Assessment of the efficacy of thymol against *Toxocara vitulorum* in experimentally infected rats

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**Abstract** The effect of thymol and ivermectin on the development and embryonation of *Toxocara vitulorum* (*T. vitulorum*) eggs, as well as their migration in albino rats was investigated both in vitro and in vivo. A total of forty male albino rats were divided into four groups for an in vivo experiment. The first group was uninfected; the second group was infected but left untreated; the third group was infected and received thymol at a dose of 40 mg/kg; and the fourth group was infected and received ivermectin (0.2 mg/kg). In vitro, thymol inhibited the development of *Toxocara* larvae within the eggs. However, ivermectin, produced inconsistent results. The in vivo results indicated that the recovery rates of *Toxocara* larvae from the liver and lungs on day 7 post-infection were significantly lower in the thymol or *Toxocara*-treated groups than in the infected untreated control. Albumin levels were significantly increased in the thymol-treated group as compared to the positive control and ivermectin groups. Nitric oxide, IL-4, and IFN-γ levels in the serum of the thymol or ivermectin-treated groups were significantly lower than that of the positive control group. Histopathological examination demonstrated that thymol and ivermectin were effective in reducing larval load, reducing the number and size of granulomas in the absence of larvae, and improving tissue architecture. The current study concluded that thymol possessed anti-*Toxocara* activity in a rat model. Additionally, thymol possessed ovicidal properties and may be used as a disinfectant.

**Keywords** Thymol · *Toxocara vitulorum* · Ivermectin · Biochemical alterations · Cytokines · Histopathology

**Introduction**

Toxocariasis is gaining international attention and is listed by the US Centers for Disease Control and Prevention (CDC) as one of the five most neglected parasitic infections (Macpherson 2013; Holland 2017). Toxocariasis is a widespread zoonotic disease initiated by nematodes belonging to the genus *Toxocara* (family *Toxocaridae*, superfamiliy *Ascaridoidea*). Four species have been identified globally, involving *T. cati, T. canis, T. vitulorum*, and *T. malaysiensis*, with domesticated animals serving as their definitive hosts (Gasser 2013). *T. vitulorum* is an endemic infection found in tropical cattle and buffalo...
calves. *Toxocara* infection has been correlated with increased mortality and morbidity in calves, as well as uncompensated stunted growth in the survivors. It was found in the small intestines of domestic cattle (Davila et al. 2010; Rast et al. 2013). In Egypt, *T. vitulorum* infection was prevalent in cattle and buffalo calves (El-Ashram and Aboelhadid 2019). The parasite is one of the most economically significant diseases as it affects young animals (one- to three-month-old calves) because of maternal infection, frequently resulting in elevated mortality rates (Devi et al. 2000). Humans are considered an accidental host for *T. vitulorum* infections. In man, *Toxocara* larvae do not mature into adult worms. Additionally, they become infected after overwhelming the embryonated eggs found in soil, contaminated food, or encapsulated *Toxocara* larvae in improperly cooked meat of paratenic hosts, including chickens, sheep, and cattle (Macpherson 2013). Moreover, *Toxocara* larvae migrate and remain encysted in various body tissues and organs for months or years (somatic migration). Numerous clinical entities may be caused during the larval migration, involving visceral larval migrans (VLM), ocular larval migrans (OLM), as well as covert toxocariasis (Magnaval et al. 2001; Roldán et al. 2010).

Numerous medications are currently being used to treat toxocariasis in animals, with some showing efficiency against adult worms. None, however, has eliminated *Toxocara* tissue’s larval stages, which are a major source of vertical transmission (Maffrand et al. 2006; Jin et al. 2008). Larval treatment in paratenic hosts, such as humans, is also critical for interrupting the parasite’s life cycle (Fok and Kassai 1998). *T. vitulorum* larvae migrate through the liver and lungs of paratenic hosts and then spread to other organs, involving the muscles, kidney, and brain (Amerasinghe et al. 1992). Various plants have been shown to have protective efficiencies against several parasites of animal origin, with promising results in managing and breaking the parasites’ life cycle. *Thyme* (*Thymus vulgaris, T. vulgaris*) contains a lot of thymol (20.0–54.0% of the crude plant) (El-Ashram and Abdelhafez 2020). Thymol is one of the major components of *thyme* essential oils and is well known for its anti-microbial and anthelmintic properties, in addition to its superior scolicidal action (Elisondo et al. 2008). Due to progressive outcomes of toxocariasis in man and animals, along with the massive fecal egg output in diseased animals, the extreme egg resistance to adverse environmental conditions, and additionally, the ineffectiveness of the most commonly used anthelmintic medications to combat either larval/adult stages (Aydin et al. 2006). The current study was carried out to assess the protective effect of thymol in controlling zoonotic *T. vitulorum* infections using a rat model.

