Saudi Journal of Biological Sciences 30 (2023) 103763

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# Ameliorative synergistic therapeutic effect of gallic acid and albendazole against *Trichinella spiralis* muscular phase infection and assessment of their effects on hepatic and cardiac tissues in male mice

Bander Albogami

*Biology Department, College of Sciences, Taif University, Taif 21944, Saudi Arabia*

ARTICLE INFO

*Article history:*
Received 3 July 2023
Revised 20 July 2023
Accepted 29 July 2023
Available online 5 August 2023

*Keywords:*
*Trichinella*
Gallic acid
TEM
Antioxidant
Oxidative Stress

ABSTRACT

Trichinellosis is a serious food-borne parasitic disease with serious community health effects, mainly causing muscle damage with no recent approved treatment. This study aimed to assess the therapeutic effect of gallic acid (GA) as a potent antioxidant against the encysted phase of *Trichinella spiralis* in male (BALB/c) mice alone or combined with albendazole (ALB) and to detect their synergistic effects on the histology and ultrastructure of skeletal and cardiac muscles and some biochemical blood analyses. Forty male mice were randomly divided into five groups (8 mice/group). 1st group: the negative control received only distilled water, 2nd group: the positive control (infected control group without treatment), 3rd group: infected group plus treatment with ALB (50 mg $Kg^{-1}$ orally), and 4th group: infected group and then treated with GA (30 mg $Kg^{-1}$ orally) and finally 5th infected group treated with a combination of both ALB and GA. Aspartate and Alanine aminotransferase, Lactate dehydrogenase, alkaline phosphatase, C-reactive protein, Interleukin-4 and Creatine kinase were used as biochemical markers of hepatic and cardiac toxicity and inflammation. Malondialdehyde level, catalase, superoxide dismutase, and glutathione peroxidase were evaluated in heart tissue homogenates beside histological and ultrastructural examination of heart and skeletal muscles beside parasitological analyses. Results showed that the reduction % of *Trichinella* sp. larvae $g^{-1}$ in muscles of the group treated with the combination of GA and ALB showed overall reduction percentages. Oral administration of 30 mg $kg^{1}$ of GA led to infection reduction of *T. spiralis* than ALB treated group. Both administration of ALB beside GA showed the best treatment group that resulted in high infection reduction besides amelioration of both biochemical markers and restoration of histological and ultrastructures to normal state. In conclusion, GA is highly effective against *T. spiralis* which could be a promising alternative antioxidant drug and the GA effect was higher in the case of combination with ALB. This experiment provides a basis for further exploration of potent activities of other antioxidants against different phases of *T. spiralis* and the reduction of any health hazards prospectively.



## 1. Introduction

Trichinellosis, an infection caused by *Trichinella spiralis*, is a parasitic disease globally and is mainly food-borne. *Trichinella* sp. causes general health hazards in the community with many concerns related to food safety. Huge cases of human infection occur annually by eating undercooked raw pork meat containing the infective larval phase *of Trichinella* (Walter et al., 2022; Daoxiu et al., 2021).

The infection of humans with *T. spiralis* strain combines 3 main phases including the muscular phase. The parasite's infection causes mainly inflammation in the muscles of hosts. These larvae are considered key and essential causes mortality (Bruschi, 2014).

The European Center for Disease Control has categorized human trichinellosis into clinical and epidemiological cases. The clinical case should have three symptoms:fever, muscle fatigue, gastrointestinal symptoms and even retinal hemorrhages (Sumeeta et al.,2021). Currently, no effective therapeutic is available for trichinellosis, Based on (Abo Maged et al.,2023) encouraged step

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

*E-mail address:* b.boqami@tu.edu.sa

https://doi.org/10.1016/j.sjbs.2023.103763
1319-562X/

for anti-trichinellosis medication with synergistic impact by modulating inflammation and larval capsule formation.

The main aim of treatment of trichinellosis is to decline muscular damage. Albendazole (ALB) is one of the essential anthelminthic drugs but the main defect in this drug is its low water solubility which limits absorption. So, effective antioxidants and natural drugs are essentially required (Nada et al., 2018). Several studies have reported the effective use of different therapeutics for various muscle damage (Seow et al., 2021).

To keep the therapeutic efficacy of anti-parasitic drugs and then lower the resistance against these strains, several therapeutic and alternative active compounds strategies were used, including the use of alternative active compounds from different chemical groups, and the use of drug combinations (Eissa et al.,2022).

Several studies supported the use of many antioxidants to treat muscle injuries. Gallic acid (GA) is present naturally as the acid in tea, red fruits and onions (El-Megharbel and Hamza, 2022). GA is a multi-functional acid owing to its high scavenging with high metal chelating capacities (Badhani et al., 2015).

Many studies revealed that GA has several applied applications. GA inhibits the growth of many cancer cells. GA also has anti-diabetic effects (Lan et al., 2007). Additionally, GA has either anti-fungal or anti-viral activities (Jayamani and Shanmugam, 2014). Moreover, GA has been used as potent antioxidant formula (Jittawan and Siriamornpun, 2008).

GA is a potent antioxidant scavenging free radicals; protecting the biological cells from high oxidative injuries (Gao et al., 2019). Excessive production of free radicals can facilitate the incidence of the process of lipid peroxidation and cause damage to the cellular membranes and thus, induce an imbalance between the production of the reactive oxygen species and their elimination; this may cause oxidative damage that leads to incidence of different and dangerous diseases (El-Megharbel and Hamza, 2022). Recently, humanities need more potent antioxidants for alleviation of severe stress and competing against severe diseases.

To my knowledge, the effect of GA either alone or in combination with ALB on infection by *Trichinella* sp., especially treatment of the muscle phases, has not yet been examined. Many prospective studies are needed to evaluate the therapeutic different effects of GA doses as alternative therapies. Thus, the current aim of this experimental study was to evaluate the efficacy of GA either alone or combined with ALB in *T. spiralis*-infected mice.

## 2. Material and methods

### 2.1. Determination of the sample's size

The current sample size was accurately calculated by using the G-Power program, which justified the use of seven to eight mice in each group. The 40 male mice were spitted into 5 groups. Group I: healthy control non-treated group (negative control) mice; Group II: infected non-treated; Group III, IV, and V: Infected groups, treated with ALB, GA, and a combination of ALB and GA respectively. (Scheme 1). Each mouse in Groups III to V was orally inoculated with 200 ~ 300 *T. spiralis* larvae (Siriyasatien and Yingyourd, 2003).

### 2.2. Experimental animals and models

This experimental study lasted from June 2021 to May 2022. The experimental study was performed on 40 male, BALB/c mice, which are pathogen and parasite-free, weighing 25 to 30 g and 7–8 weeks age. Mice were obtained from Theodore Bilharz Research Institute, Giza, Egypt upon ethical approval of the research institute and ethical approval committe for animal care ZU-IACUC and were kept under suitable light and temperature with standard food and water *ad libitum*. For evaluation of the muscular infection, 32 mice were orally infected with *T. spiralis* larvae 200 ~ 300 / mice; 8 male mice were kept as non-infected healthy control.

### 2.3. Parasites and induction of infection

*T. spiralis* strains were extracted from infected meat samples with proven infections (Gamble,1996). Heavily infected meat samples were completely digested in ~ 1% pepsin for 24 hr. at 37 °C. Free larvae were collected and washed several times by sedimentation and then normal physiological saline 0.9%, respectively. Live larvae were counted $ml^{-1}$. (Denham, 1965) and infected mice were orally infected with 200 ~ 300 coiled, active, and motile larvae (Siriyasatien and Yingyourd, 2003).

