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The potential effect of *Leiurus quinquestriatus* scorpion venom as an anti-parasitic medication for trichinellosis

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Abstract

Trichinella spiralis is a parasitic nematode with a complex life cycle and is recognized as a significant public health concern. Although Albendazole (ABZ) and Mebendazole are widely used to treat trichinellosis, natural products may offer safer and more effective alternatives. This study evaluated the efficacy of *Leiurus quinquestriatus* venom (LQV) as an anti-parasitic agent against *T. spiralis* infection in mice. The median lethal dose (LD_{50}) of LQV was first determined in adult male CD1 mice, sixty mice were randomly assigned to four groups (n = 15): non-infected, non-treated controls; infected, untreated controls; infected mice treated orally with ABZ (50 mg/kg); and infected mice treated intraperitoneally with LQV (0.03 mg/kg; 1/10 LD_{50}) for 15 days. At 7- and 35-day post-infection, adult worm and muscle larval burdens were evaluated. Serum interleukin-4 (IL-4) and interleukin-10 (IL-10) levels were measured, and histopathological and immunohistochemical analyses were performed on intestinal and skeletal muscle tissues. Compared to untreated infected controls, LQV treatment led to a 55% reduction in adult worm burden and a 49% reduction in muscle larvae. Both ABZ and LQV significantly lowered IL-4 and IL-10 levels. Histological and immunohistochemical findings showed that LQV ameliorated infection-induced tissue inflammation and pathology. These results suggest that LQV exerts notable antiparasitic and immunomodulatory effects, potentially representing a promising natural alternative to conventional therapies for trichinellosis.

Keywords: Adult parasite Interleukins, Larvae, *Leiurus quinquestriatus* venom, Trichinellosis

Introduction

Trichinosis is a parasitic disease that affects humans and animals. *Trichinella spiralis*, a parasitic nematode, causes it. *T. spiralis* is distinguished by a complex life cycle. Trichinosis is defined as a public health concern, especially when pork meat is actually consumed without proper cooking (1, 2). It is widely spread in areas with less stringent food safety regulations, but with improved practices, infections have significantly decreased. The parasite's life cycle begins with encysted larvae in the tissue of the host, then develops into an adult type in the host's intestine. Furthermore, migration of the larval stages to the muscular tissues causes discomfort, fever, and other symptoms (3). Trichinosis is diagnosed using muscle biopsies and serological examinations. Essentially, prevention of trichinosis depends mainly on the proper cooking of meat products to remove the larvae (4). Normally, the treatment involves anti-parasitic medications

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such as albendazole (ABZ) or mebendazole, which are able to kill the worms and larvae in the small intestine (5). In recent years, natural products (NPs) have been found to have anti-parasitic agents that could be able to reduce the parasitic infection (6). Animal venoms as NPs possess a variety of toxins/proteins/peptides which act as ionic channel inhibitors and target several vital physiological processes in the parasite. This led to an increase in their target specificity as anti-parasitic agents (7, 8). There are many different kinds of scorpions. The most lethal scorpion species for humans are found in the Buthidae family, which includes *Leiurus quinquestratus* (9, 10). Components of scorpion venom (SV) have been investigated as medicines, and other uses in the creation of pharmaceutical products have been documented (11). *Leiurus quinquestriatus* venom (LQV) could have a therapeutic effect, which in turn led to its application in a drug product (12). LQV is soluble in water, composed of oligopeptides, nucleotides, amino acids, and other organic compounds. It contains enzymes such as phospholipases, hyaluronidase, serotonin, histamine, and protease inhibitors (13). The potential therapeutic value of different LQV compounds is being increasingly investigated, as these compounds may represent promising leads for the development of new pharmaceuticals, for instance, anti-microbial, anti-leishmanial, anti-malarial, anti-arthritic, anti-inflammatory, anti-diabetic, in vitro, and in vivo antitumor effects (11, 14-17). Furthermore, LQV has a wide range of effects that significantly impact biological effects, particularly cancer, and a varied number of parasites (18). This venom includes several peptides that show anti-carcinogenic activities. As an example, chlorotoxin is a peptide that specifically induces apoptosis in cancerous cells (19). Recently, LQV showed novel and promising benefits against the pathological alterations caused by *Toxocara canis* larval infection in mouse models (20). However, its relationship with *T. spiralis* and its therapeutic potential require further empirical research to illuminate the specifics of such interactions. This study was conducted to quantify the ability of the anti-parasitic action of LQV against the adult and larval stages of *T. spiralis*.

Materials and methods

Experimental mice

Adult male CD1 mice of 8 weeks of age with an average weight of 22± 3 grams were purchased from the animal husbandry department at Alexandria University. Animals were kept at room temperature (25˚C) with a set 12-hour light/dark cycle at the Faculty of Science, Tanta University. Mice were resting for at least two weeks before executing the experimental plan. Experimental mice were allowed unrestricted water and food access.

