



Efficacy of levamisole-loaded nanoparticles in the treatment of *Trichinella spiralis* infection in mice

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[Eficácia das nanopartículas carregadas com levamisole no tratamento da infecção por *Trichinella spiralis* em camundongos]

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ABSTRACT

Trichinosis still poses a threat to both humans and animals. The current study compared the efficacy of levamisole medication loaded on polylactic acid nanoparticles (PLANs) to albendazole in treating intestinal and encapsulated muscle larvae of murine *Trichinella spiralis* infection. The mice were categorized into two main groups which further divided into four subgroups including: the infected control group, the infected group treated with PLANs, levamisole-loaded PLANs, and albendazole. The mice were all infected orally with 250 *T. spiralis* larvae. A notable and significant increase in the levels of ALT, AST, ALP, TP, and GGT were observed in the groups infected with 250 *T. spiralis* larvae and slaughtered at both 7- and 32-days post-infection, illustrating the altered biochemical reactions to the invasion of *T. spiralis* isolates against the treated subgroups. Changes that showed at 32 dpi revealed the presence of large numbers of cystic *T. spiralis* larvae scattered in the sarcoplasm and many chronic inflammatory cells. The histopathological finding on the seventh dpi revealed the presence of adult worm sections within the mucosa along with chronic inflammatory cells infiltrating the mucosa and submucosa. In conclusion, a noticeable improvement was observed in *T. spiralis* infected mice using PLANs.

Keywords: *Trichinella spiralis*, levamisole, biochemical parameters, histopathology, polylactic acid nanoparticles (PLANs)

RESUMO

A triquinose ainda representa uma ameaça tanto para os seres humanos quanto para os animais. Este estudo comparou a eficácia do medicamento levamisole carregado em nanopartículas de ácido polilático (PLANs) com a eficácia do albendazol no tratamento de larvas intestinais e musculares encapsuladas da infecção murina por *Trichinella spiralis*. Os camundongos foram categorizados em dois grupos principais que, por sua vez, foram divididos em quatro subgrupos, incluindo: grupo de controle infectado, grupo infectado tratado com PLANs, PLANs carregados com levamisole e albendazol. Todos os camundongos foram infectados oralmente com 250 larvas de *T. spiralis*. Um aumento notável e significativo nos níveis de ALT, AST, ALP, TP e GGT foi observado nos grupos infectados com 250 larvas de *T. spiralis* e abatidos aos 7 e 32 dias após a infecção, ilustrando as reações bioquímicas alteradas à invasão de isolados de *T. spiralis* contra os subgrupos tratados. As alterações que apareceram aos 32 dpi revelaram a presença de um grande número de larvas císticas de *T. spiralis* espalhadas no sarcoplasma e um grande número de células inflamatórias crônicas. O achado histopatológico no sétimo dpi revelou a presença de seções de vermes adultos na mucosa junto com células inflamatórias crônicas infiltradas na mucosa e na submucosa. Em conclusão, foi observada uma melhora notável em camundongos infectados com *T. spiralis* usando PLANs.

Palavras-chave: *Trichinella spiralis*, levamisole, parâmetros bioquímicos, histopatologia, nanopartículas de ácido polilático (PLANs)

INTRODUCTION

Trichinellosis is a common zoonotic disease caused by consuming undercooked pork or other meat products contaminated with infectious encysted larvae of *Trichinella* spp., with *Trichinella spiralis* being the primary cause of human infections (Malone *et al.*, 2024). Around ten million individuals worldwide are impacted by the illness, which is still a major public health concern (Chávez-Ruvalcaba *et al.*, 2021). According to several reports from around the world, human trichinellosis is a neglected tropical illness that has a negative influence on import-export trade, the slaughter business, and public health (Pozio and Zarlenga, 2013, Song *et al.*, 2018). According to (Gómez-Morales *et al.*, 2018), human trichinellosis can range in severity from asymptomatic to deadly. Gastrointestinal issues, including diarrhea, vomiting, nausea, and abdominal discomfort, are the initial clinical indications of trichinellosis and often start two to seven days after consuming raw or undercooked meat (Muñoz-Carrillo *et al.*, 2018, Ortiz *et al.*, 2024).

The reason for these symptoms is that *T. spiralis* larvae invade intestinal epithelial cells, where they mature into adults, reproduce, and give birth to new larvae three to seven days post infection (dpi) (Muñoz-Carrillo *et al.*, 2017). According to studies, adult *T. spiralis* is eliminated from the colon 10–17 days post infection. The initial 17-day period known as the intestinal phase of the disease has a critical role in shaping the progression and outcome of trichinellosis (Ding *et al.*, 2016). To reduce the main dangers to the public's health, trichinellosis must be diagnosed and treated as soon as possible (Intirach *et al.*, 2024). When the larvae of *T. spiralis* break free of the cyst and enter the small intestine, the infection process starts. The larvae eventually mature into adult worms. The young larvae that adult female worms release after mating go to skeletal muscle cells via the circulatory system, where they encyst and grow into muscle larvae (Lee and Best, 1983).