### Materials and methods

#### Animals

Forty male albino rats weighing 120–160 g were obtained from the Laboratory Animal Unit of Beni-Suef University’s Faculty of Pharmacy. The rats were divided into four groups (ten rats each), and they were housed in plastic cages. Rats were kept in the Parasitology Laboratory, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. Ambient conditions including temperature (20–25 °C), relative humidity (55.0%), as well as 12 h light–dark cycle, with the lights turned off at 7 p.m, were maintained during the experiment period. The animals were fed standard rodent pellets and were given unlimited access to water.

#### Chemicals

The purified thymol used in the present study was purchased from Sigma- Aldrich (CAS Number: 89–83-8; St. Louis MO, USA). The commercial anthelmintic ivermectin was obtained from Pharma Swede Ph. Comp. (Egypt).

#### Embryonation of Toxocara eggs

Adult females *T. vitulorum* were gathered from naturally infected buffalo calves brought to random veterinary clinics, Beni-Suef Province, Egypt. Gravid female worms were collected and thoroughly washed in 0.85% normal saline before the gravid uteri were removed with fine scissors. The eggs were sieved, washed, and precipitated several times with 1.0% formal saline prior to storage in a sufficient solution at 4 °C as an egg stock (Amerasinghe et al. 1992). In addition, the collected eggs were placed in clean Petri dishes (90 mm in diameter) containing formal saline (1.0%). The dishes were incubated at 28 °C for 14–18 days, with the solution changed every two days, aerated, and examined under a microscope to notice how the embryonic cells developed. After about 10 days of incubation, the second-stage larvae begin to emerge from these eggs.

#### In vitro assessment of thymol and ivermectin on Toxocara eggs

Using dimethyl sulfoxide (DMSO) as an emulsifier, different concentrations of thymol (10, 5, 2.5, 1.25, and 0.625 mg/ml) were prepared. To make the emulsifier, 120 mg of purified thymol was dissolved in 600 μl DMSO (10.0%). In a microtiter plate (96 well), double-fold serial dilutions in saline solution (NaCl 0.9%) were performed to
a final concentration of 0.625 mg/100 µl. To each well, one hundred *T. vitulorum* eggs in formal saline were added to achieve a final concentration ranging from 10.0% to 0.625% (Arafa et al. 2020). Ivermectin was diluted in distilled water to obtain five concentrations of 0.08, 0.04, 0.02, 0.01 mg/ml, and 0.005 mg/ml. As a negative control, 1.0% formal saline was used. The plates were incubated at 28 °C for 10 days before being examined under a light microscope to determine the percentage of larval development. All treatments were carried out in triplicate, and experiments were independently repeated thrice.

**In vivo assessment of thymol and ivermectin on Toxocara migration in liver and lungs**

**Experimental design**

The experiment was divided into four groups (ten rats each). Throughout the experiment, a negative control group was given phosphate buffer saline orally. A positive control group of rats was infected but not treated. The rats in the thymol-treated group received thymol in their drinking water at a dose of 40.00 mg/kg body weight for seven days prior to the challenge (Arafa et al. 2020). The final group (ivermectin-treated group) received ivermectin (0.2 mg/kg) in the drinking water for seven days prior to infection. All groups (except the negative control group) were inoculated intraperitoneally with 2500 embryonated eggs per rat via a gastric tube (Barriga and Omar 1992). The experiment was carried out to determine the percentage of larval development. All treatments were carried out in triplicate, and experiments were independently repeated thrice.

The liver and lung organs were obtained from all groups under experiment on the 7th day post-infection with *T. vitulorum* eggs. Organs of all groups were trimmed into small pieces of 2–3 mm thickness and fixed in Bouin’s fluid for 72 h. The fixed samples were dehydrated in ethyl alcohol, cleared in Xylol, and embedded in Paraplast®. 4–5 µm thick sections were obtained on clean and dry glass slides then stained with Hematoxylin and Eosin (H&E) as a general stain, Periodic-acid Schiff (PAS) for detection of mucin and Crossmon’s trichrom stain for detection of collagen fibers. All techniques and stains were used according to Suvarna et al. (2019). In addition, the measurement of the granuloma diameter of the rat’s liver and lung and its counting were performed in five fields for each section (i.e., fifteen fields for each group). The Image J analysis software program (NIH, Bethesda, Maryland) and LEICA (DFC290 HD system digital camera, Heerbrug, Switzerland) connected to the light microscopy (10X objective lens) were used to score histopathological lesions in the liver and lung stained with H&E in terms of the degree of cell damage (Gibson-Corley et al. 2013).

