Veterinary Integrative Sciences 2025; 23(1): e2025028-1-23. DOI: 10.12982/VIS.2025.028

Veterinary Integrative Sciences
ISSN: 2629-9968 (online)

Research article

Therapeutic role of Thymol, Propolis and *Balanites aegyptiaca* against experimental *Toxocara vitulorum* infection

Huda Mohammed Kuraa[1,*], Safaa Sayed Malek[2], Iman Abdel-Raheem Mahmoud Abdel-Rahman[3] and Abeer H.M. El Hendy[4]

[1] Parasitology Department, Animal Health Research Institute, Agriculture Research Center, Ministry of Agriculture, Assiut Governorate 71511, Egypt.
[2] Infectious Diseases, Department of animal Medicine, Faculty of Veterinary Medicine, Assiut University, 71511. Egypt.
[3] Department of Pharmacognosy, Faculty of Pharmacy, South Valley University, Qena 83523, Egypt
[4] Pathology Department, Animal Health Research Institute, Agriculture Research Center, Ministry of Agriculture, Assiut Governorate 71511, Egypt.

Abstract

Toxocara vitulorum (*T. vitulorum*) is a significant intestinal nematode that has led to a large global economic loss for farm animals. While there is a paucity of safe and effective anthelmintic drug development against it. Thus, this work aimed to estimate the antiparasitic effect of Thymol, *Balanites aegyptiaca* (*B. aegyptiaca*), and propolis as an alternative treatments against *T. vitulorum* larvae in vivo. To achieve this purpose, the embryonation of *T. vitulorum* eggs were done in vitro. Then, a total of thirty female wester rats were divided into six groups (5 each). Infected rats were orally inoculated by 2500 eggs containing third stage larvae. These groups were (control negative), (control positive), treated with (piperazine citrate 300 mg/kg), (thymol 40 mg/kg), (*B. aegyptiaca* 250 mg/kg), and (propolis 100 mg/kg). Parasitological and histopathological responses of all treated groups were evaluated at 7 and 14 days post infection (dpi). Microscopical examination of lung, liver (pepsin digested organs) and brain (squashed method) were used for counting of larvae. The numbers of larvae reduced at 14 dpi and accomplished the highest reduction in Thymol group (75%) followed by Propolis group (55.8%) and *B. aegyptiaca* group (53.8%) than in control positive group. Histopathological examination proved that thymol followed by propolis and *B. aegyptiaca* extracts were effective in improving the pathological changes and had a good effect on organ damage protection that was caused by *T. vitulorum* larvae. So, they can be used as an alternative, safe, and effective treatment of *T. vitulorum* infection.

Keywords: *Balanites aegyptiaca*, Histopathology, Propolis, Thymol, *Toxocara vitulorum*

[*]**Corresponding author**: Huda Mohammed Kuraa, Parasitology Department, Animal Health Research Institute, Agriculture Research Center, Ministry of Agriculture, Assiut Governorate 71511, Egypt. E-mail: huda5380@yahoo.com.

Article history; *received manuscript: 18 March 2024,*
revised manuscript: 10 May 2024,
accepted manuscript: 8 June 2024,
published online: 24 June 2024,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Toxocariasis was listed by the "US Centers for Disease Control and Prevention (CDC)" as one of the most five parasite infections that people ignore (Holland, 2017). It is a wide pervasive zoonotic disease originated by nematodes (Gasser, 2013). Of all *Toxocara* species, *Toxocara vitulorum* (*T. vitulorum*) has the least zoonotic importance (Moyo, 2002). Human beings are deemed an accidental host for *T. vitulorum* infections, in which its larvae never mature into adult worms (Macpherson, 2013). Humans are infected through the ingestion of eggs containing third stage larva from a variety of environmental sources polluted with host feces (Ma et al., 2018). Furthermore, the existence of *T. vitulorum* larvae in milk is a risk agent for visceral larvae migrans as a result of drinking unpasteurized milk from diseased animals (Glickman et al., 1987).

