

Assessment 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 *in vitro*. 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 received thymol at a dose of 40 mg/kg; and the fourth group received ivermectin (0.2 mg/kg). Thymol inhibited the development of *Toxocara* larvae within the eggs *in vitro*. Ivermectin; however, 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 ivermectin-treated group than in the infected untreated 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 architectures. Albumin levels were significantly increased in the thymol-treated group. Nitric oxide, IL-4, and IFN- γ levels were significantly decreased in the serum of the thymol- or ivermectin-treated group. 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.

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*, superfamily *Ascaridoidea*). Four species have been identified globally, involving *T. cati*, *T. canis*, *T. vitulorum*, as well as *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 an increased mortality and morbidity in calves, as well as uncompensated stunted growth in the survivors. *T. vitulorum* was found worldwide in the small intestines of domestic cattle and buffalo (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). Human beings are considered an aberrant or accidental host for *T. vitulorum* infections. In man, *Toxocara* larvae do not mature into adult worms (Macpherson 2013). Additionally, humans became infected after consuming embryonated eggs found in soil, contaminated food, or encapsulated *Toxocara* larvae located in improperly cooked meat of paratenic hosts, such as 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 by larval migration, including visceral larval migrans (VLM), ocular larval migrans (OLM), and covert toxocariasis (Magnaval et al. 2001; Roldán et al. 2010).

Numerous medications are currently being used to treat toxocariasis in animals, with some showing promise 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, as humans, is also critical for interrupting the parasite's life cycle (Fok and Kassai 1998). *T. vitulorum* larvae migrate through the liver and lung 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 medicinal properties against several parasites of animal origin, with promising results in managing and breaking the parasites' life cycle. *Thyme* (*Thymus vulgaris*) contains a lot of thymol (20.0–54.0% of the crude plant) (El-Ashram and Abdelhafez2020). Thymol is one of the major components of thyme essential oils and is well known for its anti-microbial and anthelmintic efficiencies, in addition to its superior scolicidal action (Elissondo et al. 2008). The massive fecal egg output in diseased animals, the extreme egg resistance to adverse environmental conditions, the ineffectiveness of the most used anthelmintic medications to combat either larval/adult stages, the potential threat to animal health, as well as the progressive outcomes in man are altogether significant issues contributing to the complexity of human and animal toxocariasis (Aydin et al. 2006). The current study was carried out to assess the protective effect of thymol in controlling zoonotic *T. vitulorum* infections in 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 of ten rats each, and they were housed in plastic cages. Rats were kept in the Laboratory of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, at an ambient temperature of 20-25 °C, with a relative humidity of 55.0%, on a 12 h light-dark cycle, with the lights turned off at 7 p.m. 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 brought from Pharma Swede Ph. Comp. (Egypt).

Embryonation of *Toxocara* eggs

Adult female *T. vitulorum* were obtained from naturally infected buffalo calves brought to veterinary clinics. Gravid female worms were collected and thoroughly washed in 0.85% normal saline before gravid uteri were removed with fine scissors. The eggs were sieved, washed, and precipitated several times with 1.0% formal-saline before being stored in sufficient solution in the refrigerator 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 see how the embryonic cells developed. After about 10 days of incubation, the second-stage larvae began to emerge from these eggs.

***In vitro* effect 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 formol 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% formol 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* effect of thymol and ivermectin on *Toxocara* migration in liver and lungs**

Experimental design

The experiment was divided into four groups of 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 the drinking water at a dose of 40.00 mg/kg body weight for seven days prior to challenge (via stomach tube) (Arafa et al. 2020). For seven days prior to infection, the final group (ivermectin-treated group) received ivermectin (0.2 mg/kg) in the drinking water. All groups (except the negative control group) were inoculated orally with 2500 embryonated eggs per rat via a gastric tube (Barriga and Omar 1992). The experiment was continued until day 7 post-infection, at which point all rats were euthanized via intraperitoneal barbiturate injection (Dutton 2019). Immediately prior to this, blood samples from the medial canthus of the eye were taken for serum biochemical and cytokine analyses. Macro- and microscopically, the liver and lung were examined for larval migration. The ethical standards for animal regulations were followed and approved by Beni-Suef University's Faculty of Veterinary Medicine (BSU/0256/2019).

