The Curative Effect of Zingiber Officinale and Cinnamomum Zeylanicum Extracts on Experimental Trichinella Spiralis Infection

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Abstract

Trichinellosis is a re-emerging zoonotic disease that has become a public health concern since its reported human outbreaks in many countries. The traditional therapy has many adverse effects in addition to the developing resistance. So, this necessitates finding effective natural alternatives. The current study targeted to assess the potential therapeutic effects of *Zingiber officinale* and *Cinnamomum zeylanicum* in comparison to albendazole, a conventional therapy for treatment of trichinosis. Sixty mice were classified into five groups (12 mice each), non-infected control, infected control, combined albendazole and prednisolone, *Zingiber officinale*, and *Cinnamomum zeylanicum* treated groups. Mice sacrifice was performed on the 7^{th} and 35^{th} days post infection for intestinal and muscular phases respectively. Efficiency of the used preparations was assessed by parasitological, histopathological, immunohistochemical, biochemical studies in addition to ultrastructural evaluation using transmission electron microscopy. A significant reduction in the mean number of *T. spiralis* adults and larvae was observed in *Zingiber officinale* and *Cinnamomum zeylanicum* treated groups, (64.5%, 50.8%) and (68%, 54.6%) respectively. Also, both extracts showed moderate cytoplasmic reactivity for TGF-β1, (69.3% & 67.8%) respectively. The highest reduction in serum TNF- α level was observed in *Zingiber officinale* treated group during the muscle phase (58.4%) while in the intestinal phase was 50%. The ultrastructural study revealed degenerative effects on both adults and larvae in addition to obvious improvement of the histopathological changes in the small intestine and muscles. We concluded that these herbal extracts especially *Zingiber officinale* can be considered a practical and successful alternative for the treatment of trichinellosis.

Introduction

Trichinellosis is a zoonotic disease caused mainly by the nematode parasite, *Trichinella spiralis*. It affects a wide range of hosts including man (Ashour and Elbakary 2011). Trichinellosis is distributed almost all over the world. It has a burden of approximately 10,000 people per year and 0.2% mortality rate (García et al. 2014). It is transmitted by ingestion of raw or insufficiently cooked pork meat. The life cycle of *T. spiralis* consists of enteral and parenteral phases (Abou Rayia et al. 2017). Enteral or intestinal phase clinically presents by abdominal symptoms as diarrhea and abdominal pain (Wilson et al. 2015). Parenteral or muscular phase presents by periorbital edema, myalgia and muscle weakness (Gottstein et al. 2009).

Benzimidazoles as mebendazole and albendazole are commonly used for treatment of trichinellosis. However, these drugs are not fully effective against *T. spiralis* larvae (García et al. 2014). This may be attributed to poor bioavailability after oral administration which leads to poor oral absorption (Piccirilli et al. 2014). Moreover, some of these drugs are contraindicated in pregnancy and children under three years (Yadav and Temjenmongla 2012), other drugs are thought to be carcinogenic (Shalaby et al., 2010). Thus, there is an increasing need for safe and effective drugs, especially those derived from herbs (Yadav and Temjenmongla 2012), as they are less toxic and almost have no adverse effects (Basyoni and El-Sabaa, 2013).

Zingiber officinale or ginger belongs to the family *Zingiberaceae*. It is used worldwide as a spice and flavoring agent. Also, it has an antioxidant effect which augments the immune response, allowing the body to naturally fight infections. Furthermore, ginger has the ability to increase digestive fluids and counteract toxins. These effects may help in parasite clearance (Ghosh et al. 2011). Few studies investigated the anthelmintic activity of ginger and its constituents (Iqbal et al. 2006). They showed a larvicidal agent against *Angiostrongylus cantonensis* (Lin et al. 2010a) and *Anisakis simplex* (Lin et al. 2010b). *Cinnamomum zeylanicum* (cinnamon) is derived from a Greek word that means sweet wood and comes from the inner bark of tropical cinnamon trees (Vinitha and Ballal 2008). Cinnamon showed antiparasitic effect against *Anisakis* larvae (Trabelsi et al. 2019), *Babesia* and *Theileria* (Batiha et al. 2020). The present study was carried out to assess the parasiticidal and both pro and anti-inflammatory effects of *Zingiber officinale* and *Cinnamomum zeylanicum* extracts against *T. spiralis* in experimentally infected mice in enteral and parenteral phases.

Material And Methods

Animals and parasite

A total of sixty parasite free, laboratory-bred, male Swiss Albino mice 5 weeks old, weighing about 20-25gm each, were used. Mice were obtained from animal house of Theodor Bilharz Research Institute (Giza, Egypt) and maintained in accordance with institutional and national guidelines. Mice were infected orally with 200-250 *T. spiralis* L1 larvae per mouse (Dunn and Wright 1985).

Experimental design

Animals were divided into 5 groups (12 mice each) as following: G (1) Non-infected control group (healthy control), G (2): Infected untreated (infected control), G (3): Infected then treated with albendazole and prednisolone, G (4): Infected then treated with *Zingiber officinale* extract and G (5): Infected then treated with *Cinnamomum zeylanicum* extract.

On the 7^{th} day p.i., six mice from each group were sacrificed and blood samples were collected for determination of serum TNF-α. The abdominal skin was sterilized then incised and the peritoneum was opened to dissect the small intestine which is then preserved in formalin 10% for histopathological examination and immunohistochemical study. The rest of the intestine was used for *T. spiralis* adult worm counting. Adults were then preserved in fresh modified Karnovsky's fixative to be examined later on under electron microscopy. On the 35^{th} d.p.i., 6 mice from each group were sacrificed. Blood samples were collected for the determination of serum TNF-α. The peritoneum was opened and the diaphragm was carefully dissected for histopathological and ultrastructure studies. The rest of the muscle samples were digested for total larval count.

Drugs

Albendazole was used as Alzental suspension (EIPICO), 20 mg/ml. Each mouse received a dose of 50 mg/kg orally for 3 consecutive days starting from the 3rd day post infection according to Attia et al. (2015). Prednisolone was available as Predsol suspension, 5 mg/ml. It was given in a dose of 0.7 mg/kg orally for 3 successive days starting from the 3rd day post infection (Manzur et al. 2008).

