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Dual anti-parasitic and immune-modulatory role of Eugenol in experimental trichinellosis: A Comparative study with Albendazole

Lamia Bakr*, Yasser A. Dar, Mostafa H. El Mehlawy, Mai El Ghannam

Zoology Department, Faculty of Science, Tanta University, Egypt.

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Corresponding author:

Lamia Bakr, Ph. D.

E-mail:

lamiaa.bakr@science.tanta.edu.eg

Mobile: (+2)01128488487

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ABSTRACT

Trichinella spiralis is a worm that causes trichinellosis, a neglected zoonotic disease acquired by eating undercooked pork. Albendazole (ABZ) is the preferred medication for treating trichinellosis, however its effectiveness is restricted during the muscular phase. It is also necessary to find alternate therapies because ABZ has the potential to be harmful. Clove oil contains a phenolic component called Eugenol (EUG), has demonstrated as anti-inflammatory and anti-parasitic agent. The purpose of this study was to assess the in vivo effectiveness of EUG versus ABZ treatment in experimentally *T.spiralis* infected mice. After being infected with *T. spiralis*, 80 Swiss albino mice were split into two groups and given either ABZ or EUG for the duration of the infection's intestinal and muscular phases. Analysis was done using parasitological, biochemical, histological, and immunohistochemical methods. The results demonstrated that while ABZ treatment was more effective during the muscular phase, EUG therapy dramatically decreased the adult worm burden in the intestinal phase more than ABZ. Serum levels of IL-4 and IL-10 were considerably reduced by both ABZ and EUG treatments; however, in the early phases of infection, EUG showed a stronger suppression of inflammatory markers. EUG's anti-inflammatory and tissue-preserving properties were validated by histological results, and decreased TNF-α expression in muscle tissue. In conclusion, treatment with EUG showed both immunomodulatory and anti-parasitic properties, which lessen tissue changes and the parasite load. These results imply that EUG may be used as an adjuvant or natural therapeutic substitute for ABZ in the treatment of trichinellosis.

Keywords: Albendazole; Anti-parasitic activity; Cytokines; Eugenol; Histopathology; Parasite burden; *Trichinella spiralis*

1. Introduction

One of the primary anthelmintic medications used to treat trichinellosis is albendazole (ABZ), a benzimidazole derivative. ABZ has immunomodulatory effects in addition to its direct anti-parasitic action, which enhances therapeutic results. By binding to β-tubulin, ABZ prevents microtubule polymerization in parasite cells, which impairs glucose absorption and depletes energy. The parasite becomes immobile and dies as a result (Lacey 1990). Research on experimental murine trichinellosis has repeatedly demonstrated that ABZ shown to act effectively against encysted muscle larvae as well as adult worms in the intestines during *Trichinella spiralis* infection, with complete elimination of early-stage larvae and significant reduction of muscle-encysted larvae in mice (Li et al., 2012). Although ABZ has been the recommended treatment for a long time, its poor efficacy during the encysted muscle phase, growing concerns about toxicity and drug resistance underscore the urgent need for safer, more effective alternatives.

In this context, natural products have gained renewed attention due to their diverse bioactive properties and relative safety. Eugenol (EUG) is a naturally occurring phenolic chemical that is mostly present in bay leaves, basil, clove oil (*Syzygium aromaticum*), and cinnamon. The

antibacterial, anti-inflammatory, antioxidant and anti-parasitic properties of EUG are well-established (Tavvabi-Kashani et al., 2024). It has a significant promise in its immune-modulatory and anti-parasitic qualities. Significant anti-parasitic activity is demonstrated by EUG against a range of helminthic and protozoan parasites. Its mechanisms include interference with essential enzymes, breakdown of parasite cell membranes, and inhibition of energy metabolism. EUG, for instance, has shown effective against the causal agent of visceral leishmaniasis, *Leishmania donovani*. By causing oxidative stress and mitochondrial malfunction, it causes promastigotes to die in a manner similar to apoptosis (Dutta et al., 2007). Furthermore, via changing mitochondrial potential and membrane integrity, it has been shown to inhibit *Trypanosoma cruzi*, the causative agent of Chagas disease (Santoro et al., 2007). Furthermore, EUG has been demonstrated to lower worm load and egg production in infected mice with helminths such as *Schistosoma mansoni*. When used with praziquantel, it disrupts the integrity of the tegument and may have a synergistic impact (de Oliveira et al., 2014).