### 2.4. Study design

40 (8 mice/each group) male albino mice were spitted into 5 treated groups, simulating muscular phase. G1: control (-ve control group), received distilled water (1 ml/Kg), G2: infected control group (infected without treatment), G3: infected group and then treated with ALB (50 mg/Kg), G4: infected group and then treated with GA (30 mg/Kg) and finally, G5: infected group and then treated with a combination of both ALB and GA. For parasitological, histopathological, and biochemical assessments, heart, diaphragm, and thigh specimens were collected from mice sacrificed on the 31th-day post-infection (Scheme:1).

### 2.5. Euthanasia

All treated mice were euthanized using intraperitoneal (I.P) anesthesia. Mice received light anti-coagulant anesthesia based on animal care international ethics (HIN) (500 mg $kg^{-1}$ thiopental I.P) (Liang et al.,1987). Samples of the heart, skeletal, and tongue muscles were all removed and all samples were histopathologically examined (Shalaby et al., 2010). Samples of the examined muscles were used for recording and counting the mean number of T. *spiralis* larvae (Carrillo et al.,2018).

### 2.6. Evaluation of drug effects

#### 2.6.1. Lipid biomarkers and hepatic functions

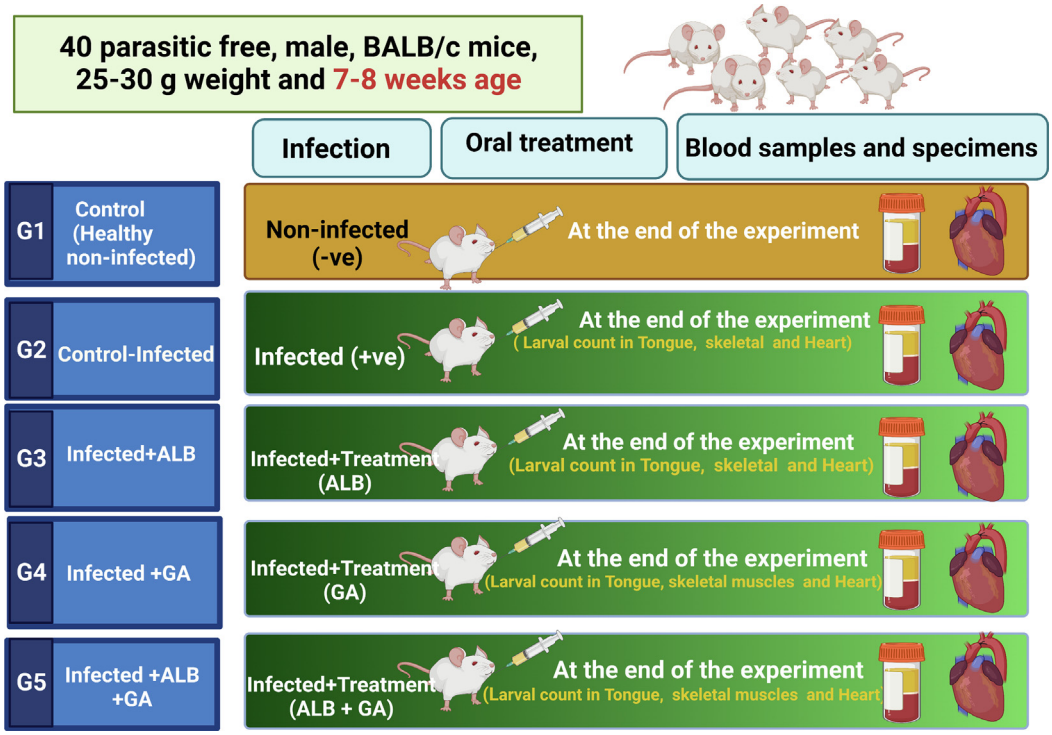
Alanine and aspartate aminotransferase (AST and ALT) were evaluated based on kits (Spinreact, Girona, Spain), while lactate dehydrogenase (LDH) serum level was measured using LDH reagent/assay kit (GmbH Schigraben, Hannover, Germany), alkaline phosphatase (ALP) levels were measured using commercial kits.

#### 2.6.2. Heart antioxidant enzymatic biomarkers

Catalase (CAT) activity (Aebi, 1984), superoxide dismutase (SOD) (Sun et al., 1988), malondialdehyde levels (MDA) (Ohkawa et al., 1979), and glutathione peroxidase (GPx) (Paglia et al.,1967) were evaluated.

#### 2.6.3. Counting the larvae in muscles

The excised muscles from all mice after the end of treatment, were precisely weighed and then digested by acidic pepsin by addition of hydrochloric acid to emit larvae, they were basically counted at magnification × 40 via using a stereomicroscope. The larval count was then calculated as the mean number of larvae $g^{-1}$ of the digested muscles and was compared to define the reduction % in larval count in different treated groups as previously explained by García et al.,2013.




**Scheme 1.** Experimental design.

*2.6.4. Histopathological examination*

Excised heart tissues, skeletal (thigh) and tongue muscles, from each mouse in all groups, were all fixed by neutral buffered formalin (10 %) and defined based on standard methods of histology examination. Obtained Sections were then deeply stained with (H&E) staining (Rtail et al., 2020). Inflammatory markers were divided into 4 grades:

(− − − −) Non-inflammation, (+---) minimal (1%:14% elevation in inflammation), (++--) mild (15%: 35% elevation in inflammation), (+++-) moderate (36%: 70% elevation in inflammation), and (++++) severe (71%: 100% elevation in inflammation) as compared to the normal control group.

*2.7. Statistical analysis*

Data were analyzed by using the statistical analysis software package (SPSS) version 26 (IBM, USA). Data were expressed as "mean ± standard error (SE)". for categorical variables. ANOVA with post hoc test and Duncan tests were used. P values were considered as significant at $P < 0.05$ (Chan, 2003).

## 3. Results

*3.1. Parasitological assessment of different treated groups*

*3.1.1. Effect of GA and ALB as compared to the non-treated infected group*

Non-larvae were detected in the normal control non-infected group in the thoracicskeletal , heart and tongue muscles that were excised from the animals after the end of the experimental digestion of muscles. The mean number of larvae $g^{-1}$ muscles in both the non-infected group and infected treated groups was shown in Table.S4 and Fig.S1.

As shown in Table 1, the maximum reduction of larvae mean number in tongue, skeletal , and heart muscles were recorded in the infected treated group with a combination of both ALB and GA followed by the infected group treated with GA and this record proved the synergistic mechanism of action between both ALB and GA.

In the non-infected treated group, the number of larvae/g in diaphragm muscles was higher than in heart, tongue, and thigh muscles. The lowest number of larvae/g were recorded in the infected group treated with both ALB and GA in tongue muscles followed by increments in heart, thigh, and eventually diaphragm muscles.

*3.1.2. Histopathological assessment of therapeutic potential activity of both ALB and GA*

Examination of muscular tissues of the heart, skeletal and tongue muscles Figs. 1-3 shows the incidence of severe infection in infected non-treated groups, Meanwhile, the disappearing of these encysted larvae and replacement by eosinophilic exudates in case of treatment with either ALB and GA and the best therapeutic potential was observed in GA group and the best replacement of cellular infiltration, Meanwhile, complete disappearing of any histopathological signs in combined therapeutic treated group by ALB and GA.

Expression of inflammation in the Infected non-treated group showed a high percentage of larvae cysts of *T. spiralis* in the case of the tongue, skeletal , and heart muscles, Meanwhile, the lowest treated group showed mild cellular infiltration with thin cyst capsule, the infected group treated with a combination of both GA and ALB and showed better therapeutic results than each infected group treated with only ALB or GA each alone as table (S5).

