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ORIGINAL ARTICLE

The Impact of Nitazoxanide Loaded on Solid Lipid Nanoparticles on Experimental Trichinellosis

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ABSTRACT

Background: Trichinellosis is a worldwide re-emerging parasitic disease, affecting humans. This study aimed to assess the efficacy of nitazoxanide (NTZ) loaded on solid lipid nanoparticles (SLNs) for the treatment of murine *Trichinella spiralis* infection in comparison with the crude nitazoxanide and ivermectin.

Methods: 160 Male Swiss albino mice were divided into eight groups. Each group was further subdivided into the intestinal phase & muscular phase; **G1:** healthy not infected control, **G2:** infected control group, **G3:** infected and treated with SLNs, **G4:** infected and treated with ivermectin, **G5:** infected and treated with NTZ, **G6:** infected and treated with NTZ-SLNs, **G7:** infected and treated with ivermectin combined with NTZ and **G8:** infected then treated with ivermectin combined with NTZ-SLNs. All groups except G1 were orally infected with 250-300 *T. spiralis* larvae. Assessment of the treatment efficacy was performed parasitologically, histopathologically and serologically.

Results: A significant reduction in the number of intestinal adult worm and encysted muscular larvae in all treated groups was found with highest reduction rate in G8 (99.1%,89%). These results were confirmed by histopathological improvement of the targeted groups.

Conclusion: Although NTZ was inferior to ivermectin in the treatment of trichinellosis during the intestinal phase, it was superior to it in the muscular phase. Loading NTZ on SLNs resulted in increasing its efficacy against both intestinal and muscular phases. The combinations between ivermectin and the crude and loaded NTZ showed higher efficacies. ELISA results were non-significant, being not valid for the follow up of the treatment efficacy.

Keywords: Ivermectin; Muscular Disease; Nitazoxanide; Solid Lipid Nanoparticles; Trichinellosis.

INTRODUCTION

Trichinellosis is a worldwide parasitic disease caused by ingestion of raw or undercooked meat containing the infective larvae of *Trichinella spiralis*[1]. The main sources of infection are the pork and its products[2]. *T. spiralis* has a unique ability to transform the infected muscle cell, creating a

new type of cell in the host body, called nurse cell[3]. Mebendazole, the traditional treatment for trichinosis, has limited bioavailability, high degree of resistance, and weak activity against the encapsulated larvae[4]. Ivermectin is effective against both muscular and intestinal phases of trichinellosis and future resistance is usually expected.

Therefore, there is an urgent need to find new alternative treatments[5].

Nitazoxanide (NTZ) is an anti-parasitic drug, used against intestinal cestodes, protozoa and nematodes[6]. Ashour *et al.*[7] reported its promising activity against enteral and more effect on the parenteral phases of trichinellosis. It is also a safe drug because it is neither mutagenic nor teratogenic[8]. The wide spectrum of NTZ against parasites suggests that its effect does not depend on a parasite-specific mechanism but more likely it has an immunomodulatory action[9]. Although oral administration of drugs has many advantages over the other routes, but the drug solubility and dissolution rate still have a vital role in its absorption[10].

Nanomedicine offers an improvement of oral delivery by bioavailability enhancement, adverse-effect minimization and food-effect mitigation[11]. Solid lipid nanoparticles (SLNs), a type of nanocarriers, are at the forefront of the potential application in oral drug delivery systems[12]. SLNs are widely applicable for carrying anti-infection drugs to treat bacterial, fungal, viral and parasitic infection[13]. NTZ could be successfully incorporated in a stable biocompatible liposome (NTZ-SLNs) using a modified thin film hydration technique that showed high capture efficacy and high *in vitro* drug release rates[14].

This study aimed to assess the efficacy of nitazoxanide loaded on solid lipid nanoparticles in the treatment of intestinal and muscular phase of murine *Trichinella spiralis* infection in comparison with the crude nitazoxanide and ivermectin.

MATERIALS AND METHODS

Ethical clearance:

The experiment was done following the ARRIVE guidelines and in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The experiment was approved by Institutional Animal Care and Use Committee-Zagazig

University (IACUC-ZU), approval no. 4297 on 4th February 2018.

Parasites and experiment animals:

T. spiralis strain was obtained as laboratory bred infected albino mice from Medical Parasitology Department, Faculty of Medicine, Tanta University, Egypt. 160 Male Swiss albino mice (six-week-old) were purchased from Animal House, Faculty of Medicine, Zagazig University, Egypt. These mice were kept on standard commercial pelleted diet with free accessible water all over the time of the study in Animal House.