Currently, several benzimidazole derivatives, including mebendazole (MBZ), albendazole, and flubendazole, are used to treat human trichinellosis (Chai *et al.*, 2021; Pavel *et al.*, 2023). Due to their limited bioavailability, none of these medications was thought to be 100%

effective against *T. spiralis* larvae that had just been born or that had been encapsulated. Some of these remedies should not be administered to children under the age of five due to their reduced efficacy and/or resistance to encapsulated parasite larvae (Tagboto and Townson, 2001; Caner *et al.*, 2008). Some are also contraindicated during pregnancy. Both medications have low water solubility, which restricts their absorption across the intestinal lumen and results in decreased efficacy, in addition to imperfect effectiveness against encapsulated larvae and rising drug resistance (Rayia *et al.*, 2022). As a result, taking large dosages of the medication causes a variety of adverse effects, the majority of which are gastrointestinal.

Moreover, experiments have shown that MBZ has the potential to cause teratogenic effects in rats and mice (Allam *et al.*, 2021). Hence, there is an urgent requirement for innovative and secure anti-*Trichinella* drugs, namely those that can effectively combat the muscular phase of the infection, which can lead to severe myopathy and chronic forms of myositis (Allam *et al.*, 2021, Rayia *et al.*, 2022). Levamisole, a synthetic compound derived from imidazothiazole, is employed as a treatment to combat viral and bacterial infections. It is widely employed as an anthelmintic drug in both people and animals (Guo *et al.*, 2023). The antiparasitic medication levamisole exerts its mechanism of action by producing anesthetic effects on L-subtype nicotinic acetylcholine receptors in nematode muscle. This agonistic activity inhibits the male's capacity for copulation and weakens his control over his reproductive muscles. The Food and Drug Administration has granted authorization for the use of polylactic acid (PLA), a remarkably versatile material known for its advantageous properties such as biocompatibility and biodegradability. Consequently, PLA has undergone scrutiny for multiplicity therapeutic applications, particularly as a means of delivering medications and antigens in nanoparticle form (Essa *et al.*, 2013, Legaz *et al.*, 2016). By assessing the parasitological, biochemical, and histopathological parameters, the current experimental study aimed to determine how well the drug levamisole loaded on polylactic acid nanoparticles (PLANs) treats intestinal and encapsulated muscle larvae of

murine *T. spiralis* infection in comparison to albendazole.

MATERIALS AND METHODS

Male albino mice (112 mice/6–8-weeks old) weighing 20–25g apparently healthy and free of parasites were purchased from the Schistosome biological supply center, Theodore Bilharz Research Institute Giza, Egypt (SBSP/TBRI), where mice were bred till the end of the study. These mice were kept on standard environmentally controlled conditions, kept under standard laboratory care (21°C 45–55% humidity), filtered drinking water, 24% protein, 4% fat and 4.5% fiber diet. The handling of the experimental animals was performed according to the international ethical conditions and valid guidelines adopted by Theodore Bilharz Research Institute.

As previously mentioned by (Dunn and Wright 1985), larvae were extracted from the muscles of mice that were infected with *T. spiralis*. In short, the infected muscles were digested by immersing them in a digestive solution. (1,000 mL of saline with 20mL of strong HCl and 20g of pepsin) for 12 hours at 37°C while being stirred by a machine. The suspension release was centrifuged for two minutes at 1,000 rpm to let out the larvae. The substance was centrifuged once more after being cleaned with regular saline (0.9% NaCl). The larvae were enumerated using hemocytometer to determine the precise quantity of inoculum required for mice infection. In preparation for the animal trials, the sediment containing the larvae was mixed with saline solution that included 1.5% gelatin. To create a stable solution, the sediment larvae were then resuspended in 1.5% gelatin in saline. The necessary inoculum size for each target number of larvae was modified following multiple hemocytometer counts. Prior to infection, the mice underwent a twelve-hour period of fasting. Subsequently, the larvae were administered to the mice using a tuberculin syringe, directly into their stomach. Each mouse's inoculum was tuned to hold roughly 250 larvae.

The drug Albendazole (ALB) was obtained from Pharma Cure Pharmaceutical Co. as a 200mg/5 mL suspension. It was given at a dosage of 50 mg per kilogram each day. Kahira Pharmaceuticals & Chemical Industries

Company produced levamisole (LEVA, 40mg, 3 tablet), which was taken once day at a dose of 25mg/kg. The poly (D, L-lactide) polymer (PDLLA) was acquired from Sigma-Aldrich Corporation (St. Louis, MO, USA) and has an intrinsic viscosity of 0.68 dL/g. Its mean molecular weight (MW) was determined by gel permeation chromatography/size exclusion chromatography (GPC/SEC) analysis.

A 2mg/mL concentration of Poly (D, L-lactide) polymer (PDLLA) was dissolved in acetone. Using a high-speed homogenizer set to 13,000 rpm, NPs were spontaneously generated by dropping 4.5mL of PDLLA solution dropwise into 13.5mL of aqueous solution (pyrogen-free water) containing 1% Pluronic R F68 Prill (BASF Corporation, Ludwigshafen, Germany). Following the addition of the complete PDLLA solution, homogenization was maintained for a further two minutes. After centrifuging PLANP at 13,000 g for 20 minutes at 10°C, it was resuspended in water free of pyrogen and condensed. After doing this process twice more, each batch was finally concentrated to a final amount of 2mL (ilva *et al.*, 2019).