Plant material and preparation of extracts

Briefly, 300 g of fresh ginger rhizome and 250 g of dried *Cinnamomum zeylanicum* bark were cut into small pieces, macerated into 90% ethyl alcohol and left for 10 days. Alcohol was replaced by fresh one every 3 days. The extract was filtrated and ethanol was removed via rotary evaporator at 50^{o} C under reduced pressure to obtain viscous residues of crude plant extracts. These residues were then subjected to lyophilization to afford 40 gm of powdered extract of the plant. The suspension of the lyophilized extract was prepared for oral administration using 0.5% Tween-80 (ADWIC, Egypt) in normal saline. The concentration was adjusted that each 0.1 ml of the prepared suspension contains 0.3 mg of the plant extract (Mahmoud et al. 2014). Extracts were orally administered at a dose of 100 mg/kg (Hsiang et al. 2015) and 50 µg/g body weight (Kwon et al. 2011) once daily for *Zingiber officinale* and *Cinnamomum zeylanicum* respectively starting from the 1st day of infection till the day of sacrifice.

Parasitological study

Isolation and counting of adult worms

Mice were sacrificed and the small intestine was taken off. After the intestine was washed with physiological saline, it was split longitudinally along its entire length and divided into 2-cm portions, then placed in a beaker full of physiological saline for 3-4 hrs at 37°C. The intestine was shaken well in the saline, rinsed in a similar amount of saline and finally removed and discarded. Adults were allowed to sediment by standing in the container for half an hour. The supernatant was removed; the sediment was reconstituted in 3–5 drops of physiological saline and poured into a petri-dish. The number of adults was counted under the dissecting microscope at X 20 power (Shin et al. 2008).

Total larval burden in muscles

Mice were sacrificed on the 35th day p.i. Muscle larvae counts in whole carcasses were determined according to the method described by Martínez-Gómez et al (2009). Briefly, each mouse was skinned and eviscerated. Teeth, tail and ears were removed and the rest of the animal was cut into small pieces by scissors and digested in 1% pepsin-hydrochloride in 200 ml distilled water. Following incubation of the

mixture at 37°C for 2 hrs with continuous stirring by an electromagnetic stirrer, encysted larvae were collected via the sedimentation technique and washed several times in distilled water. The number of larvae was counted microscopically using a McMaster counting chamber.

Histopathological study

The formol-saline preserved intestinal and muscle tissue specimens were processed in an automated tissue processor in two steps; fixation and dehydration. Initially, tissue samples were immersed in 10% buffered formalin for 48 hrs, after that the fixative was removed in distilled water for 30 minutes. Dehydration process was then carried out by running the tissues through a graded series of alcohol (70%, 90 %and 100%). Tissue specimens were then cleared in several changes of xylene, then the samples were impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 µm) were stained with hematoxylin and eosin (Suvarna et al. 2013).

Immunohistochemistry

The standard immunohistochemical methods were adopted with Bancroft and Gamble (2008). Briefly, sections were mounted on positively charged glass slides (Biogenex, USA), and deparaffinized in xylene. After xylene was removed by absolute ethanol, slides were placed in an unsealed plastic container filled with sufficient antigen retrieval solution (Citrate buffer solution, pH 6) and microwaved for 5 minutes at power 10. The container was removed and allowed to cool for 15 minutes and the slides were washed in deionized water for several times then placed in phosphate buffer saline (PBS) for 5 minutes. Tissue sections were incubated with an endogenous peroxidase blocking reagent containing hydrogen peroxide and sodium azide (DAKO peroxidase blocking reagent, Cat. No.S 2001). Excess buffer was blotted off, and the slides were allowed to dry except for the tissue section. The supersensitive primary monoclonal antibody against transforming growth factor-beta 1 (TGF-β1) was added to the sections and incubated for 60 min at room temperature then, the slides were rinsed in PBS, incubated with biotin-streptavidin (BSA) system. After that, 1-2 drops of the ready-to-use DAKO EnVision + system were applied for 20 minutes at room temperature and rinsed again with PBS. Diaminobenzidine (DAB) was used as a chromogen. The slides were mixed for 10-20 min until a desirable brown color was obtained then counterstained with Mayer's hematoxylin.

Quantitative scoring method

TGF-β1 immuno-activity quantitation was made by digital image analysis through the image J analysis software on five fields from each slide. This software could measure the total positive stained brown color/areas throughout the unstained cells. From this data an index (positively stained cells per a total of 1000 cells) can be computed.

Determination of serum TNF-α

The concentrations of serum TNF-α were quantitatively determined in all groups, on days 7 and 35 p.i. using Quantikine®ELISA kits, Cat. No. MTA00B (R&D systems, USA). All ELISA techniques were performed according to the manufacturer's instructions. The absorbance of the serum samples was read within 10 min at a wave length of 450 nm using an ELISA micro-titer plate reader.

Transmission electron microscopy

Adults

The collected adults were centrifuged at 7000 rpm for 1 min, and then the parasites were resuspended in fresh modified Karunovsky's fixative and fixed at 4°C for 3 days. Fixative was then removed and the parasites were post-fixed for 1 hour in 2% osmium tetroxide. Adults were then dehydrated in ethanol followed by acetone series solutions of increasing concentration (Karunovsky 1965). The material was embedded in the epoxy resin Epon 812 according to Luft's standard method (Luft 1961). After hardening, blocks were removed and sections were cut using an ultramicrotome. Ultrathin sections were examined using a JEM 2100 transmission electron microscope (TEM).

Muscle samples

1 mm^3 diaphragm muscle samples were cut then fixed within 5 minutes. Muscle samples were then prepared by the same steps as adults.

Ethical consideration

The study protocol was approved by the ethical committee of the Institutional Review Board (IRB) Unit, Faculty of Medicine, Zagazig University (approval number: 4002).

Statistical analysis

Quantitative values of the measured parameters were expressed as mean ± standard deviation (SD). Data were analyzed by one way-ANOVA to determine significance of differences between studied groups using Statistical Package for Social Sciences (SPSS), version 18.0. All statistical tests were considered significant at $p < 0.05$ and highly significant at $p < 0.01$.