Toxocariasis in children is more common than in adults due to a lack of personal hygiene (Carvalho and Rocha, 2014). Larvae of *T. vitulorum* migrate through the lung and liver of paratenic hosts and subsequently spread to other organs (brain, kidney, and muscles) (Shehata et al., 2022). Then it encysted in different body organs and tissues for months or years (somatic migration), leading to ocular larval migrans (OLM), visceral larva migrans (VLM), neurotoxocariasis, and covert toxocariasis (Ma et al., 2018). *Toxocara vitulorum* is a species of nematode Toxocaridae that inhabits cattle and buffaloes small intestines worldwide and is especially common in subtropical and tropical geographical areas (Roberts, 1990; Raza et al., 2013; Venjakob et al., 2017; Dewair and Bessat, 2020). Young animals are more susceptible to the infection that causes one of the most economically important diseases of young calves (1–3 months old) (Singh et al., 2008; Amin and El-Kabany, 2013). In Egypt, *T. vitulorum* infection was widespread in buffalo and cattle calves and deemed a common clinical problem resulting in economic losses (Sultan et al., 2015). It is transmitted from the dam to the calf through colostrum milk as a main source of infection, and then calves start to shed the eggs in feces at day 22 after birth (Srivastava and Sharma, 1981; Roberts et al., 1990; Aydin et al., 2006; Avcioğlu and Balkaya, 2011) causing severe harm to the intestinal mucous membrane (Singh et al., 2008). This infection has been associated with increased morbidity and mortality in calves, along with stunted uncompensated growth in the survivors (Shehata et al., 2022).

Dewair and Bessat (2020) discovered *T. vitulorum* infection in the colostrum milk of buffaloes and cows through the postparturient period. The wall of the small intestines of calves is penetrated by larvae “2–8 hours” later and goes direct to the liver through the portal vein, whereas a few arrive at the mesenteric lymph nodes. Then, the infection propagates to other body organs, including the lungs, brain, kidneys, muscles and peripheral lymph nodes (Rizk et al., 2018). In general, *T. vitulorum* infection is often exhibited by diarrhea, constipation, pica, poor growth rate, bad performance, and intestinal obstruction as a result of heavy infestation (Rizk et al., 2018). The infection is subclinical, although heavy infections by a huge number of worms lead to diarrhea and severe enteritis (Roberts, 1990). Without a correct diagnosis and suitable treatment, high mortality rates in bovine calves cause severe economic losses (Dewair and Bessat, 2020). The huge fecal egg production in diseased animals, the extreme resistance of eggs to adverse ecological conditions, and the most commonly utilized anthelmintic drugs were ineffective in combat either larval or adult stages, that led to a probable threat to animal health (Aydin et al., 2006). So, searching for more safe and effective treatments against the *Toxocara* infection is an urgent issue.

Toxocariasis therapy is established largely on chemical drugs (Hassan, et al. 2016) which create severe side effects (resistant parasites, chemical residues in host tissues and environmental pollution). Such problems diverted the attention of researchers to the development of alternative approaches for treatment (Iqbal et al., 2003). While, El-Nahas et al. (2020) mentioned that there is no satisfactory drugs

against toxocariasis till now. Currently, there are several medications that are used to treat animal toxocariasis, with some showing potential against adult worms. However, none have eradicated the tissue's larval stages of *Toxocara*, which are a main source of vertical transmission, and treatment of larvae in paratenic hosts is also essential to interrupting the life cycle of the parasite (Shehata et al., 2022).

Phytotherapy is well-defined as a treatment created with plants and attracts the attention of researchers as an alternate treatment in recent years (Duru et al., 2023). Thyme "*Thymus vulgaris* (*T. vulgaris*)" comprises a lot of thymol (20–54% of the crude plant) which is one of the main constituents of thyme essential oils and has anthelmintic and anti-microbial efficiencies (Kowalczyk et al., 2020). Also, *Balanites aegyptiaca* is a member of family Zygophyllaceae and is popularly well-known as “desert date” that reflects its edible fruits and could be of value in helminthes elimination (Shalaby et al., 2012; Murthy et al., 2020). Propolis is a resinous substance that collected by bees from numerous plant sources, which is a gummy, sticky, and balsamic substance; bees use it to seal the bee-hive holes. It is utilized in traditional medicine and has at least 300 chemical composites (Hossain et al., 2022). Its composition varies according to plant source, geography, seasons, climate, and environmental circumstances (Hossain et al., 2022). Moreover, it showed anthelmintic activity against the adult worm of *Fasciola gigantica* (Hegazi et al., 2007).

The present study was carried out to evaluate the parasitological and pathological effects of the Thymol, *B. aegyptiaca* methanolic extract, Propolis ethanolic extract as alternative treatments of *T. vitulorum* in the experimentally infected rats.

MATERIALS AND METHODS

Ethics Statement

The ethical standards for animal regulations were followed and approved by Faculty of Veterinary Medicine, Assiut University (06/2023/0137).