To conjugate PLANPs with LEVA drug, about 1gram of the LEVA drug was added to 5mL pH adjusted PLANPs solution. The mixture was mixed and magnetically stirred at 500 rpm for 30 min. Subsequently, 0.1mL of 10% Bovine Serum Albumin (BSA) solution was added to block the residual surface of the PLANPs. The mixture was then incubated for 20 min. at room temperature before being centrifuged at 13,000 rpm for 45 min at 4°C for three times. Following the last centrifugation, the pellets were mixed again in 2mL of phosphate buffer (pH 7.2, 0.01 M) containing 1% BSA and 0.05% sodium azide. The PLANPs -Lac was refrigerated at a temperature of 4 degrees Celsius prior to its usage (Karve *et al.*, 2011).

Mice were randomized into 2 main groups (A-Intestinal phase group and B-Muscle phase group) each group divided into 5 subgroups of 8 mice infected with 250 *T. spiralis* larvae.

A. Intestinal phase

Group 1: Infected mice not treated

Group 2: Infected treated with 50 mg/kg ALB at 1 dpi for 3 days

Group 3: Infected treated with 25 mg/kg/day Levamisole at 1 dpi for 3 days

Group 4: Infected treated with 50 µg/mouse of PLAN at 1 dpi for 3 days

Group 5: Infected treated with 50 µg/mouse of PLAN loaded with 25 mg/kg/day levamisole at 1 dpi for 3 days

All mice in intestinal phase subgroups were scarify 7 dpi. In parallel 5 uninfected healthy mice were scarifying at the same time served as negative control for intestinal phase subgroups

B. Muscle phase

Group 1: Infected mice not treated

Group 2: Infected treated with 50mg/kg ALB at 30 dpi for 3 days.

Group 3: Infected treated with 25mg/kg/day levamisole at 30 dpi for 3 days.

Group 4: Infected treated with 50µg/mouse of PLAN at 30 dpi for 3 days.

Group 5: Infected treated with 50µg/mouse of PLAN loaded with 25 mg/kg/day levamisole at 30 dpi for 3 days. All mice in muscle phase subgroups were scarify 34 dpi. In parallel 5 uninfected healthy mice were scarified at the same time served as negative control for muscle phase subgroups.

Each mouse's small intestine was used to extract adult *T. spiralis* worms, which were then counted in accordance with description (Wassom *et al.*, 1988). Each animal's small intestine was lengthwise dissected, sliced into segments measuring 5cm, and then incubated for two hours at 37°C in 0.9% saline. After that, the intestines were cultured for six hours at 5°C in sodium hydroxide (0.05%). The intestines were then cleaned with water and the resulting liquid was filtered through a 200-mesh sieve to gather the worms. Using a Pasteur pipette, the gathered material was distributed onto 2 × 3-inch glass slides after being cleaned with distilled water. Under a dissecting microscope, the total number of worms in each of the fish's five-centimeter intestinal segments was counted. The average number of worms per group was then computed to allow for comparisons across other groups.

Each mouse's diseased muscles were cut before being digested into a 1% pepsin solution in 0.1NHCl in order to count the larvae. By incubating the mixture for two hours at 37°C and stirring with a magnetic stirrer, digestion was

achieved. To separate the coarse particles, a 50mesh/inch screen was used. To remove any larvae that had become adsorbed, the coarse particles were repeatedly washed with tap water. Before decanting, the filtrate was allowed to settle for 30 minutes to allow the larvae to settle. Using a McMaster counting chamber, sediment larvae were counted under a microscope (Ashour *et al.*, 2022; Matar *et al.*, 2023).

At 7 and 32 dpi, blood samples were collected from retro-orbital vein of each mouse in the studied groups which was taken in Eppendorf tubes to separate the serum for chemical analysis Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), gamma-glutamyl transferase (GGT) and total protein. The blood biochemical parameters were quantitatively measured using the 5010 V5+ semi-automatic photometer, a model spectrophotometer manufactured by RIELE in Germany. The indicated procedures were employed for the measurements. The Bio diagnostic kits (Dokki, Giza, Egypt) were used for the determination of serum aminotransferase enzymes (AST and ALT) activities by kinetic method total protein and alkaline phosphatase (ALP) using colorimetric method as described in the manufacturer's instructions of kit. Spectrum kit (Obour City, Cairo, Egypt) was utilized to determine GGT by colorimetric method described in the manufacturer's instructions of kit.

Intestinal samples (1cm from the small intestine at the junction of the proximal 1/3 and distal 2/3) were collected from mice killed at 7 dpi, while skeletal muscle samples from the hind legs were obtained from mice killed on dpi 7 and dpi 32. These samples were fixed in 10% formalin, dehydrated, clarified, and then embedded in paraffin blocks. Five-µm-thick paraffin sections were collected, stained with hematoxylin and eosin (H and E), and examined microscopically (Nassef *et al.*, 2018).