**Statistical analysis**

One-way analysis of variance (ANOVA) was used for the statistical analysis, followed by the Tukey multiple comparison post-hoc test. *P* values less than 0.05 were considered significant when the obtained data were expressed as a mean ± SE. Statistical Package for the Social Sciences 22 was used to perform all calculations (SPSS, Chicago, Illinois, USA). The median inhibitory
concentration (IC50) for each treatment was estimated using a Probit statistical model (Finney, 1952).

**Results**

**In vitro effect of ivermectin and thymol on *Toxocara* egg development**

Eggs containing developed larvae made up 53.6% of the negative control, while non-developed eggs made up to 24.66%. In contrast to the negative control, the egg containing developed larvae was significantly ($P < 0.05$) reduced in the thymol-treated group (Fig. 1). Thymol completely stopped the embryonation of *Toxocara* eggs at higher concentrations (5 mg and 10 mg/ml). Thymol had an IC50 of 0.80 mg/ml and ivermectin had an IC50 of 0.023 mg/ml. Ivermectin concentrations of 0.04 mg and 0.08 mg/ml resulted in significant egg embryonation stopping (Table 1).

**Protective effect of ivermectin and thymol against *Toxocara* in a rat model (in vivo)**

**Postmortem inspection of liver and lungs**

All the infected group’s affected livers appeared pale in color, with the lungs appearing slightly congested. The liver and lungs in the negative control group were of normal color and texture. At day 7 post-infection, the thymol or ivermectin-treated rat had a significantly lower number of recovered larvae from the liver and lung than the positive control rat (Table 2). When compared to the positive control (603 and 330), ivermectin and thymol administration resulted in a significant decrease in the number of larvae in the liver (203 and 90) and lungs (250 and 110) at day 7 post-infection, with a protection rate of (66.33% and 58.54%) in the liver and (72.73% and 66.67%) in the lungs in the ivermectin-treated group and in the thymol-treated group, respectively.

**Biochemical and cytokine parameters**

In the current study, all infected groups had a significant increase ($P < 0.05$) in liver enzyme activities (ALT and AST) when compared to the negative control. In comparison to the positive control, thymol- and ivermectin-treated rats reduced the negative effects of *Toxocara* on hepatic tissues. Total protein and globulin levels didn’t exhibit significant changes between all experimental groups. While albumin values showed a significant decrease in the positive control group when compared to other experimental groups except the ivermectin treated group. Thymol treated group showed significant ($P < 0.05$) elevated albumin value when compared to control infected and ivermectin

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**Fig. 1** Egg’s inhibition; **A** Negative control (uninfected untreated) eggs with a normal larval development. **B** Ivermectin-treated eggs showing larval development and cell division. **C** Thymol-treated eggs showing only cell division.
treated groups however there was non-significant changes incomparable with control negative group (Table 3). In the current study, serum NO, IL-4, and IFN-γ levels in all infected groups were significantly \((P \leq 0.05)\) higher than in the negative control group. However, when compared to the positive control, the thymol- or ivermectin-treated groups showed significant reductions (Table 4).

**Histopathological findings**

In the negative control group, histopathological examination of lung tissues revealed a normal histological picture (Fig. 2A). In the positive control, a widespread multiple large-sized fibrocellular granuloma containing *Toxocara* larvae and heavy inflammatory cellular infiltration caused thickening of the interalveolar septa (Fig. 2B). A collagenous connective tissue capsule encased these granulomas (Fig. 2C). Furthermore, *Toxocara* larvae were found in the lung parenchyma (Fig. 2D). The alveoli collapsed due to excessive leucocytic infiltration in the interalveolar septa (Fig. 2D1). A fibrous capsule encased the *Toxocara* larva in the large cellular granuloma (Fig. 2D2). The number and size of granulomas were significantly reduced in the ivermectin-treated group, as well as the absence of larvae and improved lung tissue (Table 5, Fig. 2E). In the thymol-treated group, the number and size of granulomas