ELISA kit:

Anti-mouse *Trichinella spiralis* IgG kit, manufactured by NOVA Company, Daxing Industry Zone, Beijing, China was purchased from Biovision Company, Egypt.

Drug treatment:

Ivermectin (Iverzine ®, UNI Pharma) was given as a single oral dose of 0.2 mg/kg body weight on the 5th and the 35th days post infection (PI)[15]. Nitazoxanide (Nanazoxid, 100 mg suspension, Medizen for Utopia Pharmaceuticals, Egypt) was administered orally in a dose of 50 mg/kg/day on the 2nd day PI for 3 successive days and on the 30th days PI for 14 days[7]. SLNs were administered in a dose of 1.25mg/mouse. The groups receiving the combined drugs, have been given full dose of drugs.

The preparation of NTZ-SLNs:

It passed through two steps; The microemulsion (ME) step in which stearic acid, the lipid matrix, was weighed and heated to 70°C. The lipophilic drug NTZ, 0.5 gram, was added to the melted stearic acid and various amounts of emulsifiers either tween 20 or pluronic F 127 or lithocholic acid or lecithin or sometimes combination of them were dissolved in water and heated to the same temperature. The hot aqueous phase was added to the lipid phase and magnetically stirred at 500 rpm for 10 min. to form transparent and thermodynamically stable microemulsion. Then, hot ME was added into the water at 2-3°C for 2 hours to solidify/crystallize then subjected the emulsion to high shear dispersion (HSD) with a homogenizer at 10,000-15,000 rounds per minute for 10-40 minutes[16]. The lyophilization step was performed at

Biochemistry Department, Faculty of Agriculture, Zagazig University, in which SLNs dispersions were freeze-dried to remove water. In order to increase the particle concentration, glucose (3%) was used in the lyophilization process as a cryo-protectant.

Characterization methods of SLNs:

It was performed at National Institute of Research, Egypt. The development of SLNs have been evaluated using Ultraviolet visible Spectroscopy (UV-vis scan) (Jasco V630) at range (200-800 nm) for each reaction mixture and were recorded using 2 µl of solution[17]. The size of SLNs was determined by using Zeta sizer nano series (Nano ZS -Malvern, UK) by dynamic light scattering (DLS) using Milli-Q water as baseline. All measurements were performed at 26°C. The recorded correlation functions and measured particle motilities were converted into size distributions and Zeta sizer, respectively, using the Malvern Dispersion Software[18]. Scanning electron microscopy (SEM) (SU1510 model; Hitachi Ltd., Tokyo, Japan) was used to detect the morphological appearance of the nanoparticles[19].

Experimental design:

160 Male Swiss albino mice were included in this study then divided into eight main groups of 20 mice each. Each group was further subdivided into 2 subgroups of 10 mice each (group A: for intestinal phase & group B: for muscular phase) as follows: G1: healthy not infected control mice, G2: infected control group, G3: infected and treated with SLNs, G4: infected and treated with ivermectin, G5: infected and treated with NTZ, G6: infected and treated with NTZ-SLNs, G7: infected and treated with ivermectin combined with NTZ and G8: infected and treated with ivermectin combined with NTZ-SLNs. All groups except G1 were infected orally with 250-300 *T. spiralis* ML according to Wassom *et al.*[20].

Collection of larvae:

Each mouse was dissected, and its muscles were digested in 1% pepsin and 1% concentrated HCl in 200 ml distilled water. The mixture was incubated at 37°C for two hours under continuous agitation using an electric stirrer. The digested product was passed through a 50-mesh/inch sieve to

remove the coarse particles. Encysted larvae were collected on a 200-mesh/inch sieve, washed twice with tap water and then suspended in 150 ml of tap water in a conical flask. The supernatant fluid was then discarded and the larvae in the sediment were counted microscopically using a McMaster counting chamber[21].

Collection of serum samples:

Blood samples were collected from retro-orbital blood vessels of infected mice at the 21th using capillary tube and neck veins at the 45th days PI then left for 10-20 minutes to clot at room temperature. Serum samples were obtained by removal of these clots by centrifugation at 2000-3000 rpm for 20 minutes.

Parasitological assessment:

- a) The intestinal phase: collection and counting the adult worms of *Trichinella spiralis* according to Wakelin and Lioyed[22] was done on the 7th day PI.
- b) The muscular phase: collection and counting the encysted muscle larvae according to Denham[21] was done on the 45th day PI.