Results

Parasitological assessments

A significant reduction in the mean number of *T. spiralis* adults was observed among the treated groups compared with the infected untreated group. Mice treated with albendazole & prednisolone showed the highest reduction followed by *Zingiber officinale* and *Cinnamomum zeylanicum* treated groups (93.5%, 64.5%, and 50.8%). Regarding larvae count, there was a significant decrease in mean number of total larvae in muscles in treated groups compared with the positive control group. The highest reduction was observed in albendazole & prednisolone treated group (90.6%), followed by *Zingiber officinale* and *Cinnamomum zeylanicum* with reduction percentages of 68% and 54.6% respectively (Table 1).

Table 1
Mean *T. spiralis* adult count in the small intestine and larvae count in muscles

	Adult worms count/mouse Muscle larvae count/mouse					
	(n = 6) (n = 6)					
	Mean ± SD	**Range**	**R %**	**Mean ± SD**	**Range**	**R %**
Positive control	41.33 ± 2.08	39–43		4232 ± 632.1	3515–4710	
Albendazole & prednisolone	2.66 ± 1.52	1–4	93.5%	397.3 ± 45.94	347–437	90.6%
Zingiber officinale extract	14.67 ± 2.5	12–17	64.5%	1353 ± 310.1	1000–1580	68%
Cinnamomum zeylanicum extract	20.33 ± 1.5	19–21	50.8%	1920 ± 81.85	1830–1990	54.6%
F-Test	218.8			63.19		
p-value	<0.001**			<0.001**		

n = number of studied mice in each group, SD = standard deviation, F = ANOVA test, *p* value = probability, **Highly significant difference.

Histopathological findings

Small intestine

Dense inflammatory cellular infiltrate was observed in infected non-treated group predominantly in the core of the villi and extending into submucosa with flattening of villi and sloughing of the villous tip. Cut sections in *T. spiralis* adults could also be noticed. On the other hand, a significant reduction in the intensity of the inflammation and prolonged villi was observed in *Zingiber officinale* group. *Cinnamomum zeylanicum* treated group showed moderate inflammatory cells infiltration with improvement of

architecture and length of the villi compared with the infected non-treated group. Mice treated with albendazole and prednisolone exhibited mild to moderate inflammation in the core of villi (Fig. 1).

Skeletal muscles

Histopathological examination of skeletal muscle of the infected control group showed encysted *T. spiralis* larvae within a nurse cell, collagenous capsule and dense inflammatory cellular infiltrations. An alteration in archticture of the muscle fibres was clearly detected. The marked reduction in the number of deposited larvae with a significant decrease of inflammatory cellular infiltrate around the larvae was evident in *Zingiber officinale* group. *Cinnamomum zeylanicum* treated group showed moderate inflammation while albendazole and prednisolone treated group displayed mild to moderate inflammatory cellular infiltrate around the deposited larvae (Fig. 2).

Immunohistochemical assessment

Investigated immune-stained sections from different segments of the small intestinal tract of the studied groups revealed a weak cytoplasmic reactivity for the used marker (TGF-β1) in negative control group; positive cells showed light brown-colored cytoplasmic contents. A strong cytoplasmic reactivity for the used marker was observed in infected control group and albendazole and prednisolone treated group, while there was moderate cytoplasmic reactivity for the used marker in *Zingiber officinale* and *Cinnamomum zeylanicum* treated groups. Positive cells were encountered in the mucosal and submucosal stromal cells, vascular and microvascular endothelial cells (Fig. 3). Imaging analysis technique estimated the positive cells to be (25.56%, 73.5%, 76.1%, 69.3% & 67.8%) of the total cellular contents of the studied groups respectively (Table 2).

Table 2
TGF-β1 positive cells (%)

Groups	TGF-β1 positive cells (%)
Negative control	25.56 %
Positive control	73.5 %
Albendazole & prednisolone	76.1 %
Zingiber officinale extract	69.3 %
Cinnamomum zeylanicum extract	67.8 %

Serum level of TNF-α

A significant reduction in serum TNF-α on the 7th and 35th days p.i. was obseved in targeted groups treated with the herbal extracts. During intestinal phase, *Zingiber officinale* and *Cinnamomum zeylanicum* treated groups displayed reduction percentages of 50% and 33.5% respectively while

albendazole & prednisolone treated group exhibited a percentage of (51.5%). *Zingiber officinale* extract showed the highest reduction during muscle phase (58.4%) followed by albendazole & prednisolone (51.9%) and *Cinnamomum zeylanicum* extract (41%) (Table 3).

Table 3
TNF-α level in serum

Groups	TNF-α (pg/ml)					
	7th d.p.i. (n = 6)			35th d.p.i. (n = 6)		
	Mean ± SD	Range	R %	Mean ± SD	Range	R %
Negative control	230.7 ± 4.041	227–235		232.3 ± 11.59	220–243	
Positive control	937.7 ± 24.79	911–960		949.3 ± 21.13	927–969	
Albendazole & prednisolone	454.3 ± 32.04	428–490	51.5%	451.7 ± 34.03	425–490	51.9%
Zingiber officinale extract	466.0 ± 7.937	460–475	50%	390.3 ± 25.54	370–419	58.4%
Cinnamomum zeylanicum extract	622.7 ± 20.74	604–619	33.5%	551.7 ± 26.31	528–580	41%
F-Test	476.9			334		
p-value	<0.001**			<0.001**		

n = number of studied mice in each group, SD = standard deviation, F = ANOVA test, *p* value = probability, **Highly significant difference.

Transmission electron microscopy

Regarding the ultrastructural examination of *T. spiralis* adults, multiple cuticular depressions and disturbed continuity were evident in *Zingiber officinale* group. *Cinnamomum zeylanicum* group showed loss of epicuticular waviness and lack of discrimination of cuticle layers (Fig.4). EM examination of the diaphragm in *Zingiber officinale* group showed a marked reduction in the matrix and inflammatory zone and closer appearance of normal muscle with regular light and dark bands. Degenerative changes in the larvae and separation of superficial layers of the cuticle in a wide area were also detected. The *Cinnamomum zeylanicum* group also showed a marked reduction in the inflammatory zone together with separation and blebbing of superficial cuticular layers (Fig. 5).