Data were presented as mean + standard error values. The treatment efficacy was calculated from the percentage of reduction in the adult worm or larval count. This employed the following equation (Ashour *et al.*, 2022) Efficacy of treatment (%) = [(Nc-Nt) /Nc] x 100 Where Nc is the mean number recovered in controls and Nt is the mean number recovered in treated mice.

The statistical analysis employed one-way ANOVA test followed by Tukey's multiple comparison as post hoc test. This was used to assess the significance of the difference between groups in worms and larvae count in different stages of treatment. The tests were performed using the Statistical Package for Social Sciences (SPSS) software, version 2, developed by SPSS Inc. in Chicago, Illinois, USA.

RESULTS

On the seventh day post-infection, adult *T. spiralis* worms were counted in the small intestine of the sacrificed mice. Fig. 1 displays

the average number of live adult *T. spiralis* worms per mouse found in the small intestine. In the small intestine of mice infected with 250 *T. spiralis* larvae and slaughtered at 7 days post-infection, the mean value of living *T. spiralis* adult worm count was (158.7 ± 11.7 , 111.2 ± 10.5 , 132.9 ± 10.9 , 98.3 ± 6.7 and 28.2 ± 3.5) in the control group, LEVA, PLAN, LEVA loaded with PLAN and ALB treated groups respectively. The subgroup of mice treated with ALB showed the most significant decrease in the total number of *T. spiralis* larvae (82.2 % reduction), followed by the subgroup treated with LEVA loaded with PLAN (38.1% reduction).

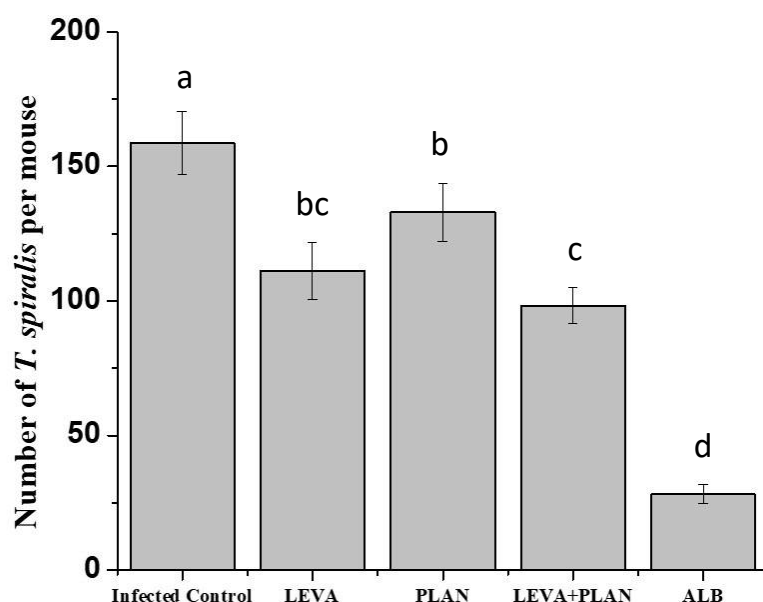


Figure 1. The mean number of living *T. spiralis* adult worms at the 7th day post-infection (dpi). Data are presented as mean $\pm$ standard deviation of three independent experiments $F = 85.44$; $df = 4$. Different letters indicate a statistically significant difference between the values ($P < 0.05$).

Concerning the muscle phase, the mean number of larval stages of *T. spiralis* was estimated on day 34 pi and presented (Fig. 2). The mean value of encysted larvae counts in the muscle of mice infected with 250 *T. spiralis* larvae and sacrificed at 34th dpi was (2149.5 ± 231.2 , 1691.2 ± 219 , 1812.3 ± 228.9 , 724.7 ± 196 and 589.7 ± 144.5) in the control group, LEVA, PLAN, LEVA loaded with PLAN and ALB treated groups respectively. The highest significantly decreased in the total number of encysted larvae was observed in subgroup of mice treated with ALB (72.6 % reduction)

followed by subgroup treated with LEVA loaded with PLAN (66.3 % reduction).

Table 1 displays the albumin level and enzyme levels of the infected as well as control groups of mice with respect to the biochemical changes in the group of mice infected with *T. spiralis* larvae. After being infected with *T. spiralis* larvae and sacrificed at 7 days post-infection, the ALT levels in the normal control group increased to 61 ± 4.9 (U/L), while the infected control group saw an increase to 99.5 ± 12.3 , 92.4 ± 12.1 , 96.2 ± 11.8 , 81.4 ± 10.3 , and 73.5 ± 9.4 (U/L) in the groups

treated with LEVA, PLAN, ALB, and LEVA loaded with LPAN, respectively. Similarly, mice infected with *T. spiralis* larvae and sacrificed at 7 showed a significant increase in AST levels: AST levels increased from 54 ± 3.6 (U/L) in normal mice to 92.1 ± 9.7 , 89.4 ± 11.8 , 90.8 ± 9.1 , 77.4 ± 8.8 and 66.7 ± 7.6 (U/L) in infected control group, group treated with LEVA, group treated with PLAN, group treated with LEVA loaded with LPAN, and group treated with ALB, respectively. From 107.4 ± 18.7 (U/L) in the control group to 153.67 ± 21.6 , 147.7 ± 20.9 , 149.3 ± 19.8 , 122.4 ± 14.6 , and 119.7 ± 7.6 (U/L) in the infected control group, group treated with LEVA, group treated with PLAN, group treated with LEVA loaded with LPAN, and group treated