Sandwich-ELISA:

According to the manufacturer's instructions, Sandwich-ELISA technique was used for qualitative detection of antibodies using *T. spiralis* anti-mouse IgG ELISA. Microtiter plate wells were coated with *T. spiralis* excretory/secretory (E/S) antigens partially purified in conditions capable of maintaining the antigens' native form. About 50 µl negative and positive control solutions were added to the negative and the positive control wells respectively. In sample wells, 40 µl of sample dilution solution and 10 µl of serum samples were added and mixed well with gentle shaking. Incubation at 37°C for 30 minutes after sealed with closure plate membrane. The concentrated washing buffer with distilled water was diluted. The closure membrane was peeled off carefully, then the wash solution was aspirated and refilled in the wells. The 50 µl horse radish peroxidase (HRP) conjugate reagent was added to each well except the blank control one.

Test effectiveness was indicated by the average value of positive control ≥ 1.00 and the average value of negative control ≤ 0.10 . The

presence of *Trichinella* IgG was determined by comparing with the CUT OFF value. The CUT OFF value was calculated on the basis of the average OD of the negative serum samples plus three standard deviations[23]. The critical value (CUT OFF) = the average value of negative control + 0.15, so negative judgment is obtained when O.D. value < CUT OFF and positive judgment is obtained when O.D. value \geq CUT OFF.

Histopathological examination:

In the intestinal phase, one centimeter from the mid intestinal region was taken at the 7th day PI then fixed in 10% formol-saline. In the muscular phase, the tongue, the diaphragm and a piece of the thigh muscle were taken from infected mice at the 45th day PI then fixed in 10% formol-saline[24].

Statistical analysis:

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 22 and ANOVA F test to evaluate the possible differences between the studied groups. The P value < 0.05 was considered significant, and P value of >0.05 indicates non-significant results. The quantitative data were expressed as mean \pm SD (standard deviation). The reduction percentage (R %): $R\% = A - B/A \times 100$ Where A represents the mean value of the control group results, B represents the mean of treated group results. Serological results were determined by chi-square test using total numbers of serum samples obtained from treated groups after 21- and 45-days PI.

RESULTS

Characterization of SLNs:

The graphs showed UV-vis scan of SLNs, NTZ-SLNs and NTZ in Fig.1 (A, B, C). The wavelength of maximum absorbance of NTZ-SLNs was 220 nm. By Nano ZS, the particle size and size distribution of SLNs with mean diameter of 272.6 nm and standard deviation of 135.2 nm (49.6%). Cumulative result showed particle size of SLNs as follow: 25 % of distribution < 185.6 nm, 50 % of distribution < 272.6 nm, 75 % of distribution < 365.3 nm, 90 % of distribution < 483.2 nm, 99 % of distribution < 795.3 nm and 80 % of distribution < 394.1 nm (Fig .1D). The FTIR chromatogram showed that there was no major

shifting and no loss of functional peaks between the spectra of NTZ-SLNs, SLNs and NTZ which indicated no interaction between NTZ and lipid in addition to loading the NTZ on SLNs (Fig.1E). Fig.(S1A) showed SEM images of the drug-loaded SLNs at $\times 400$ whereas, Fig.(S1B) showed $\times 4000$ magnification that the nano-formulated NTZ-SLNs obtained were seen to be spherical in shape with smooth surface.

Parasitological results:

In table (1), the mean number of adult worms in the small intestine was highly decreased in G8 infected and treated with ivermectin combined with NTZ-SLNs, followed by G7 infected and treated with ivermectin combined with NTZ with the reduction percentages of 93.3% and 85.8% respectively with high statistically significant difference ($P < 0.001$). Adult worms count in G4 infected and treated with ivermectin, G5 infected and treated with NTZ and G6 infected and treated with NTZ-SLNs was decreased also with reduction percentages of 80.2%, 70.6% and 79.7% respectively. The count of encysted larvae in muscles was highly decreased in G8 (89%) followed by G7 (84%) with high statistically significant difference ($P < 0.001$). The count of encysted larvae in muscles was moderately decreased in G5(73.7%) and G6(78.9%) with high statistically significant difference ($P < 0.001$) but the reduction percentages in those groups were less than G7 & G8. On the other hand, G4 showed the least reduction in encysted larvae count than other treated groups (54.1%). There was statistical significance between all tested groups ($P < 0.001$). There was no significant reduction in larvae count in G3 infected and treated with SLNs compared to the infected control group (G2).