Discussion

Trichinellosis is a worldwide severe zoonotic disease that involves the activation of inflammatory cells. It is also accompanied by prominent expression of proinflammatory cytokines in the affected host (Xu et al. 2019). Many experts are calling for the use of natural remedies, since most synthetic products have harmful effects and some of them have been found to be cancerous. Thus, an alternate, healthy, and effective herbal remedy is required to treat both enteral and parenteral phases of *T. spiralis* (Shalaby et al. 2010). For our knowledge, no study has been conducted assessing the effect of *Zingiber officinale* and *Cinnamomum zeylanicum* on *Trichinella spiralis* infection.

Concerning the anti-parasitic effect, the existing finding revealed a significant reduction in the adult worms and larvae counts in *Zingiber officinale* (64.5%, 68%) and *Cinnamomum zeylanicum* (50.8%, 54.6%) groups compared with the infected nontreated group. Albendazole and prednisolone treated group exhibited a lower efficacy on *T. spiralis* larvae than that on adults (90.6% vs 93.5%) due to the low bioavailability and water solubility after oral administration **(Caner et al. 2008).** Our findings are in accordance with Attia et al. (2015) and Shalaby et al. (2010), who recorded reduction in adult count, nevertheless, the larval reduction rate was 26.4%, also, Shoheib et al. (2006) recorded decreased effectiveness of albendazole against the encysted larvae with a reduction rate of 65.2%. Interestingly, *Zingiber officinale* and *Cinnamomum zeylanicum* extracts displayed a higher parasiticidal effect against larvae compared with their effect on adults. Therefore, the use of the same concentration with increasing the duration of treatment may improve the outcome of treatment and increase the parasite mortality.

The parasiticidal effect of *Zingiber officinale* may be due to its anticholinergic effect (Qian and Liu 1992), since, acetylcholine is the neurotransmitter in nematode muscles (Neal 2012). Furthermore, its potent proteolytic enzyme “zingibain” could be responsible for its antiparasitic effect (Khalil and El-houseny 2013). Similarly, **El-Sayed (2017)**, stated antiparasitic effect of *Zingiber officinale* on *Toxocara canis*, and, Merawin et al. (2010) reported that *Zingiber officinale* rhizome extract was effective against *Dirofilaria immitis.*The antiparasitic effect of *Cinnamomum zeylanicum* could be elucidated by its abundant content of trans-cinnamaldehyde and proanthocyanidin tannins (Williams et al. 2015). In agreement with our finding was that of Trabelsi et al. (2019) who studied the anti-parasitic effect of cinnamon extract on *Anisakis* and reported its lethal effect on *Anisakis* larvae type 1 both in vivo and in vitro.

Regarding the histopathological studies of the small intestine, a significant decrease in the intensity of inflammation and reconstructed villi architecture were evident in *Zingiber officinale* group compared with the positive control group in which, a dense inflammatory cellular infiltrate was evident mainly in the core of the villi and lamina propria with shortening of the villi and flattening of their tips and with *Cinnamomum zeylanicum* treated group that showed moderate inflammation in the core of villi. Concerning histopathological examination of skeletal muscles, *Zingiber officinale* group displayed an obvious reduction in larvae deposition with significant reduction of inflammatory cellular infiltrate around them. However, in *Cinnamomum zeylanicum* group there was a reasonable decrease in muscle larvae count with moderate inflammation around the larvae compared to the positive control.

TGF-β1 is a cytokine that plays a role in pro-inflammatory as well as in anti-inflammatory responses. The concomitant presence of TGF-β1 and IL6 favors a pro-inflammatory environment mediated by Th17 (Grainger et al. 2010). Th17 cells play a critical role in defense against some extracellular pathogens and are involved in numerous inflammatory and autoimmune diseases (Bedoya et al. 2013; Jafarzadeh et al. 2018). Production of TGF-β1 by antigen presenting cells leads to the differentiation of induced regulatory T cells (iTregs) **(Chen et al. 2010)** which inhibit the protective T cell responses of the host and contributes to disease progression. This fact highlights the importance of this cytokine during parasitic infections (Bhattacharya et al. 2014).

Our study revealed moderate cytoplasmic reactivity for TGF-β1 in *Zingiber officinale* and *Cinnamomum zeylanicum* treated groups compared to a weak cytoplasmic reactivity in negative control group and strong cytoplasmic reactivity in infected control group and albendazole & prednisolone treated group. In agreement with our results was that of Gungor et al (2020) who studied the effect of zingerone on induced lung fibrosis and reported reduction of TGF-β1 in lung tissue. Abdi et al. (2020) found that *Zingiber officinale* downregulated expression of TGF-β1 genes in comparison to diabetic group. Also, Zheng et al. (2011) stated that cinnamaldehyde prevents diabetic nephritis by inhibition of oxidative damage and downregulation of TGF-β1.

As regard the biochemical studies, *Zingiber officinale* treated group displayed a significant reduction in the level of TNF-α on the 7th & 35th d.p.i. (50%, 58.4%) while *Cinnamomum zeylanicum* (33.5%, 41%), compared to albendazole & prednisolone treated group (51.5%, 51.9%).“Zingerone”, one of the components of *Zingiber officinale* could be responsible for the reduction of TNF-α level. Our findings are supported with that of Ueda et al. (2010) who investigated the effect of squeezed ginger extract on many cytokines including TNF-α and reported a significant reduction in its level in peritoneal cells. Rehman et al, (2019), reported that zingerone can inhibit mRNA expression of TNF-α, consequently interfered with cell signaling pathway and suppress TNF-α expression. Furthermore, Haniadka et al. (2013) attributed the anti-inflammatory effect of *Zingiber officinale* to its ability to inhibit COX-1 and COX-2 with subsequent prostaglandins synthesis suppression.