*3.2. TEM assessment of therapeutic potential activity of both ALB and GA*

Examination of muscular tissues of the tongue, skeletal, and heart muscles showing the incidence of severe infection in infected non-treated groups. Meanwhile, the disappearance of these encysted larvae and replacement by eosinophilic exudates in case

**Table 1**
Changes in liver enzyme functions and marker of hepatic damage in control infected non-treated group and infected treated groups with either ALB, GA or their combination (mean ± S.E).

| Treated groups | | ALT(U/L) | AST(U/L) | ALP (U/L) | LDH (U/L) |
|---|---|---|---|---|---|
| Group (I) | (Control group) (-ve control) | 13.63 ± 0.38$^{e}$ | 13.52 ± 0.57$^{e}$ | 22.37 ± 0.79$^{d}$ | 108.72 ± 2.53$^{d}$ |
| Group (II) | (Infected treated group) (+ve control) | 173.02 ± 10.58$^{a}$ | 269.38 ± 4.19$^{a}$ | 195.54 ± 1.70$^{a}$ | 583.94 ± 33.06$^{a}$ |
| Group (III) | Infected group + ALB (50 mg/Kg) | 115.28 ± 3.51$^{b}$ | 114.36 ± 1.98$^{b}$ | 92.94 ± 3.00$^{b}$ | 339.48 ± 14.30$^{b}$ |
| Group (IV) | Infected group + GA (30 mg/Kg) | 103.69 ± 4.52$^{c}$ | 70.47 ± 4.24$^{c}$ | 46.81 ± 2.61$^{c}$ | 167.17 ± 13.14$^{c}$ |
| Group (V) | Infected group + ALP + GA | 51.64 ± 7.59$^{d}$ | 33.59 ± 3.32d | 21.39 ± 0.51 | 163.19 ± 10.56 |

Means carry a lot of different letters which are indicators of significant levels at ($P \leq 0.05$) using Duncan's multiple range test, the highest mean value has symbol (a), and decreasing were assigned alphabetically.

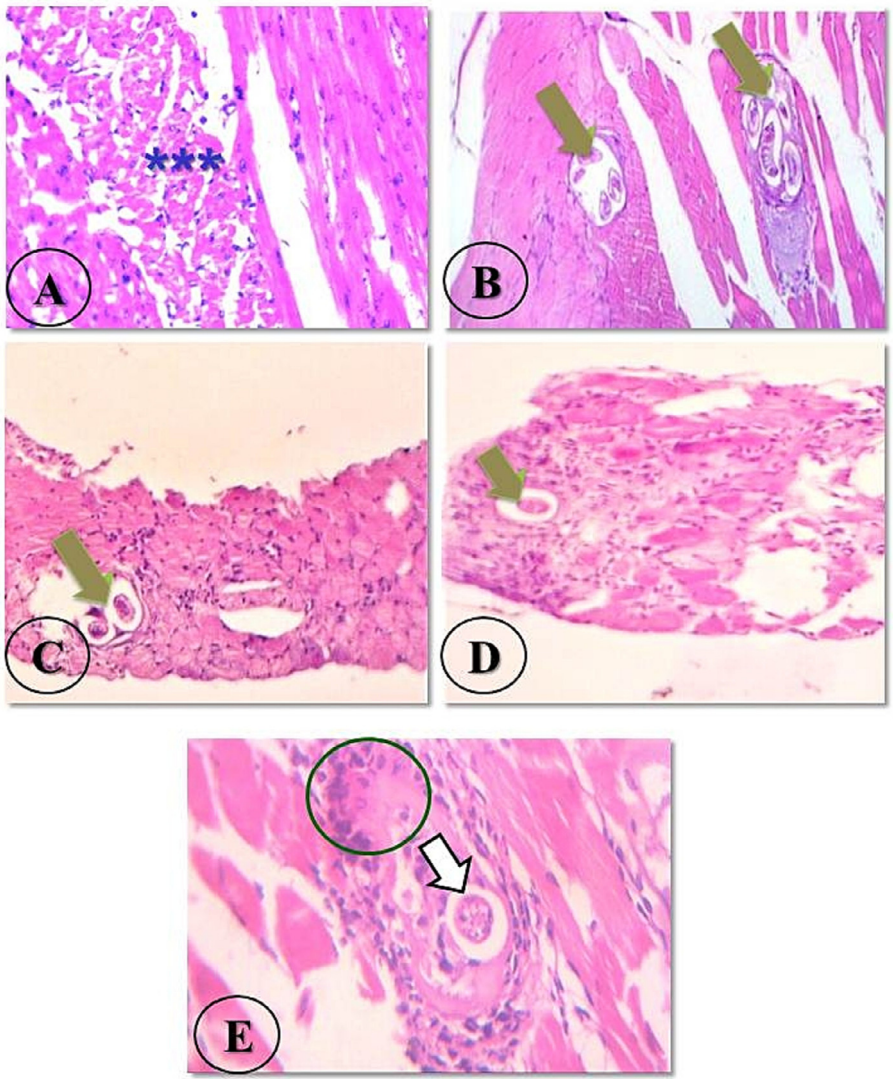



**Fig. 1.** Heart muscle sections from the healthy control group (-ve control) and infected non-treated and treated groups. (A) Control group (-ve control group): showing normal and regular heart muscle fibers with non-incidence of *T. spiralis* larval infection (H&EX400). (B) Infected non-treated: Heart muscles of the infected non-treated group showed the presence of encysted larvae in the sarcomere of the muscle of the heart surrounded by intense inflammatory cellular infiltrate (H&EX400). (C) Infected group treated group with ALB : showing homogenized vacuolation larvae, and then splitting of the tight capsule (Green arrow) and converted to the thin layers (Green arrow) with diffuse inflammatory infiltration that invading the capsule (H&EX400). (D) Infected group and treated with GA: showing *T. spiralis larvae* (Green arrow) accompanied by mild and fibrotic inflammatory infiltration (H&EX400). (E) Infected group was treated with the combination of ALB and GA showing the replacement of multiple *T. spiralis* larvae with eosinophilic exudates and great reduction of larval encyst with fibrotic cellular infiltration (White arrow) (H&EX400).

of treatment with either GA alone and in combination with ALB was better than ALB only treated group, and the best therapeutic potential was observed in the synergistic effect of both ALB and GA-group and showing the best replacement of cellular infiltration, Meanwhile, complete disappearing of any histopathological signs in combined therapeutic treated group by ALB and GA as shown in Figs. 4,5 and 6.

### *3.3. Liver functions in infected non-treated group and different infected treated groups*

Treatment of the infected group with either ALB or GA did not induce hepatic toxicity and this was represented by lowering the level of all liver functions and markers of hepatic safety as LDH and ALP. Results obtained as in Table 1 and Fig (S2) showed a high