EUG is a potential treatment for inflammatory and immunological-related disorders since it regulates both innate and adaptive immune responses. By suppressing pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, mostly by blocking the NF-κB signaling pathway, it demonstrates anti-inflammatory qualities (Thakur and Pitre, 2009). EUG has been demonstrated to promote macrophage phagocytic activity and lymphocyte proliferation in murine models. According to Kaur et al. (2010), it also encourages a balance between Th1 and Th2 immune responses, indicating a regulatory function in adaptive immunity. In parasitic infections, these immune-modulatory effects may enhance host defenses while minimizing tissue-damaging inflammation.

For example, EUG has been shown to lower parasite burden in *Toxoplasma gondii* infection models while also regulating the immune response to avoid excessive inflammation (Mohamed et al., 2020). Only a small number of researches, nevertheless, have assessed its therapeutic effectiveness against helminthic illnesses like trichinellosis. In a mouse model. So the purpose of this study is to assess EUG's potential as a natural substance that is safer and more successful than ABZ, a common anthelmintic drug, in the treatment of trichinellosis

2. Materials and methods

Drugs and chemicals

Eugenol was obtained from Fuzhou Farwell Import and Export Co. (Hong Kong, China). ABZ-suspension (400 mg/10 mL) was purchased from Pharma Cure Pharmaceutical Industries, Egypt. Interleukin-4 (IL-4), and IL-10 Kits were purchased from Thermo-Fisher Scientific, USA.

Experimental mice

Eighty male Swiss albino mice (5–6 weeks old, and 20–25 g) were obtained from the Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Mice were kept in conventional conditions, fed a commercial food, and their tap water was free of chlorine. For a week, they were acclimated. This study was approved by Tanta University's Faculty of Science's institutional animal ethics committee under ethical procedure number IACUC-SCI-TU-0042.

Parasite and mice infection

The pig strain *T. spiralis* was obtained from naturally infected pigs at the Cairo butcher.

Afterwards, it was kept at Tanta University's Zoology Department's Laboratory of Parasitology unit. There were multiple passes through donor albino rats.

Two hundreds *T. spiralis* larvae were orally given to each mouse by stomach tube after 12 hours starvation according to the method described by Abou Rayia et al. (2017) and Nassef et al., 2019). Mice were divided into four groups; 20 animals each: Group A: neither Negative control, not infected nor receiving treatment. Group B: Infected and untreated positive control. Group C: ABZ-treated (infected and receiving oral ABZ treatment at a dose of 50 mg/kg/day). Group D: Infected and receiving oral EUG treatment at a dose of 500 µg/kg/day. Two subgroups (a and b, each with ten mice) were created from groups C and D:

Subgroup a: Received treatment from 3–5 days post-infection (D.P.I.) during the intestinal phase and sacrificed on day 7. Subgroup b: Received treatment between 31 and 33 D.P.I., during the muscular phase and sacrificed on day 35.

Parasitological assessments

The pepsin-HCl digestion method was used to measure the muscular larval burden on day 35. The number of larvae per gram of digested tissue was used to express the larval burden. According to Ozkoc et al. (2009), *T. spiralis* adults and muscle larvae were extracted from the infected mice. Seven days post infection, *adult T. spiralis* worms were extracted from the small intestines of infected mice. To allow the worms to move out of the tissue, the intestine was washed, split into small pieces, each measuring 2 cm, and opened lengthwise along its whole length. It was then left in normal saline at 37° C for three to four hours (Wakelin and Wilson, 1980).

For recovery of muscle larvae, mice were sacrificed on 35 D.P.I. In a conical flask, the skinned mouse was minced to facilitate larval recovery and was incubated for the entire night at 37 C° with artificial digestive fluid (1% pepsin, W/V and 1% HCL, V/V). The larvae were collected from the bottom of the conical flask, washed multiple times with PBS (pH: 7.4), suspended for 30 minutes to allow sedimentation, and their number per milliliter was counted under a light microscope after being filtered with a thieve to remove the debris. They were then washed in phosphate buffer saline PBS (pH: 7.4) (Abd-Elrahman et al., 2020).

Cytokines assessment

Blood was drawn from the mouse eye orbital plexus and centrifuged to separate serum. Following the manufacturer's instructions, the concentrations of the IL-4 and IL-10 were measured using commercial enzyme-linked immunosorbent (ELISA) kits (R and D Systems, Minneapolis, MN, United States).