In *Cinnamomum zeylanicum* treated group, we supposed that the significant decrease in TNF-α level could be linked to “cinnamaldehyde”, the principal constituent of cinnamon as reported by Liao et al. (2012). Rathi et al. (2013), added that, the antioxidant components of cinnamon can inhibit Nuclear Factor kappa activation with the subsequent inhibition of pro-inflammatory cytokines including TNF-α. The administration of glucocorticoids for treatment of immediate hypersensitivity associated with trichinosis can inhibit cellular immune response (mainly lymphocytes) and reduce the cytokines production, including TNF-α as reported by Kisiel & Kaszuba (2011). This clarifies the reduction of TNF-α in albendazole & prednisolone treated group.

Concerning the electron microscopy examination of both adult worms and muscle larvae samples, remarkable degenerative changes were observed in tested herbs groups compared with the control group. An obvious blunting of epicuticle, with a marked reduction in the inflammatory zone, and separation of

superficial layers of the cuticle of the larvae were detected. These changes were more evident in *Zingiber officinale* treated group. The *Cinnamomum zeylanicum* group also showed blebbing of superficial cuticular layers. We suppose that *Zingiber officinale* potent proteolytic enzyme “Zingibain” is responsible for the resulted cuticular damage as reported by Khalil and El-houseny (2013**).** The Integrity of nematode cuticle is crucial for nutrition and defensive function as well as to maintain shape. Cuticular damage can extremely affect *T. spiralis* adults and larvae since it is considered as a safeguard against physical and immunological harm, and plays a role in osmoregulation **(**Roberts et al. 2013).

In conclusion, *Zingiber officinale* followed by *Cinnamomum zeylanicum* ethanolic extracts have a therapeutic and anti-inflammatory effects on *T. spiralis* infection. These extracts may be promising alternatives in treatment of trichinellosis. We are looking forward to assess the efficacy of the active ingredients of these remedies with evaluation of their effects on the genetic diversity after treatment.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

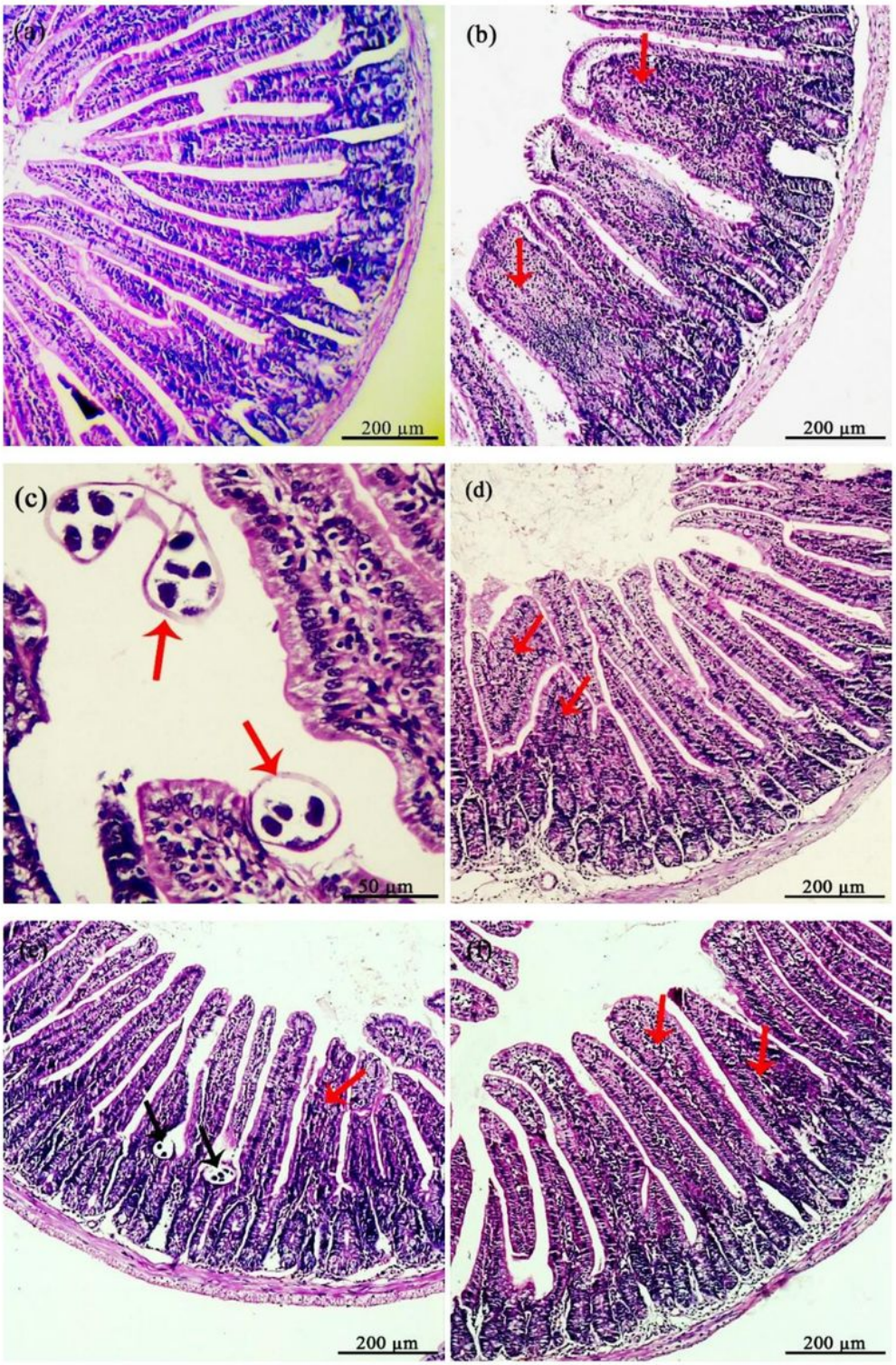

Figure 1

Sections of small intestine of studied groups on the 7th d.p.i. a) normal control group showing intestinal villi with normal architecture and length (X200), b) obvious inflammatory cellular infiltration (arrows) in submucosa & the core of the villi of infected non-treated group (X200), c) cut section in T. spiralis adults seen both intramucosal and in the lumen (arrows) (X400), d) albendazole and prednisolone treated group, with decrease in inflammatory infiltration in the core of villi (arrows) (X200). e) Zingiber officinale treated

mice with evident reduction in the intensity of the inflammation (red arrow), but cut sections in Trichinella spiralis adults were observed (black arrows) (X200). f) Cinnamomum zeylanicum treated mice with moderate inflammatory cell infiltrates (red arrows) (X200).

