Silver Nano Particles Improve the Therapeutic Effect of Mebendazole Treatment during the Muscular Phase of Experimental Trichinellosis

Manal Ahmed EL-Melegy[1], Nagwa Saad Ghoneim[2], Nermeen Mohamed Noor EL-dien[3], Mohamed Soliman Rizk[4]

[1]Department of Parasitology, Faculty of Medicine, Menoufia University, Egypt
[2]Department of Histology & Molecular Biology, Faculty of Medicine, Menoufia University, Egypt
[3]Department of Anatomy, Faculty of Medicine, Menoufia University, Egypt
[4]Department of Biochemistry, Faculty of Medicine, Menoufia University, Egypt
nermeennooreldien@gmail.com

Abstract: Background: Mebendazole is one of the Benzimidazole drugs that are used for treatment of trichinellosis, but they have a restricted effect against encysted *Trichinella spiralis (T. spiralis)* larvae in muscles. **Aim:** Our aim wasto improve the anthelminthic effect ofmebendazole; so we used mebendazole loaded to silver nano particles (AgNPs) to evaluate its effect on encysted *T. spiralis*larvae in muscles compared to the treatment with mebendazole alone and AgNPs alone. **Methods:** Thirty (30) female Swiss albino micewereused in this study & equally divided into five groups. Group1 (G1): served as control negative group (non infected non treated), while the other 4 groups (G2, G3, G4 & G5) were infected with 400*T. spiralis*larvae. G2: served as control positive group (infected non treated), G3: treated with mebendazole (50mg /kg / day), G4: treated with AgNPs (50mg / kg / day) & G5: treated with mebendazole (20mg / kg / day) loaded to AgNPs. Treatment of mice in G3, G4 & G5 started on 35thday post infection for 5 consecutive days. The duration of the experiment was 40days All mice were sacrificed at the end of the experiment. Evaluation of the treatment was done by counting *T. spiralis* larvae, histological examination of skeletal muscle tissue using Hx& E and Mallory stains, immunohistochemical staining of muscle tissue using cyclooxygenase-2 (COX-2), scanning electron microscopic study and biochemical measuring of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and creatinine phosphokinase (CPK). **Results:** Our results revealed that each of the three lines of treatment (mebendazole alone, AgNPs alone and mebendazole loaded to AgNPs) showed significant ($p \leq 0.001$) reduction in the mean larval count compared to the infected control. There was significant ($p \leq 0.001$) reduction in the mean larval count with mebendazole loaded to AgNPs comparing with the other treated groups. The percentage of reduction was (40.18%,38.46%,92.25%) respectively. Inthe present work treatment with mebendazole 20mg loaded to AgNPsshowed restoration of nearly normal appearance of skeletal muscle fibers with marked reduction of inflammatory reaction and COX-2 expression. while treatment with mebendazole alone showedmoderate damage and necrosis ofmuscle fibers, moderate inflammatory reaction and COX-2 expression. In AgNPstreated group there wasmild damage and necrosis of skeletal muscle fibers and mildinflammatory reaction and COX-2 expression. Biochemical results showeda highly significant ($p \leq 0.0001$) reduction of AST, ALT, creatinine and CPK levels in all treated mice compared to infected control group, with highly significant reduction ($p \leq 0.0001$) in mebendazole loaded AgNPs treated group compared to the other treated groups. **Conclusion**: AgNPs improves the anthelminthic effect of mebendazole in treatment of muscular phase of experimental trichinellosis.

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Key wards: AgNano, mebendazole, Tricinella, COX-2

1-Introduction

Trichinellosis is a zoonotic disease that transmitted through ingestion of incompletely cooked pork meat containing infective *T. spiralis* larvae **(1)** It has 2 phases in life cycle the intestinal phase (enteral phase)where the adults reside in the small intestine while inmuscular phase (parenteral phase)the larvae encysted in skeletal muscle forming nurse cells complex) within the same host **(2).** Subsequently, migrating larvae and their metabolites provokean immediate reaction, with an inflammatory and allergic response. Muscle weakness and myalgia are the common manifestations resulting from muscle damage due to nurse cell formation by the infecting larvae **(3)**. Benzimidazole drugs are anthelmintic drugs for the treatment of trichinellosis **(4)**. However, they have a limited effect against encapsulated larval stages of *T. spiralis* **(5)**. Nevertheless, benzimidazole derivatives have alimited effect by poor water solubility and the consequent low bioavailability, **(6).** Therefore, a high dose of mebendazole (MBZ) is used for helminthic infections with many adverse effects. Hepatic and

haematologic toxicities are the most common severe side effects. Several studies have shown evidence of teratogenic effects of MBZ in rats and mice **(7).** So it becomes mandatory the development of new pharmaceutical formulations. Recent advances in nanoscience and nanotechnology improve the way we diagnose, treat, and prevent various diseases be causet hey have characteristic biological effects depends on the structure and size **(8)**. Silver nanoparticles (AgNPs) are one of the most important nanomaterials that are involved in nanomedicine **(9).** They have been widely used as antibacterial agents, biomedical applications, and also as antiangiogenic **(10)** and anticancer agents **(11) (12).** AgNPs achieved a great attention in biomedical applications due to their unique physicochemical properties **(13)**. So in the current work we selected AgNPsto assess its effect in combination with mebendazole on the muscular phase of trichinellosis to improve drugabsorbance and reduce drug toxicity. We evaluate its effect through parasitological, histological, immunohistochemical, scanning electron microscopic and biochemical studies.