Histopathological and immunohistochemical examinations

On 7 and 35 D.P.I, skeletal muscles and portions of the small intestine from every group were fixed in 10% formalin for 24 hours, washed in water for 12 hours then dehydrated in ascending concentrations of ethanol, and then cleared in xylene. Pure soft paraffin was used for the two-hour impregnation process at 55°C. A microtome was then used to cut hard paraffin sections that were 5 µm thick. Hematoxylin and an eosin stain were applied to the sections (Carleton et al., 1980).

Sections of paraffin-embedded skeletal muscle (5 µm) were stained for TNF-α on a Dako Autostainer using an anti-TNF antibody (YPA2155, Chongqing Biospes, China). DAB substrate and heat-induced epitope retrieval were employed. TNF-α staining intensity was used to semi-quantitatively grade the sections.

Statistical Analysis

Graph-Pad Prism 7 was used to perform statistical analyses in this study. Data were presented as the mean ± standard deviation (SD), and the significance of the differences between mean values of experimental and control groups was analyzed by multiple t-tests. Statistical significance was defined as $p<0.05$.

3. Results

EUG-treatment decreased the number of adults *T. spiralis* and muscle larvae

In the infected non-treated group, the mean number of adults *T. spiralis* which collected from the intestinal fluid was 33.33 ± 7.638/ml, while the number of these adults in the ABZ-treated group was 4.33 ± 1.52/ml, with 87% reduction, which was statistically significant ($p<0.05$) compared to the control positive group. Moreover, in EUG-treated group, the number of adult worms was 3.3 ± 1.52 /ml with 90% reduction, which was statistically significant ($p<0.05$) compared to the control positive group (Fig. 1).

The mean number of muscle larvae of *T. spiralis* of carcass digested was 12225 ± 1793/ml in the infected non-treated group, while in the ABZ-treated group, it was 3900 ± 2182/ml, with 68.1% reduction, which was statistically significant ($p<0.05$) in comparison to the control positive group. Furthermore, in EUG-treated group, it was 6650 ± 1965/ml with (45.6%) percentage of reduction, which was statistically significant ($p<0.05$) in comparison to the control positive group (Fig. 2).

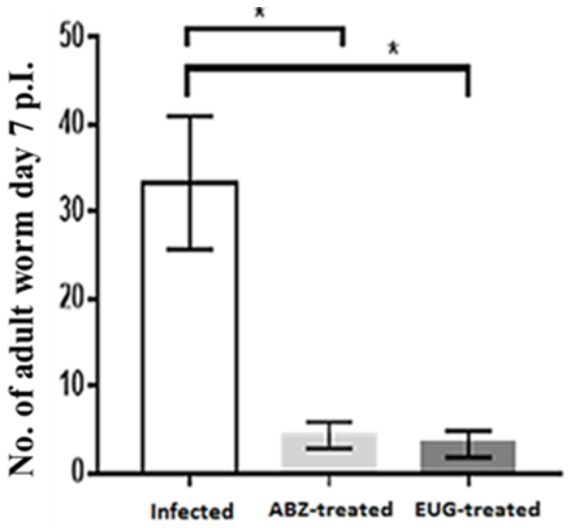

Fig. 1. Number of adult *T. spiralis in vivo* on 7 D.P.I. among studied groups. EUG-treated group had the lowest *T. spiralis* adult count, followed by ABZ treated group, compared to the positive control group.*: Significant at $p \leq 0.05$.

Treatment with EUG augmented IL-4 and IL-10 levels

The results showed a significant increase in IL-4 and IL-10 levels in the sera of infected non-treated mice compared to the control group ($p<0.05$). However, the infected-treated groups showed significant reduction in IL-4 levels, which was 80% in the ABZ-treated group. In the EUG-treated group, the reduction was 83% (Fig. 3). There is a significant reduction in IL-10 levels to 63% and 54.5% in ABZ- treated and EUG-treated groups, respectively (Fig. 3). The measurements of these biochemical markers in different groups were statistically significant ($p<0.05$). At day 35 P.I, the results showed significant increase in IL-4 and IL-10 in the sera of infected non-treated mice. However, the infected treated groups showed significant reduction in IL-4 levels, especially in the ABZ-treated group with reduction percentage of 68.7% followed by EUG-treated group (54.56%).

There was a significant reduction in IL-4 levels by 65.9% and 56.08% in ABZ-treated group and EUG-treated group, respectively (Fig. 4). Measurements of these biochemical markers in different groups were statistically significant ($p<0.05$).

There was no significant change in IL-4 and IL-10 levels in the negative control group between day 7 and day 35. However, in the positive control and treated groups, IL-4 and IL-10 levels were significantly higher on day 35 ($p<0.05$) (Table 1-2).