Collection of *T. vitulorum* adult worms

It was collected from naturally infected cattle calve feces (2-months old) who administered a dosage of an anthelmintic (piperazine citrate) and purgative (magnesium sulphate). The adult worms that were expelled in the feces were immediately collected and transferred in 0.9% physiological saline to the laboratory (Venjakob et al., 2017). Then washed for removal of any adherent fecal materials for several times and the adult worms were differentiated females from males based on their morphological features (Soulsby and Mönnig, 1968). Females were sustained as a source of eggs and the males were discarded laterly (Hassan et al., 2016; Dewair and Bessat, 2020).

Collection of *T. vitulorum* eggs and embryonation

The eggs were extracted by slit-cutting along the uterine regions of worms (Fan and Su, 2004) and left in distilled water-filled plastic containers at room temperature (28˚C ± 4˚C) overnight. The eggs were released by gently squeezing the worm's body using smooth-toothed forceps. After that, egg samples were examined microscopically to check for the extracted eggs quality. For embryonation, the eggs were sieved, washed in distilled water 2–3 times and then precipitated by centrifugation at (800 rpm for 5 minutes) several times. Then the eggs were incubated in 50 ml falcon tubes with distilled water at temperature (28˚C ± 4˚C) with the size of water to the egg mass not more than 5:1. Then, the water changed and aerated every 2 days. Incubation was continued for 4 weeks with periodical evaluation of eggs with light microscope for embryonic development (Dewair and Bessat, 2020).

Piperazine citrate

The commercial anthelmintic piperazine citrate (100%) was brought from (El-Road for Chem. & Med. Preps. Company Egypt).

Thymol

The pure crystallized thymol (Prolabo-N°de code 20728.23, RHÔNE-POULENC- 12. Rue Pelée. 75- Paris) were used. Purified thymol was dissolved in 10% Dimethyl sulphoxide LR (DMSO) (ALPHA CHEMIKA – INDIA) as an emulsifier (Shehata et al., 2022).

Preparation of *Balanites aegyptiaca* methanolic extract

Fresh fruits of *B. aegyptiaca* were gathered from a cultivated field nearby "South Valley University". Identifying the collected fruits was conducted and a voucher specimen of the plant (code: Ba.50) was preserved in the herbarium "Department of Pharmacognosy, Faculty of Pharmacy, South Valley University, Egypt".

The fruits were washed thoroughly to remove any debris; The kernels of fruits were discarded and the remaining mesocarp were dried up at room temperature prior ground to giving a fine powder. Then, the extract was prepared via maceration (500 gm of dried mesocarp were soaked in methanol 90% for 72 h) and then subjected to the extraction in "Soxhlet apparatus" till complete exhaustion. After that, the extract was filtered using a filter paper (Whatman No.1), and evaporated by a rotary evaporator at 40°C under reduced pressure. Then, the solvent-free residue was kept at 4°C for consequent preparation of the required doses (Shalaby et al., 2010).

Preparation of propolis ethanolic extract

50 grams of Bee Propolis (Imtenan Healthy Shop, Obour City, Egypt) was extracted using 250 ml of 80% ethanol for two days while being stirred frequently, then filtration. The extraction process was then repeated three times. After that, the alcoholic extract evaporated underneath vacuum at 40°C, until it was completely dry. The solvent-free residue was kept at 4°C until the required dosage was prepared (Hassan et al., 2016).

Experimental design and protocol

Thirty female wister rats, 6-8 weeks, weighing 120-150 g were obtained and reared in the animal house of "Faculty of Medicine, Assiut University". Rats were divided into six groups (each of five rats) and were placed in cages. These rats were kept at a surrounded temperature (20-25°C), with a relative humidity of 55%, with the lights turned off at 7.00 PM and a 12 hour light-dark cycle. The animals were nourished by standard food and water under appropriate environmental conditions (Shehata et al., 2022). The rats were treated and handled in accordance with "international guidelines and ethical committee of Faculty of Veterinary Medicine".

All groups except the negative control group were inoculated orally with 2500 embryonated *T. vitulorum* eggs which suspended in (0.5 mL) saline solution through a gastric tube to each rat (Shehata et al., 2022), followed by the administration of (0.5 ml) distilled water to the rats in order to flush out any remnant eggs in the syringe (Llanes et al., 2019; Shehata et al., 2022). During the experiment, a negative control group was given orally (one ml) of distilled water. The rats of positive control group were infected without treatment. The rats administrated piperazine citrate (300 mg/kg Bw) in the drinking water (piperazine citrate treated group). The rats received thymol (40 mg/kg Bw) in the drinking water (thymol treated group) (Shehata et al., 2022). The rats given propolis (100 mg/kg Bw) in the drinking water (propolis treated group) (Hassan et al., 2016). The rats received *B. aegyptiaca* (250 mg/kg Bw) in the drinking water (*B. aegyptiaca* treated group) (Shalaby et al., 2012). All treated groups received treatment once daily for 7 sequential days beginning

from the first day of infection (El-Nahas et al., 2020). This experiment was sustained till day 14 post-infection.