2-Material & methods:

2.1.-Experimental animals & infection

Thirty (30) female Swiss albino mice 7-8 weeks age weighting about 20-25gm. were obtained from Theodor Bilharz Research Institute (Giza, Egypt). They were laboratory bred. All animals received human care in compliance with the Public Health Service Policy on Human Care and Use of Laboratory Animals, published by the National Institutes of Health and were approved by the Ethical Committee of the College of Medicine, Menoufiya University, Egypt. They were divided into equally five groups (6 mice / group). The experiment lasted 40 days. *T. spiralis* strain was previously genotyped as *T. spiralis* by the European Union Reference Laboratory for Parasites. Superior Institute of Health, Rome, Italy was obtained from Tanta faculty of medicine by consecutive passages through rats and mice. The infected skeletal muscles takenfrom the diaphragm muscle were cut excised (~2 cm), then digested with pepsin solution (Pepsin 1g / HCl 1ml / distilled water to 100ml) for 12 hours **(14).** The freshly isolated *T. spiralis* larvae were counted under a stereomicroscope. Each mouse in the control positive & experimental (infected) groups was infected with400 *T. spiralis* live larvae per oral feeding gavage tube **(14).**

Animal groups:

G1: non infected non treated (control negative)

G2: infected non treated (control positive)

G3: infected & treated with mebendazole 50 mg/kg/day for 5 days **(15).**

G4: infected & treated with AgNPs50mg/kg/day for 5 days **(16).**

G5: infected & treated with mebendazole 20 mg/kg loaded to AgNPs for 5 days.

2.2 Drug:

Mebendazole: was purchased as tablets (Alexandria CO. for pharmacuticals & chemical industries. Alexandria-Egypt.). One tablet (100 mg) was dissolved in 50ml distilled water and given orally in a dose of 50 mg/kg/day for 5consecutive days staring on the 35th day post infection (dpi**) (15).**

2.3. Synthesis of Silver Nanoparticles

The synthesis procedure was done at Theodor Bilharz Research Institute (Giza, Egypt) using chemical reduction method according to **(17)**. Briefly, 30 mL of 0.002M sodium Borohydride (NaBH4) (Sigma-Aldrich) were added in an Erlenmeyer flask with magnetic stir bar. The flask was putin an ice bath on a stir plate for about 20 minutes. 2mL of 0.001M silver nitrate (AgNO3) (Sigma-Aldrich) was added into the stirring NaBH4 solution. Few drops of 1.5 M sodium chloride (NaCl) were dripped to the solution. A small portion of the solution was transferred to a test tube. A drop of 0.3% PVP (Sigma-Aldrich) was added. The sample was transferred to toaster oven for 30 minutes. The morphology and UV-absorbance of AgNPs was analyzed by using transmission electron microscopy (JEOL, Japan). UV/Visible spectroscopy (Labomedspectro UV-VIS 2700, USA)was used for structural characterization of silver nanoparticles. The particle size was in the range of (~40nm) and spherical in shape.

2.4. Mebendazole loaded to AgNPS: (18)

Mebendazole 20 mg was loaded into silver nanoparticles by swelling equilibrium method. After removing the nanoparticles from the drug solution. The loading efficiency of drug in the nanoparticles is monitored spectrophotometrically, at kmax 491.2 nm.

2.5. Counting *Trichinella spiralis* larvae:

Mice were sacrificed and larvae were obtained fromskeletal muscle biopsy using1 % pepsin/1 % HCl for digestion **(19).** larvae were collected after artificial digestion of muscles, the collected larvae were washed two to three times with tap water and suspended in a conical flask for half an hour to help sedimentation. Supernatant fluid was discarded and sediment larvae were microscopically counted using stereomicroscope. The total number of the larvae were counted for every mouse.

2.6. Histological & immunological examination:

For light microscopic examination the specimens were fixed in 10% formalin and processed to prepare 5 µm thick paraffin sections for H&E staining, Mallory trichrome staining, immunohistochemical stain for cox 2 **(20) (21)**. For preparation of semi thin sections, small pieces of skeletal muscles of each group were

fixed immediately in 5% gluteraldehyde for 24h. The specimens were processed and semithin sections were prepared and stained with toluidine blue stain **(22).**

2.7. Scanning Electron microscopic (SEM) examination:

(SEM) processing and examination were carried out at electron microscopy unit. Faculty of Medicine, Tanta University, using electron microscope (JEOL, Japan). according to **(23**) (**24**).

Photographs were recorded on Kodak electron image plates **(25).**

2.8. Biochemical study:

Blood samples were withdrawn at the end of the of experiment in plain tubes. They were left at room temperature for 30 minutes, then centrifuged at 3000 rpm for 15 min. aliquoted and stored at – 20 ºC. Alanine transaminase (ALT),aspartate transaminase (AST), creatinephosphokinas (CPK) and lactate dehydogenes (LDH) enzymes were measured by spectrophotometic methods by using Cobas-C while total immunoglobin E was measured by automated immunoassay analyzer Cobas e 411 (Rochecompany – USA).

2.9. Statistical analysis

Data were analyzed and compared by student's t-test. The p-value was used to test the significant change in the experimental animals among the different groups. The data collected were tabulated as mean ± SD and analyzed using statistical package for the Social Science Software (SPSS) software (version 17.0 on an IBM compatible computer; SPSS Inc., Chicago, Illinois, USA). P value was set at 0.05, P>0.05 non-significant, P value<0.05 significant and P value <0.001 highly significant **(26).**

3-Results:

Parasitological results:

Larval count:

Coiled larvae were seen in the different groups after digestion of skeletal muscles **(Fig.1).**

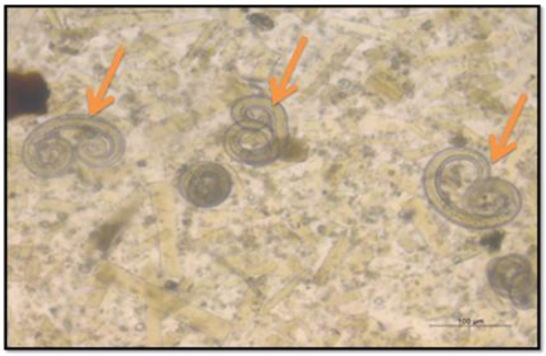


Fig (1): *Trichenilla spiralis* larvae after muscle digestion X 100

The mean number of totallarvae countin the treated groups (G3, G4, G5) was significantly (p≤ 0.001) reduced comparing to the infected non treated group G2.