with ALB, respectively, the ALP levels increased in the exposed groups. Comparably, ALB levels in the infected control group, group treated with LEVA, group treated with PLAN, group treated with LEVA loaded with LPAN, and group treated with ALB all significantly decreased from 5.5 ± 0.6 (g/dl) to 3.3 ± 0.7 , 3.9 ± 0.9 , 3.5 ± 0.4 , 4.3 ± 0.5 , and 5.2 ± 0.2 (g/dl), respectively. The levels of GGT in the exposed groups also dropped, going from 9.5 ± 1.7 (U/L) in the control group to 4.6 ± 2.6 , 5.2 ± 2.2 , 4.9 ± 2.3 , 5.6 ± 2.8 , and 8.8 ± 3.1 (U/L) in the groups that were infected, treated with LEVA, treated with PLAN, treated with LEVA loaded with LPAN, and treated with ALB, respectively.

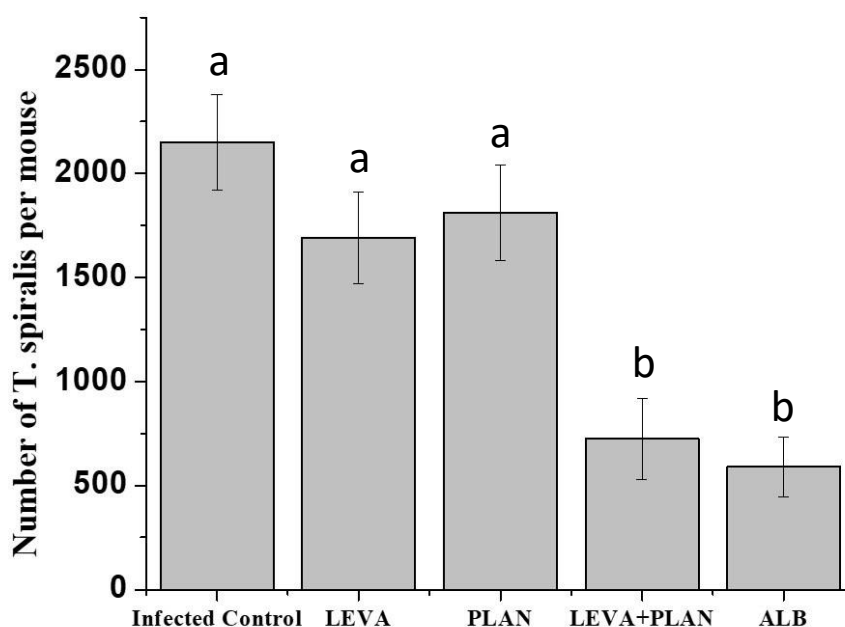


Figure 2. The mean number of encysted larvae of *Trichinella spiralis* at the 34th day post-infection (dpi). Data are presented as mean $\pm$ standard deviation of three independent experiments $F = 33.94$; $df = 4$. Different letters indicate a statistically significant difference between the values ($P < 0.05$).

Table 1. Biochemical alterations in intestinal phase subgroups sacrificed at 7 dpi

Group	Normal control	Infected control	Leva treatment	Plan treatment	Leva + plan TT	ABZ treatment	F
ALT	$61 \pm 4.9c$	$99.5 \pm 12.3a$	$92.4 \pm 12.1a$	$96.2 \pm 11.8a$	$81.4 \pm 10.3ab$	$73.5 \pm 9.4bc$	6.07
AST	$54 \pm 3.6c$	$92.1 \pm 9.7a$	$89.4 \pm 11.5bc$	$90.8 \pm 9.1a$	$77.4 \pm 8.8abc$	$66.7 \pm 7.6bc$	9.41
ALP	$107.4 \pm 18.7a$	$153.67 \pm 21.6a$	$147.7 \pm 20.9a$	$149.3 \pm 19.8a$	$122.4 \pm 14.6a$	$119.7 \pm 7.6a$	3.66
ALB	$5.5 \pm 0.6a$	$3.3 \pm 0.7b$	$3.9 \pm 0.9b$	$3.5 \pm 0.4b$	$4.3 \pm 0.4ab$	$5.2 \pm 0.2a$	11.79
GGT	$9.5 \pm 1.7a$	$4.6 \pm 0.6b$	$5.2 \pm 0.2b$	$4.9 \pm 0.3b$	$5.6 \pm 0.8b$	$8.8 \pm 0.3a$	20.55

$df = 5$. Different letters horizontally indicate a statistically significant difference between the values ($P < 0.05$).