Collection and processing of samples

The rats were euthanized “2 rats from each group on days 7 post infection” and “3 rats from each group on days 14 post infection” and the tissues samples (lungs, liver and brain) were gathered for parasitological and histopathological inspections (Llanes et al., 2019). Each sample of liver, lungs was divided into two parts, one part for artificial digestion and the other part for histopathological examination (Cardillo et al., 2009).

Parasitological examination

Each liver and lungs samples were minced and digested completely. Subsequently, the tissues were placed into the digestive solution (pepsin, 5 g; Hcl 37%, 10 ml in 1,000 ml/water) "Pepsin 1:3000 ex. Porcine Stomach Mucosa, 0.8 Anson U/mg. Batch No 8993790 (Sisco research laboratories Pvt. Ltd., India)" and preserved at 37°C overnight to recover the residual larvae. The digested suspension was sieved using a filter. The Sedimental liquid was centrifuged at 1,500 rpm for 2 min. The supernatant was discarded, leaving only about 2 ml of the sediments which mixed thoroughly and the samples were examined microscopically (100 x magnification) for the existence of larvae (Zibaei et al., 2010; Llanes et al., 2019). Each brain was divided into two parts for parasitological and histopathological observations. The brain tissues were cut into small portions and without any digestion squashed between two slides, and larvae were examined and counted directly via light microscope (100 x and 400 x magnification) (Llanes et al., 2019; El-Nahas et al., 2020).

The percentage of larval reduction after treatment was calculated in accordance with the following formula: $\text{Reduction } (\%) = \frac{C - T}{C} \times 100$ (El-Nahas et al., 2020) (C is the number of recovered larvae from positive control group and T is the number of recovered larvae from treated group).

Histopathological examination

At 7 and 14 days post-infection, rats of each group were sacrificed and necropsied and the organs (liver, lungs and brain) examined grossly for pathological lesions. Then, the tissues specimens were taken from each rat in groups then were processed and stained with “Hematoxylin and eosin (H&E)” for histopathological inspection according to (Bancroft and Gambl, 2008). This inspection using light microscope (10X, 40X objective lens) to evaluate the effects of the different treatments on the histopathological picture (Cardillo et al., 2009; Shehata et al., 2022). Examination of histological slide preparations of lung, liver, and brain was performed using a light microscope with (10x objective lens) of five different fields of view for each sample to score histopathological lesions (Gibson-Corley et al., 2013).

Statistical analysis

The statistical analysis was performed using “One-way analysis of variance (ANOVA)”, followed by the “Tukey post-hoc test”. P values “less than 0.05” were considered significant difference between treated groups via “the statistical software SPSS (Version 16; SPSS Inc., Chicago, USA)” (Shehata et al., 2022).

RESULTS

The adult worms were identified as *T. vitulorum* depending on its morphological characters and distinguished into male adult worms (17 – 21 cm length) with the mean ± SD were (19.00 ± 2.83) and female adult worms (27 - 31

cm length) with the mean ± SD were (26.25 ± 3.78) (Figure 1). The extracted eggs from adult females were (ovoid to spherical) with average measured 75 × 81.4 µm (70.2×80.6 µm to 83×84.8 µm) with the mean ± SD were (74.95 ± 4.52 × 81.4 ± 3.21) and revealed successful embryonic development in the laboratory conditions, wherein eggs developed from unembryonated stage to eggs containing fully developed larvae passing through different embryonic developmental stages that recognized inside the eggs with microscopic examination. Some larvae were emerged naturally from a tiny hole in the eggshell (Figure 2).