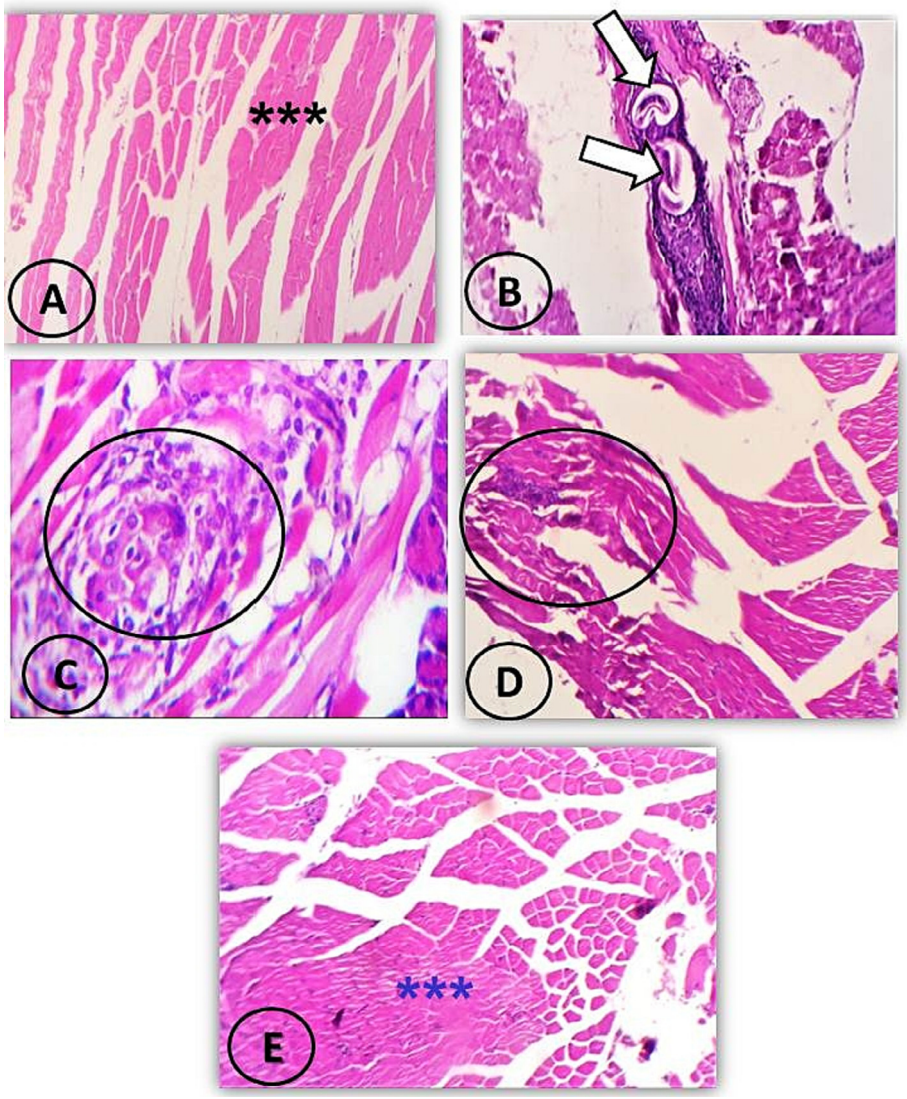



**Fig. 2.** Skeletal muscles from the non-infected control group (-ve control) and non-treated infected treated group. (A) Control group: healthy normal control group showing normal and regular muscular bundles (***) with normal nuclei and thin fibrous stroma (H&EX400). (B) Non-treated Infected group: this group revealed the presence of multiple-encysted larvae of *T. spiralis* that were tightly surrounded by intense inflammatory infiltrates (White arrows) (H&EX400). (C, D) Infected treated with ALB or GA: infiltrates with replacement of the larvae by eosinophilic exudates as in ALB group (Black circle), meanwhile even reduction of eosinophilic exudates in GA group (Black circle). (H&EX400). (E) Infected treated with the combination of ALB and GA , respectively: showing complete restoration of the normal status of skeletal muscles with no histopathological changes (***) were observed in skeletal muscle specimens (H&EX400).

elevation of hepatic enzymes and marker of hepatic damage (LDH) in the infected no-treated group, Meanwhile, these values were restored in groups treated with either GA or ALB and this value was best restored in combined treated group with both ALB and GA with the best treatment results.

### *3.4. Inflammatory markers in infected non-treated group and different infected treated groups*

Treatment of the infected group with either ALB or GA did not induce inflammation markers and this was represented by lowering the level of two main inflammation markers (CRP and IL-6). Results obtained as in Table 2. and Fig (S3) showed a high elevation of both CRP and IL-6 in the infected non-treated group, Meanwhile, these values were restored in groups treated with either GA or ALB and this value was best restored in combined treated group with both ALB and GA with the best treatment and therapeutic results and lowering the inflammatory markers.

### *3.5. Changes in oxidative stress in cardiac tissues in the infected non-treated group and different infected treated groups*

Treatment of the infected group with either ALB or GA induced a marked reduction in the final marker of lipid peroxidation (MDA) which means a reduction in the level of oxidative stress and induced marked elevation of antioxidant enzymes (SOD, CAT, and GPx) enzymes in cardiac tissue homogenates. Results obtained as shown in Table 3. showed a high elevation of all antioxidant enzymes in groups treated with either ALB and/or GA and the best elevation rate was recorded in ALB and GA-treated groups. Combined treatment with both ALB and GA with the best treatment and therapeutic results and lowering the oxidative stress markers.

## 4. Discussion

The slogan of the world now is how to combat the silent killer (Oxidative stress) and using of potent antioxidant agents with high