The protective effect of thymol and ivermectin against *Toxocara* larvae in vivo

Postmortem inspection of internal organs

The liver and lung were minced separately and digested in a pepsin-HCl solution (20 times their volume) (0.7 g pepsin and 0.7 ml HCl in normal saline) for 24 h at 37 °C (Horiuchi et al. 2005). A sieve was used to filter the digested suspension. Centrifuge the filtrate for 2 min at 1,500 rpm. The pellets were then collected and examined for the presence of *T. vitulorum* larvae. At a magnification of 10X, the larvae were counted using a light microscope. The efficacy percentages of thymol and ivermectin treatments were

determined by the number of recovered larvae counted (Amerasinghe et al. 1992), with the following formula:

Efficacy (%) of either thymol or ivermectin treatments = $100 \times (\text{the mean number of recovered larvae in the negative control} - \text{the mean number of recovered larvae in the treated group}) / (\text{the mean number of recovered larvae in the negative control})$.

Biochemical and cytokine parameters

On the seventh day after infection, five rats were bled, and blood was collected. Serum samples were used to determine biochemical parameters. The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the method described by Elissondo et al. (2008). Protein and albumin levels were estimated as previously denoted by Bergmeyer et al. (1985). Serum nitric oxide (NO) value was determined using the colorimetric method as stated by Bories and Bories (1995). Moreover, cytokines IL-4 and IFN- γ levels were measured in the serum of rats using Sandwich ELISA with anti-cytokine antibodies (Stenken JA, Poschenrieder 2014) and following the manufacturer's instructions (Phar Mingen, San Diego, USA).

Histopathological examination

At day 7 after infection, all groups had their liver and lungs removed. The isolated organs were cut into small pieces with a thickness of 2-3 mm and fixed in Bouin's fluid for 72 h. The fixed samples were dehydrated in ethyl alcohol in increasing concentrations, cleared in xylene, and embedded in Paraplast®. Hematoxylin and eosin (H&E) for general histological examination (El-Ashram et al. 2017; El-Ashram et al. 2020), Periodic-acid Schiff (PAS) for mucin detection, and Crossmon's trichrom stain for collagen fiber detection were used to cut and mount μm 4-5 m thick sections on clean and dry glass slides (Suvarna et al. 2019). In addition, the size and number of granulomas in each section of the rat liver and lung were measured in five fields (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 \pm SD. Statistical Package for the Social Sciences 22 was used to perform all calculations (SPSS, Chicago, Illinois, USA).

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 24.66%. In contrast to the negative control, the egg containing developed larvae was significantly ($P \leq 0.05$) 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 (Table 1). Similarly, when compared to the negative control, ivermectin significantly reduced the egg development rate. 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).

Table 1
In vitro inhibitory effect of thymol or ivermectin on *Toxocara* eggs embryonation

Egg status		Egg contains larvae	Early developed eggs	Non developed eggs	IC50 (95% CI)
Treatment Concentrations		Mean \pm Std. Error			
Control -		53.60 \pm 4.58e	21.66 \pm 1.76 ^c	24.66 \pm 2.02 ^a	
Thymol	6.25 mg/mL	21.67 \pm 1.76 ^c	46.3 \pm 1.45 ^e	32.33 \pm 3.17 ^b	0.8 (0.12–2.6)
	12.5 mg/mL	16.33 \pm 2.02 ^b	28.66 \pm 1.48 ^d	57.00 \pm 5.29 ^c	
	25.0 mg/mL	4 \pm 1.7 ^a	12.66 \pm 1.76 ^b	83.33 \pm 1.21 ^d	
	50.0 mg/mL	.00 \pm .00 ^a	10.00 \pm 1.15 ^b	90.00 \pm 1.14 ^e	
	100.0 mg/mL	.00 \pm .00 ^a	.00 \pm .00 ^a	100.00 \pm .00 ^f	
Ivermectin	0.005mg/mL	40.00 \pm .057 ^d	27.66 \pm 1.54 ^d	32.33 \pm 1.20 ^b	0.023 (0.003–0.075)
	0.01	40.33 \pm 2.18 ^d	27.33 \pm 1.76 ^d	35.00 \pm 1.20 ^b	
	0.02	37.66 \pm 0.33 ^d	32.66 \pm 1.02 ^d	29.66 \pm 1.45 ^a	
	0.04	24.67 \pm 1.47 ^c	52.00 \pm 1.15 ^f	23.33 \pm 1.45 ^a	
	0.08	18.33 \pm 0.88 ^b	43.66 \pm .088 ^e	38 \pm 1.52 ^b	