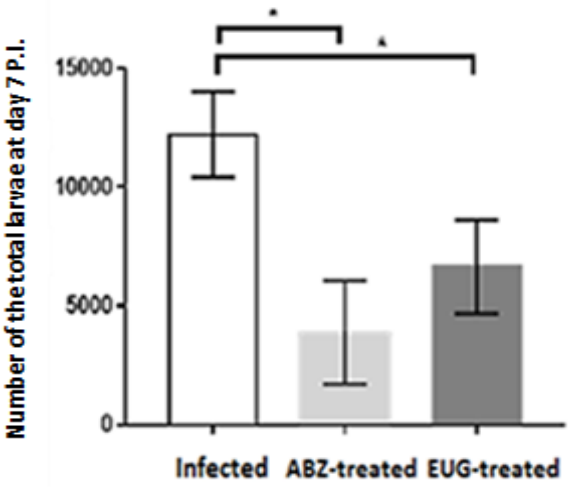

Fig. 2. Number of ML of *T. spiralis* in vivo on 7 D.P.I. among studied groups. ABZ-treated group had the lowest *T. spiralis* larval count, followed by EUG-treated group, compared to the positive control.*: Significant at $p \leq 0.05$.

Table 1. Serum levels of IL4 and IL-10 on 7 D.P.I

Groups	IL-4 level Pg/ml	IL-10 level Pg/ml
–ve Control	9.567 ± 0.56	15.97 ± 0.20
+ve Control	54.17 ± 4.6*	74 ± 1.27*
ABZ-treated	16.93 ± 0.8*	25.23 ± 2.15*
EGU-treated	24.67 ± 3.3*	32.5 ± 0.9*

*: Significant vs. –ve control at $p \leq 0.05$

Table 2. Serum levels of IL4 and IL-10 on 35D. P.I

Groups	IL-4 level Pg/ml	IL-10 level Pg/ml
– ve Control	9.4 ± 0.16	15.9 ± 0.09
+ ve Control	43.2 ± 15.4*	54.3 ± 27.7*
ABZ-treated	11.6 ± 7.4*	19 ± 8.8*
EGU-treated	15.0 ± 13.6*	24.1 ± 11.7*

*: Significant vs. –ve control at $p \leq 0.05$.

Histopathological investigations

Histopathological examination of sections in the small intestine stained with Hematoxylin and eosin revealed that the control negative group had normal architecture with a regular villous pattern (Fig. 5 A). No histopathological changes were observed in the intestinal sections of the uninfected, untreated control group. The infected -untreated group showed that there was a distorted villous pattern with

enlargement and expansion by inflammatory cells. There was also dense intravillous inflammatory cellular infiltration, mainly consisting of mononuclear cellular infiltrate in the form of lymphocytes and plasma cells (Fig. 5 B). While the ABZ-treated group revealed that there was a distorted villous pattern with broadening and focal expansion by mononuclear inflammatory cells, as shown in (Fig. 5C). The EUG-treated group showed remarkable improvement of the histopathological changes of the intestine, with a return to the normal villous pattern. An evident decrease in the intensity of the inflammatory cellular infiltration was observed (Fig. 5 D).

Histopathological examination of skeletal muscles revealed that control negative groups had no observed histopathological changes in muscular sections (Fig. 6 A). The infected untreated group has a massive number of encysted *T. spiralis* larvae diffusely present in muscles sarcoplasm and massive number of chronic inflammatory cells infiltrating in muscle bundles and surrounding the encysted larvae. Muscles showed many encysted *T. spiralis* larvae, and each one was surrounded by a thick, intact or degenerated capsule (Fig. 6 B-C). However, the ABZ-treated group revealed a slight decrease in the number of *T. spiralis* larvae and showed fewer encysted larvae than those in the infected untreated group, with heavy inflammatory cellular infiltration surrounding them (Fig. 6 D and E).

The capsule appeared thick and complete. It also showed degeneration of the larvae in the form of fragmentation and invasion by inflammatory cellular infiltrate.

The EUG–treated group (Fig. 6 F and G) showed a moderate degree of larval deposition. Most of them showed fragmentation, degenerated capsules surrounded by moderate mononuclear inflammatory cells, and it also showed many cysts with degenerated capsules rimmed by few inflammatory cells (mostly macrophages).