Ethical approval:

The Institutional Animal Ethical Committee's (IAEC) protocols were followed for all animal research, and the animals were given at least two weeks to adjust before the experimentation. All experiments and procedures were performed following ARRIVE guidelines and were consented to by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Tanta University, with the approval number (IACUC-SCI-TU-0452)

Scorpion collection and venom preparation

From the southern Aswan governorate in Egypt, *L. quinquestriatus* scorpions were collected. Scorpions were transferred to the Invertebrate Zoology Department, Faculty of Science, Tanta University. The scorpions were identified by taxonomists according to Abdel-Rahman and colleagues (21). Electrical stimulation (12- 17V) was used to collect the venom from the telson of scorpions. The venom was lyophilized in Corporate Serum and Vaccine (VACSERA). Different concentrations were prepared from the lyophilized venom, and the sub-lethal doses were prepared according to Salama (22).

Determination of the median lethal dose of LQV

Thirty-six male CD1 mice were divided into six groups (n = 6). Assessment of the median lethal dose (LD_{50}) was assessed after the intraperitoneal injection (i.p) with a single dose of *L. quinquestriatus* venom (LQV) (0.1–5 mg/kg). Mice were observed for 24 hours. The arithmetic approach was used to calculate the LD_{50} value as early as described by Aliu and Nwude (23).

LD_{50}= LDy-∑ (Dd ×md)/N Where: LD y = Highest dose (LD_{100}), N = Number of animals per group, Dd= Dose difference, Md = Mean dead (Table 1).

Parasites and infection

The strain of *T. spiralis* is a pig strain originally isolated from naturally infected pigs at Cairo abattoir and routinely maintained in the laboratory of the Zoology Department, Faculty of Science, Tanta University by consecutive passages through donor albino rats. Subsequently, the transfection of the larvae into the experimental mice was 200 larvae /mouse. The mice were given tap water, a standard meal 2 hours before infection, as formerly done by Abou Rayia et al. (24).

Drugs and experimental design

Suspension of ABZ in a concentration of 400 mg/10 mL was supplied by Pharma Cure, Pharmaceutical Industries in Egypt. Before infection, all mice were fasted for 12 hours. A tuberculin syringe has been used to directly inject 200 larvae of *T. spiralis* into the stomach (25). The groups of 60 mice were four, each group consisting of fifteen animals (n=15). The 1st group (Gp1) served as non-infected non-treated mice (Negative control). Gp2 had infected and untreated mice (Positive control). Gp3 had been infected and orally treated with 50 mg/kg of ABZ. Gp4 had been infected and daily oral treatment with 0.03 mg/kg b.wt of LQV for 15 days. Animals in Gp1 to Gp4 were divided into subgroups, and a scarification was performed at 7 days and 35 post-infections.

Detection of *T. spiralis* adult worms and muscle larvae burden

Evaluation of the drug reliability on the intestinal phase and muscular phase was executed by sacrificing the subgroups (Gp1 -Gp4) on the 7th and 35th days, respectively. The small intestine was prepared as Wakelin and Wilson had previously described (26). The pepsin digestion process was then used to isolate the muscle larvae, and then the worm reduction rate was calculated (27).

Determination of the serum levels of interleukin-4 and interleukin-10

The anti-inflammatory cytokines IL-4 and IL-10 were measured in serum samples using commercial sandwich ELISA kits (Boster Biological Technology Co., Ltd.).

Histopathological investigations

On days 7 and 35 post-infection, the tissues of the small intestine and skeletal muscles were reserved and fixed in 10% formalin for 24 hours. Dehydrated, rinsed in water, dehydrated, and then cleaned in xylene. Hematoxylin and eosin staining was applied to paraffin sections that were cut to a thickness of 5µm following Carleton (28).

Immunohistochemical studies

All sections were undergoing deparaffinization, rehydration, and antigen retrieval in a citrate buffer at pH 6.0. Samples were incubated in a humid chamber overnight at 4°C with Anti-FOXP3 as a primary antibody, which was purchased from Biopsies Company (Catalog#YPA2193, rabbit polyclonal IgG, China). Biotin-streptavidin was used as a secondary antibody and was diluted to 1:100 (DAKO Company). The antigen was localized by the addition of 3,3'-diaminobenzidine tetrahydrochloride (DAB) as a substrate chromogen solution (DAKO Envision, Denmark). Slides were counterstained with hematoxylin, dehydrated in alcohol, and mounted. FOXP3 expression was defined with brownish coloration. Using High Power Field at 5X magnification, positive FOXP3 overexpression was determined (29).

Statistical analysis

Data were presented as mean ± SD and were analyzed using one–way analysis of variance (ANOVA), followed by Dunnet test, and $p < 0.05$ *or* $p < 0.01$ were statistically significant.

Results

The median lethal dose (LD_{50}) of LQV

The arithmetic approach was used to calculate the LD_{50} value of LQV in CD-1 male mice. Different doses of LQV (0.05 to 0.3 mg/Kg b.wt) were injected i.p. to different groups of mice. Based on the data presented in table 1, the LD_{50} of LQV was 0.3mg/Kg b.wt (Table 1).