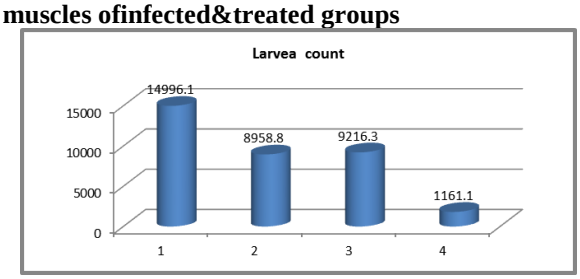
While G5 showed asignificant (p≤0.001)reduction in the mean larval count compared to the other treated groups (G3, G4). The percentage of reduction in the meanlarval count in (G3, G4, G5) was 40.18%,38.46% and92.25%respectively **(Graph.1).**

Histological results:

Haematoxylin and Eosin stain

Haematoxylin and Eosin stained sections of skeletal muscle of G1 showed elongated skeletal muscle fibers with acidophilic cytoplasm, multiple peripheral basophilic nuclei and transverse striations **(Fig.2. A &Fig 3. A).** Sections of G2 showed heavy infestation by *T. spirallis* encapsulated larvae, necrosis of muscle fibers with loss of striation and nuclei, heavy lymphocytic infiltration **(Figs.2. B & 3. B)**. Sections of G3showed also multiple encapsulated larvae with thickened capsule with partial fragmentation of its internal structure, heavy lymphocytic infiltration, loss of striation and nuclei of most of fibers with vaculation **(Fig. 2. C & Fig 3. C1,C2)**, while sections of G4 showed thinning of larvae capsule, more necrosis of larvae internal structure, less lymphocytic infiltration around larvae and less area of necrosis of muscle fibers **(Fig.2. D & Fig. 3. D1, D2)**. Sections of G5 showed marked improvement in the form of marked thinning and destruction of larvae capsule with decrease in size of larvae, destruction and lysis of larvae's internal structure, empty spaces at site of dead larvae, more or less nearly normal skeletal muscle fibers with peripheral nuclei and striation & no lymphocytic infiltration **(Fig.2. E & Fig 3. E1, E 2).**

Graph.1: The mean number of totallarvae in muscles ofinfected&treated groups



SD= the standard deviation. ** Highly significant (p<0.001).

Histological results:

Haematoxylin and Eosin stain

Haematoxylin and Eosin stained sections of skeletal muscle of G1 showed elongated skeletal muscle fibers with acidophilic cytoplasm, multiple peripheral basophilic nuclei and transverse striations

(Figs.2. A &Fig.3. A). Sections of G2 showed heavy infestation by *T.spirallis* encapsulated larvae, necrosis of muscle fibers with loss of striation and nuclei, heavy lymphocytic infiltration **(Figs.2. B &Fig.3. B)**. Sections of G3showed also multiple encapsulated larvae with thickened capsule with partial fragmentation of its internal structure, heavy lymphocytic infiltration, loss of striation and nuclei of most of fibers with vaculation **(Fig.2. C &Fig. 3. C1, C2)**, while sections of G4 showed thinning of larvae capsule, more necrosis of larvae internal structure, less lymphocytic infiltration around larvae and less area of necrosis of muscle fibers **(Fig.2. D & Fig. 3 (D1, D2)**. Sections of G5 showed marked improvement in the form of marked thinning and destruction of larvae capsule with decrease in size of larvae, destruction and lysis of larvae's internal structure, empty spaces at site of dead larvae, more or less nearly normal skeletal muscle fibers with peripheral nuclei and striation & no lymphocytic infiltration **(Fig.2. E & Fig. 3 E1, E2).**

Mallory trichrome stain:

Mallory trichrome stained Sections of G1showed minimal amount of collagen fibers between skeletal muscle fibers **(Fig.4. A)**. G2 & G3 showed excessive deposition of collagen fibers around larvae and between muscle fibers **(Figs.4. B & C)**, whileG4 showed moderate deposition of collagen fibers **(Fig.4. D)**. G5 showed deposition of mild amount of collagen fibers around empty spaces of dead larvae and between muscle fibers **(Fig.4. E)**.

Immunohistochemical stain (COX-2):

Cox 2 stained sections of G1 showed weak reaction **(Fig.5. A)** while G2 showed strong positive reaction around heavy infested larvae and between muscle fibers **(Fig.5. B)**. G3 showed moderate positive reaction around capsule and larvae and between muscle fibers **(Fig.5. C)**, G4 showed mild reaction of the stain **(Fig.5. D)**& G5 showed mild to weak reaction between muscle fibers and around empty spaces of dead larvae **(Fig.5. E)**.

Toluidine blue stain (Semithin sections)

Semithin sections of G1 stained with toluidine blue revealed normal skeletal muscle fibers **(Fig.6. A)**.Sections of G2 revealed part of larvae with thick capsule, part of its internal structure, congested blood vessels, hemorrhage with extra vasation of RBCs, inflammatory cells, adjacent muscle fibers showed degeneration of some nuclei, some nuclei were hyperchromatic, other nuclei show karyolysis **(Fig.6. B)**. Sections of G3showed picture more or less near to G2with fragmentation of internal structure of larvae **(Fig.6. C)**, while sections of G4 showed marked thinning of larvae capsule with decrease of size of its internal structure with some inflammatory cells **(Fig.6. D)** and degeneration of muscles in both groups **(Fig.6. C & D)**. On the other hand, G5 revealed marked improvement with destruction and lamination of capsule of larvae andlysis of its internal structure, less inflammatory cells & skeletal muscle fibers appeared within normal **(Fig.6. E1 , E2)**.

Scanning Electron microscope (SEM) results:

Scanning electron microscope of *T.spiralis*larvae of G2 showed normal cuticle with transverse creases and longitudinal ridges (**(Fig.7. B1-B2**). There was marked morphological changes in the larvae of treated groups . The larvae in G3 showed multiple vesicles and blebs on the cuticle (**(Fig.7. C1, C2**). while the larvae in G4 showedsloughing of some areas of the cuticle with fissures and small blebs (**(Fig.7. D1, D2**). On the other hand the larvae in G5 showed destruction and deformity of the cuticle with formation of a cauliflower masses (**(Fig.7. E1, E2**).