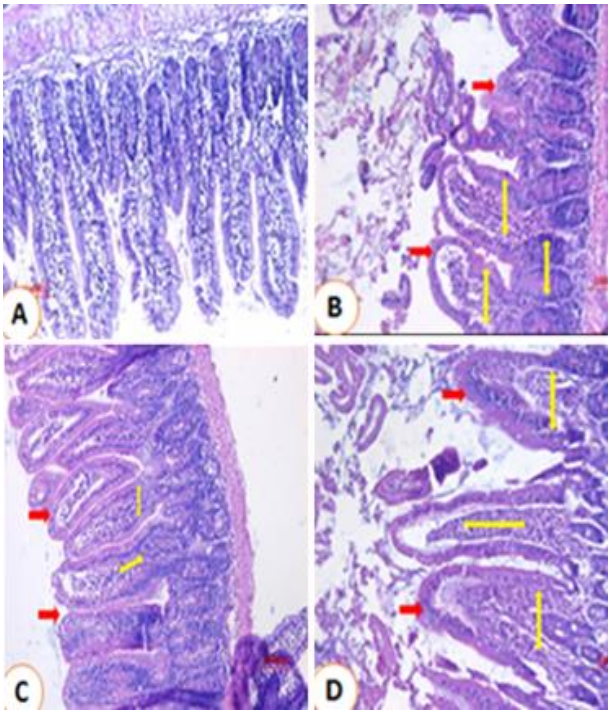

Fig. 5. Photomicrographs of sections in the small intestine of: The control negative section showing a regular villous pattern and mild focal expansion by mononuclear inflammatory cells (H and E stain, X100) (**A)**. The control positive section showing a distorted villous pattern (red arrows) with broadening and expansion by inflammatory cells (yellow arrows) (Hematoxylin and Eosin stain, X100) (**B)**. ABZ-treated section showing distorted villous pattern (red arrows) and focal expansion by mononuclear inflammatory cells (yellow arrows). (H and E stain, X100) (**C)**. EUG-treated section showing mostly preserved villous architecture (red arrows) with mild broadening of villous pattern and mild focal expansion by mononuclear inflammatory cells (yellow arrows). (H and E stain, X100) **(D).**

Immunohistochemical investigations

Fig. 7 revealed that the negative control mouse (non-infected, non-treated) had a negative expression of TNF-α (Fig. 7 A). While the infected positive control revealed mild expression of TNF-α in inflammatory cells around intact *T. spiralis* cysts and dense expression of TNF-α in inflammatory cells around degenerated *Trichinella* cysts (Fig. 7 B and C).