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 organs (liver and lung) 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 mice had a significantly lower number of recovered larvae from the liver and lung than the positive control mice (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.

Table 2
Larval counts in the liver and lung of the treated and control groups at day 7 post-infection in rat model

Organ Group	Liver	Lung	Protection percent
Negative control	-	-	100
Positive control	603	330	0
Thymol-treated	250	110	58.54 (liver) 66.67 (lung)
Ivermectin-treated	203	90	66.33 (liver) 72.73 (lung)

Biochemical and cytokine parameters

In the current study, the positive control had a significant increase in liver enzyme activities (ALT and AST) when compared to the negative control. When compared to the positive control, thymol- and ivermectin-treated rats reduced the negative effects of *Toxocara* on hepatic tissues. When total protein and globulin levels were compared to the other experimental groups, the positive control showed a non-significant decrease. When compared to the positive control and ivermectin-treated groups, the thymol-treated group had a significantly higher albumin value. However, there were no significant differences between the positive and negative controls (Table 3). In the current study, serum NO, IL-4, and IFN- γ levels in the positive control and thymol/ivermectin-treated groups were significantly 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).

Table 3
Serum biochemical parameters in different experimental groups (Means \pm SE)

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

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

Table 4
Serum nitric oxide (NO), IL-4, and IFN- γ levels in different experimental groups (Means \pm SE)

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

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

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. 2D1). 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 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 and D). The negative control had a normal lobular architecture in the livers, with a central vein 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. 4). The most pronounced histopathological picture of the liver in the ivermectin-treated group was moderate degenerative changes of hepatocytes and the absence of granuloma (Fig. 4). Dilated central veins and a moderate improvement in hepatocytes with granuloma formation were seen in the thymol-treated group (Fig. 4F).

Table 5

Effect of thymol or Ivermectin treatment on granuloma numbers and diameters and histopathological changes in rat liver and lung stained with H&E based on scoring severity of injury

Groups	Organs	Lesion type	Negative control	Positive control	Ivermectin-treated	Thymol-treated
Pathological lesions						
Size of granuloma /μm			0	1065.2	315.46	363.66
(Mean \pm SD)				± 55.33	± 22.78	± 23.23
No. of granuloma/microscopic field	Liver		0	2.60	1.10	1.60
				± 0.12	± 0.10	± 0.12
(Mean \pm SD)	Lung		0	5.500	2.30	3.47
				± 0.26	± 0.35	± 0.57
Lesion score	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
	Liver	Congestion	0	3	1	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
Tissue injury in the liver and lung is scored in terms of degree of cell damage as follows: 0 = no change; 1 = < 25% cell damage; 2 = 26–50% cell damage; 3 = 51–75% cell damage; and 4 = 76–100% cell damage.						