Biochemical results:

Revealed that there was a highly (P<0.0001) significant decrease in serum levels of, ALT, AST, CPK, Creatinine and LDH in the serum of the treated groups G3, G4, G5 compared to the infected control G2. There was a highly significant (P<0.0001) reduction in the mean levels of AST, ALT, CPK, Creatinine and LDH in G5 as compared with their levels in G2, G3, G4 (Table 1).

Table 1: Serum biochemical parameters in the different groups

	G1	G2	G3	G4	G5	F	P. value
ALT: (U/ml)	**30.35±0.65**	**69.67±2.66**	**57.27±3.88**	**58.71±4.66**	**47.1±4.1**	284.3	**0.000**
AST: (U/ml)	**121.64±1.34**	**170.55±4.72**	**160.15±5.20**	**160.54±6.70**	**147.9±11.53**	227.8	**0.000**
CPK: (IU/L)	**52.58±1.16**	**500.30±14.27**	**384.50±21.67**	**210.00±64.17**	**94.6±16.2**	421.5	**0.000**
Creat: (mg/dl)	**1.53±0.13**	**2.96±0.28**	**2.75±0.45**	**2.50±0.14**	**1.97±0.19**	56.3	**0.000**
LDH: (mg/dl)	**151.2± 3.6**	**455.6±5.68**	**397.7±11.9**	**204.9±8.3**	**165.5±8.3**	**3072.1**	**0.001**

Highly significant (p<0.001).

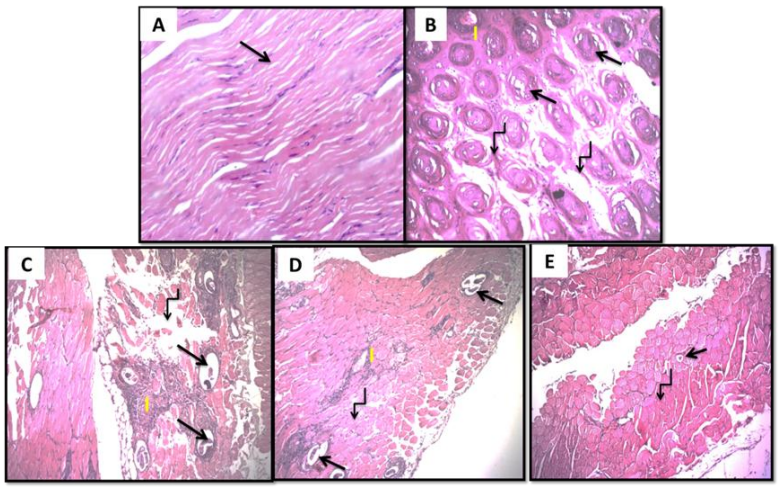


Fig (2): A) photomicrograph of Hx & E stained Section of skeletal muscle of (A): (G1) showing normal elongated skeletal muscle fibers (arrow) with acidophilic cytoplasm, multiple peripheral basophilic nuclei and transverse striations. (B): (G2) showing heavy infestation of *Trichinella spiralis* encysted larvae (arrow). Area of necrosis of muscle tissue (curved arrow) & heavy lymphocytic infiltration (I): (C) (G3) showing multiple encysted larvae with partial necrosis (arrow), heavy lymphocytic infiltration (I), area of necrosis in muscle (curved arrow). (D): (G4) showing few larvae with mild necrosis (arrows), mild lymphocytic infiltration (I) & minimum degeneration in muscle fibers (curved arrow). (E): (G5) showing very few small larvae (arrow), nearly normal skeletal muscle fibers (curved arrow) & no lymphocytic infiltration (H & E x100).