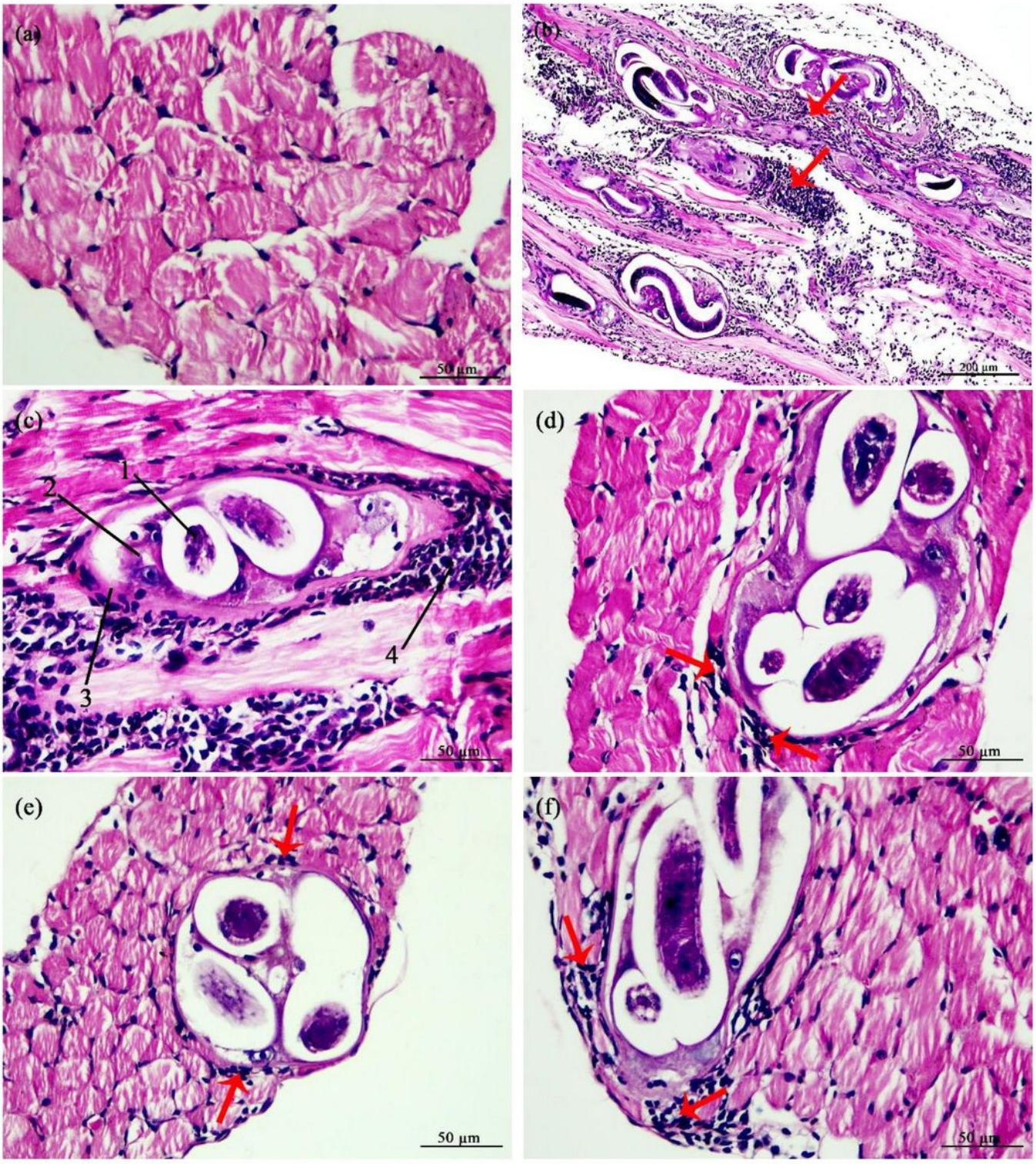

Figure 2

Sections of diaphragm of studied groups on the 35th d.p.i. a) normal control group with normal diaphragm muscle fibres (X400), b) diffuse degenerative changes all over the muscle bundles with

multiple encysted T. spiralis larvae in infected non-treated group (X200), c) higher magnification showing 1) T. spiralis larva 2) Nurse cell 3) Collagen capsule 4) Marked inflammatory infiltrate (X400), d) mice treated with albendazole and prednisolone showing mild to moderate inflammatory infiltrate (arrows) around T. spiralis larvae (X400), e) Zingiber officinale group showing mild inflammatory infiltrate (arrows) around the larva (X400), f) Cinnamomum zeylanicum group showing larva surrounded by moderate inflammatory infiltrate (arrow) (X400).