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 researchers 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 ($P \leq 0.05$) at concentrations less than 5% 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 90.8–100.0%. Thymol's effect on *Haemonchus contortus* confirmed the *in vitro* result, as thymol inhibited larval hatching by 98.0% and killed newly hatched larvae at a rate of 90.8–100.0% Boubaker (Elandalousi et al. 2013; André et al. 2107). Albendazole and thymol in combination demonstrated increased inhibition efficiency when used to treat experimental alveolar echinococcosis in mice (Albani et al. 2015). 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 group 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 research (Abo-Shehada MN, 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 enzymes were significantly reduced in groups treated with thymol or ivermectin compared to the infected untreated group. This could be because of ivermectin or thymol on larval development. Notably, thymol treatment reduced the severity of inflammation and damage to the liver, as well as the percentage of eosinophils, because of thymol's anti-oxidative and anti-inflammatory properties. 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). The results indicated a significant decrease in albumin levels in untreated infected rats compared to untreated 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 P, 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% 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 (Khambu 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. NO has the potential to be toxic to DNA and may dissociate from hydroxyl radicals, resulting in DNA oxidation/strand breaks (Peng et al. 2003). 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 destruction of bio-structures in many organs. The infected untreated group had a significant increase in NO levels. This finding is consistent with the findings of El-Din et al. (2014), who found a significant increase in serum NO in the infected untreated group 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.

In this study, IFN- γ , a Th1 cytokine, may play important roles in controlling the *Toxocara* infection. IFN- γ stimulates IgG2a and IgG3 antibody secretion and improves macrophage differentiation. Furthermore, 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 2012; Faz-López et al. 2013). It was discovered that IFN- γ increased gradually with puppyhood age in puppies infected with *T. canis* (Cuéllar et al. 2001). 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 that infiltration of mononuclear cells (MNCs) and formation of multiple granulomatous lesions in the liver of *T. canis*-infected rabbits.

The use of thymol or ivermectin improved the liver tissues and histopathological picture. In the treated groups, the number and size of granulomas, as well as the severity of injury, were reduced. Furthermore, the treated groups exhibited goblet cells and mucin in a moderately similar manner to the untreated 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 lung in experimentally infected rats revealed a massive mononuclear cell infiltration, which 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 foci that were distributed in hepatic parenchyma and portal areas and Sommerfelt et al. (2014) who reported periportal and perilobular hepatitis in pig's liver 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 rat. These results agree with those observed in *T. canis* egg-infected mink (Klockiewicz et al., 2019). 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 liver and lung. 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 diameters 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 *T. Vulgaris* extract's ability 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 agreed 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 lung. Furthermore, in the treated groups, the oxidative profile and interleukins were found to be better and less harmful. Finally, thymol could be used as a disinfectant to prevent egg development, lowering the risk of infections, environmental contamination, and cross transmission.

Declarations

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Authors' contribution

Author 1: shearing the conception, design of the study acquisition of data, drafting the manuscript and final approving of the manuscript. Author 2: operating the in-vitro part of the study. Author 2: helping in design of the study acquisition of data, perform the biochemical analysis of data and perform the statistical analysis. Author 3: make the histopathological examination and interpretation of samples. Author 4: helping during the in-vivo part of the study. Author 5: collecting the adult worm from the buffalo calves. Authors 6, 7 and 8: assisting in the scientific writing, editing, and drafting the manuscript.

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Ethics Statement

The ethical standards for animal regulations were followed and approved by Beni-Suef University's Faculty of Veterinary Medicine (BSU/0256/2019). The ARRIVE guidelines 2.0: author checklist is included as a supplementary file.