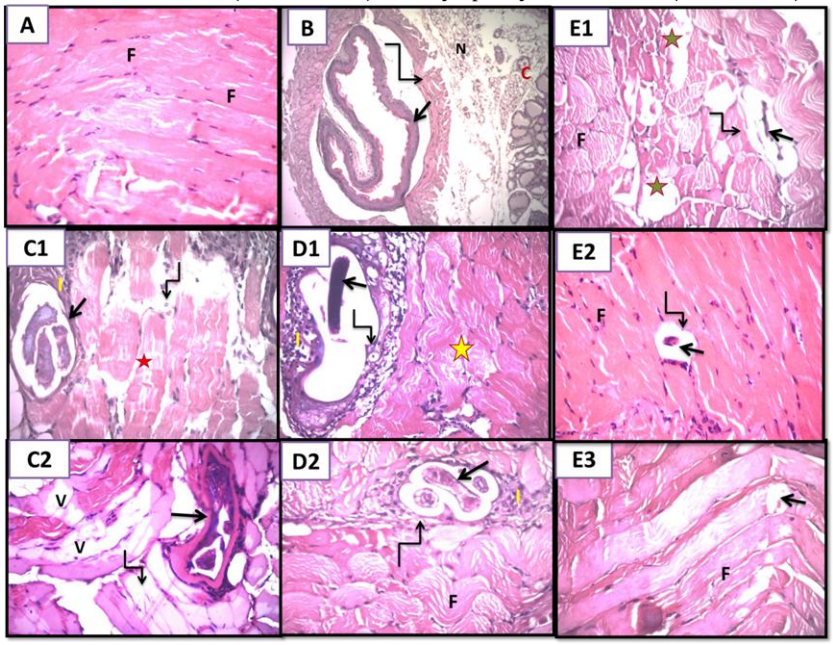


Fig 3: A photomicrograph of Hx & E stained sections of skeletal muscle of:(G1: A) showing normal elongated skeletal muscle fibers (F) with acidophilic cytoplasm, multiple peripheral basophilic nuclei and transverses striations. (G2: B) showing complete encysted larva (arrow), with thick wall (curved arrow), ghosts of necrotized tissue (N)&congested blood vessels (C). (G3: C1, C2) showing: Larva with thickened capsule and fragmentation of its internal structure (arrow), lymphocytic infiltration (I), necrotized tissue (curved arrow), loss of nuclei (asterisk). (C2): showing larvae with thickened capsule (arrow), loss of striations and nuclei of muscle fibers (curved arrow) & vacuolation of some muscle fibers (V).(G4: D1, D2) (D1): showing thinking of larvae capsule (curved arrow), partial necrosis of larva (arrow), heavy lymphocytic infiltration around larvae (I)&areas of necrosis in muscle fibers (asterisk). (D2): showing larva with some necrosis () arrow) & thinned capsule(curved arrow), moderate lymphocytic infiltration (I)&mild degradation in some muscle fibers (F). (G5: E1, E2, E3). (E1): showing larvae with very thin partially destructed capsule (curved arrow), marked lysis of larvae (arrow), empty spaces at site of dead larvae (asterisk), muscle fibers more or less near normal & nuclei start to appear (F). (E2): showing small size larva (curved arrow) with marked lysis of its internal structure (arrow) and more or less normal skeletal muscle fibers with peripheral nuclei & striations (F). (E3): showing very small shrunken larva with very thin capsule (arrow), muscle fibers appear nearly normal & nuclei starts or appear (F) (Hz&Ex400).

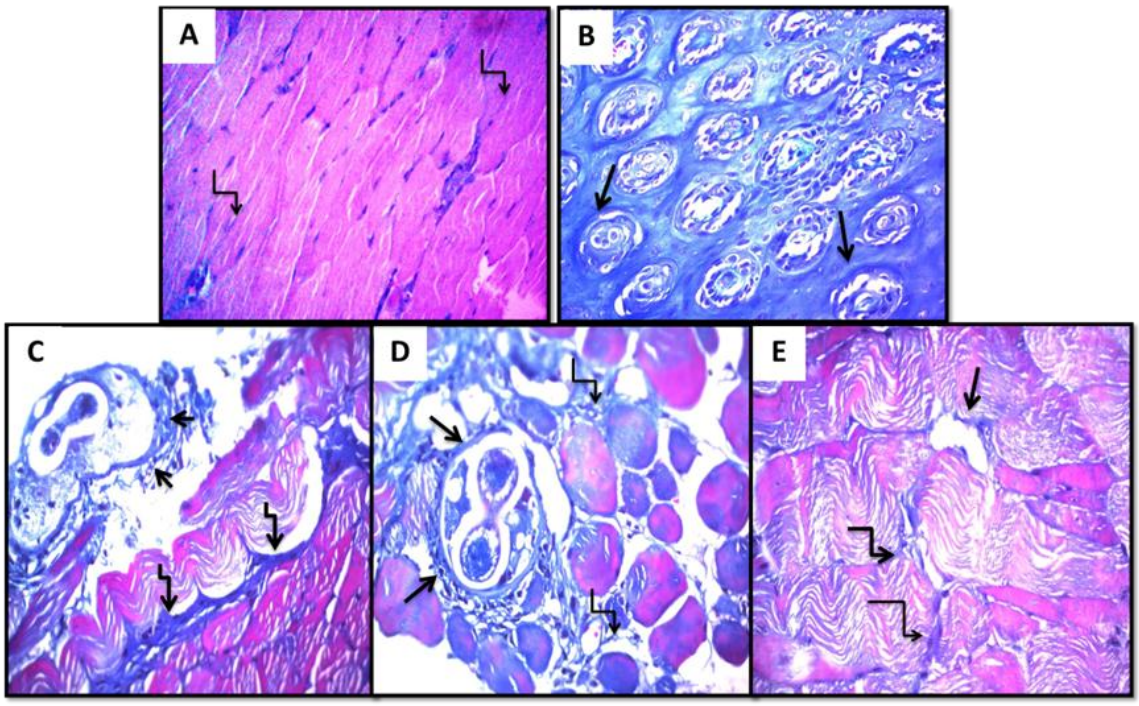


Fig. (4): A photomicrograph of Mallory trichrome stained sections of skeletal muscle of (G1: A): showing normal elongated skeletal muscle fibers (red) with minimal amount of blue collagen fibers between the muscle fibers (curved arrows). (G2: B): showing massive deposition of blue collagen fibers around larvae and between muscle fibers (arrows) (G3: C): showing marked deposition of collagen fibers around larvae (arrows) and between muscle fibers (curved arrows). (G4: D): showing moderate deposition of collagen fibers around larvae (arrows)&in between muscle fibers (curved arrows)/(G5: E): showing mild amount of collagen fibers around empty larvae (arrow) and in between muscle fibers (curved arrows) (Mallory x200)