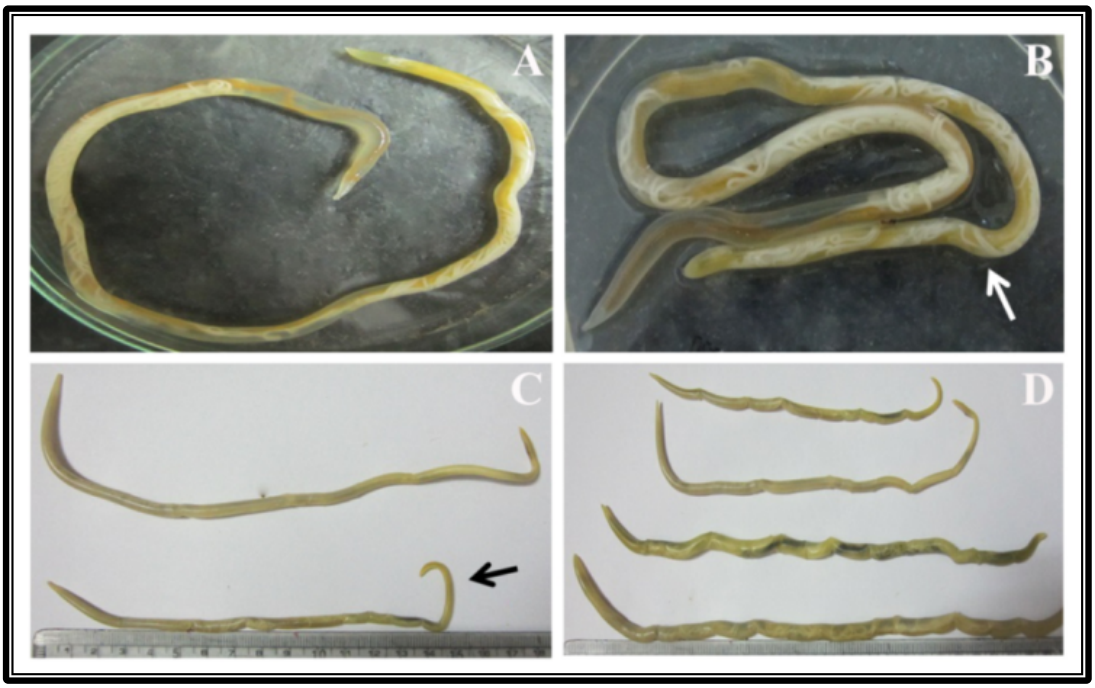

Figure 1 Showing *Toxocara vitulorum* adult worms collected from naturally infected calves. White arrow: adult female, black arrow: adult male.