| Table 1 | In vitro inhibitory effect of thymol or ivermectin on *Toxocara* eggs embryonation (Means ± SE) |
|---------|-------------------------------------------------------------------------------------------------------------------------------------|
| Treatment | Concentration (mg/ml) | Egg larvae | Early developed eggs | Non developed eggs | IC50 (95% CI) |
| Control  | 53.60 ± 4.58\(^a\) | 21.66 ± 1.76\(^a\) | 24.66 ± 2.02\(^a\) |
| Thymol  | 6.25 | 21.67 ± 1.76\(^b\) | 46.3 ± 1.45\(^b\) | 32.33 ± 3.17\(^b\) | 0.8 (0.12–2.6) |
| 12.5 | 16.33 ± 2.02\(^c\) | 28.66 ± 0.48\(^c\) | 57.00 ± 5.29\(^c\) |
| 2.05 | 4.00 ± 1.76\(^d\) | 12.66 ± 1.76\(^d\) | 83.33 ± 1.21\(^d\) |
| 50.0 | 0.00 ± 0.00\(^e\) | 10.00 ± 1.15\(^e\) | 90.00 ± 1.14\(^e\) |
| 100.0 | 0.00 ± 0.00\(^f\) | 00.00 ± 00.00\(^f\) | 100.00 ± 00.00\(^f\) |
| Ivermectin  | 0.005 | 40.00 ± 0.57\(^g\) | 27.66 ± 1.54\(^g\) | 32.33 ± 1.20\(^b\) | 0.023 (0.003–0.075) |
| 0.01 | 40.33 ± 2.18\(^h\) | 27.33 ± 1.76\(^h\) | 35.00 ± 1.20\(^h\) |
| 0.02 | 37.66 ± 0.33\(^i\) | 32.66 ± 1.02\(^i\) | 29.66 ± 1.45\(^i\) |
| 0.04 | 24.67 ± 1.47\(^j\) | 52.00 ± 1.15\(^j\) | 23.33 ± 1.45\(^j\) |
| 0.08 | 18.33 ± 0.88\(^k\) | 43.66 ± 0.88\(^k\) | 38 ± 1.52\(^k\) |

Means with superscripts (\(a, b, c, d, e, f\)) within a column are significantly different at \(P < 0.05\)

| Table 2 | Larval counts in the liver and lung of the treated and control groups at day 7 post-infection in rat model |
|---------|-------------------------------------------------------------------------------------------------------------------------------------|
| Group | Liver | Lung | Protection percent |
|        |        |        | Liver | Lung |
| Negative control | – | – | 100 | 100 |
| Positive control | 603 | 330 | 00.00 | 00.00 |
| Thymol-treated | 250 | 110 | 58.54 | 66.67 |
| Ivermectin-treated | 203 | 90 | 66.33 | 72.73 |

| Table 3 | Serum biochemical parameters in different experimental groups (Means ± SE) |
|---------|-------------------------------------------------------------------------------------------------------------------------------------|
| Group | ALT (U/L) | AST (U/L) | Total protein (g/dl) | Albumin (g/dl) | Globulins (g/dl) |
| Negative control | 22.15 ± 1.53\(^a\) | 50.05 ± 1.19\(^a\) | 5.86 ± 0.27\(^a\) | 2.25 ± 0.09\(^a\) | 3.62 ± 0.31\(^a\) |
| Positive control | 60.80 ± 1.51\(^b\) | 131.33 ± 2.40\(^b\) | 5.43 ± 0.28\(^a\) | 1.99 ± 0.009\(^b\) | 3.44 ± 0.29\(^a\) |
| Thymol treated | 35.18 ± 2.36\(^c\) | 96.33 ± 2.03\(^c\) | 6.27 ± 0.12\(^a\) | 2.41 ± 0.04\(^ac\) | 3.86 ± 0.14\(^a\) |
| Ivermectin treated | 41.50 ± 2.56\(^c\) | 100.00 ± 2.89\(^c\) | 6.25 ± 0.24\(^a\) | 2.07 ± 0.006\(^ab\) | 4.18 ± 0.24\(^a\) |

Means with different superscripts (\(a, b, c\) and \(d\)) within a column are significantly different at \(P < 0.05\)
decreased with the absence of larvae in most sections and a moderate perivascular leucocytic infiltrate (Fig. 2F). In the negative control, histochemical analysis of the lungs with PAS staining revealed a normal distribution of bronchial goblet cells and normal mucin production (Fig. 3A). There was a lot of goblet cell metaplasia and a lot of mucin production in the infected group (Fig. 3B). The number of goblet cells and mucin production in the thymol or ivermectin-treated group were both moderate (Fig. 3C, D). The negative control had a normal lobular architecture in the