Treating the infected mice with LQV reduced the adult worms at day 7 and the larval burden at day 35 post-infection

In order to detect the presence of *T. spiralis* adult and larvae, infected mice were sacrificed on the 7 and 35 – days respectively following infection. The study cleared those infected untreated mice (Positive control) had a significantly high worm burden of *T. spiralis* adult worms, while ABZ-treated mice showed near complete elimination of adult *T. spiralis* worms. However, LQV-treated mice showed a moderate reduction in worm numbers. As a result, the larval count at day 35 post-infection in infected untreated mice (positive control) was significantly high (Table 2). However, no *T. spiralis* larvae were detected in ABZ-treated mice. LQV-treated mice show a moderate reduction in the number of larvae per gram (Table 2).

Table 1: The median lethal dose (LD50) of *Leiurus quinquestriatus* venom (LQV) in albino mice

LQV (mg/kg b.w.)	No. of deaths in 1st exp. (Death/N)	No. of deaths in the 2nd exp. (Death/N)	Md	Dd	Md× Dd
0.05	0/6	0/6	0	0	0
0.1	0/6	1/6	0.5	0.05	0.025
0.15	1/6	1/6	1	0.05	0.05
0.2	2/6	2/6	2	0.05	0.10
0.25	3/6	2/6	2.5	0.05	0.12
0.3	4/6	3/6	3.5	0.05	0.17
0.35	5/6	5/6	5	0.05	0.20
0.4	6/6	6/6	6	0.05	0.30
∑					0.965

LQV: *Leiurus quinquestriatus* venom; LDy is the highest dose (LD_{100}), N is the number of animals/group, Dd: Dose difference, Md: Mean death.

Table 2. Number of adult worms recovered at day 7 and larvae burden at day 35-day post-infection

Groups	Number of adult worms	Larvae burden
	7 days post-infection	35 days post-infection
Infected alone	69.8 ± 7.3^a	13850 ± 980^a
Infected /ABZ	0.6 ± 0.23^b	0 ± 0^b
Infected /LQV	35.4 ± 4.2^c	6320 ± 453^c

ABZ: Albendazole; LQV: *Leiurus quinquestriatus* venom

Treating the infected mice with LQV reduced the IL-4 and IL-10 levels

Following a parasitic infection, the levels of IL-4 and IL-10 were analyzed to evaluate immune modulation, as shown in Fig. 1. The current data showed a significant decrease in IL-4 levels in the groups of infected mice that were treated with ABZ ($P<0.001$). Also, IL-4 levels were slightly decreased in the LQV-treated group ($P = 0.002$) compared to the infected control group at 7^{th} and 35^{th} days post-infection (Fig. 1A). Moreover, data show a significant decrease of IL-10 levels ($P <0.001$) in the ABZ-treated group (Fig. 1B). In the LQV-treated group, the IL-10 levels were decreased compared to the infected control group at 7^{th} and $35^{`1+th}$ days post-infection (Fig. 1B).

LQV-treatment decreased the pathological changes in the intestinal and skeletal muscles of the infected mice

As shown in the intestinal tissue sections (Figure 2 A-G), a significant increase of *T. spiralis* larvae in the intestinal mucosa (black arrows) in untreated infected mice (Positive control) compared to untreated -uninfected group (-ve) (Fig 2 A), also observed a severe villous atrophy and tissue disruption, indicating direct parasitic damage. While the red arrows indicate dense inflammatory infiltrates, which may indicate an immunological response (Fig. 2 B and C). In ABZ-treated mice, it was reported that a partial repair of villi, but there is still some disarray in some places (red arrow) (Fig. 2D and E). The present study indicates that LQV-treated mice showed a significant recovery of villous architecture (yellow arrows), notice, and a decreased inflammatory infiltrate, as a sign of successful immunological resolution and parasite removal.as well as areas of regenerating villi (black arrows) and a slight infiltration of inflammation (Fig. 2F and G). In the skeletal muscle sections (Figure 3 A-G), a significant increase in larval encystation of T. *spiralis* in Gp2 with surrounding inflammatory responses (Fig. 3 B and C) referred to the untreated-uninfected group (-ve) (Fig. 3 A), also notice reduction in muscle larvae in LQV-treated mice (Fig. 3 D and E), while in ABZ-treated mice showed a complete elimination of encysted larvae (Fig. 3 F and G).

LQV-treatment decreased Fox-P3 expression in the intestinal and skeletal muscles of the infected mice

In the intestinal tissue sections (Fig. 4A-G), compared to untreated -uninfected group (-ve) (Fig 4 A), the positive control group showed a significant increase in Fox-P3 expression, indicative of regulatory T-cell activity, possibly to control excessive inflammation (Fig. 4B and C), also it was reported that a moderate Fox-P3 expression and focal minimal Fox-P3 expression in both ABZ-treated mice (Fig. 4D and E) and LQV-treated mice, respectively demonstrating continued immunological regulation brought on by lingering parasites (Fig. 4 F and G). In the skeletal muscle sections (Fig. 5 A-E), the results showed that in LQV-treated group had FoxP3-negative immune cells, suggesting impaired immune suppression (Fig. 5 C) as compared to the positive control group, which shows a significant dense positive expression of Fox-P3 in the inflammatory cells surrounding *T. spiralis* cysts (Fig.5 B). Also, the ABZ-treated group had degenerated cysts with Fox-P3 negative inflammatory cells, confirming successful parasite clearance (Fig. 5D and E).