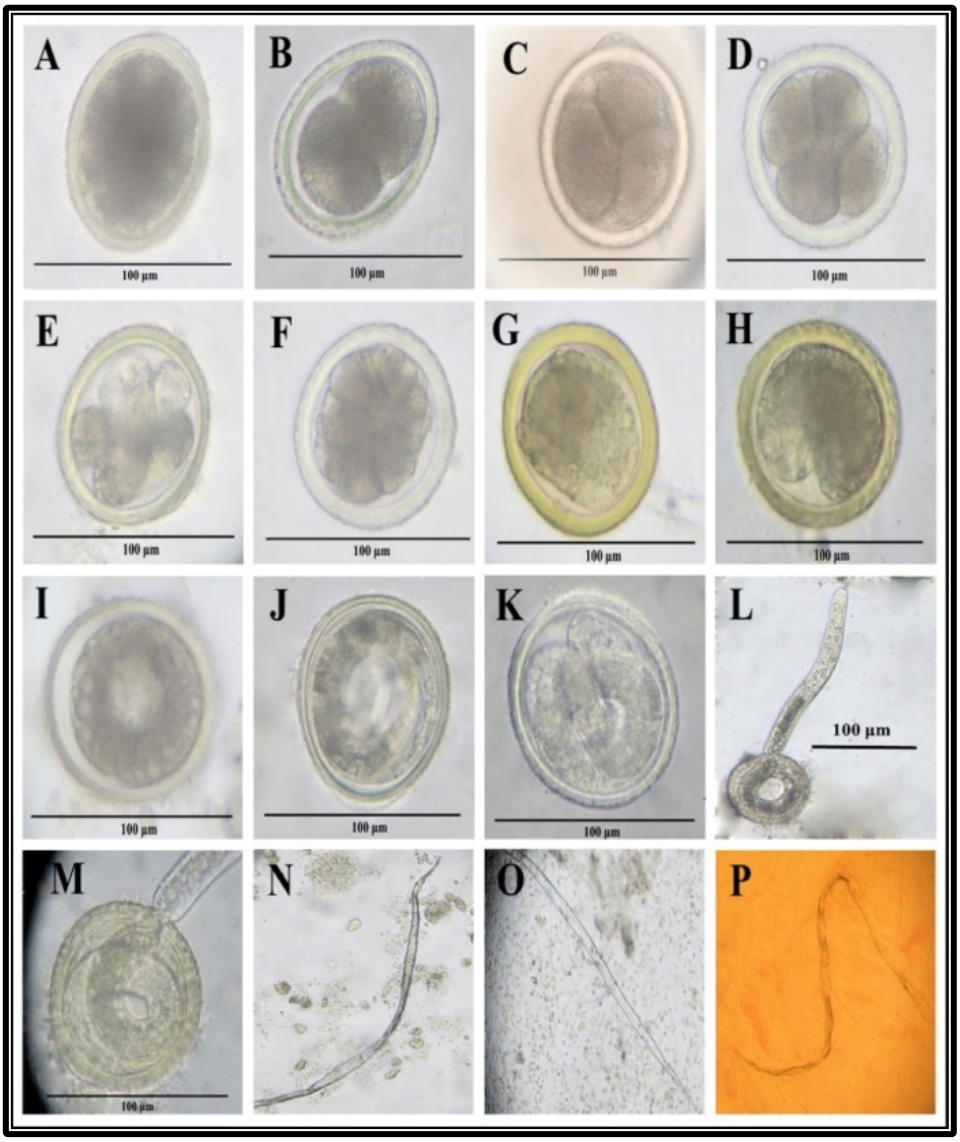

Figure 2 Showing *Toxocara vitulorum* eggs developmental stages: (A) One cell, (B) Two cell, (C) Three cell, (D) Four cell, (E) early-morula, (F) late-morula, (G) blastula, (H) gastrula, (I) The embryo becomes long enough allowing the two ends of the "U" to meet each other and the embryo forms a close ring, (J) Second-stage larva, (K) Third stage larva, (L, M) Larva naturally emerged from a small hole in the egg shell and (N, O, P) *T. vitulorum* larvae from infected rats tissues, (N) A larva in the liver (artificial tissue digestion), (O) A larva in the lung (artificial tissue digestion) and (P) A larva in the brain (squash method).

At day 7 post infection, the control positive group revealed 41 larvae in lung and liver. With introduction of all kinds of treatments at day 7 post infection, the larval number decreased and achieved the highest reduction in Thymol group (61%) followed by Propolis group (24.4%) and *B. aegyptiaca* group (9.8%). Whereas, no reduction in larvae in piperazine citrate group. On the other hand, only one larva was found in the brain of *B. aegyptiaca* group. There was significant difference between larval recovery in lung, liver and brain at day 7 post-infection between control positive group and Thymol group, *B. aegyptiaca* group and Propolis group (P = 0.00) with no significant difference between control positive group and piperazine citrate group (P = 1.00) (Table 1).

At day 14 post infection, the maximum number of *T. vitulorum* larvae was 52 that observed in the lung and liver and brain of control positive group. While, the

larval number reduced and achieved the highest reduction (84.6%) in piperazine citrate group followed by Thymol group (75%), Propolis group (55.8%), and *B. aegyptiaca* group (53.8%). The larvae numbers declined from the examined liver samples. Whereas, larvae were observed in some brain tissues examined in all groups except the Propolis group. There was significant difference between larval recovery in lung, liver and brain at day 14 post-infection between control positive group and piperazine citrate group, Thymol group, *B. aegyptiaca* group and Propolis group (P = 0.000). No significant difference was found between *B. aegyptiaca* group and Propolis group (P = 0.738) and the number of recovered larvae was recorded in (Figure 2, Table 2).

Table 1 Effects of alternative treatments on experimentally infected two rats with *T. vitulorum* embryonated eggs at day 7 post infection

Groups	No. of larvae recovered from			Total No. of larvae	Reduction percent (%)		
	Lung	Liver	Brain		Lung	Liver	Total
Control positive group	30^{a}	11^{a}	0	41^{a}	0	0	0
Piperazine citrate group	26^{b}	15^{b}	0	41^{a}	13.3	0	0
Thymol group	13^{c}	3^{c}	0	16^{b}	56.7	72.7	61
Balanites aegyptiaca group	16^{d}	20^{d}	1	37^{c}	46.7	0	9.8
Propolis group	15^{cd}	16^{b}	0	31^{d}	50	0	24.4

^{a,b,c,d}: Different superscript letters in the same column indicate significantly different values for a given parameter ($p < 0.05$ by post hoc Tukey's test) (P = 0.00 and 0.03 of Lung; P = 0.00 of Liver; P = 0.00 of total larvae number); same superscript letters in the same column indicate not significantly different values for a given parameter ($p > 0.05$ by post hoc Tukey's test) (P = 0.74 and 0.18 of Lung; P = 0.74 of Liver; P = 1.00 of total larvae number).