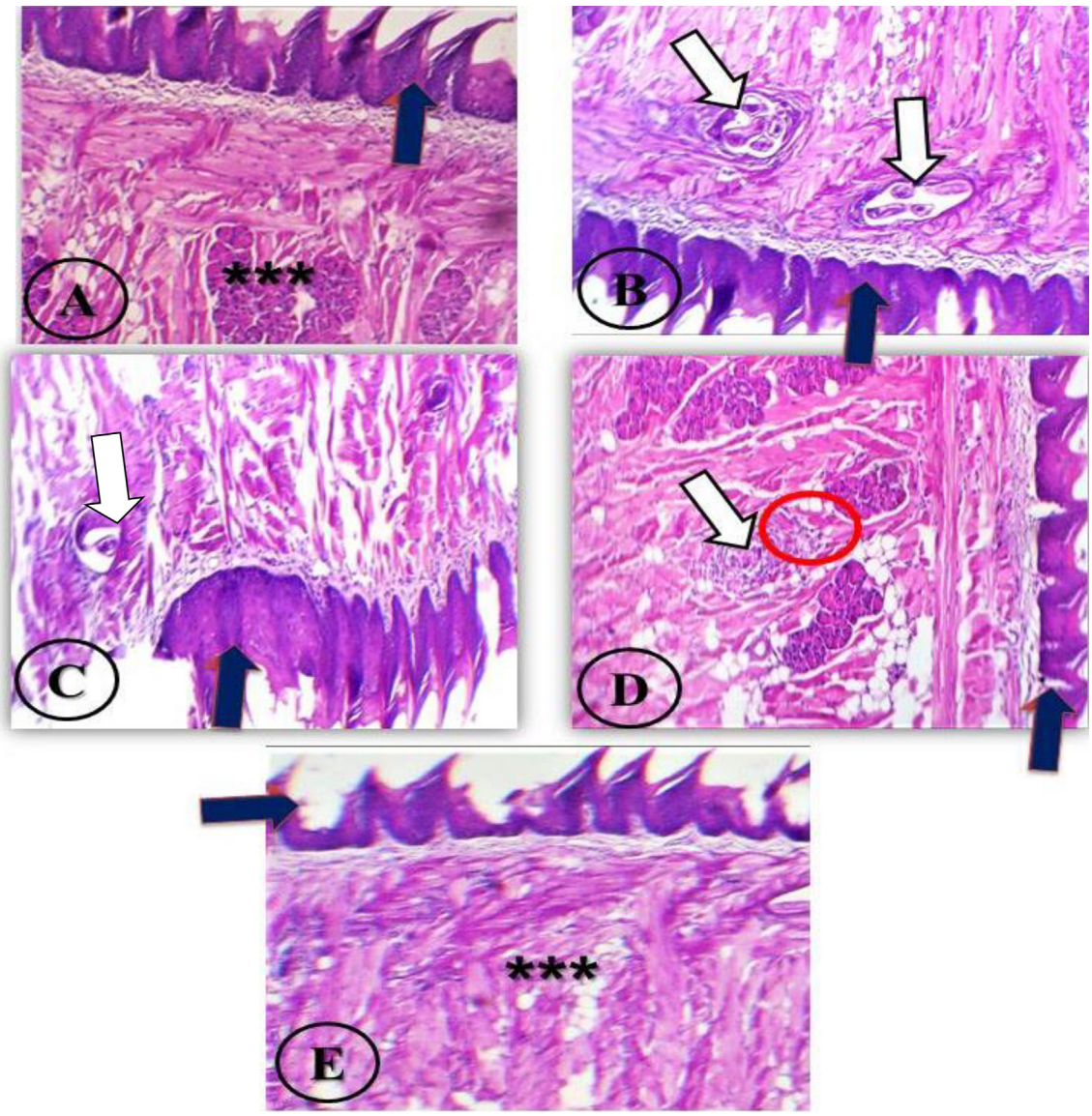



**Fig. 3.** Tongue muscle sections from (A) control group showing the healthy control group (-ve control) and infected non-treated group showed normal tongue muscle with no presence of encysted larvae of *T. spiralis* (H&EX400). (B) Infected non-treated group: showing high infection rate by incidence of two vacuolated larval capsules with severe and intense inflammatory cellular infiltration (White arrow) (H&EX400). (C) Infected group treated with ALB revealed of reduction of larval cysts of *T. spiralis* (White arrow). (D) Infected group treated with GA: showing partial replacement of larval cysts with eosinophilic exudates in tongue muscles (Red circle) 9H&EX400). (E) Infected group treated with the combination of ALB and GA showed high restoration of normal tongue structure with very minimized larval cysts (***) and no incidence of histopathological infections (Blue arrow) (H&EX400).

abilities to alleviate oxidative stress and parasitic infections which may represent health hazards to the general community.

The current study intended to assess the antiparasitic action of either ALB or GA each alone or in combination against trichinellosis as was reported previously the anti-parasitic action of ALB against *T. spiralis* (Eissa et al., 2022) and GA was previously reported by its potent antioxidant activities and its high ability in scavenging free radicals. So, the current concept was to combine the two agents to achieve a high anti-parasitic effect concurrent with antioxidant activity.

Concerning the parasitological assessment, the highest larval stage of *T. spiralis* was recorded in the diaphragm of the infected male mice followed by heart muscles, thigh, and tongue muscles, which aligns with the findings of Eissa et al.,2022, this confirmed the accuracy of infection level in the experimental infected male mice and thus right evaluation of therapeutic efficacy of the therapeutic drug and active compound.

The infected groups treated with either ALB or GA or a combination of ALB and GA afforded a marked decline in the larvae of *T. spiralis* as compared to the non-treated infected group with high elimination and eventual eradication of larvae of *T. spiralis*, the reduction percentage, especially in tongue and diaphragm, were as follows: For the diaphragm, the larval load was reduced by 98.40% in combined treated group with a combination of ALB and GA, followed by the infected treated group with GA by 96.81% and he last infected one treated with ALB by 94.08% percentage and this proved the high anti-parasitic effect of the combination of both ALB and GA and about to eradicate the larval stage of *T. spiralis*.

Regarding tongue muscles, the percentage of larval reduction in infected groups treated by a combination of ALB and GA, then treated by GA and then treated by ALB, were as follows: 98.53, 87.18 and 83.82% respectively confirming the high therapeutic efficacy of the combination of ALB and GA against infection induced by *T. spiralis*.

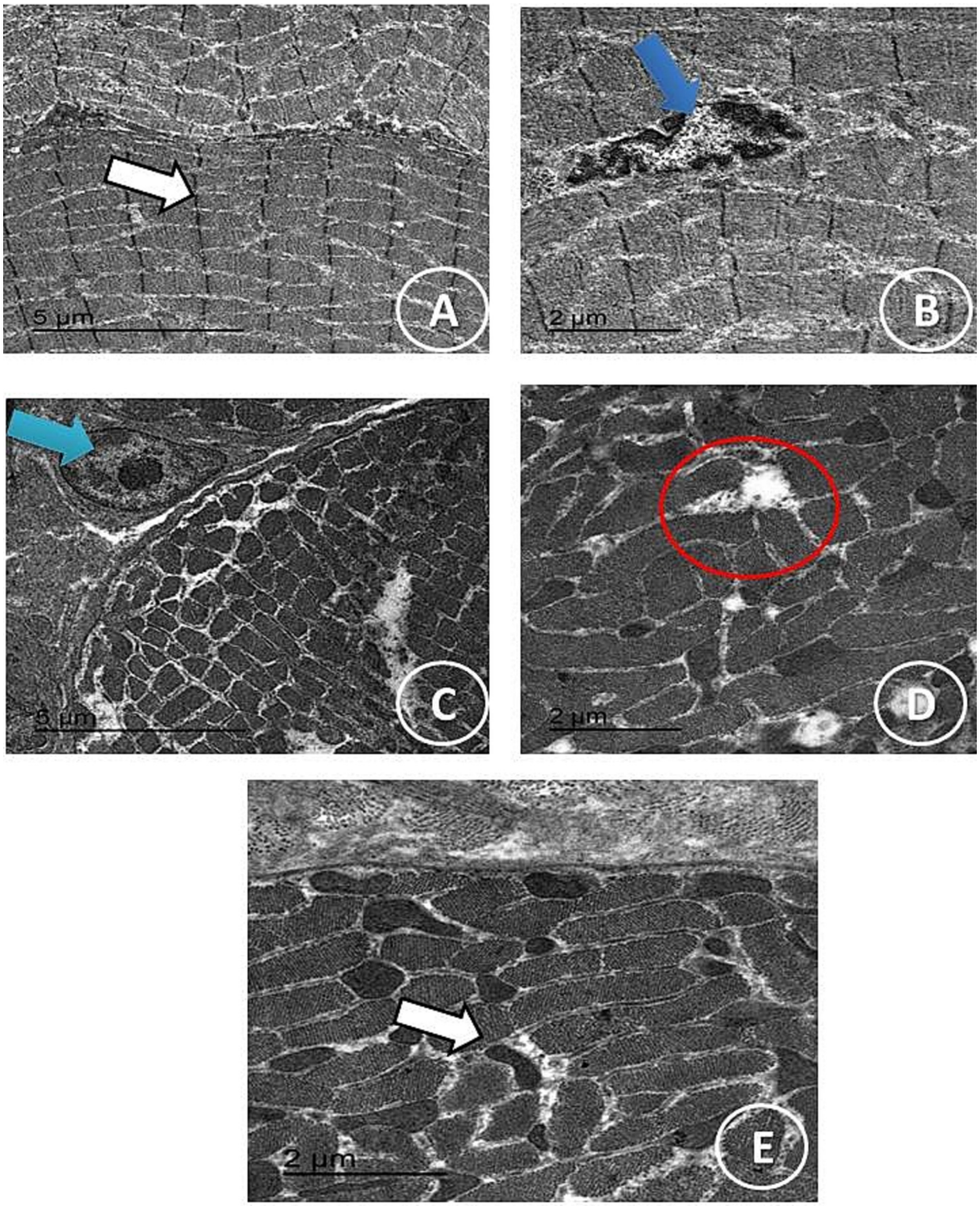



**Fig. 4.** TEM of skeletal muscles (A) control non–infected treated group (-ve control group) showing intact skeletal muscles with intact septa (White arrow) (Scale bar = 5 µm). (B) Infected non-treated group (+ve control group) showing the appearance of dense vacuolization of *T. spiralis* larvae (Blue arrows) with intense cellular infiltration (Scale bar = 2 µm). (C) Infected group treated with ALB showing the presence of reduced larval cysts with a thin membrane (Blue arrow) (Scale bar = 5 µm). (D) Infected group treated with GA showing replacement of larvae cysts with eosinophilic extrudes (Red circle) with restoration of normal cardiac structure (Scale bar = 2 µm). (E) group treated with the combination of ALB and GA showed great restoration of normal cardiac septa and cardiac muscles with the absence of any parasitological infections (White arrow) (Scale bar = 2 µm).