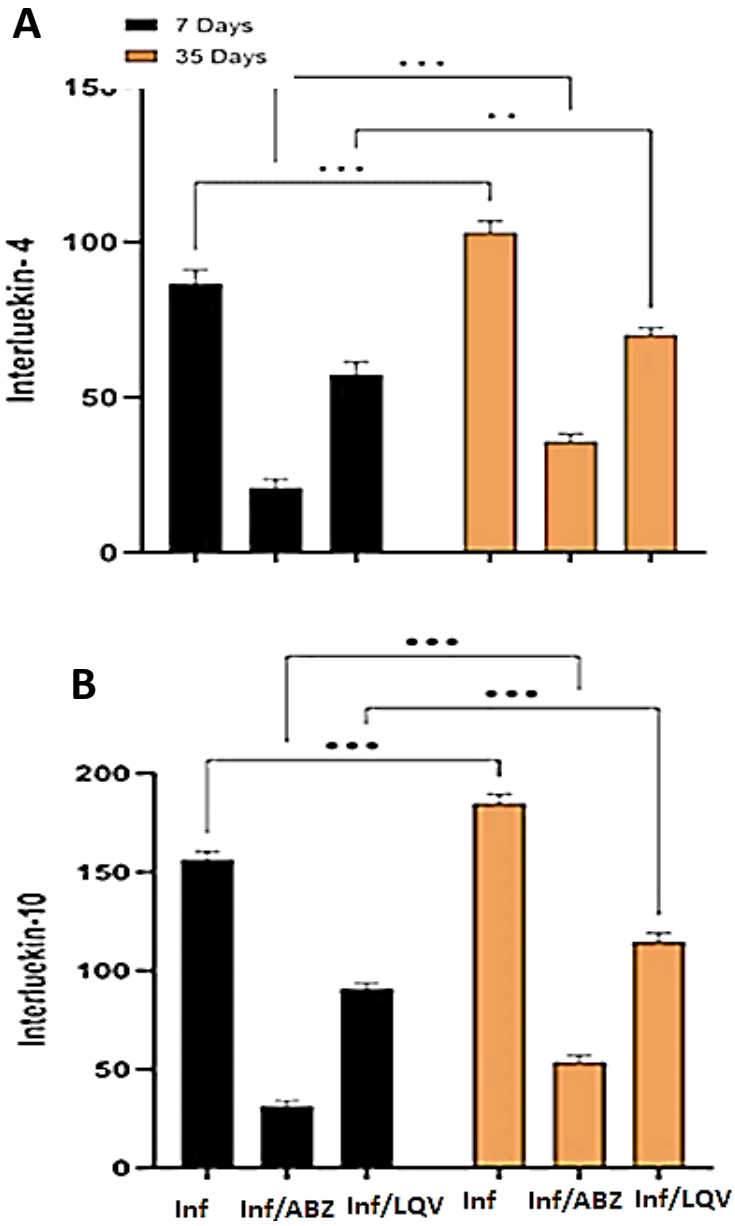

Figure 1. A) Showing overexpression of IL-4 in the groups that were treated with ABZ and the LQV compared to the positive control at 7th and 35th days post-infection. **B)** Showing higher overexpression of IL-10 in the groups that were treated with LQV and ABZ compared to the positive control at 7th and 35th days post-infection.