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Figures

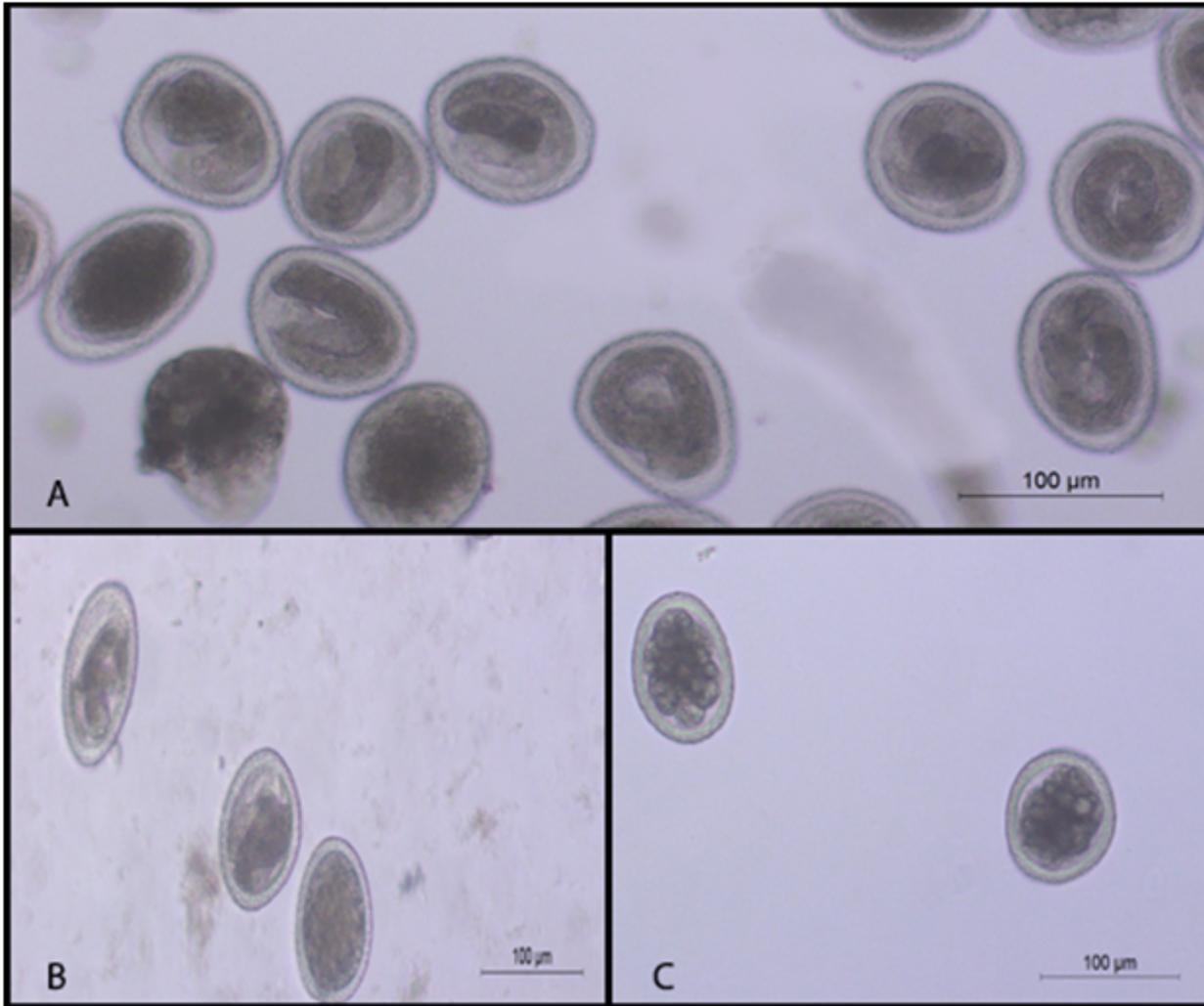


Figure 1

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.