The biochemical alterations in groups of mice sacrificed at 34 dpi were shown in table 2, there was a rise in ALT from 75 ± 6.7 (U/L) in the normal control group to 114.2 ± 9.4 , 102.2 ± 13.4 , 109.5 ± 14.1 , 85.3 ± 11.9 and 79.2 ± 8.7 (U/L) in infected control group, group treated with levamisole, group treated with PLAN, group treated with LEVA loaded with LPAN and group treated with ALB respectively. The same significant increases in the level of AST in mice infected with *T. spiralis* larvae and sacrificed at 34 dpi where the AST level was an increase from 59 ± 3.9 (U/L) in normal mice to 106.1 ± 9.9 , 94.5 ± 11.2 , 101.1 ± 10.7 , 90.3 ± 9.2 and 78.7 ± 7.1 (U/L) in the infected control group, group treated with LEVA, group treated with PLAN, group treated with LEVA loaded with LPAN and group treated with ALB respectively. The ALP levels also increased in the exposed groups from 114.4 ± 22.9 (U/L) in the control group to 187.7 ± 44.2

44.2 , 172.5 ± 32.9 , 179.8 ± 35.4 , 141.3 ± 22.6 and 127.8 ± 19.4 (U/L) in infected control group, group treated with LEVA, group treated with PLAN, group treated with LEVA loaded with LPAN and group treated with ALB respectively. ALB levels significantly decreased from 6.1 ± 0.4 (g/dl) in the control group to 3.1 ± 0.5 , 3.7 ± 0.4 , 3.6 ± 0.3 , 4.1 ± 0.3 and 5.5 ± 0.4 (g/dl) in infected control group, group treated with LEVA, group treated with PLAN, group treated with LEVA loaded with LPAN and group treated with ALB respectively. The GGT levels also decreased in the exposed groups from 10.2 ± 1.4 (U/L) in the control group to 4.0 ± 0.1 , 5.1 ± 0.3 , 4.7 ± 0.3 , 6.4 ± 0.6 and 9.3 ± 0.4 (U/L) in infected control group, group treated with LEVA, group treated with PLAN, group treated with LEVA loaded with LPAN and group treated with ALB respectively.

Table 2. Biochemical alterations in muscle phase subgroups sacrificed 34 dpi

Group	Normal control	Infected control	Leva treatment	Plan treatment	Leva + plan TT	ABZ treatment	F
ALT	$75 \pm 6.7b$	$114.2 \pm 9.4a$	$102.2 \pm 13.4ab$	$109.5 \pm 14.1a$	$85.3 \pm 11.9ab$	$79.2 \pm 8.7b$	6.77
AST	$59 \pm 3.9c$	$106.1 \pm 9.9a$	$94.5 \pm 11.2ab$	$101.1 \pm 10.7ab$	$90.3 \pm 9.2ab$	$78.7 \pm 7.1bc$	10.89
ALP	$114.4 \pm 22.9a$	$187.7 \pm 44.2a$	$172.5 \pm 32.9a$	$179.8 \pm 35.4a$	$141.3 \pm 22.6a$	$127.8 \pm 19.4a$	2.88
ALB	$6.1 \pm 0.4a$	$3.1 \pm 0.5b$	$3.7 \pm 0.4b$	$3.6 \pm 0.3b$	$4.1 \pm 0.3b$	$5.5 \pm 0.4a$	27.67
GGT	$10.2 \pm 1.4a$	$4.0 \pm 0.1c$	$5.1 \pm 0.3bc$	$4.7 \pm 0.3bc$	$6.4 \pm 0.6b$	$9.3 \pm 0.4a$	44.37

df = 5. Different letters horizontally indicate a statistically significant difference between the values ($P < 0.05$).

The intestine and muscular portions of the uninfected and untreated control group showed no histological alterations. As well as adult worm sections in the mucosa, intestinal sections from the untreated infected group at the seventh day post-infection revealed the presence of chronic inflammatory cells infiltrating both the mucosa and submucosa. When comparing the intestinal portions of the mice treated with ABZ to the untreated infected group, there was a noticeable decrease in inflammatory infiltration, while the mice treated with LEVA alone showed mild to moderate cell infiltration. The groups who received both LEVA and PLAN treatment showed the greatest decrease in inflammatory infiltrates (Fig. 3).

The muscular diaphragms of infected, untreated mice displayed a huge inflammatory cellular infiltration with atrophy surrounding the encapsulated larvae; in most cases, the capsule seemed thick and complete. Fig. 4 depicts the histopathological alterations in the muscle diaphragms. Following ABZ treatment, there were fewer encysted larvae and less inflammatory cells in the muscle diaphragm tissue. Following treatment with LEVA loaded with PLAN, the muscle sections of the mice displayed significantly fewer encysted larvae; instead, most of them displayed degenerative changes, areas of breakdown, vacuolization, and invasion by an inflammatory cellular infiltrate.