Table 4 Serum nitric oxide, IL-4, and IFN-γ levels in different experimental groups (Means ± SE)

| Group               | NO (nmol/ml) | IL-4 (pg/ml) | IFN-γ (pg/ml) |
|---------------------|--------------|--------------|---------------|
| Negative control    | 13.40 ± 1.68a| 29.45 ± 2.03a| 34.2 ± 1.12a  |
| Positive control    | 47.18 ± 1.11b| 83.96 ± 3.51b| 122.10 ± 1.74b|
| Thymol-treated      | 19.60 ± 1.10c| 60.80 ± 4.10c| 69.43 ± 2.19c |
| Ivermectin-treated  | 22.23 ± 1.04c| 56.43 ± 2.58c| 74.70 ± 3.06c |

Means with different superscripts (a, b, c, and d) within a column are significantly different at P < 0.05

Fig. 2 Rat’s lung affections: A Control negative group showing a normal histological picture of lung alveoli (A) and bronchiole (B). H&E stain; B–D Infected group with T. vitulorum eggs at day 7 post-infection (control positive group): B Large cellular granulomas (G), Toxocara larva embedded in granuloma (arrowhead) and thickening of the interalveolar wall (IA) due to an intense polymorphnuclear inflammatory infiltrate composed primarily of eosinophils. H&E stain; C Large fibrocellular granuloma (G) surrounded by a collagenous connective tissue capsule (arrows). Crossmon’s trichrom stain; D1 Toxocara vitulorum larvae (arrowhead) within the lung parenchyma, an excessive leucocytic infiltration in the interalveolar septa (IA) and collapsed alveoli. H&E stain; D2 Large cellular granuloma (G) containing Toxocara vitulorum larvae (L) surrounded by a fibrous capsule (arrows). Crossmon’s trichrom stain; E Ivermectin-treated group showing a medium-sized peri-bronchial fibrocellular granuloma (G) surrounded by a fibrous capsule (arrow), small-sized cellular granulomas (arrowheads) within the lung parenchyma without Toxocara vitulorum larva and moderate improvement of lung tissues (LT). H&E stain; F Thymol- treated group showing a medium-sized granuloma (G) containing larvae (arrow), and moderate improvement of lung tissues (LT). H&E stain. Scale bars: 100 μm, 100 μm, 200 μm, 100 μm, 200 μm, 200 μm, 200 μm, respectively.
livers, with normal central veins and radiating hepatic cords (Fig. 4A). The rats in the positive control group had severe hepatocyte degeneration, blood vessel congestion, and edema (Fig. 4B). We also found large cellular granulomas embedded in the hepatic parenchyma, as well as excessive fibrous connective tissue proliferation in the hepatic parenchyma (Fig. 4C) and portal area (Fig. 4D). The most pronounced histopathological picture of the liver in the ivermectin-treated group was moderate degenerative changes of hepatocytes and the absence of granuloma.

### Table 5
Effect of thymol and Ivermectin treatment on granuloma number and diameter and histopathological changes in rat liver and lung stained with H&E based on scoring severity of injury

| Groups Lesions | Organ | Negative control | Positive control | Ivermectin-treated | Thymol-treated |
|----------------|-------|------------------|------------------|--------------------|----------------|
| Size of granuloma/μm | Liver | 0 | 1065.2 ± 55.33 | 315.46 ± 22.78 | 363.66 ± 23.23 |
| No. of granuloma/ microscopic field | Liver | 0 | 2.60 ± 0.12 | 1.10 ± 0.10 | 1.60 ± 0.12 |
| | Lung | 0 | 5.50 ± 0.26 | 2.30 ± 0.35 | 3.47 ± 0.57 |
| Lesion score | Liver | Congestion | 0 | 3 | 1 |
| | Degeneration | 0 | 2 | 1 | 1 |
| | Necrosis | 0 | 2 | 1 | 1 |
| | Inflammation | 0 | 3 | 1 | 1 |
| | Fibrosis | 0 | 2 | 1 | 1 |
| | Infiltration | 0 | 2 | 1 | 1 |
| | Lung | Bronchiolitis | 0 | 2 | 1 | 1 |
| | Edema | 0 | 2 | 1 | 1 |
| | Epithelial thickening | 0 | 1 | 1 | 2 |
| | Epithelial degeneration | 0 | 2 | 1 | 2 |
| | Fibrosis | 0 | 2 | 1 | 2 |
| | Interstitial pneumonia | 0 | 1 | 1 | 2 |

Tissue injury in the liver and lung was scored in terms of degree of cell damage as following: 0 = no change; 1 = < 25% cell damage; 2 = 26–50% cell damage; 3 = 51–75% cell damage; and 4 = 76–100% cell damage.