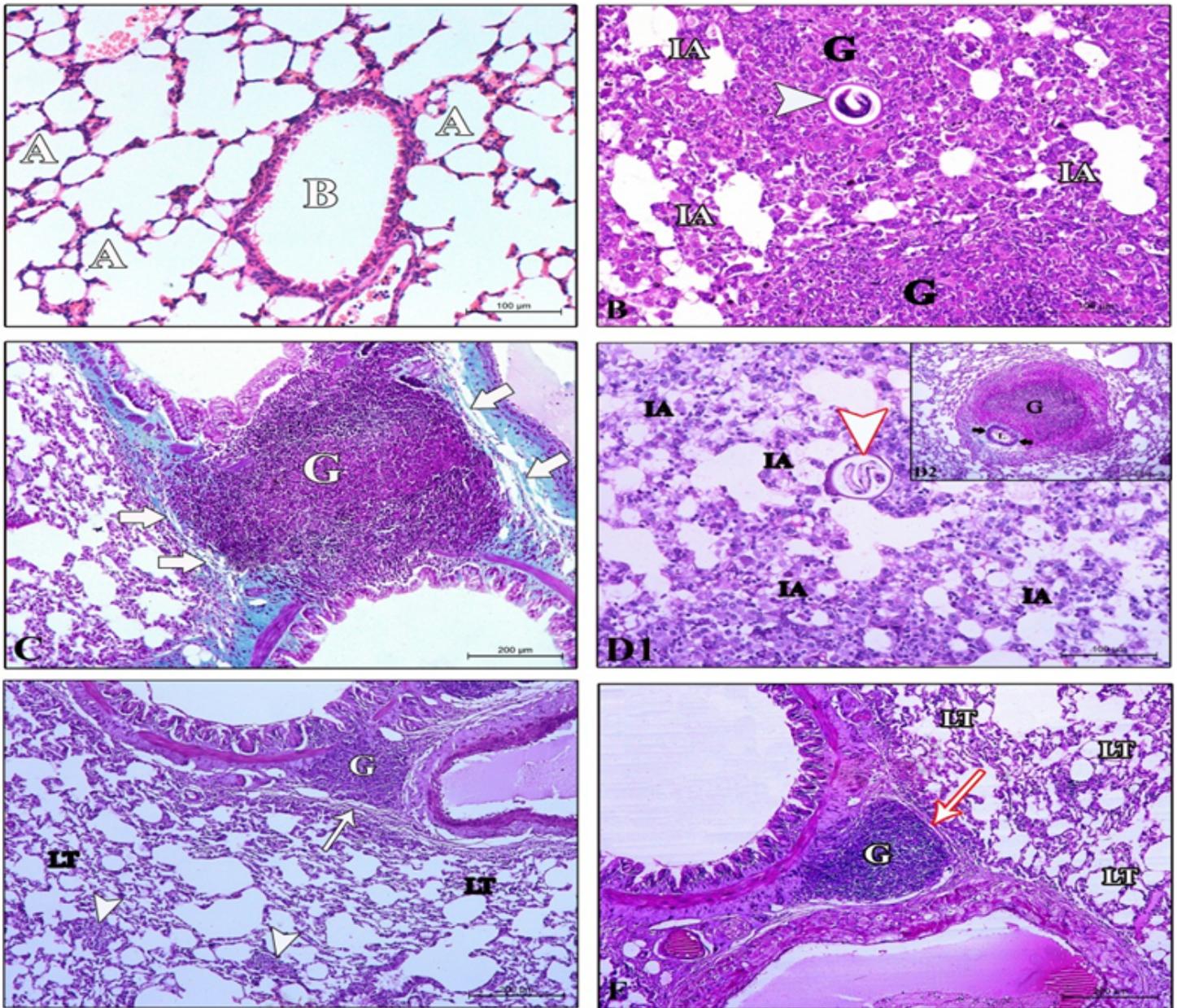


Figure 2

Rat's lung affections: A) Control negative group showing 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 (arrow head) and thickening of the interalveolar wall (IA) due to an intense inflammatory infiltration. H&E stain; C) Large fibrocellular granuloma (G) surrounded by a collagenous connective tissue capsule (arrows). Crossmon's trichrom stain; D1) *T. vitulorum* larvae (arrow head) 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 *T. 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 (arrow heads)

within the lung parenchyma without *T. vitulorum* larva and moderate improvement of lung tissues (LT). H&E stain; F) Thymol- treated group showing a medium-sized peri-bronchial fibrocellular granuloma (G) surrounded by a fibrous capsule (arrow), and moderate improvement of lung tissues (LT). H&E stain. Scale bar: 100µm, 100µm, 200µm, 100µm, 200µm, 200µm, 200µm respectively.