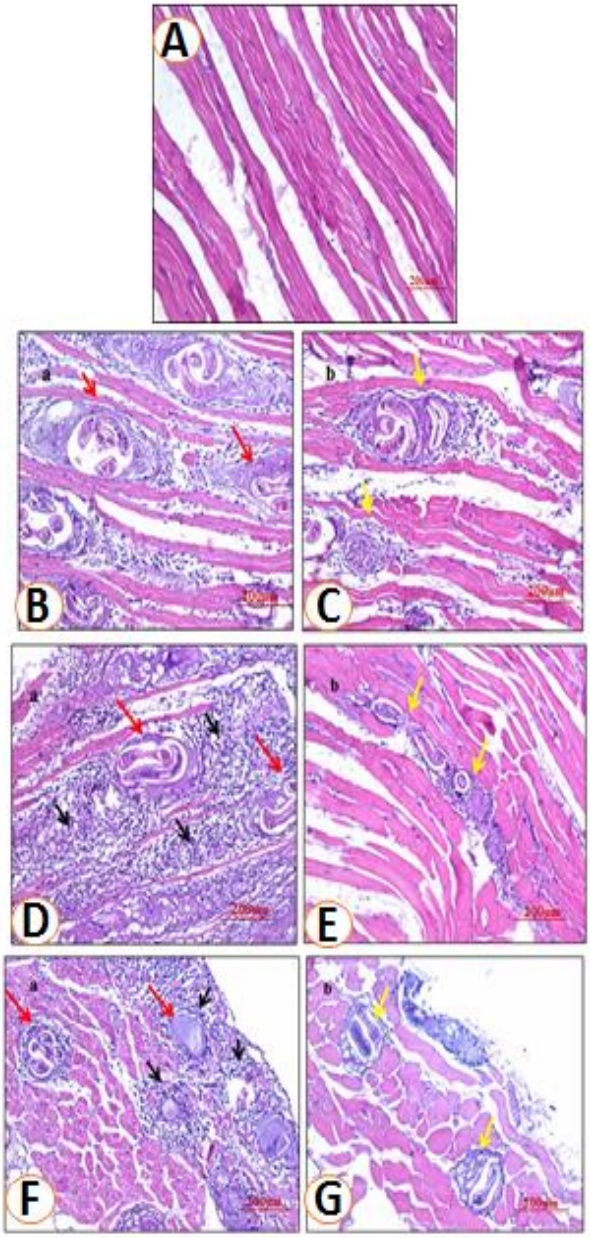

Fig. 6 (A-F). Photomicrographs of sections in skeletal muscle of: Control negative, group showing normal architecture (H&E stain, X200) **(A).** Control positive showing many *Trichinella* cysts with intact capsules and contents (red arrows) **(B)** and degenerated capsule (yellow arrows) **(C)** surrounded by moderate number of mononuclear inflammatory cells (H&E stain, X200). ABZ-treated section showing many *Trichinella* cysts with degenerated capsules (red arrows) **(D)** surrounded by dense mononuclear inflammatory cells (black arrows). It is also showing a remnant of degenerated cyst **(E)** surrounded by a few inflammatory cells (mostly macrophages) (yellow arrows) (H&E stain, X200) EUG-treated section showing many *Trichinella* cysts with degenerated capsules (red arrows) **(F)** surrounded by moderate mononuclear inflammatory cells (black arrows) and it is also showing many cysts with degenerated capsules **(G)** rimmed by few inflammatory cells (mostly macrophages) (yellow arrows) (H&E stain, X200)**.**

In ABZ-treated mice, immunohistochemical expression of TNF-α in the muscular tissues was dense in the inflammatory cells around *Trichinella* cyst as well as within larvae of degenerated cyst (Fig. 7 D and E). Also, the EUG-treated mice revealed mild expression of TNF-α in inflammatory cell around degenerated *Trichinella* cyst (Fig. 7 F and G). In ABZ-treated mice, immunohistochemical expression of TNF-α in the muscular tissues was dense in the inflammatory cells around *Trichinella* cyst as well as within larvae of degenerated cyst (Fig. 7 D&E). Also, the EUG-treated mice revealed mild expression of TNF-α in inflammatory cell around degenerated *Trichinella* cyst (Fig. 7 F and G).

The use of medicinal plants to treat parasite diseases has received a lot of attention lately because to its low side effects when compared to synthetic medications (Nisar et al., 2021). One of clove oil's main ingredients, EUG, exhibits anti-parasitic properties against helminths and protozoa (El-Kady et al., 2019; Nasiou and Giannakou, 2020). Given ABZ's negative consequences (Bucková et al., 2018), EUG might be a good substitute. Compared to ABZ, EUG was more effective in this in vivo experimental investigation at lowering the adult *T. spiralis* worm burden in the intestines of infected mice. ABZ, on the other hand, showed a better ability to lessen the load of muscle larvae (ML).

The current findings were examined in light of earlier research employing various natural products, using ABZ as a reference medication, because there is a dearth of information on in vivo studies employing EUG. ABZ's effectiveness against *T. spiralis* has been extensively documented, although the results have been inconsistent (Chung et al., 2001; Siriyasatien et al., 2003; Huang et al., 2020; El-Wakil et al., 2022). This could be due to variations in the dosage, timing, and length of treatment (Siriyasatien et al., 2003). When ABZ and graviola extract were combined, El-Wakil et al. (2021) recorded a considerable decrease in the number of adult worms, and the current findings were consistent with the reduction in muscle larvae.