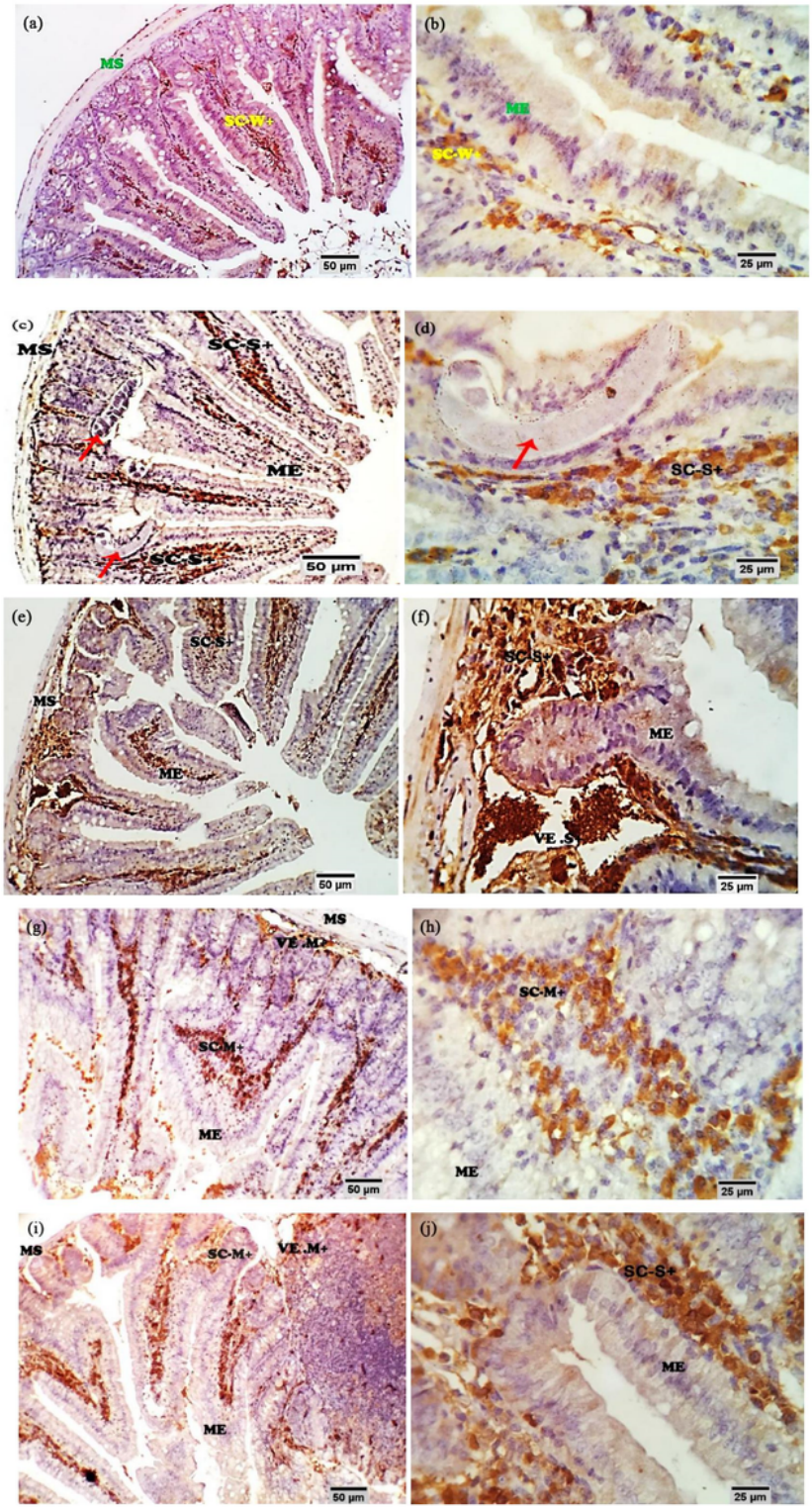

Figure 3

Immunostained sections of small intestine of studied groups on the 7th d.p.i. a, b) negative control group shows very weak cytoplasmic reactivity for the used TGF-β1 marker (W+), Positive cells show light brown-colored cytoplasmic contents seen in the mucosal and submucosal stromal cells (SC); c, d) positive control group and e, f) albendazole & prednisolone treated group show a strong cytoplasmic reactivity for the used marker (S+). Positive stromal cells show dark brown-colored cytoplasmic contents (SC-S+), red arrows point to cut sections in T. spiralis adults (g, h) Zingiber officinale treated mice and i, j) Cinnamomum zeylanicum treated mice, immune-staining showing a moderate cytoplasmic reactivity for the used marker (M+). Positive cells show dark brown-colored cytoplasmic contents (MS=musculosa, ME= mucosal epithelium, SC=stromal cells, VE= vascular endothelial cells) (a, c, e, g and i, X200 & b, d, f, h and j, the same photomicrograph with higher magnification X400).