Table 2 Effects of alternative treatments on experimentally infected three rats with *T. vitulorum* embryonated eggs at day 14 post infection

Groups	No. of larvae per gm recovered from			Total No. of larvae	Reduction percent (%)		
	Lung	Liver	Brain		Lung	Liver	Total
Control positive group	43^{a}	8^{a}	1	52^{a}	0	0	0
Piperazine citrate group	5^{b}	2^{b}	1	8^{b}	88.4	75	84.6
Thymol group	6^{b}	6^{a}	1	13^{c}	86	25	75
Balanites aegyptiaca group	20^{c}	2^{b}	2	24^{d}	53.5	75	53.8
Propolis group	17^{d}	6^{a}	0	23^{d}	60.5	25	55.8

^{a,b,c,d}: Different superscript letters in the same column indicate significantly different values for a given parameter ($p < 0.05$ by post hoc Tukey's test) (P = 0.00 and 0.03 of Lung; P = 0.00 of Liver; P = 0.000 of total larvae number); same superscript letters in the same column indicate not significantly different values for a given parameter ($p > 0.05$ by post hoc Tukey's test) (P = 0.74 of Lung; P = 0.18 and 1.00 of Liver; P = 0.74 of total larvae number).

The gross examination of rats at 7 dpi revealed congested lungs and liver in control positive group and piperazine treated group while liver and lungs of all other infected groups appeared nearly normal in color and texture. While, inspection of necropsied rats at 14 dpi revealed normal lungs and liver in control negative group (Figure 3A) and mild congested lungs and enlarged congested liver in control positive group (Figure 3B). While, there is mild congested lungs and mild dark liver in piperazine treated group (Figure 3C) and mild congested lungs and nearly normal

liver of thymol treated group (Figure 3D). In *B. aegyptiaca* treated group showed focal hemorrhagic area on lungs with normal liver colour (Figure 3E) and in Propolis treated group, the lungs and liver were nearly normal in colour (Figure 3F).

The histological picture of rat's lungs of control negative group at 7 and 14 dpi, showed normal lung alveoli and bronchioles (Figure 4A, B). In control positive group at 7 dpi, the larvae detected nearby congested vasculature and associated with eosinophilic reaction, lymphocytes and macrophages with collapsed alveoli and thickening of inter alveolar septa (Figure 4C). While, at 14 dpi, the cross section of larvae found surrounded by eosinophils, peribronchial granulomatous reaction and degenerative changes of bronchial epithelium (Figure 4D). In piperazine treated group, the lungs tissues were remarkable eosinophilic granulomas with no recognizable larvae at 7 dpi (Figure 4E). But, at 14 dpi, the larvae detected and surrounded by eosinophilic reaction with peribronchial lymphocytic infiltrates and hyperplasia of bronchiolar epithelium (Figure 4F, Table 3).