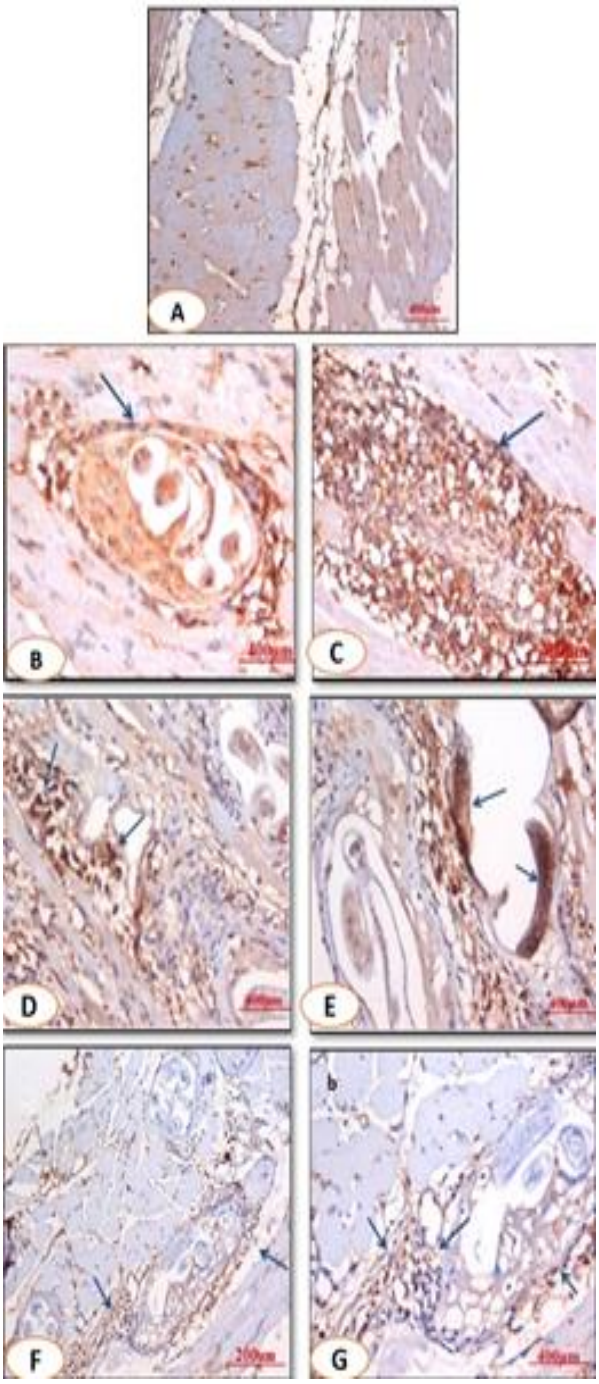

Fig. 7. (A-F). Photomicrographs of Immunohistochemical expression of TNFα in skeletal muscle sections of: **(A)** negative control section revealed negative expression of TNFα (IHC for TNF, X400). Positive control section revealed mild expression of TNF-α in inflammatory cells around intact **(B)** (black arrow) and dense expression of TNF-α in inflammatory cells around degenerated *Trichinella* cyst **(C)** (IHC for TNF-α; X400). ABZ-treated mouse revealed dense expression of TNF-α in inflammatory cells around *Trichinella* cyst **(D)** as well as within larvae of degenerated cyst **(E)** (IHC for TNF-α, X400). And section of EUG-treated mouse revealed mild expression of TNF-α in inflammatory cells around a degenerated *Trichinella* cyst (black arrow) (IHC for TNF-α, **(F)** X200; **(G)** X400).

4. Discussion

Expulsion of adult *T. spiralis* from the intestine is likely facilitated by inflammatory responses regulated by T-helper cells (Bruschi and Chiumiento, 2012). Therefore, the superior efficacy of EUG against the intestinal stage may be attributed to both its direct antiparasitic effects and its immunomodulatory properties. This is consistent with El-Kady et al. (2019), who demonstrated the anti-schistosomal efficacy of EUG. In the current study, serum levels of IL-4 and IL-10 were elevated during both early and chronic stages of infection, indicating that *T. spiralis* induces a complex Th1/Th2 immune response with a predominant Th2 polarization (Frydas et al., 1996; Li and Ko, 2001; Ilic et al., 2012; Ding et al., 2017; Farid et al., 2019; Song et al., 2019). According to Frydas et al. (2001), interactions between other cytokines and the severity of the disease may be correlated with higher cytokine levels. IL-4 and IL-10 serum levels dropped 7- and 35-days post-infection (P.I.) after EUG and ABZ therapy. EUG has been proven to have an inhibitory effect on peritoneal macrophages' production of cytokines (IL-6, IL-1β, and IL-10) in vitro (Bachiega et al., 2012), and its emulsified form has exhibited comparable effects against visceral leishmaniasis (Islamuddin et al., 2016). In the current investigation, the EUG-group's IL-4 level was lower at 7 days (P.I.) _but 35 days (P.I.) higher than that of the ABZ- group. Tissue healing and parasite ejection are mediated by IL-4, IL-9, and IL-13 (Kreider et al., 2007).