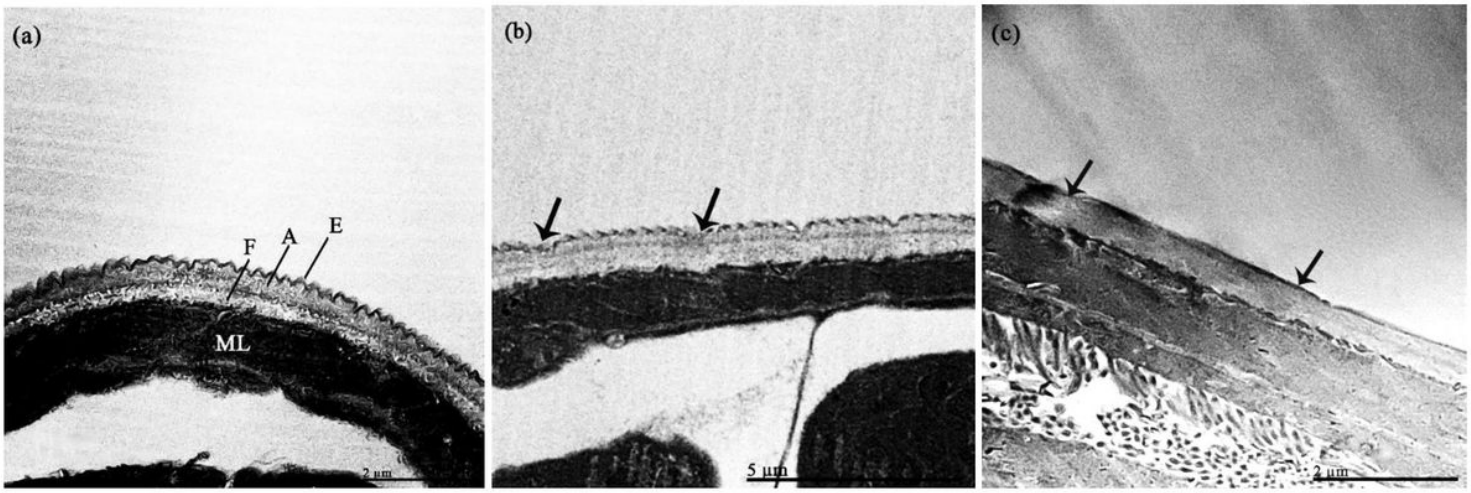

Figure 4

Transmission electron micrograph of Trichinella spiralis adult on the 7th d.p.i. (a) the infected non-treated group, showing the characteristic structure of adult cuticle; formed of two layers: the amorphous layer [A] and the fibrillar layer [F]. The cuticle is covered by the wavy epicuticle [E]. [ML] refers to the somatic muscles of the body wall (X 2000) (bar 2 µm). (b) Trichinella spiralis adult in mice treated with Zingiber officinale extract showing part of the cuticle with depressed zones and blunting of epicuticle (arrows) (X 1200) (bar 5 µm) (c) Trichinella spiralis adult in Cinnamomum zeylanicum treated mice, showing complete blunting of epicuticle (arrows) and the layers of cuticle could no longer be discriminated (X 2000) (bar 2 µm).

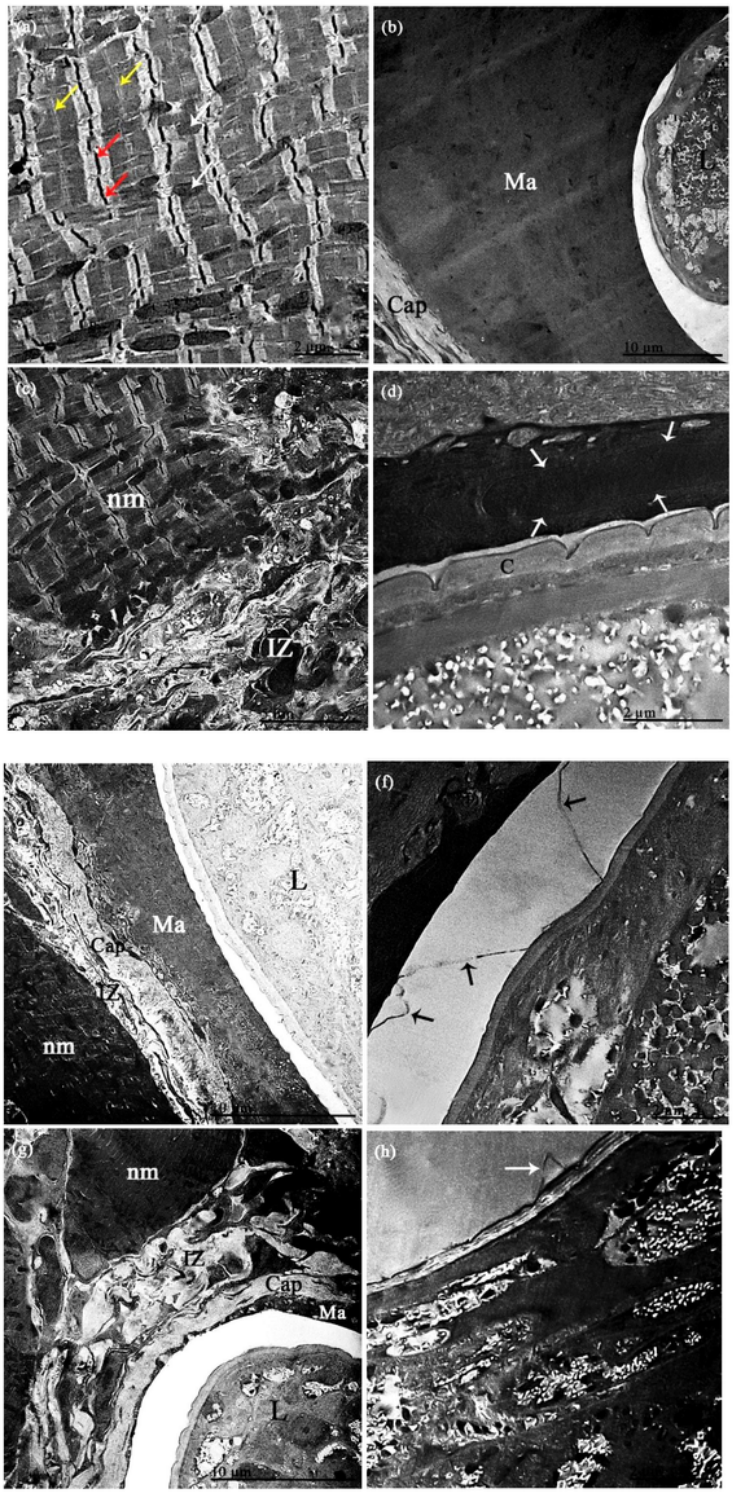

Figure 5

TEM of diaphragm on the 35th d.p.i. a) diaphragm of non-infected mouse showed the striated appearance of skeletal muscle fibres, Z line where the actin filaments are anchored (red arrows), M line, where the myosin filaments are anchored (yellow arrows) and white arrows point to mitochondria (X 1500) (bar 2 µm). b, c, d) diaphragm of infected control mice showing; b) the restructured muscle fibres (matrix) (Ma) surrounding the larva (L). A collagen capsule (Cap) is formed between the matrix and the

surrounding inflammatory zone. (X 400) (bar 10 µm) c) showing the inflammatory zone (IZ) outside the capsule (nm=normal muscle) (X 800) (bar 5 µm) d) showing larva cuticle (c) and the nurse cell nucleus (white arrows) (X 1500) (bar 2 µm). e, f) Zingiber officinale group; e)showing distinct decrease in matrix (Ma) and inflammatory zone (IZ) with closer appearance of normal muscle (nm) with regular light and dark bands (X 600) (bar 10 µm), f) showing separation of superficial layers of the cuticle in wide area (black arrows) (X 800) (bar 2 µm), g, h) Cinnamomum zeylanicum group; g) displaying the larva (L) with marked decrease in surrounding matrix (Ma) and inflammatory zone (IZ) and closer appearance of normal muscle (nm) (X 600) (bar 10 µm), h) obvious separation and blebbing of superficial layers of the cuticle (black arrow) (X 1200) (bar 2 µm).