**Fig. 3** A photomicrograph of the rat’s lungs of: A Control negative group showing normal distribution of goblet cells (arrowheads) within the bronchial epithelium; B Control positive group showing a marked increase of bronchial goblet cells (arrowheads) with an excessive mucin production; C Ivermectin-treated group showed a marked subsiding of bronchial goblet cells (arrowheads); D Thymol-treated group showing a moderate number of bronchial goblet cells (arrowheads), PAS Stain: Scale bars (A–D) = 200 μm
Fig. 4  Rat’s liver:  A Control negative group showing a normal histological picture of central veins (arrows) and surrounding hepatic cords (HC). H&E stain;  B-D Infected group with *Toxocara vitulorum* eggs at day 7 post-infection (control positive group) H&E stain;  B Marked severe degenerative changes of hepatocytes (H), congestion of blood vessels (arrowhead), and edema (E). H&E stain;  C Large cellular granuloma (G) containing larvae (arrowheads) embedded in the hepatic parenchyma (H), marked fibrosis (F) and congestion of blood vessels (arrows). H&E stain;  D Excessive proliferation of fibrous connective tissues (C) of the portal area. Crossmon’s trichrom stain;  E Ivermectin-treated group showing normal central veins (arrowheads), moderate degenerative changes of hepatocytes (H) with no granuloma formation. H&E stain;  F Thymol-treated group showing dilated central veins (CV), moderate improvement of hepatocytes (H) with a granuloma formation (G). H&E stain. Scale bar (A-F) = 200 μm

(Fig. 4E). Dilated central veins and a moderate improvement in hepatocytes with granuloma formation were seen in the thymol-treated group (Fig. 4F).

Discussion

Medicinal plants are extensively used to treat a variety of diseases, including parasitic infections. Intensive scientific research should be conducted to promote the use of herbal medicine and plant-based drugs with antiparasitic properties, as they are less expensive, less toxic, and frequently easier to obtain than commercial anthelmintics. Additionally, the risks associated with the residues of veterinary drugs in milk and other animal by-products emphasize the importance of complementary medicines. Numerous investigators have reported on the use of thymol to treat a variety of parasitic infections (Arafa et al. 2020), but few have mentioned its use in the treatment of *T. vitulorum* infections.

The in vitro study revealed that thymol significantly reduced the rate of *Toxocara* egg development (*P < 0.05*) at concentrations less than 5.0% and completely prevented *Toxocara* egg development at 5.0% and 10.0%. *Toxocara* eggs did not develop normally at ivermectin concentrations (0.625–2.5%), reaching 37.6%–40.0%, but at higher concentrations (5.0% and 10.0%), *Toxocara* egg development was impaired by 24.6% and 18.33%, respectively. Thymol’s effect on *Haemonchus contortus* confirmed the in vitro result, as thymol inhibited larval hatching by 98
percent and killed newly hatched larvae at a rate of 90.8% to 100.0% (Elandaloussi et al. 2013; André et al. 2107). Thymol’s larvicidal activity may be a result of its interaction with the SER-2 tyramine receptor in Caenorhabditis elegans (Lei et al. 2010). This receptor regulates a variety of critical nematode processes, including locomotion, egg laying, and pharyngeal pumping (Smith et al. 2007). These findings provide additional evidence for thymol’s possible interaction with the SER-2 tyramine receptor and its larvicidal activity. It is well established that the monoterpenes in thymol impair the functional and structural integrity of the cell membrane. Due to their high lipophilicity, monoterpenes may readily penetrate the cell membrane, destabilizing the phospholipid bilayer and altering the permeability of the inner and outer mitochondrial membranes of eukaryotic cells, resulting in apoptotic sequelae (Wink 2008).