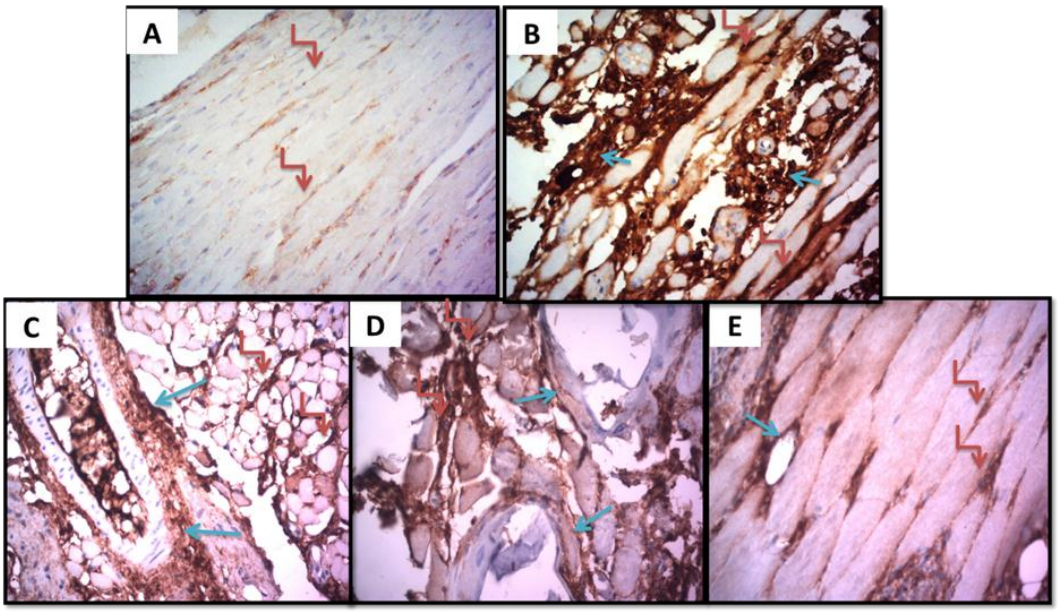


Fig. (5): A photomicrograph of COX2 immunohistochemical stained sections of skeletal muscle of (G1: A): showing weak reaction between muscle fibers (curved arrow). (G2 B): showing strong reaction around larvae (arrows) and between nuclei fibers (curved arrows). (G3: C): showing moderate reaction around capsule and no larvae (arrows) and between muscle fibers (curved arrow). (G4: D): showing mild reaction around larvae (arrows) and between muscle fibers (curved arrows). (G5: F): showing mold to weak reaction around empty larvae (arrow) and between muscle fibers (curved arrows) (COX2x400).

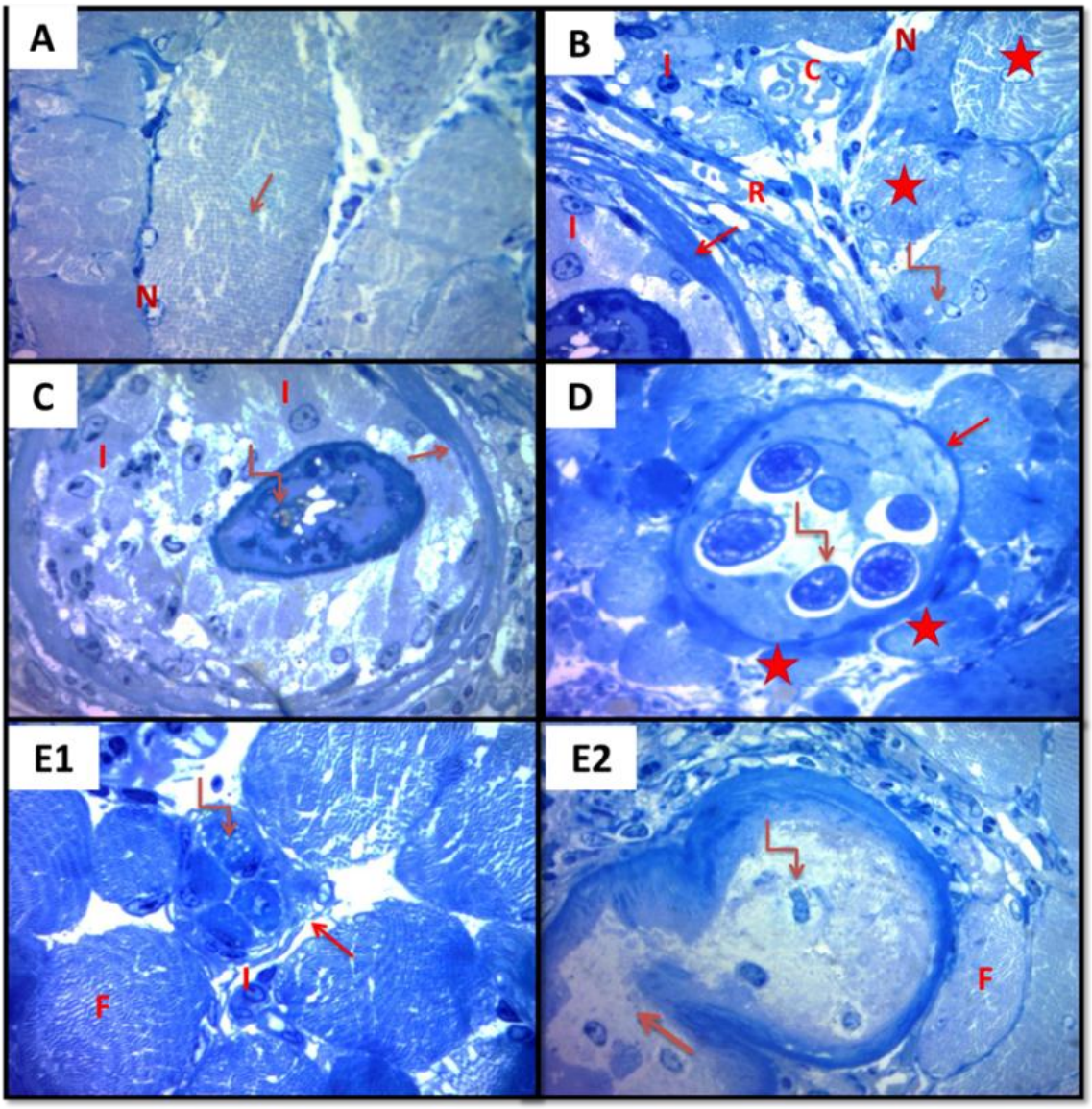


Fig (6) A photomicrograph of toludine blue stained sections of skeletal muscle of: (G1: A): showing skeletal muscle fibers with striations (arrow) & peripheral nuclei (N). (G2: B): showing part of larvae with thick capsule (arrow), elongated blood vessels (C), extra vasated RBCs (R), inflammatory cells (I), hyper chromatic nuclei (N), karolytic nuclei (curved arrow), degenerated muscle fibers (asterisks). (G3: C) showing larva with thickened capsule (arrow, small sized internal structure (curved arrow) & inflammatory cells (I). (G4: D): showing larvae with markedly thinned capsule (arrow) and small sized internal structure (curved arrow) & degenerated muscle fibers (asterisks). (G5: E1, E2): (E1): showing partial destruction of larval capsule (arrow), fragmentation of its internal structures (curved arrow), nearly skeletal muscle fibers with normal striations (F) and few inflammatory cells (I). (E): showing destruction of the wall of the capsule (arrow, marked lysis of the internal structure (curved) & nearly normal skeletal muscle fibers (F) (Toludine blue x1000).