An additional strength point for the current study, McCracken,1978 proved previously that ALB resulted in a 67% reduction in *T. spiralis* when used as a therapeutic agent against *T. spiralis* infection in infected mice, several studies confirmed this concept and the percentage of larval reduction but it was reduced greatly after combined treatment by ALB and GA.

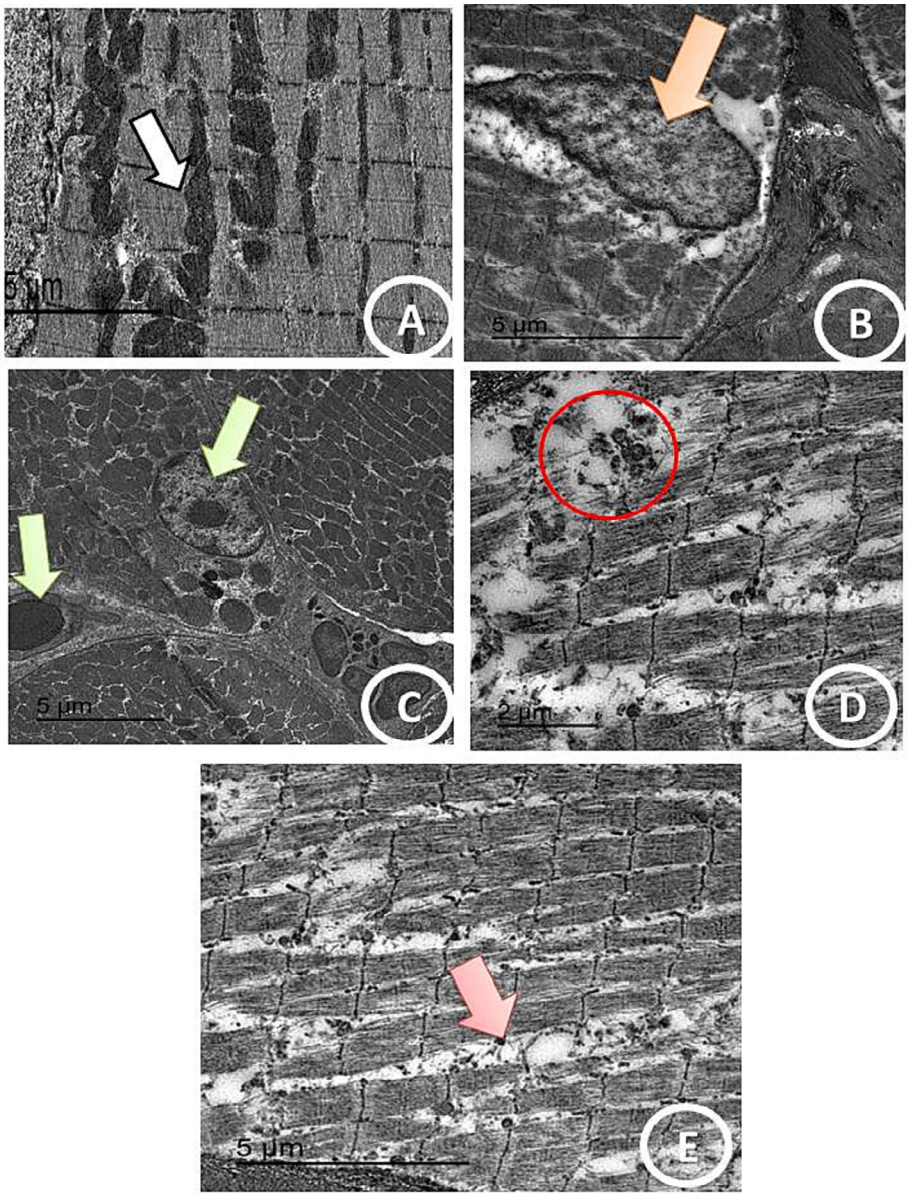



**Fig. 5.** TEM of heart muscles (A) control non–infected treated group (-ve control group) showing intact cardiac septa (White arrow) (Scale bar = 5 µm). (B) Infected non-treated group (+ve control group) showing the appearance of dense vacuolization of *T. spiralis* larvae (Orange arrows) with cellular infiltration (Scale bar = 5 µm). (C) Infected group treated with ALB showing the presence of reduced larval cysts with a thin membrane (Green arrow) (Scale bar = 5 µm). (D) Infected group treated with GA showing replacement of larvae cysts with eosinophilic extrudes (Red circle) with restoration of normal cardiac structure (Scale bar = 5 µm). (E) group treated with the combination of ALB and GA showing great restoration of normal cardiac septa and cardiac muscles with the absence of any parasitological infections (Red arrow) (Scale bar = 5 µm).

Regarding the mechanism of action of ALB, which is considered highly effective against *T. spirals* in the early stages of infection as its action is very limited to the time of treatment in early stages, ALB has the ability to disinfect *T. spiralis* larvae that are present in the small intestine or mitigating *T. spiralis* larvae from the small intestinal blood vessels to the muscles (Cohen et al., 2017), but the main defect is its low solubility that may decline it's effect greatly, thus my new trend is to strength this effect and duplicate ALB action by potent antioxidant like GA and may elevate its solubility and elongate the period of disinfection by scavenging the harmful free radicals which may help and add strength point to the action of ALB greatly as confirmed in the correct study.

In the meantime, GA was proved previously by its great ability as a potent antioxidant by different analyses (DPPH, FARAB and total antioxidant capacities) as confirmed previously by El-Megharbel and Hamza, 2022 which confirmed the oxidative stress role in the incidence of severe infection with success of this active potent antioxidant in alleviation of *T. spiralis* infection and it is the first time to confirm this synergistic effect between GA and ALB in alleviation of parasitic infection of *T. spiralis*. These new data will strengthen the idea and open a new gate for scientists to perform more studies by different dosages and evaluate new trends in the therapeutic efficacy of therapeutics and active compounds against *T. spiralis* which may be a real danger to health and infection of muscles.

The novel data of the current study related the antioxidant enzymes and markers of lipid peroxidation which confirmed the success of either GA and/or a combination of GA and ALB in the elevation of antioxidant enzymes and decline of the markers of lipid peroxidation and thus add more strength to the therapeutic efficacy of the new therapeutic combination in the alleviation of any infection induced by *T. spiralis* and this depends on the healthy