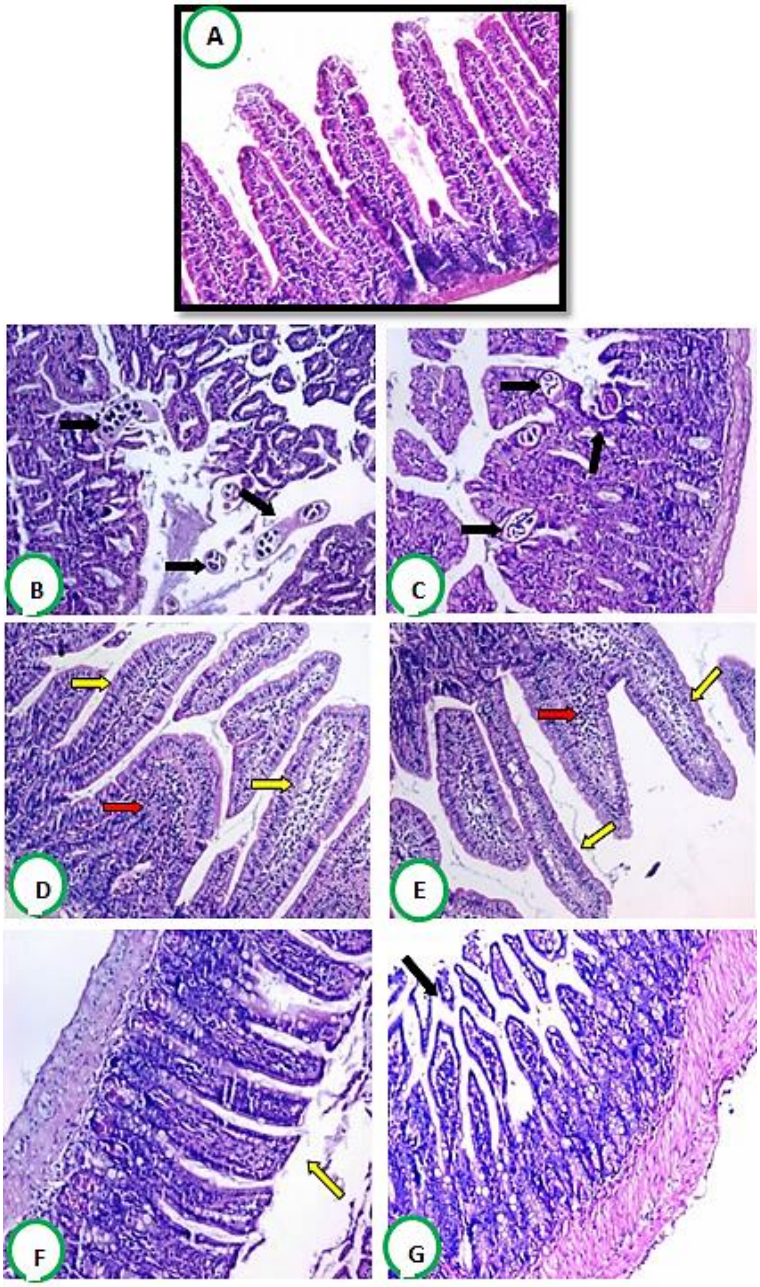

Figure 2 A-F. Sections in the intestine of infected mice showing many T.S larva (black arrows) associated with patchy inflammatory cellular infiltration (red arrow) X200 (A, B), Sections in the intestine of infected mice treated with ABZ showing more or less regular villi (yellow arrows) associated with patchy inflammatory cellular infiltration (red arrows) X-200 (C, D) and. Sections in the intestine of infected mice treated with LQV showing areas of regular villi (yellow arrow) as well as areas of regenerating villi (black arrow) X-200 (E, F)