Histopathological results:

Seven days PI, small intestine in G2 (infected control) showed dense inflammatory cellular infiltrate consisting of mainly neutrophils, eosinophils and lymphocytes in the core of the villi and extending into the submucosa. Flattening of the villi with hyperplasia of the crypts of Lieberkühn was observed. Hypertrophy and hyperplasia of musculosa were detected. The worm was present in the lumen of the intestine (Fig.2A). G3 that

received SLNs showed no significant difference in the inflammatory changes in relation to the infected control G2 as seen in (Fig.2B). G8 which received ivermectin with NTZ-SLNs, showed marked reduction in the inflammatory infiltrates with mild increase in the number of goblet cells and clearance of infection proved by the absence of adult worms as presented in (Fig.2F), followed by G7 (ivermectin + NTZ) as observed in (Fig.2E). NTZ was inferior to ivermectin in the intestinal phase as G5 (NTZ) showed moderate increase in the inflammatory infiltrates and the number of goblet cells seen in (Fig.2D). G4 receiving ivermectin and G6 taking NTZ-SLNs confirmed more improvement than G5 given NTZ with mild increase in the number of inflammatory and goblet cells and necrotic worms in the lumen of the intestine as cleared in (Fig.2C).

Forty-five days PI the cut section of muscles in G2 (control infected) showed diffuse degenerative changes all over the muscle fibers with large number of *T. spiralis* encysted larvae. Each larva was surrounded by a collagen capsule and a dense inflammatory cellular infiltration consisting mainly of histiocytes, lymphocytes, plasma cells and eosinophils with necrosis and marked fibrosis of muscle (Fig.2G). G3 (SLNs) was similar to G2 in (Fig.2H). G8 (ivermectin + NTZ-SLNs) had marked reduction in both number of

encysted larvae, inflammatory infiltrates and destruction of collagen capsules of encysted larvae with invasion of inflammatory cells, marked degeneration of larvae up to complete disappearance of them with no fibrosis of muscle as cleared in (Fig.2M). Moreover, the previous changes were also evident in G7 (ivermectin + NTZ) as shown in (Fig.2L) but less than G8 with mild fibrosis of muscle fibers. Mild inflammation, mild affection of muscle fibers, mild fibrosis with affection of capsule, invasion of inflammatory cells and moderate degeneration of larvae in G6 (NTZ-SLNs) as cleared in (Fig.2K). G5 (NTZ) had moderate inflammation, fibrosis with mild degeneration of collagen capsule and larvae, and muscle was also affected as shown in (Fig.2J). G4 (ivermectin) showed necrosis of muscles, marked inflammation and thick complete collagen capsule with the least significant reduction in number of encysted larvae and inflammatory changes in comparison to groups 5, 6, 7 and 8 as shown in (Fig.2I).

Serological results:

The detection rates of the anti-*Trichinella* IgG using ELISA for all studied groups at 21 days PI were (75-90%) and at 45 days PI were (80-100%) IgG detection rate. There was no statistically significant difference between all groups before and after treatment ($P > 0.05$) (Table 2).