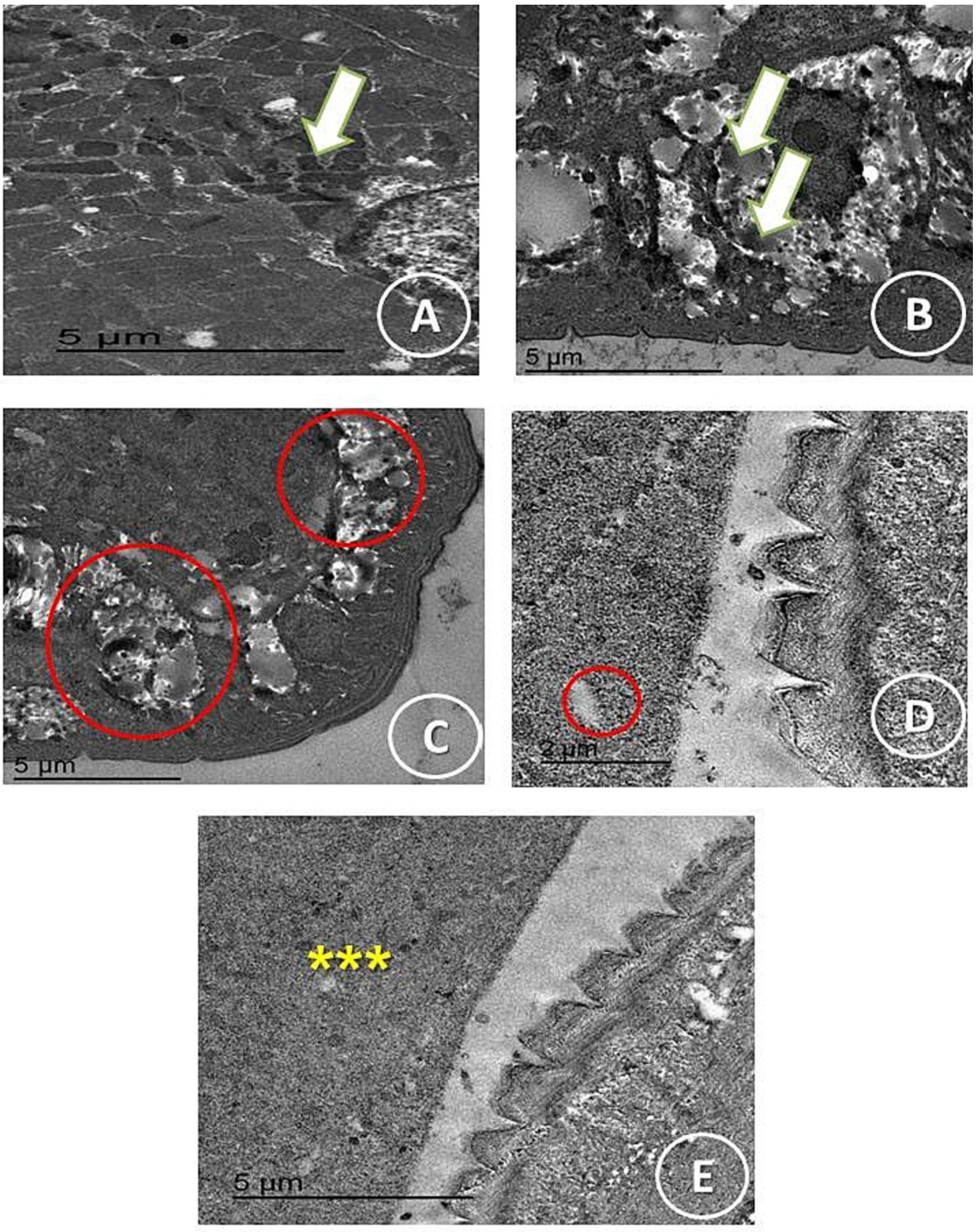



**Fig. 6.** TEM of tongue micrograph of (A) control non–infected treated group showing intact muscular filiform papillae, small filiform papillae on the body of the tongue (Green tongue) without any larval encysts (Scale bar = 5 µm). (B) Infected non-treated group (+ve control group) showing the appearance of dense and multiple larval *T. spiralis* larvae (Green arrows) (Scale bar = 5 µm). (C, D, and E) Infected treated group with ALB, then GA , and the last group treated with combination of ALB+ GA showing restoration of tongue papillae and normal muscular form with replacement of larvae cysts with eosinophilic extrudes (Red circle) in the case of (C) group treated with ALB, with great restoration of the normal muscular structure of the tongue in case of (D&E) treated groups with the combination of ALB and GA and it was the best-treated group (***) (Scale bar = 5 µm).

**Table 2**
Changes in inflammatory markers of control infected non-treated group and infected treated groups with either ALB, GA or their combination (mean ± S.E).

| Treated groups | | CRP (mg/l) | IL-6 (Pg/ml) |
|---|---|---|---|
| Group (I) | (Control group) (-ve control) | 4.980.24 | 6.45 ± 0.13 |
| Group (II) | (Infected treated group) (+ve control) | 31.85 ± 0.73 | 15.15 ± 1.51 |
| Group (III) | Infected group + ALB (50 mg/Kg) | 19.00 ± 0.500 | 12.39 ± 0.28 |
| Group (IV) | Infected group + GA (30 mg/Kg) | 15.46 ± 0.61 | 9.33 ± 0.42 |
| Group (V) | Infected group + ALP + GA | 13.70 ± 0.80 | 6.07 ± 0.33 |

Means carry a lot of different letters which are significant at ($P \leq 0.05$) by using Duncan's multiple range test, the highest mean value has symbol (a), and decreasing were assigned alphabetically.

**Table 3**
Oxidative/antioxidant of antioxidant enzymes in cardiac tissues of control non-treated infected group and infected treated groups with either ALB, GA or their combination (mean ± S.E).

| Treated groups | | MDA (U/g) | SOD (U/g) | GPx (U/g) | CAT (U/g) |
|---|---|---|---|---|---|
| Group (I) | (Control group) (-ve control) | $7.72 \pm 0.49^{e}$ | $20.16 \pm 0.39^{a}$ | $11.18 \pm 0.43^{ab}$ | $9.65 \pm 0.58^{a}$ |
| Group (II) | (Infected treated group) (+ve control) | $42.40 \pm 1.27^{a}$ | $3.28 \pm 0.36^{e}$ | $5.83 \pm 0.28^{d}$ | $1.71 \pm 0.21^{e}$ |
| Group (III) | Infected group + ALB (50 mg/Kg) | $27.10 \pm 0.92^{b}$ | $10.71 \pm 0.62^{d}$ | $8.07 \pm 0.30^{c}$ | $3.45 \pm 0.22^{d}$ |
| Group (IV) | Infected group + GA (30 mg/Kg) | $17.63 \pm 0.65^{c}$ | $12.35 \pm 0.99^{c}$ | $7.65 \pm 0.29^{c}$ | $5.32 \pm 0.21^{c}$ |
| Group (V) | Infected group + ALP + GA | $12.76 \pm 0.60^{de}$ | $15.50 \pm 0.59^{bc}$ | $9.81 \pm 0.37^{b}$ | $7.17 \pm 0.45^{b}$ |

Means carry a lot of different letters which are indicators of significant levels at ($P \leq 0.05$) using Duncan's multiple range test, the highest mean value has symbol (a), and decreasing were assigned alphabetically.

state of cells infected by these worms and establishing self-defense against these parasitic infections which is a new therapeutic trend.

Similar results were obtained regarding the histological and ultra-structural sections of different muscles such as heart, skeletal, and diaphragm muscles that revealed a sort of inflammation in the infected muscles surrounded by inflammatory cellular intense layers with the appearance of multiple vacuolization larvae, similar to Nada et al., 2018. All that proved the strength of data and accuracy of infection occurred and thus the potency of novel results by treatment by a combination of both ALB and GA which restored most of the normal structures and replacement of the larvae by eosinophilic exudes and great recovery.

Finally, the parasitological, histological, ultrastructural and biochemical investigation revealed the significant synergistic improvement induced by both ALB and GA against *T. spiralis* infection.

## 5. Conclusion

In conclusion, the present study demonstrated that GA in combination with ALB has a mitigating effect on the pathological hazard effects on the muscular phase of trichinellosis induced by *T. spiralis* larvae in infected male mice experimentally, which was highly evident with the therapeutic treatment of the combination of GA and ALB, proving that GA was more effective than single treatment by ALB alone. Various therapeutic and applied studies are recommended to investigate the potential effectiveness of different doses and schedules of synergistic therapy of both GA and ALB for the muscular phase of trichinellosis. Thus, the current study extends the therapeutic use of either ALB, GA active compound, or its combination to treat trichinellosis infection, especially as an alternative treatment for other parasitic diseases.