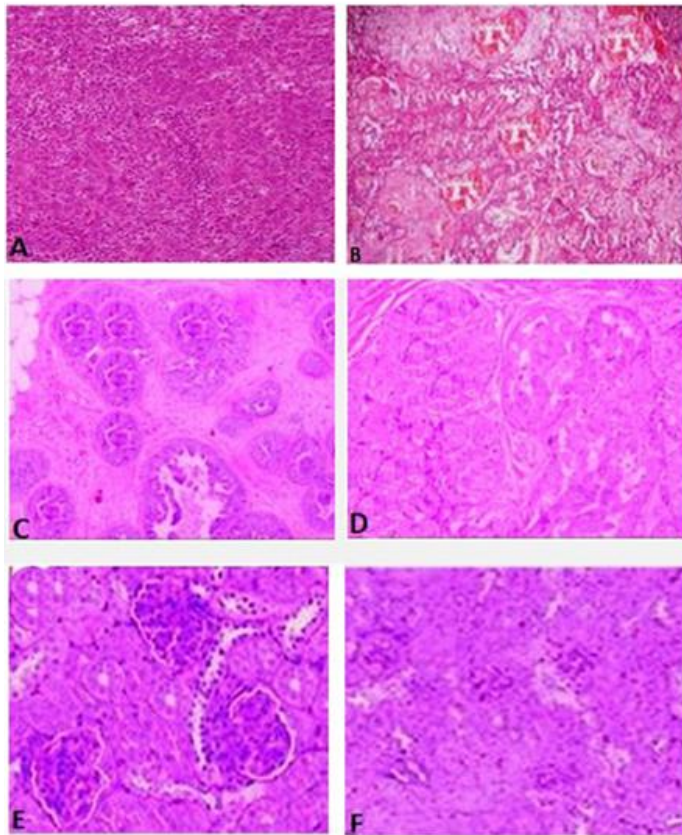


Figure 3. Histopathological changes observed in the intestinal sections at 1 dpi for 3 days. (A) Normal control. (B) Non treated infected mice. (C) Infected treated with 50 mg/kg ALB. (D) Infected treated with 25 mg/kg/day. (E) Infected treated with 50 µg/mouse of PLAN. (F) Infected treated with 50 µg/mouse of PLAN loaded with 25 mg/kg/day.

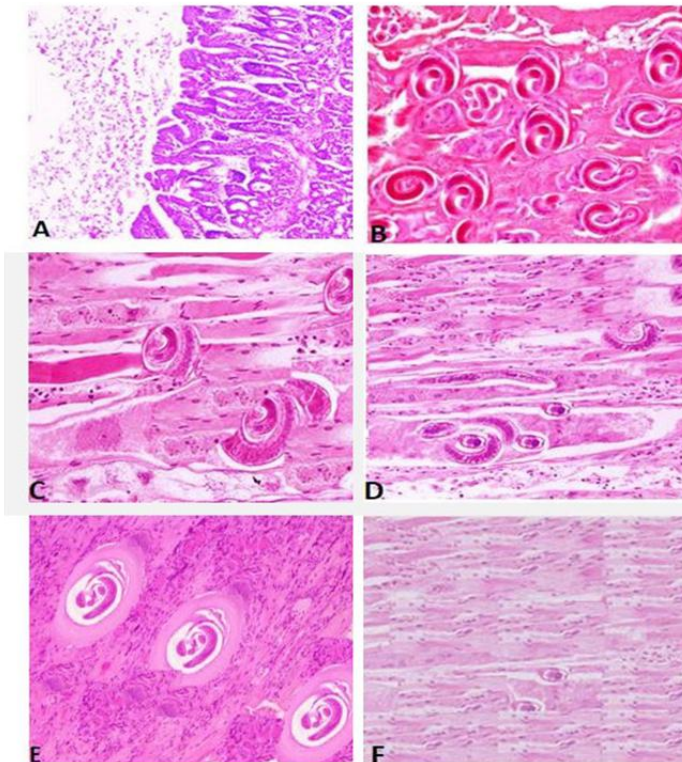


Figure 4. Histopathological changes were observed in the muscle sections at 30 dpi for 3 days. (A) Normal control. (B) Non treated infected mice. (C) Infected treated with 50 mg/kg ALB. (D) Infected treated with 25 mg/kg/day. (E) Infected treated with 50 µg/mouse of PLAN. (F) Infected treated with 50 µg/mouse of PLAN loaded with 25 mg/kg/day levamisole

DISCUSSION

The parasitic nematode (roundworm) *Trichinella spiralis* primarily infects humans but is also capable of parasitizing a wide range of other mammals, including horses, pigs, birds, and reptiles (Blaxter *et al.*, 1998; Pozio and Gomez Morales, 2023). Humans contract the disease by consuming raw or undercooked pork, horse or other domestic animal meat, and meat from wild animals like bears. There have been instances of illnesses occasionally contracted from consuming the meat of reptiles, such as turtles and lizards (Pozio and Gomez Morales, 2023). There have been no reports of person-to-person transmissions. There are an estimated 10,000 cases of trichinosis worldwide each year (Murrell and Pozio, 2011). When humans eat meat from common animals that has been tainted, cases usually arise in groups (Hailu *et al.*, 2020). Although there are other *Trichinella* species and twelve genotypes of *Trichinella* that have been reported to far, *T. spiralis* is the most frequent species that can cause illness in humans (Thanchomnang *et al.*, 2021, Pozio and Gomez Morales, 2023).