In the in vivo study, the number of recovered larvae from various organs at day 7 post-infection was significantly lower in the thymol- or ivermectin-treated groups than in the infected untreated rats. Our findings corroborated those of Lai et al. (2005), who confirmed the presence of large numbers of larvae in the liver seven days after infection. Thyme oil significantly decreased the number of Toxocara larvae in the livers of infected rats on day 7 post-infection (Amin and El-Kabany 2013). Our findings are consistent with those of previous researches (Abo-Shehada and Herbert 1984; Esatgil 2007), on ivermectin’s anthelmintic activity against T. canis larvae in mice. The current study discovered a significant increase in the activity of liver enzymes (ALT and AST) in untreated infected rats when compared to negative controls. The increased serum activity of these enzymes is associated with impaired cell membrane permeability and hepatic tissue injury, which appears to be the result of widespread Toxocara larval migration/deposition in the liver parenchyma (Kaushik et al. 1997). These findings corroborate those of Amin and El-Kabany (2013). The activities of the above-mentioned enzymes were significantly reduced in groups treated with thymol or ivermectin compared to the infected untreated group. This could be because of the effect of ivermectin or thymol on larval development. The results indicated a significant decrease in albumin levels in untreated infected rats compared to uninfected controls. Thymol treatment alleviated the effects of Toxocara infection. The observed decrease in albumin concentration could be due to a necrosis-induced decrease in the number of cells responsible for albumin synthesis in the liver (Goldwasser and Feldman 1997). Otherwise, it has been demonstrated that inflammation has a negative effect on albumin synthesis, with albumin gene expression decreasing by up to 90.0% during inflammation (Rothschild et al., 1980). Nitric oxide (NO) is produced by a variety of cell types in response to cytokine stimulation and has thus been discovered to play a role in immunologically mediated protection against an expanding list of parasites (Khabbu et al. 2007). Furthermore, NO is produced by cells other than those involved in immune response, such as hepatocytes and endothelial cells, which play an important role in the life cycle of many parasites. An increase in NO production is harmful to the cells and the tissues around them. Furthermore, activated macrophages produce a lot of NO, which can be metabolized by auto-oxidation to form peroxynitrite. In biological systems, nitric oxide is a major reactive nitrogen species. NO can react with a variety of oxidative molecules, including reactive oxygen species (ROS), molecular oxygen, thiols, and transition metals, to produce various reactive nitrogen species and, as a result, induce nitrosative stress (Soneja et al. 2005).

Nitrosative stress, which attacks biological systems and causes severe damage to biomolecules, results in irreversible changes and the destruction of bio-structures in many organs. The infected groups had a significant increase in NO levels in comparison to the control group. This finding is consistent with the findings of Nassef et al. (2014), who found a significant increase in serum NO of the T. canis infected mice when compared to the control group. These findings are consistent with those of Youdim and Deans (2000), who discovered that both thymol and thyme oil had beneficial effects on the antioxidant state of the rat brain. Thymol possesses exceptional antioxidant properties due to the presence of a phenolic hydroxyl group in its structure, which is known to be a powerful antioxidant capable of reducing the production of reactive oxygen species (Yanishlieva et al. 1999; Venu et al. 2013).

IFN-γ contributes to parasite control by enhancing the ability of phagocytic cells against the parasite (Bankoti and Stager 2012). Furthermore, IFN-γ, a Th1 cytokine, may play an important role in controlling the Toxocara infection. It stimulates IgG2a and IgG3 antibody secretion (Collins, 2016) and improves macrophage differentiation (Martinez and Gordon, 2014). Moreover, IL-4 plays a major role in the humoral immune response against infections of nematode parasites. IL-4 inducing IgE switch and regulation of worm expulsion from the intestines (Clough et al. 2011). IL-4, a Th2 cytokine, stimulates lymphocyte differentiation into plasma cells and promotes the production of specific and protective antibodies (Ho and Miaw 2016), which may be useful in combating Toxocara infection. The results revealed a significant increase in serum IL-4 and IFN-γ levels in the infected untreated group. Helminth infections frequently elicit a strong Th2 response, which is orchestrated by a wide range of cell types and cytokine secretions, including IL-4, IL-5, and IL-13 (Pulendran and Artis 2012; Faz-López et al. 2013). It was discovered that IFN-γ increased gradually with puppyhood age in puppies.
infected with *T. canis* (Torina et al., 2005). The efficacy of toxocariasis control must be evaluated by retention of larvae in the liver and lung, reduction in the hepatic eosinophilic granuloma formation, and lastly, lung resistance to the infection. Cuéllar et al. (2001) and Liljegren et al. (2003) established these criteria.