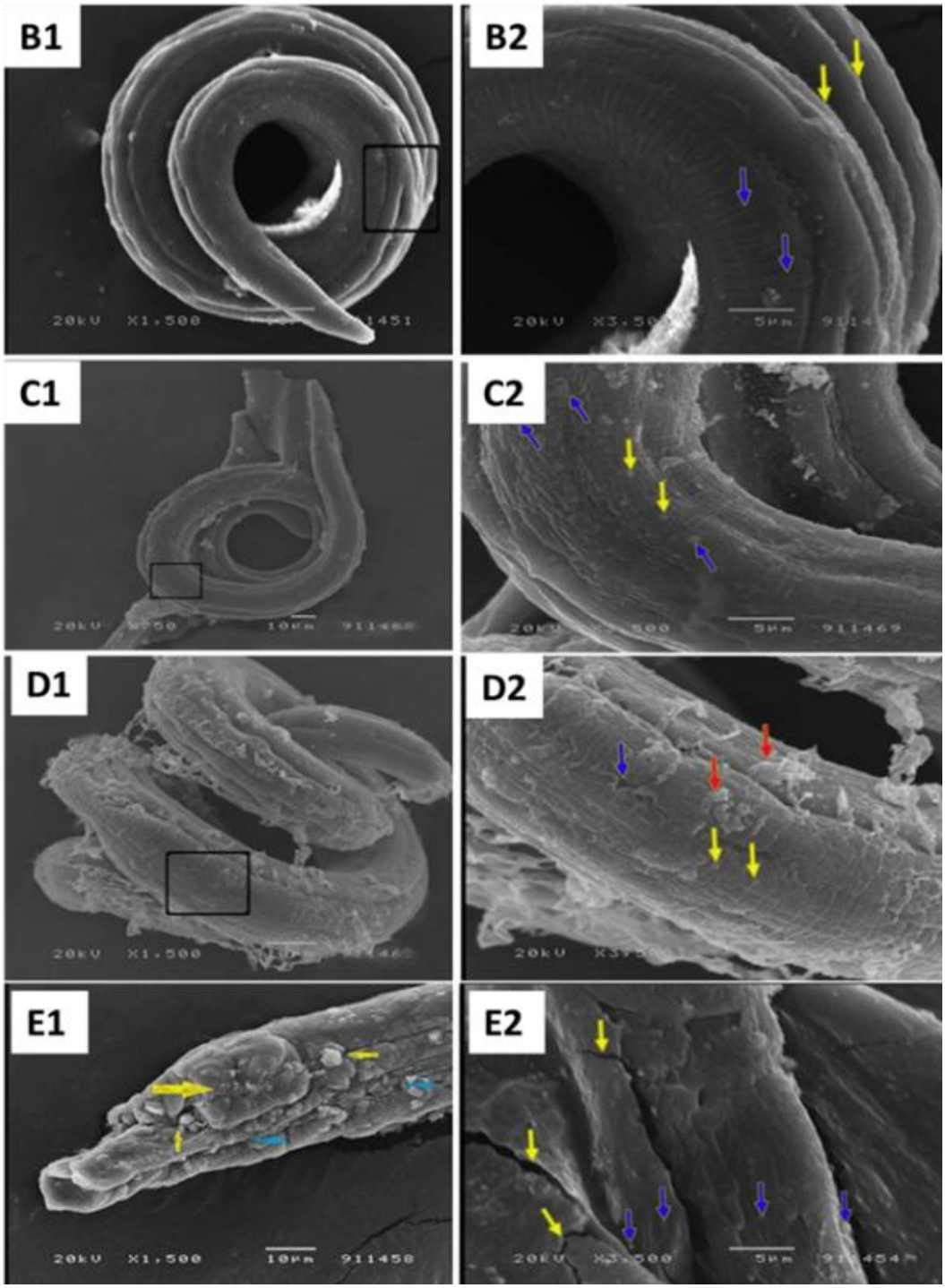


Fig (7): Scanning electron microscope of *Trichinella spiralis* larva:(G2 B1&B2): showing normal cuticle with transverse creases (blue arrow) and longitudinal ridges (yellow arrow). (G3: C1&C2): showing multiple vesicles (yellow arrow) and blebs (blue arrow). (G4: D1&D2): showing sloughing of some areas of the cuticle (red arrow) with fissures (blue arrow) and blebs (yellow arrow). (G5: E1&E2): (E1) there are large cauliflower masses (yellow arrow) on the cuticle and fissures (blue arrows). (E2) there are fissure with loss of normal annulation, destruction of the cuticle (yellow) and multiple vesicles (blue arrows).

4. Discussion

Trichinellosis is a worldwide zoonotic disease, affects mainly muscles, butit can be a serious disease with fatal complications such as myocarditis and encephalitis **(27).** The classical medical treatment includes mebendazole or albendazole had a limited effect in treating the muscular phase of *T. spiralis* **(28) (29 & 24).** Application of mebendazole at the early stage of infection may be effective but unluckily, for most cases it is diagnosed several weeks after infection, when the larvae have already established themselves in the muscles. In the current study we tried to investigate the therapeutic effect of mebendazole 50mg/kg/day, AgNPs50mg/kg/day and mebendazole20mg/kg loaded to AgNPs on encysted *T. spiralis* larvae. The better outcome of mebendazole loaded AgNPs has been proved by our results. We demonstrated that each of the three lines of treatment reduced the number of *T. spiralis* larvae in muscles but using mebendazole20 mg/kg loaded to AgNPs gave significant reduction in the mean larval count of *T. spiralis* compared to the effect of mebendazole 50mg/kg alone or AgNPs50mg/kg alone. Our results were in accordance with **(30)** who observed that there was improvement in pharmacological properties and therapeutic effect of an albendazolenano-particles in dogs. Similarly (**31)** found that brain-eating amoebae, which are almost always deadly, killed by AgNPs coated with anti-seizure drugs .

