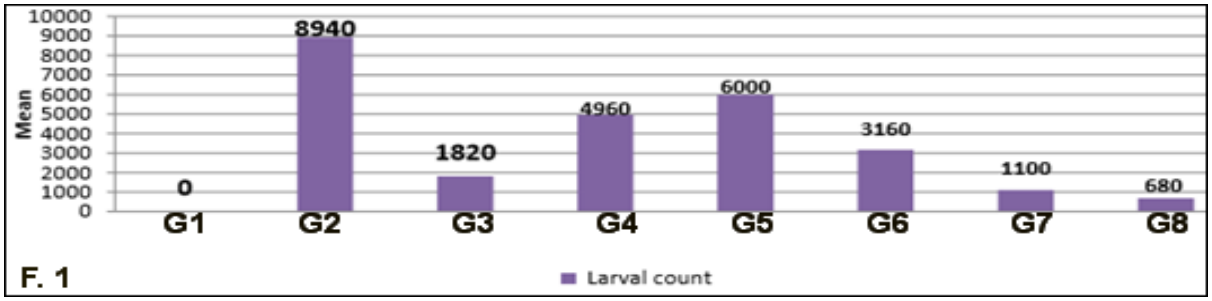
Explanation of figures

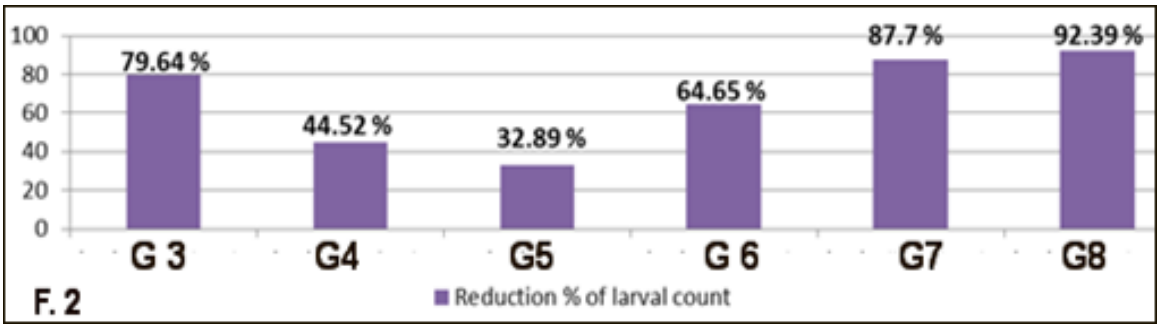
Fig. 1: Comparison between different groups regarding *Trichinella spiralis* larval count.

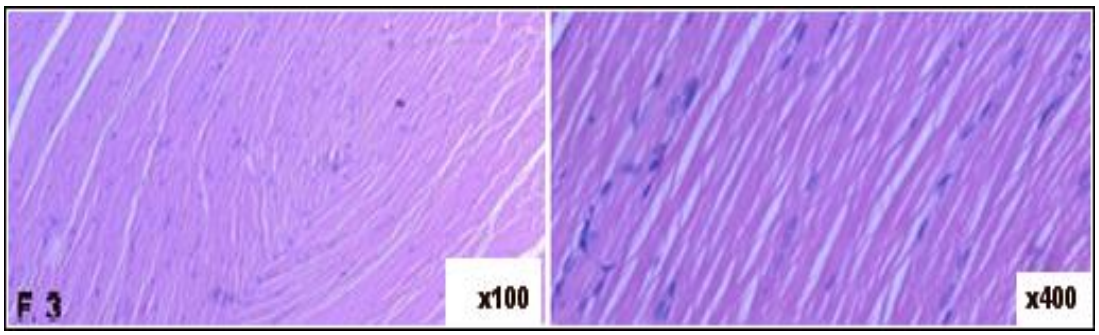
Fig. 2: Comparison between different groups regarding reduction rate of *T. spiralis* larval count.

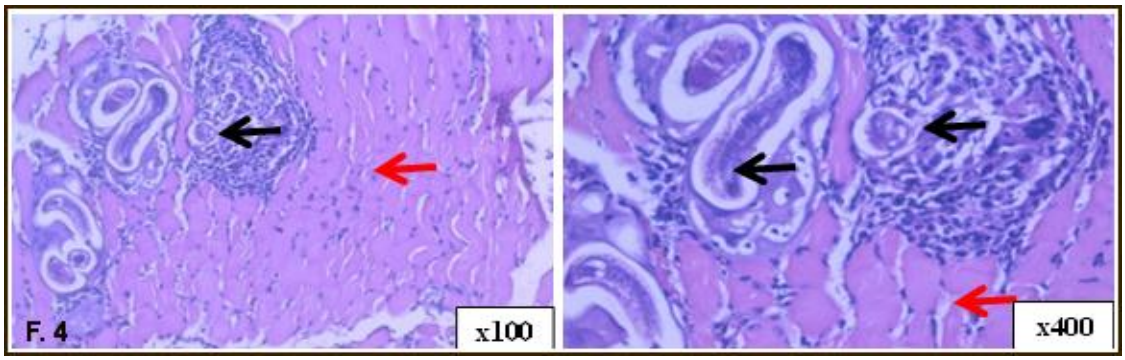
Fig. 3: Muscles section of negative control showed normal muscular architecture (H & E stained).