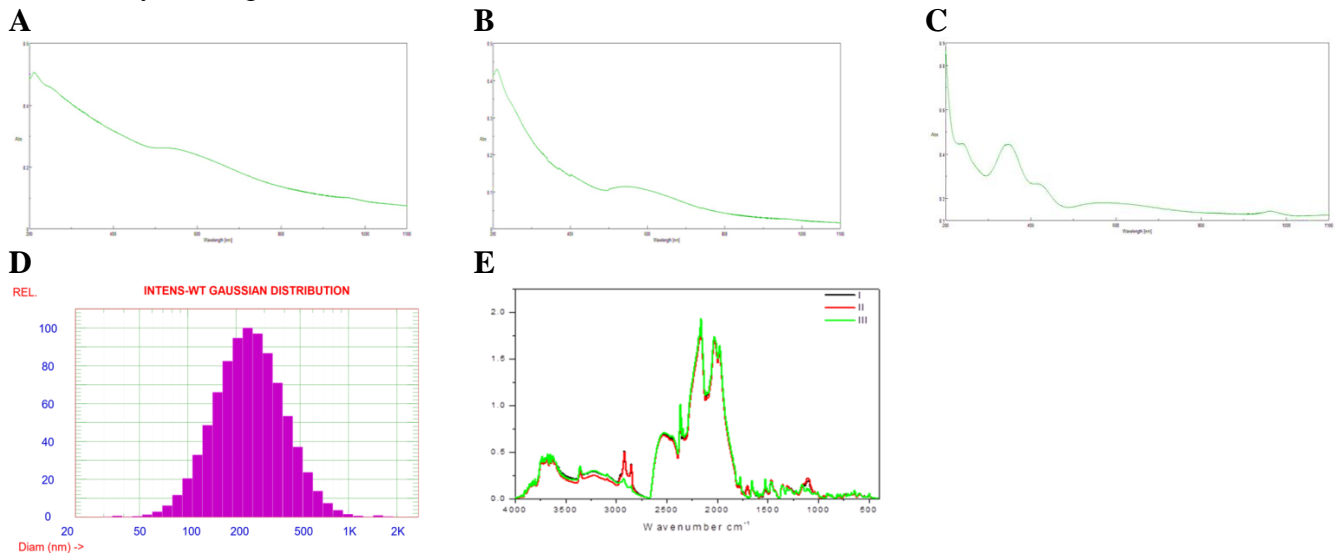
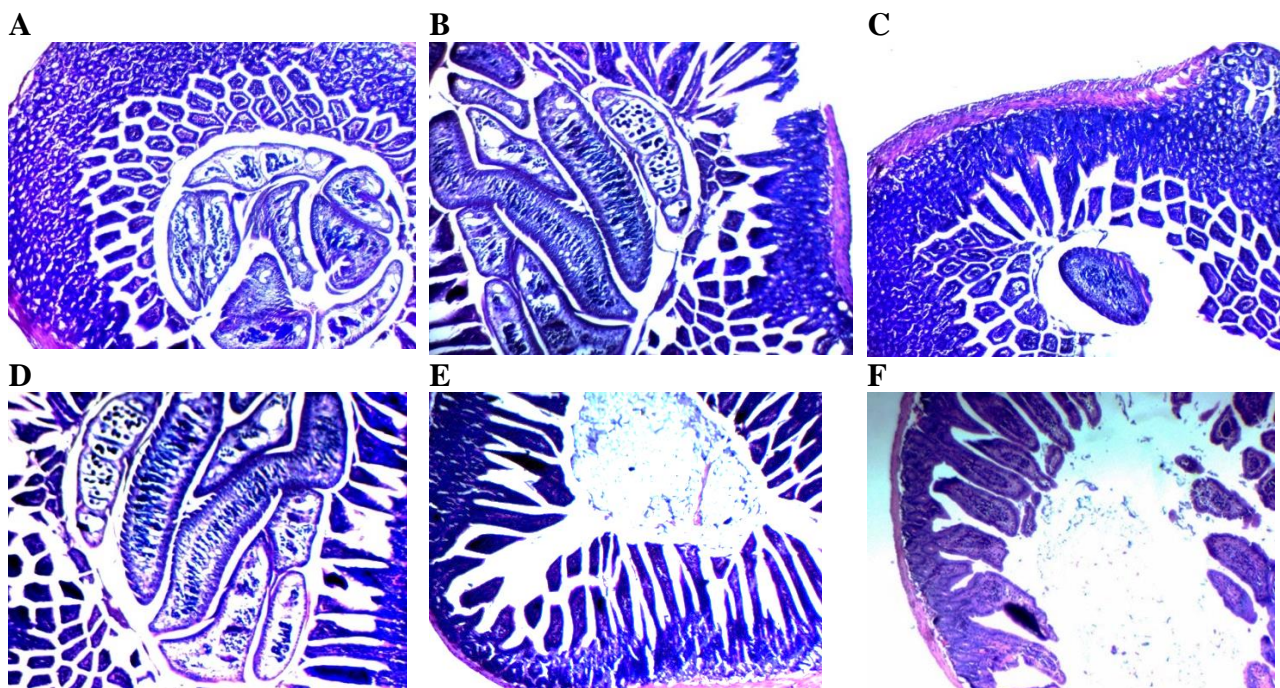
Table 1: The effect of the SLNs, NTZ, Ivermectin, NTZ-SLNs, and their combinations on the intestinal and muscular phase of *T. spiralis* among studied groups.

Groups	Worm count		Encysted muscle larvae	
	Mean \pm SD	Reduction %	Mean \pm SD	Reduction %
G1	0 \pm 0	--	0 \pm 0	---
G2	230.6 \pm 12.5	--	5462.3 \pm 211.9	---
G3	226.7 \pm 6.5	2.3	5365.6 \pm 163.2	1.8
G4	45.6 \pm 7.7	80.2	2507.1 \pm 109.9	54.1
G5	67.8 \pm 9.5	70.6	1433.9 \pm 117.0	73.7
G6	46.9 \pm 7.8	79.7	1150.2 \pm 71.4	78.9
G7	32.7 \pm 7.2	85.8	871.5 \pm 59.5	84
G8	15.4 \pm 5.6	93.3	601.4 \pm 45.7	89
ANOVA	F = 1221.5	P < 0.001 ^a	F = 2828.9	P < 0.001 ^a

^aStatistically significant.