According to Favre et al. (1990), cytokine suppression limits the recruitment of immune cells by lowering the expression of adhesion molecules on endothelial cells. In contrast, the EUG-group's IL-10 levels were consistently greater during both periods. During a *T. spiralis* infection, IL-10 is known to reduce tissue damage and immune-mediated inflammation, unintentionally aiding parasite survival (Huang et al., 2014). This could account for the enhanced tissue healing shown in histological sections from the EUG-group and the increased muscle larvae burden seen at 35 days (P.I.). In line with earlier findings, histopathological analysis of the small intestine from untreated infected mice showed villous deformation and

extensive inflammatory infiltrates (Bakr, 2014; Attia et al., 2015). Compared to ABZ, EUG-therapy greatly decreased inflammation and restored normal villous architecture.

Similar findings were reported using other plant extracts (Mohammed et al., 2022a). In skeletal muscle, untreated infected mice showed extensive encysted larvae and chronic inflammation (Ibrahim et al., 2019; Nassef et al., 2019). EUG-treatment resulted in reduced tissue damage, fewer degenerated larvae, and milder inflammation compared to ABZ, which yielded fewer larvae within intact capsules and intense inflammatory infiltration. These effects were previously observed with other natural agents (Attia et al., 2015). EUG's proven anti-inflammatory and antioxidant qualities, as well as its modulation of IL-10, may be the cause of its advantageous tissue effects (Pramod et al., 2010). Additionally, EUG may modify receptor activation or block inflammatory signaling pathways, such as TNF-α through NF-κB inhibition (Aggarwal et al., 2005; Magalhães et al., 2010). In both ABZ-treated and positive control animals, immune-histochemical examination revealed high TNF-α expression surrounding cysts, which is consistent with chronic inflammation (Xu et al., 2019). However, as was also seen with *Bassia indica* extract, moderate TNF-α expression in mice treated with EUG indicates cytokine suppression, suggesting its advantage in treatment of trichinellosis (Mohammed et al., 2022).

The present study has several limitations. Firstly, the EUG-extract was not subjected to phytochemical analysis, which restricts our knowledge of the precise bioactive substances behind its immune-modulatory and antiparasitic properties. Secondly, evaluation of relapses or maintained efficacy was not possible due to the lack of long-term follow-up beyond 35 dpi. Thirdly, the evaluation of cytokines was restricted to IL-4 and IL-10; a more complete picture of immune regulation may have been obtained by incorporating other markers such IFN-γ, IL-6, TGF-β, or regulatory T-cell markers. Furthermore, host immune gene expression and parasite load were not confirmed by molecular methods like PCR or qRT-PCR, which could have improved sensitivity and specificity. Lastly, there were no mechanistic studies conducted to confirm the mechanisms by which EUG reduces inflammation and parasites. In contrast to ABZ, the standard treatment for *T. spiralis*, this study demonstrates the encouraging anti-parasitic and immune-modulatory properties of EUG, a natural produced from clove oil. ABZ was more effective against muscle larvae, whereas EUG was more successful in lowering the adult worm burden in the intestines.

EUG's unique cytokine profile, especially its sustained IL-10 expression, may account for its protective effects on tissue architecture, even though both treatments markedly altered immune responses. Analysis of histopathology and immunohistochemistry further supported EUG's capacity to lower inflammation and suppress TNF-α expression. These results imply that EUG, which has both anti-parasitic and tissue-protective properties, is a suitable natural substitute for synthetic anthelmintics. By up-regulating anti-inflammatory cytokines like IL-10 and down-regulating pro-inflammatory cytokines like TNF-α, IL-1β, and IFN-γ, EUG regulates cytokine expression (Abdel-Ghaffar et al., 2020). Additionally, it increases the activities of antioxidant enzymes, which lowers oxidative stress indicators like malondialdehyde (MDA), which are raised during trichinellosis. These impacts help maintain tissue integrity, especially in the skeletal muscles and gut. In both the intestinal and muscular phases, EUG has demonstrated encouraging anti-parasitic action against *T. spiralis*. EUG administered orally dramatically lowers the number of adult worms in the intestines and the load of larvae in the muscular tissues, according to experimental research conducted in mouse models.

It is thought to have an anthelmintic effect since it can interfere with parasite metabolism and reproductive potential, as well as damage the integrity of parasite membranes (Abd El-Rahman et al., 2021). According to one study, mice treated with EUG showed a significant decrease in larvae counts when compared to infected controls; in the early stages of infection, this treatment was as effective as ABZ (Kamel et al., 2022). EUG-treated infected mice showed improved histological characteristics, such as less muscle necrosis,

preservation of the small intestine's villous architecture, and lower infiltration of inflammatory cells. According to biochemistry, EUG decreased levels of creatine kinase and liver enzymes, suggesting less damage to the muscles and liver (Ahmed et al., 2021).

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