The goal of the current investigation was to assess the parasitological, biochemical, and histopathological parameters to determine the effectiveness of levamisole, either by itself or in combination with polylactic acid nanoparticles (PLANs), in treating intestinal worms and the encapsulated muscle larvae of a murine *T. spiralis* infection. Mice killed seven days after infection showed a significant decrease in the number of intestinal *T. spiralis* worms in their small intestines following treatment with 50 mg/kg ABZ (82.2% reduction) and LEVA loaded with PLANs (38.1% reduction). Previous investigations (Chung *et al.*, 2001, Siriyasatien *et al.*, 2003, Shalaby *et al.*, 2010) found similar efficacies of albendazole and mebendazole. When albendazole-treated mice received the same dose for three days in a row beginning on the first dpi, there was a significant reduction of 90.9%. Levamisole and oxiclozanide combination therapy is produced as an oral suspension and is approved for the treatment of worm infestations in animals. Numerous researches have investigated the effect of LEVA on various intestinal helminths (Hamid *et al.*, 2023). Levozan® is a drug used to treat animal worm infestations. It is authorized for the

management of liver fluke infestation, verminous bronchitis, and parasitic gastroenteritis (Ahmed *et al.*, 2022).

The Food and Drug Administration has approved that the polymer polylactic acid (PLA), be a very adaptable material with appealing qualities including biocompatibility and biodegradation. Consequently, PLA has been investigated for numerous therapeutic uses, such as the administration of medications and nanoparticle antigens (Essa *et al.*, 2013, Legaz *et al.*, 2016). The biochemical responses to the invasion of *T. spiralis* being the most frequent species of *Trichinella* that can infect humans. However, *Trichinella nativa*, *Trichinella nelson*, *Trichinella britovi*, *Trichinella pseudospiralis*, *Trichinella murelli*, and *Trichinella papuae* are all species that have been linked to human illness (Ortega-Pierres *et al.*, 2000). Isolates were altered in the current study, as evidenced by a marked and significant decrease in the levels of ALT, AST, and ALP and a significant increase in TP and GGT when compared to the infected untreated group. This inspection viewed higher serum levels of ALT, AST, and ALP in treated mouse groups and lower levels of TP and GGT at 7 and 32 dpi. Consistent with our findings, (Matar *et al.*, 2023), in both the adult and larval phases, the addition demonstrated lower levels of liver enzymes (ALT & AST) in the treated groups when compared to the infected, untreated group.

Regarding the histopathological analysis, intestinal sections from the infected nontreated group on the seventh day post-infection showed the presence of persistent inflammatory cells infiltrating the mucosa and submucosa, whereas the mice treated with LEVA alone showed a significant reduction in inflammatory cellular infiltration from mild to moderate. The groups who received combined LEVA and PLAN treatment showed the most notable reduction in inflammatory infiltration. In our investigation, mice given both PLAN and LEVA treatments displayed focal necrosis, cellular infiltration inflammation, diffuse degenerative alterations, and degeneration of the encapsulated larvae, which was replaced by amorphous materials. However, when treatments were administered on day 35 as opposed to day 15 post infection, more encysted larvae were seen throughout the muscle bundles, indicating a higher level of efficacy than those earlier administered. Mebendazole has

been shown to be as effective (Yadav and Deori 2014).

Albendazole, however, has been shown in earlier research to have far less effectiveness against cystic muscle larvae (Chung *et al.*, 2001, Siriyasatien *et al.*, 2003, Zeinab S *et al.*, 2006, Shalaby *et al.*, 2010). The length, dosage, and duration of treatment all affect how effective albendazole is on the intestinal and muscular stages (Siriyasatien *et al.*, 2003, Fahmy and Diab, 2021). Inflammation and severe tissue damage in the intestine and skeletal muscles' histological sections. Reactive oxygen species (ROS), superoxide dismutase (SOD), inducible nitric oxide synthase (iNOS), upregulation of COX-2, the anti-apoptotic granules and inflammatory cells were observed in *Spiralis*-infected mice. Not all parasites create these responses (Othman *et al.*, 2016). In this study, mice euthanized at 7- and 32-days post-infection showed altered biochemical and histological markers due to *T. spiralis* is the most frequent species of *Trichinella* that can infect humans. However, *Trichinella nativa*, *nelson*, *britovi*, *pseudospiralis*, *murelli*, and *papuae* are all species that have been linked to human illness (Pozio and Gomez Morales, 2023). Treatment with LEVA loaded with PLANs resulted in considerable elimination of both adult and muscular *T. spiralis* along with improvement in biochemical and histological changes.

CONCLUSION

Mice were given an oral infection with *T. spiralis*, after which the intestinal and muscular stages were assessed in the groups given LEVA, PLAN, and LEVA loaded with LPAN. This analysis revealed improved histological and physiological features along with a significant decrease in larval population in all three groups. However, the third group treated with LEVA loaded with LPAN demonstrated a noteworthy decrease in the quantity of larvae as well as an improvement in biochemical and histological levels that were comparable to those of the control group. So, Levamisole is an effective and thoroughly researched trichinellosis treatment. But the addition of nanomaterials significantly increases the therapeutic benefits of this approach. Nevertheless, further comprehensive research is desperately needed to validate the adverse impact.

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