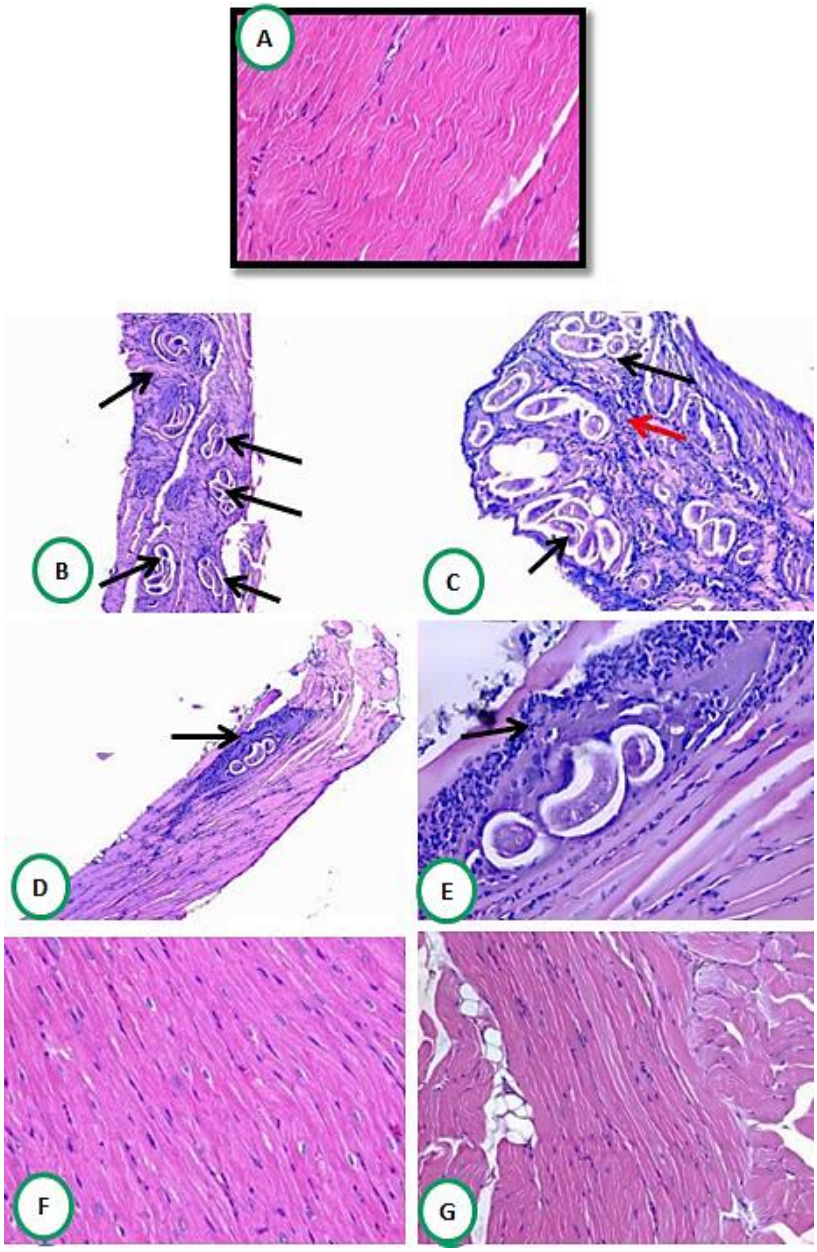

Figure 3- A-F Sections in the skeletal muscles of positive control mice showing a large number of T.S cysts (black arrows) with pericystic infiltration by chronic inflammatory cells (red arrow) **(A, B)**. Sections in the skeletal muscles of LQV-treated mice showing reduction in no. of T.S cysts (black arrows) **(C, D)**. Sections in the skeletal muscles of ABZ-treated mice showing no detectable T.S cyst (**E, F).**

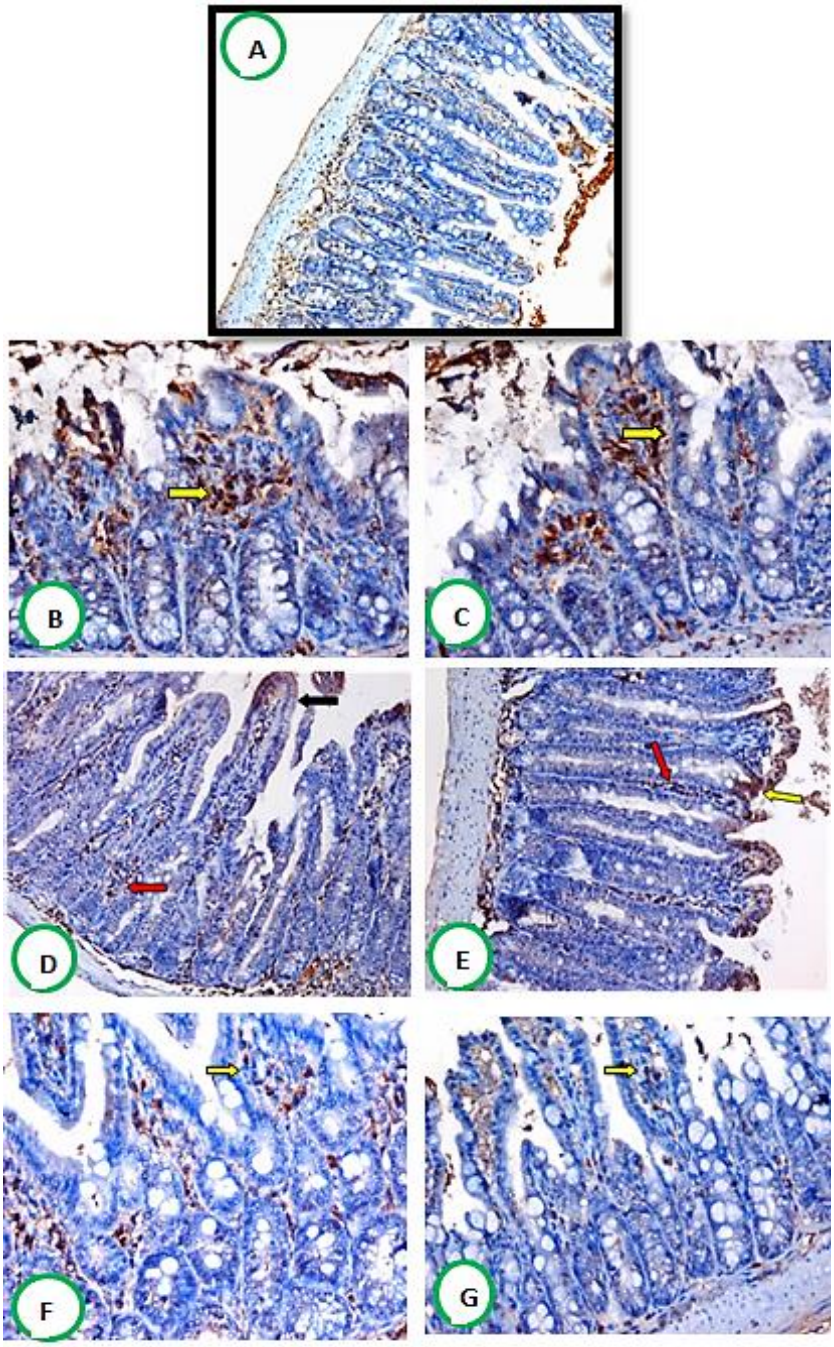

Figure 4 A-F. Section in the intestine of T.S. infected group showing diffuse dense positive expression of FoxP3 in inflammatory cells (yellow arrows) (IHC, FoxP3, X200(A, B), Section in the intestine of infected mice treated with ABZ-treatment showing few positive inflammatory cell (red arrows) and epithelial villous –tip cells (yellow arrow) (IHC for FoxP3, DAB, X200)
(C, D) and. Sections in the intestine of infected mice treated with LQV showing focal minimal positive expression of FoxP3 in inflammatory cells (yellow arrows) (IHC, Fox-P3, X200) (E, F).