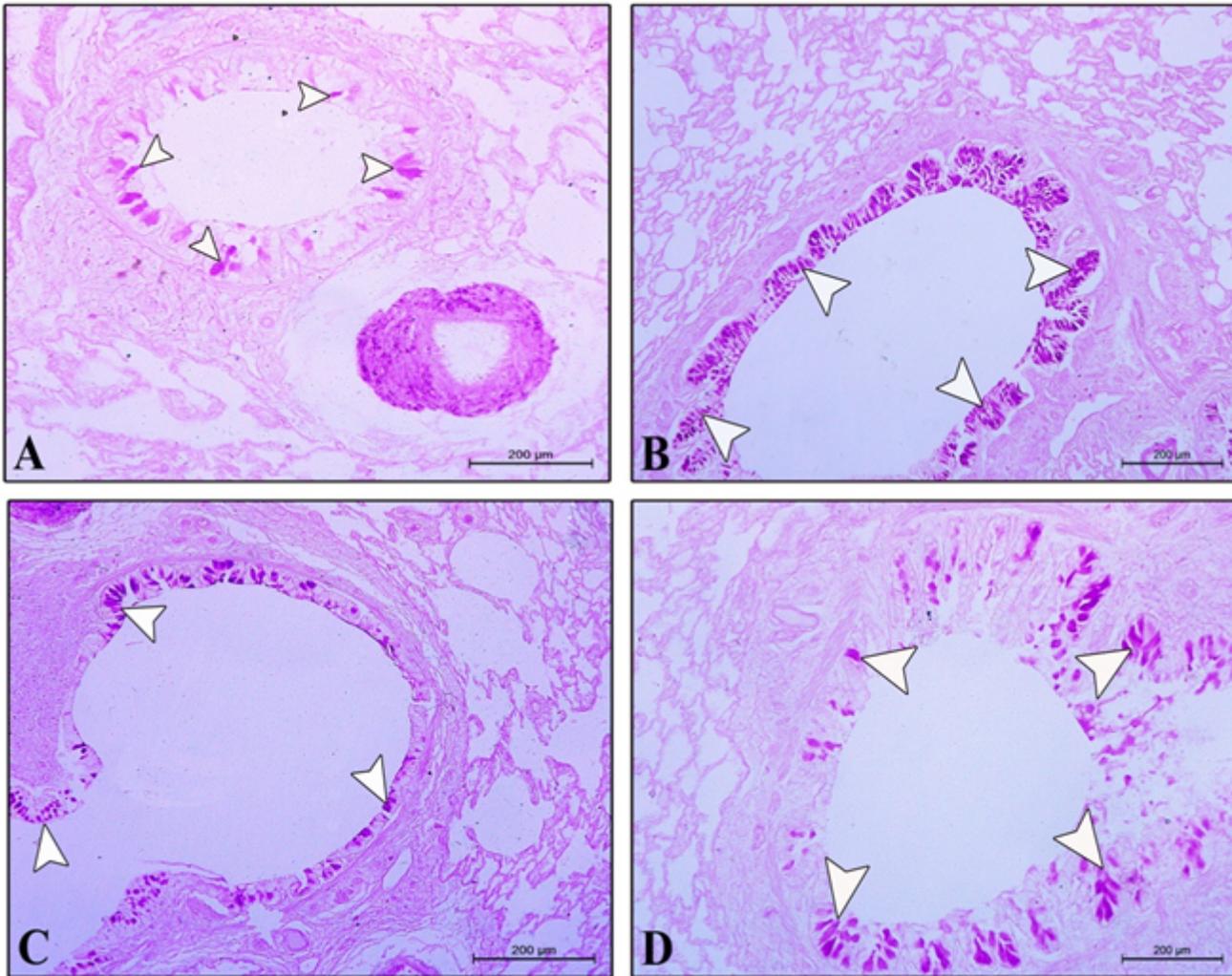


Figure 3

A photomicrograph of rat's lungs of: A) Control negative group showing normal distribution of goblet cells (arrow heads) within the bronchial epithelium; B) Control positive group showing a marked metaplasia of bronchial goblet cells (arrow heads) with an excessive mucin production, C) Ivermectin - treated group showed a marked subsiding of bronchial goblet cells (arrow heads). D) Thymol -treated group showing a moderate number of bronchial goblet cells (arrow heads), PAS Stain: Scale bar (A-E) = 200µm.