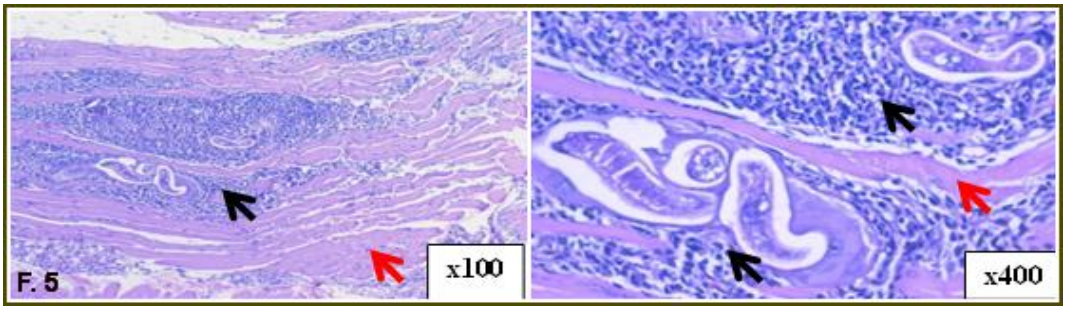
Fig. 4: Muscles section (red arrow) of infected control stained with H&E, showed *T. spiralis* encysted larvae and intact capsules surrounded by intense inflammatory cellular infiltrate (pericapsular plasma-lymphocytic inflammatory cellular infiltration), indicated by black arrows.
Fig. 5: Muscles section (red arrow) of albenazole treated mice stained with H&E, showed *T. spiralis* encysted larvae (some with degenerated capsules) surrounded by intact capsules and moderate inflammatory cellular infiltrate of mononuclear cells, mainly lymphocytes, and macrophages (black arrows).
Fig. 6: Muscles section (red arrow) of lactoferrin treated mice stained with H&E, exhibiting a decrease in number of larvae with some degeneration of the larvae capsule surrounded by mild inflammatory cellular infiltrate (black arrows).
Fig. 7: Muscles section (red arrow) of silver nanoparticles treated group stained with H&E, exhibiting fragmentation and vacuolation of the larva and capsular degeneration of most of *T. spiralis* larva with an invasion by inflammatory cells, mainly lymphocytes, and macrophages. Black arrows indicate vacuolated capsules.
Fig. 8: Muscles section (red arrow) of lactoferrin loaded on silver nanoparticles treated group stained with H&E, exhibiting a decrease in the number and a degeneration of *T. spiralis* larvae, enclosed within mild inflammation (black arrows).
Fig. 9: Muscles section (red arrow) of albendazole and lactoferrin treated group stained with H&E, showed vacuolation of larva with capsular degeneration with mild invasion by inflammatory cells (macrophages and lymphocytes) (black arrows).
Fig. 10: Muscles section (red arrow) of albendazole and lactoferrin loaded on silver nanoparticles treated group stained with H&E, showed vacuolation and degeneration larva and capsule with mild invasion by inflammatory cells (macrophages and lymphocytes) (black arrows).
Fig. 10: Muscles section (red arrow) of albendazole and lactoferrin loaded on silver nanoparticles treated group stained with H&E, showed vacuolation and degeneration larva and capsule with mild invasion by inflammatory cells (macrophages and lymphocytes) (black arrows).