Table 2: The Least significant difference (LSD) of all groups for IgG detection in muscle phase before and after treatment.

	Positive	Negative	Total
Before	61 ^c	19 ^c	80
After	64 ^c	16 ^c	80
Total	125	35	160
	$X^2 = 0.33$ (NS)		$P > 0.05^d$

^cNumber of living mice/group^dStatistically non-significant**Fig. 1:** Characterization of SLNs. (A,B,C) Comparison between UV-vis scan of SLNs, NTZ-SLNs and NTZ. (D) Intensity-weighted Gaussian distribution analysis (Solid Particle). (E) FTIR chromatogram.

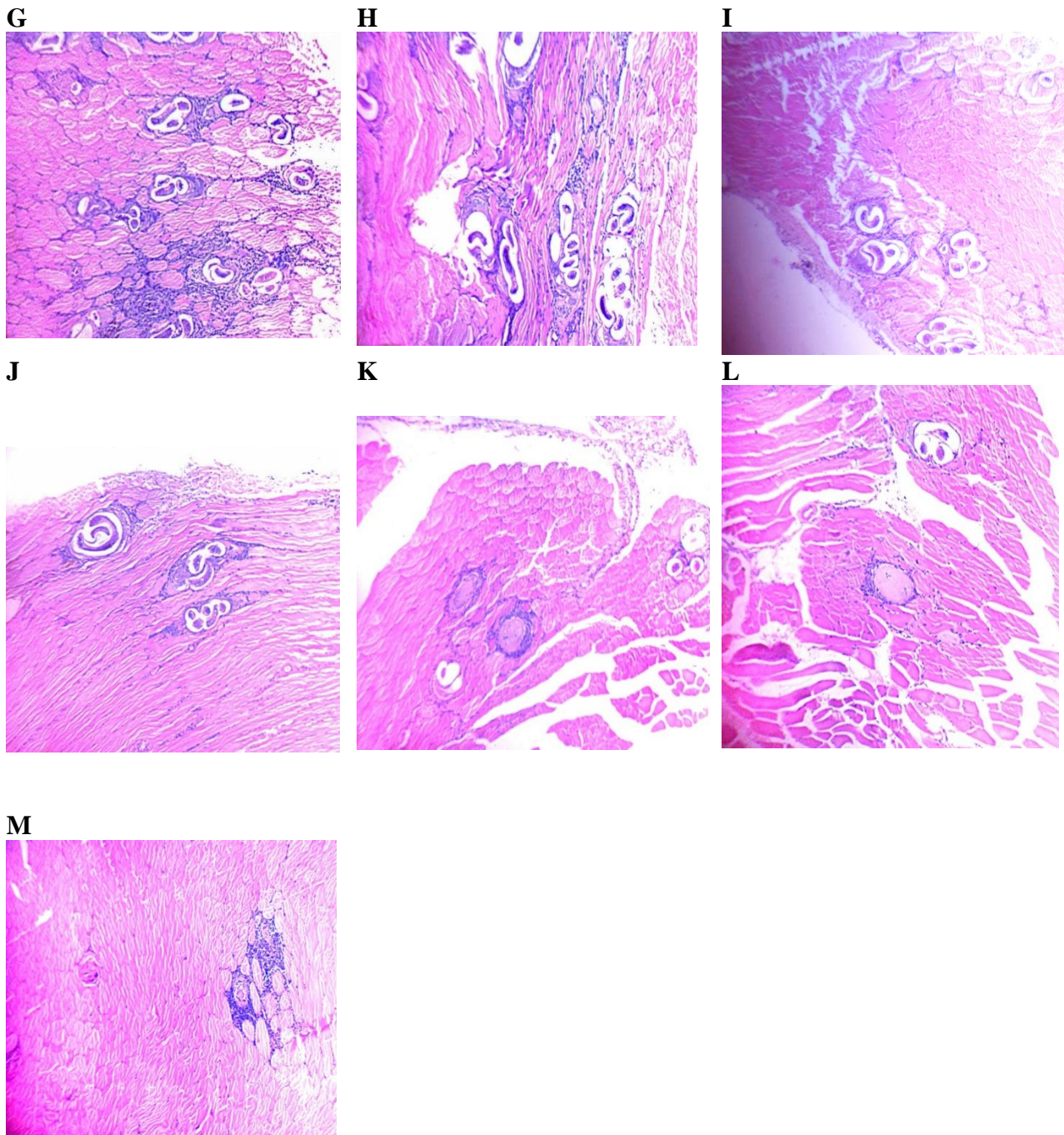


Fig. 2: Hematoxylin-eosin staining (×100) of:

- 1- Intestinal biopsy from studied groups showing (A,B) adult worm in the lumen of the intestine in G2&G3. (C) adult worm in the lumen of the intestine in G4&G6. (D) adult worm in the intestinal lumen in G5 (E) moderate reduction in the inflammatory infiltrates in G7. (F) marked reduction in the inflammatory infiltrates with mild increase in the number of goblet cells and clearance of infection proved by the absence of adult worms in G8.
- 2- muscle biopsy from studied groups showing (G, H) encysted muscle larva and inflammatory infiltrates in G2&G3. (I) encysted muscle larva, inflammatory infiltrates and intact thick capsule in G4. (J) mild degenerated muscle larva, inflammatory infiltrates and invasion of capsule by inflammatory cells (G5). (K) moderate degenerated muscle larva, invasion and complete degeneration of capsule by inflammatory cells (G6). (L) markedly degenerated muscle larva and complete degeneration and invasion of capsule by inflammatory cells in G7. (M) inflammatory infiltrate and complete degeneration and invasion of capsule by inflammatory cells in G8.

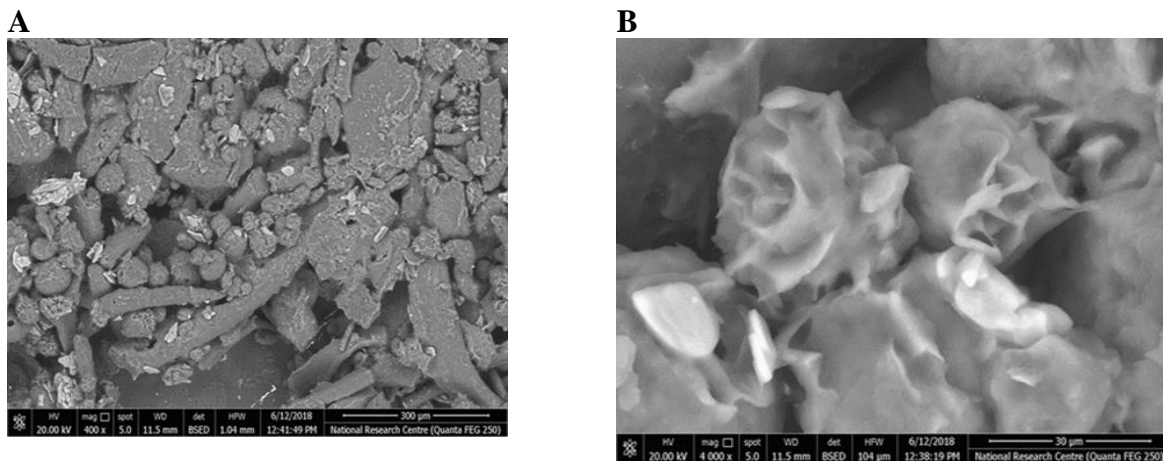


Fig. S1: SEM of the nano-formulated NTZ- SLNs (A $\times 400$, B $\times 4000$).

DISCUSSION

Trichinellosis is a food borne parasitic zoonosis widely distributed all over the world, clinically divided into intestinal, migratory and muscular phases[25],[26]. Nitazoxanide is used for treatment of *Trichinella* infection[7]. The poor water solubility of drugs, results in low bioavailability and delivery problem[27]. Moreover, the nanomedicine offers an improvement of oral delivery by bioavailability enhancement, adverse-effect minimization and food-effect mitigation[11]. Therefore, this work aimed to assess the efficacy of nitazoxanide loaded on solid lipid nanoparticles for the treatment of the intestinal and the muscular phases of *Trichinella spiralis* infection in comparison to crude nitazoxanide and ivermectin.

The SLNs, as nanocarrier, are at the forefront of the potential application in oral drug delivery systems[12]. Nitazoxanide could be successfully incorporated in a stable biocompatible liposome (NTZ-SLNs)[14]. In our research, the NTZ was incorporated into SLNs and the increase in size of particles suggests that the loaded NTZ is either adsorbed to particle surface or entangled in aliphatic chains of triglycerides.