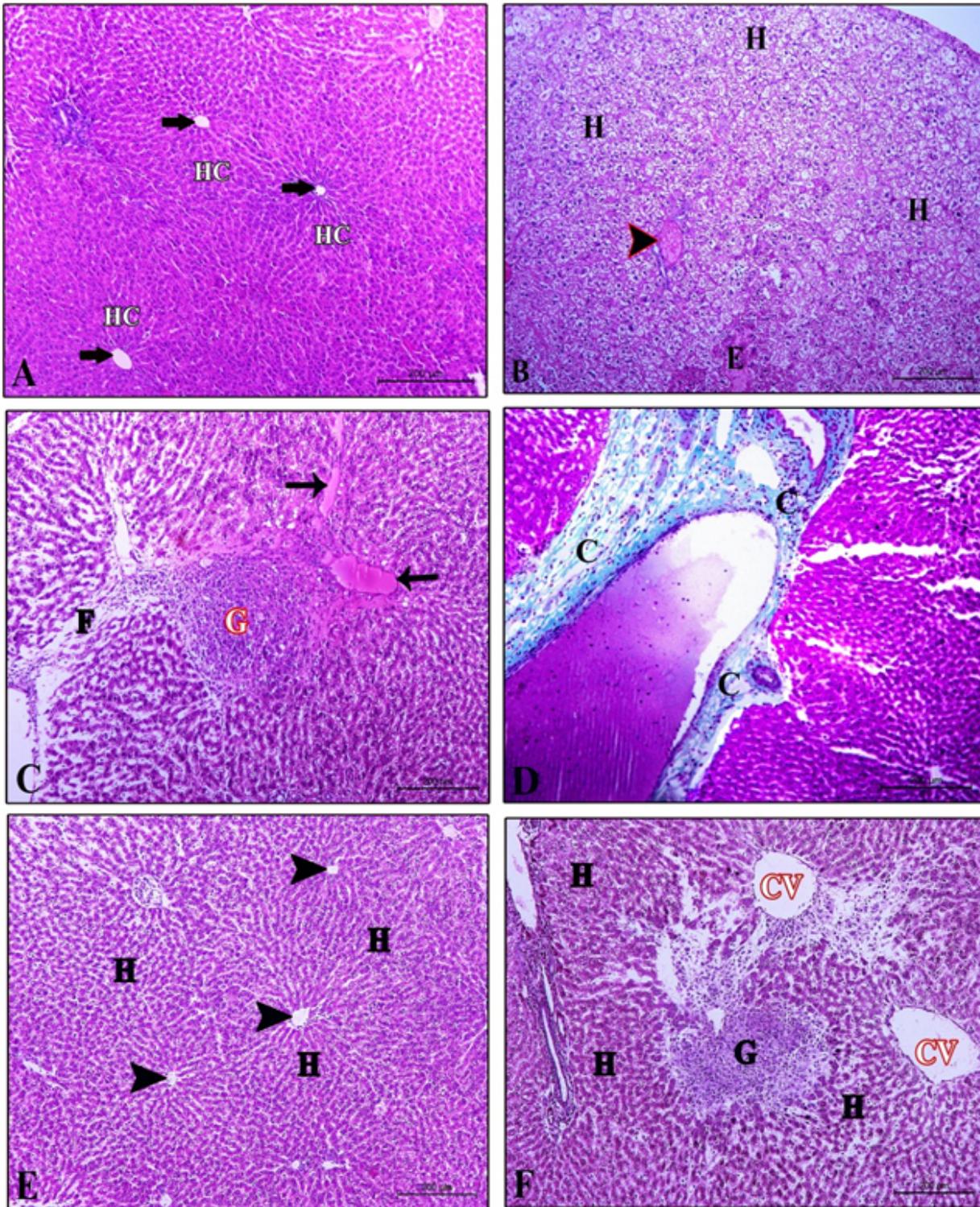


Figure 4

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 *T. 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 (arrow head), and edema (E). H&E stain; C) Large cellular granuloma (G) embedded in the hepatic parenchyma, marked fibrosis (F), and congestion of blood vessels (arrows). H&E

stain; D) Excessive proliferation of fibrous connective tissues of the portal area. Crossmon's trichrom stain; E) Ivermectin-treated group showing central veins (arrow heads), 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) =200µm.