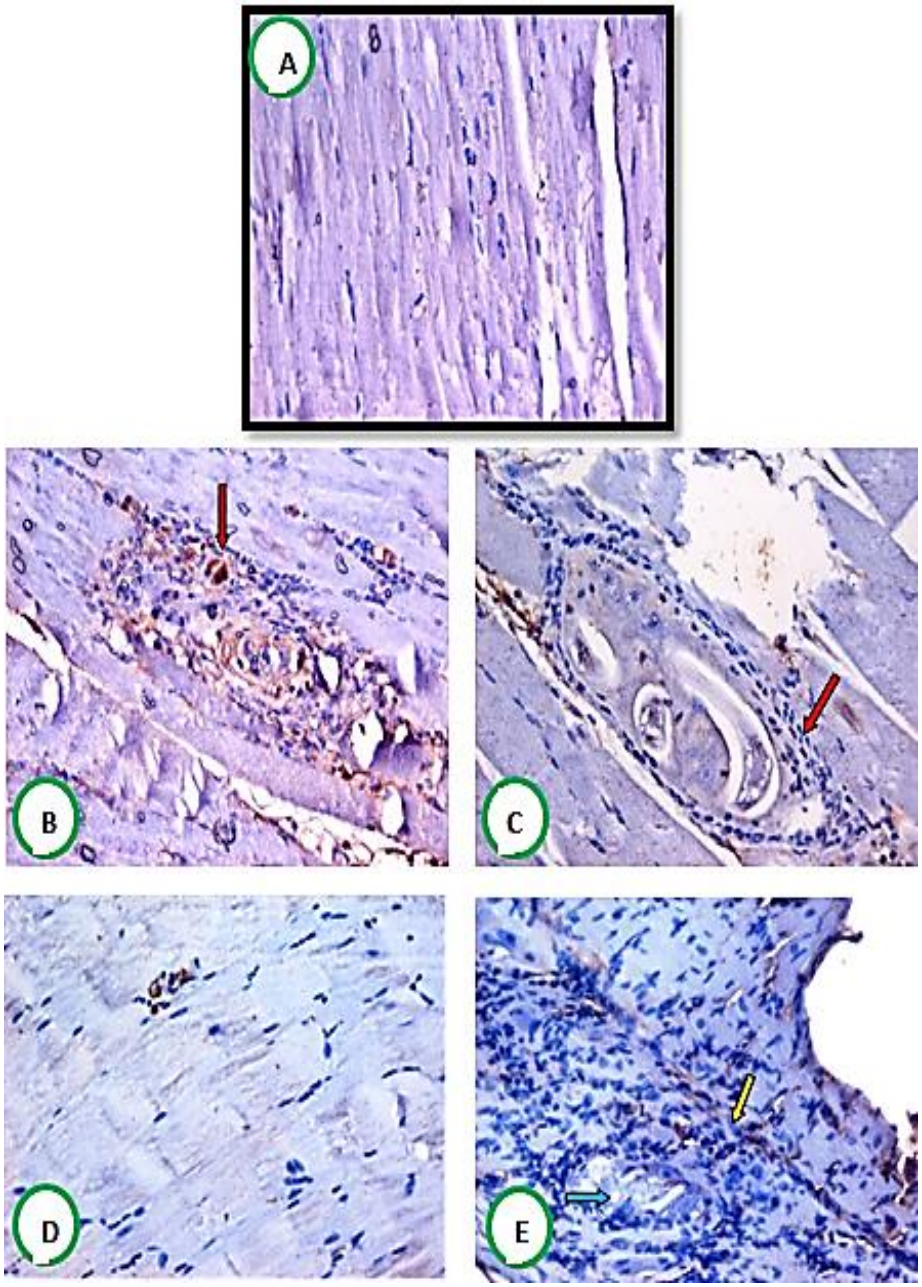

Figure 5- A-D Sections in the skeletal muscles of positive control mice showing Section in the muscle of T.S. infected mice showing focal dense positive expression of FoxP3 (red arrow) in the inflammatory cells around the T.S. cyst (IHC, FoxP3, X200) **(A)**. Sections in the skeletal muscles of LQV-treated mice showing negative expression of FoxP3 (red arrow) in the inflammatory cells around the T.S. cyst (IHC, FoxP3, X200) **(B)**.Sections in the skeletal muscles of ABZ-treated mice showing few scattered degenerated T.S. Cysts (blue arrow) with negative expression of FoxP3 in the surrounding inflammatory cells (orange arrow) (IHC for FoxP3, DAB, X200) (**C, D).**

Discussion

Trichinellosis, a parasitic disease caused by Trichinella ingestion of undercooked meat, is typically treated with anti-parasitic medications like ABZ, which is a widely used treatment for trichinellosis, a parasitic infection caused by the invasion of larvae into the host's muscle tissues (30). The current study evaluated the therapeutic efficacy of LQV against *T. spiralis* infection in mice, focusing on parasitological outcomes, immunological responses, histopathological alterations, and immunohistochemical changes in Fox-P3 expression as a marker of immunoregulation. The results demonstrate that both ABZ and LQV confer protective effects, albeit through distinct mechanisms. ABZ treatment led to near-complete elimination of adult worms and encysted larvae, consistent with previous studies that describe its broad-spectrum anthelmintic activity through inhibition of microtubule polymerization, essential for parasite survival and reproduction (31). LQV, traditionally known as a fluoroquinolone antibiotic, showed moderate antiparasitic effects by reducing both adult and larval counts. While its exact antiparasitic mechanism is not fully understood, recent research has suggested that certain antibiotics can modulate host immunity and alter parasite viability through microbiota-dependent or independent pathways (32, 33).