## Declaration of Competing Interest

The author declare that there is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment:

The researcher would like to acknowledge Deanship of Scientific Research, Taif University for funding this work.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103763.

## References

Abo Maged , A.A., Metwally, K., M., El-Menyawy, H.M., Hegab, F., El-Wakil, E. S., 2023. Assessment of the potential prophylactic and therapeutic effects of kaempferol on experimental *Trichinella spiralis* infection. J Helminthol. 97:e36. doi:10.1017/S0022149X23000184.

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126. https://doi.org/10.1016/s0076-6879(84)05016-3.

Badhani, B., Sharma, N., Kakkar, R., 2015. Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. Rsc Advances. 5(35), 27540–27557.DOI https://doi.org/10.1039/C5RA01911G.

Bruschi, D., 2014. Trichinellosis. In: Bruschi, F. (Ed.), Helminth Infections and Their Impact on Global Public Health. Springer.229–273. DOI https://doi.org/10.1007/978-3-7091-1782-8.

Carrillo, J.L.M., Maldonado-Tapia, C., Luna, A.L., Muñoz-Escobedo, J.J., De La Torre, J. A.F., Moreno-García, A., 2018. Current aspects in trichinellosis. Paras. Parasit. Dis. 65–87. https://doi.org/10.5772/intechopen.80372.

Chan., 2003. Biostatistics 102: quantitative data – parametric & non-parametric tests. Singap. Med. J. 44 (8), 391–396. PMID: 14700417

Cohen, J., Powderly, W.G., Opal, S.M., 2017. Infectious Diseases, 4th edition. Elsevier Health Sciences. ISBN: 9780702079351.

Daoxiu, X.u., Xue, B., Jing, X., Xuelin, W., Zijian, D., Wenjie, S., Fengyan, X., Yanfeng, L., Mingyuan, L., Xiaolei, L., 2021. The immune protection induced by a serine protease from the Trichinella spiralis adult against *Trichinella spiralis* infection in pigs. PLoS Negl. Trop. Dis. 15 (5), e0009408.

Denham, D.A., 1965. Studies with methyridine and Trichinella spiralis. I. Effect upon the intestinal phase in mice. Exp. Parasitol. 17 (1), 10–14. https://doi.org/10.1016/0014-4894(65)90003-2.

Eissa, F.M.A., Eassa, A.H.A., Zalat, R.S., Negm, M.S., Elmallawany, M.A., 2022. Potential therapeutic effect of platelet-rich plasma and albendazole on the muscular phase of experimental *Trichinella spiralis* infection. Food Waterborne Parasitol. 28, e00180.

El-Megharbel, S.M., Hamza, R.Z., 2022. Synthesis, spectroscopic characterizations, conductometric titration and investigation of potent antioxidant activities of gallic acid complexes with Ca (II), Cu (II), Zn(III), Cr(III) and Se (IV) metal ions. J. Mol. Liq. 358,. https://doi.org/10.1016/j.molliq.2022.119196 119196.

Gamble, H.R., 1996. Detection of trichinellosis in pigs by artificial digestion and enzyme immunoassay. J. Food Prot. 59 (3), 295–298. https://doi.org/10.4315/0362-028x-59.3.295.

Gao, J., Hu, J., Hu, D., Yang, X., 2019. A role of gallic acid in oxidative damage diseases: A comprehensive review. Nat. Prod. Commun. 1–9. https://doi.org/10.1177/1934578X19874174.

García, A., Barrera, M.G., Piccirilli, G., Vasconi, M.D., Ricardo, J.D., Leonardi, D., Hinrichsen, L.I., Lamas, M.C., 2013. Novel albendazole formulations given during the intestinal phase of *Trichinella spiralis* infection reduce effectively parasitic muscle burden in mice. Parasitol. Int. 62 (6), 568–570. https://doi.org/10.1016/j.parint.2013.08.009.

Jayamani, J., Shanmugam, G., 2014. Gallic acid, one of the components in many plant tissues, is a potential inhibitor for insulin amyloid fibril formation. Eur. J. Med. Chem. 85, 352–358. https://doi.org/10.1016/j.ejmech.2014.07.111.

Jittawan, K., Siriamornpun, S., 2008. Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. Food Chem. 110, 881–890. https://doi.org/10.1016/j.foodchem.2008.02.076.

Lan, H.C., Charn, L.Y., Chin, Y.G., Yin, C.H., 2007. Preventive effects of guava (Psidium guajava L.) leaves and its active compounds against??-dicarbonyl compounds-induced blood coagulation. Food Chem. 103, 528–535. https://doi.org/10.1016/j.foodchem.2006.08.022.

Liang, Y.S., John, B.I., Boyd, D.A., 1987. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. In Proceeding of the 1st Sino-American Symposium, 1, 34–48.

McCracken., 1978. Efficacy of mebendazole and albendazole against *Trichinella spiralis* in mice. J. Parasitol. 214–219. PMID: 641662

Nada, S.M., Mohammad, S.M., Moad, H.S.F., EL-Shafey, M.A., Al-Ghandour, A.M.F., Ibrahim, N., 2018. Therapeutic effect of *Nigella Sativa* and ivermectin versus albendazole on experimental trichinellosis in mice. J. Egypt. Soc. Parasitol. 48 (1), 85–92. Doi:10.21608/JESP.2018.77029

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Paglia, D.E., Valentive, W.N., 1967. Studies on the avantitaue and gualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169. PMID: 6066618.

Rtail, R., Maksymova, O., Viacheslav, I., Gortynska, O., Korenkov, O., Moskalenko, P., Nasser, M., Tkach, G., 2020. Improvement of skeletal muscle regeneration by platelet-rich plasma in rats with experimental chronic hyperglycemia. Biomed. Res. Int. 2020, 6980607. https://doi.org/10.1155/2020/6980607.

Seow, D., Shimozono, Y., Nazim, T., Yusof, T., Yasui, Y., Massey, A., Kennedy, J.G., 2021. Platelet-rich plasma injection for the treatment of hamstring injuries: a systematic review and meta-analysis with best-worst case analysis. Am. J. Sports Med. 49 (2), 529–537. https://doi.org/10.1177/0363546520916729.

Shalaby, M.A., Moghazy, F.M., Shalaby, H.A., Nasr, S.M., 2010. Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. Parasitol. Res. 107 (1), 17–25. https://doi.org/10.1007/s00436-010-1827-9.

Siriyasatien, P., Yingyourd, P., 2003. Nuchprayoon S. Efficacy of albendazole against early and late stage of Trichinella spiralis infection in mice. J Med Assoc Thai. 86 (Suppl2), S257-262. PMID: 12929998

Sumeeta, K., Priya, D., Bhawna, S., Charu, S., Abhishek, M., Nameirakpam, J., Rakes,h., Kumar, P., Surjit, S., Rakesh, S., 2021. Clinical and laboratory profile of trichinellosis from a non-endemic country. Indian Journal of Medical Microbiology.39(2): 235-239. https://doi.org/10.1016/j.ijmmb.2021.02.004.

Sun, Y., Oberley, L.W., Li, Y.A., 1988. A Simple method for clinical assay of superoxide dismutase. Clin. Chem. 34, 497–500. PMID: 3349599.

Walter, B., Nelson, M., Larissa, H., Christine, S., Britta, L.S., Caroline, F.F., 2022. Evaluation of the PrioCHECK™ Trichinella AAD kit to detect *Trichinella spiralis, T. britovi, and T. pseudospiralis* larvae in pork using the automated digestion method Trichomatic-35. Parasitol. Int. 86,. https://doi.org/10.1016/j.parint.2021.102449 102449.