Thehistological results revealed that treatment with mebendazole alone showed mild reduction in number of larvae, with heavy lymphocyitic infiltaration, necrosis and persistent damage of skeletal muscle fibers with loss of striations and lysis of the nuclei. The results were in agreement with **(32)** who stated that mebendazole is unable to kill encapsulated larvae of experimentally infected mice because of their low water solubility that limits its absorption. This also in agreement with **(28)** who gave mebendazole to 92 infected individual and revealed that the parasite was surrounded by inflammatory cells, no degeneration or calcifications of larvae and the capsule is markedly thickened after treatment. While in AgNPs treated group there was mild reduction in larvae count and thinning of larvae capsule, more necrosis of larvae internal structure, less lymphocytic infiltration around larvae and less area of necrosis of muscle fibers. Our results were in accordance with others **(8)** concluded that AgNPs and Chitosan NPs are considered potent therapies in the treatment of toxoplasmosis and giardiasis**. (33)** found that AgNPs has antileshmanial effect **(34)** also found antifascioliasis effect to AgNPs. Nanosilver also has antifilarial activity **(35) (36)&(37)** through reduction in ATP content of the cell, causing damage to mitochondria and increased production of Reactive Oxygen pecies (ROS). Our results revealed decrease in the amount of collagen fibers in the group treated with mebendazole loaded AgNPs compared with the other groups this was in agreement with (**38**) who revealed increase in the amount of collagen fibres due to the presence of the larvae in muscles. Death of larvae with our treatment explained the reduction of collagen fibres. Also imunohistochemical results showed that there was marked reduction in COX-2expression with mebendazole loaded to AgNPs in comparison to the other groups as COX-2 increased with infection due to damage of muscle fibers and release of prostaglandins from archidonic acid by cyclooxoginase enzyme. COX-2increased in inflammation and its reduction indicates decrease of inflammatory process within the muscles **(39).** Semithin sections and scanning electron microscope (SEM) showed pronounced thinning of the capsular wall, blebing and damage to the wall of the encysted larvae that was treated with mebendazoleloaded AgNPs. One of the hallmark effects of any anthelmintic drug was the destruction of the worm's surface **(40).** The blebbing is an attempt by the parasite to replace damaged surface membrane in response to drug action **(41).** The released positive silver ions can bind negatively charged cell membrane to interfere membrane integrity **(42).** Ag+ ions may also adhere to the membrane wall, causing holes through which they also can penetrate inside the organism **(43).** This was in agreement with **(44)** who foundmarked destruction of the cuticle of *T. spiralis* adult treated with chitosan loaded to albendazole. In a similar way **(34)** observed perforations on the egg surface of *Fasciola* treated in vitro with the triclabendazole and AgNPs. Also **(45)** reported that *Schistosoma manson i*cercariae treated with Ag NPs (10 µg/ml) 30 min after exposure showed; thinning of tegument with focal loss of spines and edematous swelling of the muscle layer. So AgNPs empower and accentuate the action of mebendazole.

In the present study, biochemical results showed a significant increase in levels of AST, ALT, Creatinine, CPK and LDH in infected control group as compared with non-infected group. This change is due to hepatic and renal damage caused by the migrating larvae **(46).** Increased levels of these enzymes were related to the number of larvae inoculated **(47).** This agreed with **(48) (2).** On the other hand, there was improvement of biochemical parameters in all treated groups. This was in agreement of **(49) (2)** who reported highly significant reduction in AST&ALT, decrease in urea and creatinine levels after the early treatment of trichinellos is with ivermectin. Our study revealed marked improvement of the biochemical parameters in G5 that received mebendazoleloaded to AgNPs compared to the other groups which attributed

to the potent larvicidal effect of the drug combination as AgNPs accentuate the larvicidal effect of mebendazoleby means of alteration in the enzyme activity of the parasite resulting in parasitic death **(50)**. (51) observed no significant changes in ALT & creatinine levels after using AgNPs in rats from the control mice On the other hand, **(52)**found a significant increase in LDH after AgNPs administration to mice in a dose of 1gm/kg/day for 30 days. Also, **(16)** observed the increasing levels of AST, ALT, ALK with AgNPs in a dose of1 mg/kg for 24 days. The difference from our results may be due to the difference in the dose and duration of AgNPs used**. (42)** stated that several investigators (**53) (54)&(55)** studied the levels of (AST) and (ALT), the markers for hepatotoxicity, in plasma after oral exposure of AgNPs and demonstrated that AST and ALT levels do not increase despite the higher oral exposure (>500mg/kg bw) of AgNPs (~60nm) for 28-day except mild enhancements of alkaline phosphatase and cholesterol levels signifying no indication of acute hepatotoxicity. The properties of AgNPs improve the effect of the antiparasitic drugsin both the systemic and local route controlling tissue distribution, cellular uptake and penetration through biological barriers **(56 & 57).**

Finally, we concluded that mebendazole loaded to AgNPs has a novel therapeutic effect in treatment of the muscular phase of trichinellosis. This was proved through its significant reduction in the mean number of larval count & its effect on the cuticle of *T. piralis* larvae causing its damage as evidenced by E/M. Additionally the marked improvement in the affected muscle in the form of its ability to reduce the expression of the inflammatory marker COX-2in muscle and decrease of collagen fibers around empty spaces of dead larvae and between muscle fibers. Further studies are required to fully understand their possible toxicity and to investigate the molecular basis for the mechanism of action.

The present study had some limitations. As, we did not evaluate the effect of this combination on the functional activity of affected skeletal muscles.

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