Salama et al. (2024) reported that LQV could be used for toxicariasis treatment because it has a lot of therapeutic activities (17). During a parasitic infection, the immune system initially increases IL-4 and IL-10 production to initiate a Th2 response against parasites (34). However, as the progress of parasitic infection progresses, the body may modulate these cytokines to shift the immune response, which reveals how the immune phenotype and function can be modulated by interleukin interaction (35). Indeed, the regulation of interleukins is crucial for regulating immune responses; they have anti-inflammatory properties and help regulate and limit immune responses, preventing excessive inflammation that can damage host tissue, as suggested by others (36). In this study, both treatments modulated the Th2 cytokines IL-4 and IL-10, which are typically elevated during helminth infections to promote humoral immunity and regulate inflammation (37). ABZ significantly suppressed both cytokines, aligning with the near-complete parasite clearance and downregulation of Th2-mediated responses. LQV, while less effective in parasite elimination, also significantly reduced IL-4 and IL-10 levels. This suggests a potential role for LQV in tempering chronic immune activation and possibly preventing tissue damage caused by prolonged Th2 responses. But ABZ presents several limitations that may hinder its long-term utility. One of the major concerns is the emergence of drug resistance, particularly due to prolonged and repeated mass drug administration programs. Resistance is primarily attributed to mutations in the β-tubulin gene of parasites, which reduce ABZ binding and impair its anthelmintic action (38). Additionally, ABZ has relatively poor oral bioavailability, which can limit its therapeutic concentrations in systemic infections, although this can be partially improved with fatty meal co-administration. Adverse effects, though generally mild, include gastrointestinal disturbances and hepatotoxicity, which may necessitate liver function monitoring during extended use. These drawbacks underscore the need for alternative or adjunctive therapies, particularly in regions with high reinfection rates or where ABZ resistance is suspected.

Histopathological examination supported parasitological and immunological findings. Untreated infected mice displayed severe villous atrophy and heavy inflammatory infiltration in intestinal tissues, along with intense larval encystation in skeletal muscle. ABZ treatment showed partial restoration of tissue architecture and complete clearance of larvae, while LQV-treated

mice showed considerable recovery of villi and reduced inflammation, suggesting its efficacy in promoting tissue repair and mitigating immune-mediated pathology (39).

Interestingly, FoxP3 expression—an indicator of regulatory T cell (Treg) activity—was upregulated in untreated infected mice, likely as a host attempt to regulate excessive inflammation (40). Both ABZ and LQV treatments resulted in reduced FoxP3 expression. The observed decrease in FoxP3+ cells in treated groups suggests diminished need for Treg-mediated control following effective parasite clearance and reduced inflammation, particularly in the case of ABZ.

Overall, while ABZ remains superior in direct antiparasitic activity, LQV demonstrated meaningful immunomodulatory and histopathological improvements. This raises the possibility of repurposing LQV as an adjunct therapy to conventional anthelmintics due to the neurotoxins affecting ion channels and possibly disrupting parasite physiology and Enzymes, which may aid in parasite penetration and disruption. This may interfere with the metabolic processes of adults and impair the development of larvae, particularly in cases of drug resistance or for reducing inflammation-driven tissue pathology.

A significant reduction of the number of ABZ-treated group by 175% compared to the positive control group. Venom extracted from deathstalker (LQV) showed less conventionality as an anti-trichinella drug, suggesting that further investigation is required to assess the safety and effectiveness of venom alongside ABZ. In conclusion, ABZ is mainly used as a regular therapy for infection with *T. spiralis*. It is significantly reducing the numbers of larval and developmental stages while improving immunological indicators. However, the combination of ABZ with additional therapeutics promotes a critical dynamic strategy for more understanding of response modulation and potential therapeutic applications. This study clearly showed the effect of the ABZ treatment and LQV treatment on parasite clearance, intestinal recovery, and immune responses. While the positive control shows a high parasite burden, severe intestinal damage, high IL-4 (Th2 response), very high IL-10 (regulatory response), and high FoxP3 (T-reg cells), the ABZ treatment results in complete parasite elimination, full intestinal restoration, suppressed IL-4, near-normal IL-10 levels, and moderate FoxP3 expression. In contrast, the LQV treatment shows partial parasite reduction, moderate intestinal improvement, partially reduced IL-4, elevated IL-10, and minimal FoxP3 expression. Overall, ABZ treatment demonstrates the most effective results across all parameters, while LQV treatment shows partial efficacy.

Conflict of interest: NIL

Funding: NIL

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