Histopathological results revealed that rats in the infected group had severe degenerative changes in hepatocytes, blood vessel congestion, and edema. Apart from a large cellular granuloma embedded in the hepatic parenchyma. The current findings were consistent with those clarified by da Silva et al. (2015) and Resende et al. (2015), who demonstrated the infiltration of mononuclear cells (MNCs) and the formation of multiple granulomatous lesions in the liver of *T. canis*-infected rabbits.

Currently, the use of thymol and ivermectin revealed improvement in the histopathological picture of hepatic tissue; the treated groups showed a marked reduction in the number and size of granulomas, as well as the severity of injury. In addition, the lungs of treated groups exhibited goblet cells and mucin secretions in a moderately similar manner to that of the control rats. This improvement in the thymol-treated group could be attributed to thymol’s ovicidal effect on *Toxocara* eggs, as demonstrated in an in vitro study. Thymol also has an anthelmintic effect on larvae (André et al. 2017), as well as antioxidant and anti-inflammatory properties that increase liver and lung tissue resistance to larval migration.

Histopathological examinations of the liver and lungs in experimentally infected rats revealed a massive mononuclear cell infiltration, that was not seen in controls. As a result, the appearance of these cells could be interpreted as a local hepatic tissue reaction to migrating larvae. Similar results were reported by Othman et al. (2013) who detected the presence of CD4 + and CD8 + T lymphocytes within *Toxocara*-induced granulomas and in a widespread inflammatory focus that was distributed in hepatic parenchyma and portal areas and Sommerfelt et al. (2014) who reported periportal and peri-lobular hepatitis in pigs’ livers experimentally infected with *T. cati*. Klockiewicz et al. (2014) added that, these mononuclear cells, including macrophages, are involved in the destruction of parasitic larval stages that have been found dead in the tissue organ. Moreover, focal hepatocytic necrosis and fibrosis were reported in experimentally infected rats. These results agree with those observed in *T. canis* egg-infected mink (Klockiewicz et al., 2014). The presence of focal areas of necrosis and fibrosis may be attributed to a hepatic tissue reaction to disrupted *T. canis* larvae. Furthermore, Amin and El-Kabany (2013) concluded that the proteolytic activities of enzymes produced by *Toxocara* larvae induced moderate to severe histopathological changes in hepatic parenchyma and a mild alteration in kidney and heart.

*Toxocara* larvae were recovered in small numbers from rat livers and lungs. These findings were consistent with those of Amin and El-Kabany (2013), who found a significant reduction in *T. vitulorum* larval count in the livers of experimentally infected rats treated with thyme oil (38.2 percent) on day 7 post-infection. The histopathological examination in the current study revealed a statistically significant reduction in the number and diameter of liver and lung granulomas in the two treated groups when compared to the positive control. In line with these findings, Ismail et al. (2019) investigated the effect of *T. Vulgaris* extract on experimental schistosomiasis and discovered that *T. Vulgaris* extract reduced the number and size of liver granulomatous inflammatory infiltrations when compared to infected untreated animals. They attributed this reduction to the ability of *T. Vulgaris* extract to stimulate the activity of natural killer cells as well as the release of tumor necrosis factor and nitric oxide from macrophages. The current study found that the thymol-treated group had a significant improvement in hepatic and lung tissues, as well as a reduction in the degree of inflammation. These findings agree with those of Abou El-Nour et al. (2005).

**Conclusion**

Thymol treatment could diminish and reduce the negative effect of *Toxocara* hepatopulmonary migration in rats, as evidenced by a lower number of recovered larvae, granulomas, and lesion score in the treated rats’ liver and lungs. Furthermore, in the treated groups, it improves the oxidative status and cytokine release. Finally, thymol could be used as a disinfectant.

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**Authors’ contribution** OS, assisting in the study design, data gathering, biochemical and the statistical analyses. SMA, organizing the study design, collecting data, drafting and final approval of the publication. WMA, operating during the in vivo part of the study. UKM, helped during the in vivo part of the study and achieved the histopathological examination. SAA, co-designing the study, drafting, and final approval of the manuscript. KHH, collect the adult worm from the buffalo calves. MIA, SEA and SSAG, assisted with scientific writing and paper editing.

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**Declarations**

**Conflict of interest** The authors have declared that no competing interests exist.

**Ethical Statement** The ethical standards for animal regulations were followed and approved by Beni-Suef University’s Faculty of...
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