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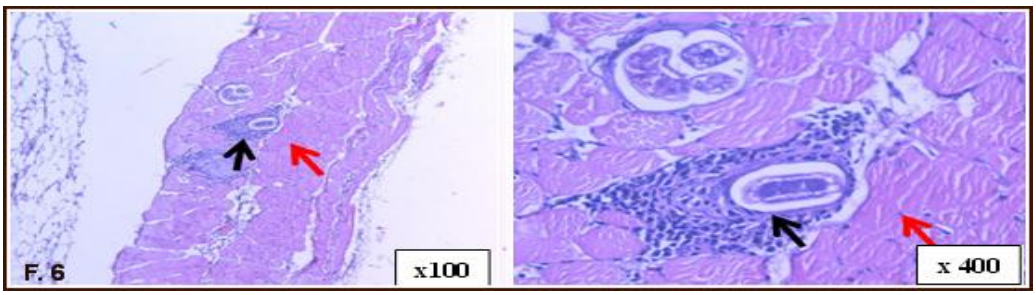
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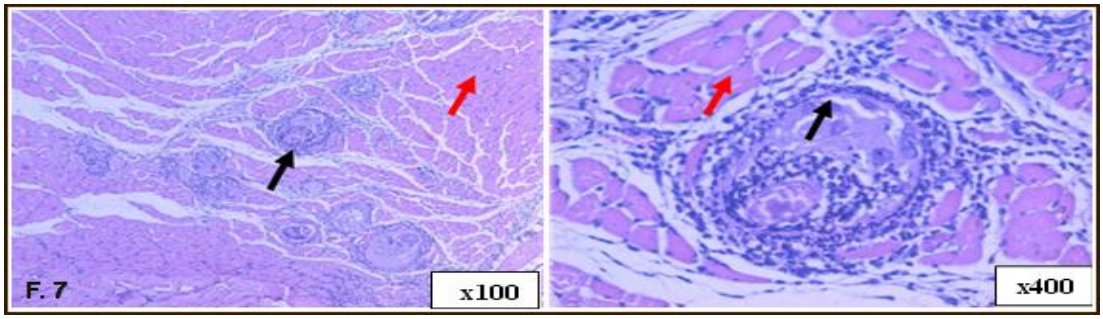
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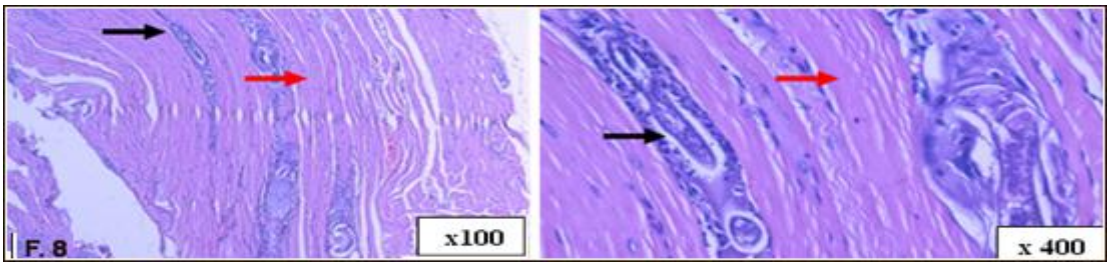
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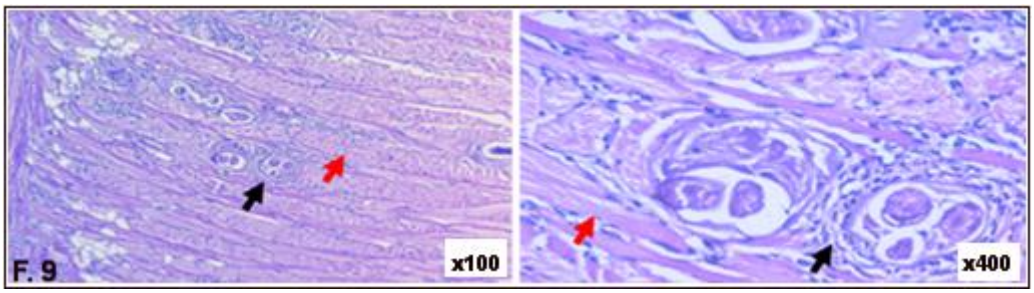
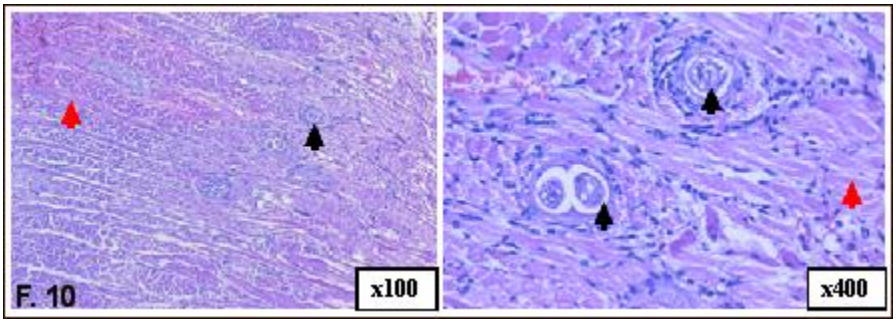

F. 9
x100
x400


F. 10
x100
x400