In the intestinal phase of the current study, the percentage reduction of the worm count in treated groups G3, G4, G5 and G6 was (2.3, 80.2, 70.6 and 79.7) respectively compared to the control infected group (G2). The best reduction (93.3%) of the adult worm count was obtained in G8 (ivermectin + NTZ-SLNs) followed by G7 (ivermectin + NTZ) (85.8%). An explanation for these results that

ivermectin, combined with either, free or loaded NTZ has synergistic effect[28]. Our results suggest the greater efficacy of the formulation compared with the pure drug. The enhanced effectiveness was probably a consequence of improved drug bioavailability resulting from the increased solubility and dissolution rate, which lead to a better absorption. Our results agree with Ashour *et al.*[7] who found that NTZ alone decreased *T. spiralis* adult count by 66.6% and with Basyoni & El-Sabaa[5] who proved that ivermectin cause worm count reduction (80.2%). This was explained by El-Azzouni[29] who implied that ivermectin has a direct effect on adults evidenced by topographic changes leading to degeneration and destruction of adults. The effect of ivermectin may be due to neurotransmitters blockage in the parasitic somatic neuromuscular system that interferes with the function of the locomotive apparatus, resulting in their paralysis or due to modulating gamma-aminobutyric acid-gated chloride channels which are more accessible in nematodes[5,30].

Concerning the muscular phase, the percentages reduction of encysted larvae in the treated groups G3, G4, G5 and G6 were (1.8, 54.1, 73.7 and 78.9%) respectively compared to the infected control group. The best reduction (89.0%) was obtained in G8 (ivermectin + NTZ-SLNs) followed by G7 (ivermectin + NTZ) (84.0%). The G6 given NTZ-SLNs showed more significant reduction in larvae count than G5 given NTZ alone. This is because loaded NTZ is better than free form. The obtained difference may also be due to the increase in the bioavailability and the slow

release of loaded drug in the small intestine. Our results agreed with Sedighi *et al.*[31] who found NTZ-SLNs more effective than crude drug for control of experimental *Cryptosporidium*. Their data indicated that nano-nitazoxanide preparations had strong anti-*Cryptosporidium* activity without side effects. The ivermectin alone in G4 showed lesser reduction in the encysted muscle larvae count than other treated groups and had a less effect on the muscular phase (54.1%) than in the intestinal phase (80.2%). These findings agreed with El-Azzouni[29] who used ivermectin to reduce larval count but disagree with Kedsamrong[32] who found that ivermectin had low efficacy on the muscular phase of *Trichnella*. The variation in results may be due to the different doses, sacrifice time and the inoculation count. In the present experiment, the combination between ivermectin and NTZ-SLNs showed the highest percentage reduction in the adult worm and encysted larvae among studied groups (93.3%,89%) respectively and this could attain the desired effect of conventional medicines[5,33].

The histopathological changes in our research, were significantly decreased in all groups during the intestinal and muscular phases compared to the control infected group except in G3 (SLNs) (Fig 2B). In the intestinal phase, marked improvement was obtained with high reduction in the inflammatory changes and goblet cells in G8 (Fig.2L) followed by G7. In the muscular phase, there was marked reduction in the number of encysted larvae up to complete degeneration and absence of them in G8 (Fig.2M) with no inflammatory changes then G7 that showed mild inflammation. In this work, the loaded NTZ showed better histopathological results than the crude NTZ in both muscular and intestinal stages. Moreover, combined forms of drugs (ivermectin + NTZ alone or with SLNs) had better results than non-combined forms on both muscular and intestinal stages due to synergism. Ivermectin was better than NTZ on intestinal phase and this agreed with Gonzalez Canga *et al.*[30] who approved that ivermectin is reacting with parasites within the lumen of the small intestine, but NTZ was better than ivermectin

on the muscular phase. These results agreed with Ashour *et al.*[7] who recovered that NTZ-treated group showed degeneration of the larva and some areas of the capsule and Basyoni & El-Sabaa[5] who stated that ivermectin showed improvement in histopathological changes of *Trichnella spiralis* infected groups.

Sandwich-ELISA technique was used in the present research for qualitative detection of antibodies using *T. spiralis* anti-mouse IgG ELISA. There was no significant difference between groups when serum samples collected before (21 days PI) and after treatment (45 days PI) were tested for anti *T. spiralis* specific IgG (Table 2). The positivity of specific anti-*T. spiralis* IgG began after larvae encystment in muscle (muscular phase). That is because ELISA with the ML ES antigens did not permit detection of the infected mice before 12 days PI[34].

CONCLUSIONS

Although NTZ was inferior to ivermectin in the treatment of trichinellosis during the intestinal phase, it was superior to it in the muscular phase. Loading NTZ on SLNs resulted in increasing its efficacy against both intestinal and muscular phases of *Trichinella spiralis* with better results than the crude NTZ. Using drug combinations like ivermectin with crude and loaded NTZ showed higher efficacies than using individual drugs. ELISA results were non-significant being not valid for the follow up of the efficacy of treatment.

Conflict of interest: The authors declare that they have no competing interests.

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