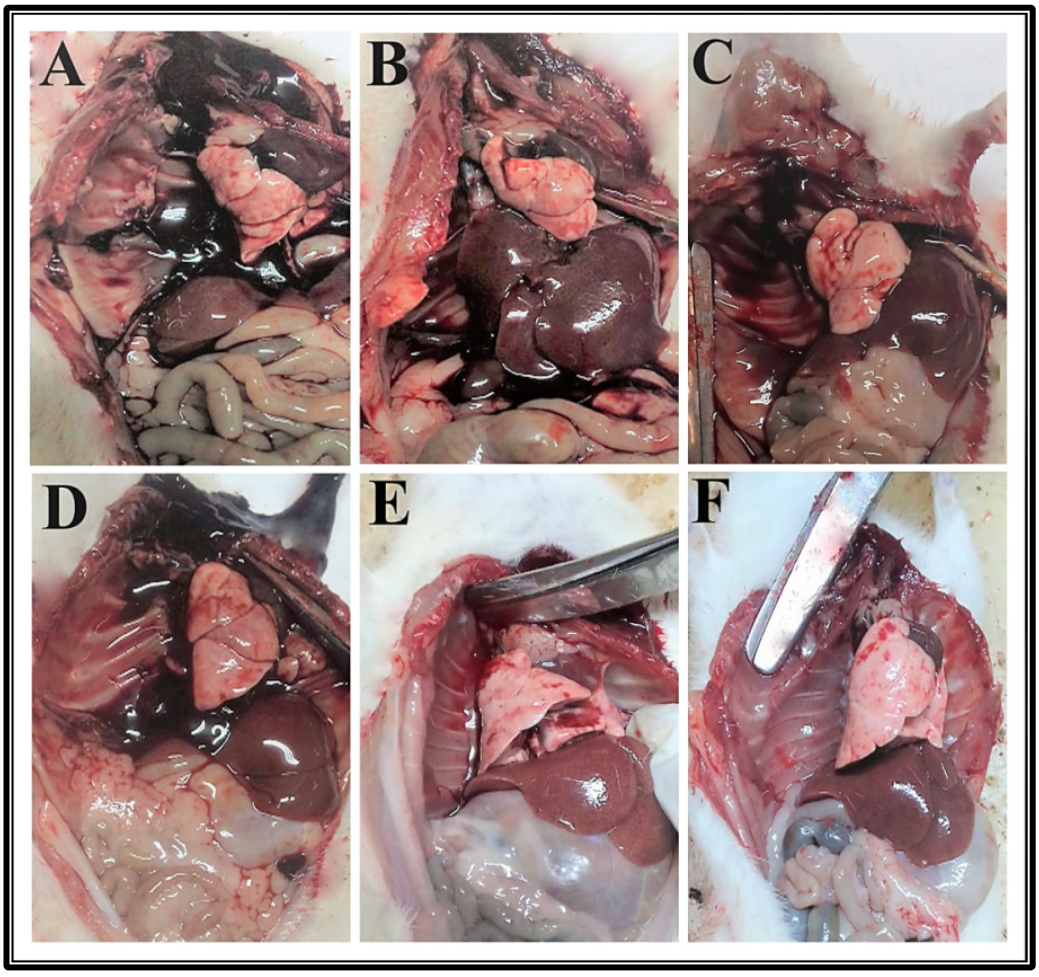

Figure 3 Macroscopic pictures of rats necropsy in different groups at 14 dpi showing: (A) Control negative group: normal lungs and liver. (B) Control positive group: mild congested lungs and enlarged congested liver. (C) Piperazine treated group: mild congested lungs and mild dark liver. (D) Thymol treated group: mild congested lungs and nearly normal liver. (E) *Balanites aegyptiaca* treated group: focal hemorrhagic area on normal lungs and normal liver colour. (F) Propolis treated group: nearly normal lungs and liver.

The rat's lungs of Thymol treated group at 7 dpi, the larvae detected and surrounded by eosinophil with lymphocytic infiltration and hyperplasia of bronchiolar epithelium (Figure 4G). Also, at 14 dpi, the larvae noticed with

lymphocytes, periphery macrophage reaction and mild bronchiolar epithelium hyperplasia (Figure 4H). Whereas, *B. aegyptiaca* group, at 7 dpi revealed eosinophil, lymphocytic reaction around larval cross section and congested blood artery (Figure 4I). At 14 dpi, *B. aegyptiaca* group, showed marked eosinophilic granuloma surround larvae with perivascular edema and hemorrhages (Figure 4J). In Propolis group, at 7 dpi, the larvae observed with eosinophils and lymphocytes infiltration, peribronchial granuloma and increased bronchial goblet cells with intrabronchial sloughed cells and mucinous material (Figure 4K). Whereas, at 14 dpi, the Propolis treated group revealed improvement in lung tissues even though presence of the larva between bronchiole and blood vessel accompanied by congested vein and perivascular edema were noticed (Figure 4L, Table 3).

Histopathological examination of the rat's liver in the control negative group at 7 and 14 dpi showed normal hepatic architecture at the central vein and portal area (Figure 5A, B). while, at 7 dpi in control positive group, we noticed remarkable periportal inflammatory cells infiltration (mainly eosinophils and lymphocytes) with portal vascular congestion and hydropic hepatic degeneration, but there were not recognizable larvae surrounding portal area (Figure 5C). While, at 14 dpi larvae cross sections observed in periportal area associated with mild lymphocytic reaction and vacuolar degenerative changes of hepatocytes were persisted (Figure 5D). While, livers tissues in piperazine group there were cross sections of larvae near the bile duct surrounded by lymphocytic reaction, congested hepatic vein and degenerative changes of hepatocytes around the portal area (Figure 5E). However, at 14 dpi, the larvae persist detected in portal area associated with mild lymphocytic reaction with minimal cytoplasmic fat vacuoles of periportal hepatocytes (Figure 5F). In Thymol treated group at 7 dpi larvae were observed surrounded by lymphocytic infiltration accompanied by congested hepatic vein but adjacent hepatocytes appeared within normal (Figure 5G). At 14 dpi revealed lymphocytes and periphery macrophage reaction with no larva detection (Figure 5H). In *B. aegyptiaca* group, at 7 dpi noticed mild lymphocytic reaction around portal area without larval detection (Figure 5I). While, at 14 dpi there were marked lymphocytes and macrophages infiltration surrounded the larva with mutable newly formed bile ductules and sporadic hepatocytes necrosis (Figure 5J). Whereas, in Propolis group at 7 dpi revealed cross sections of larvae, with minimal lymphocytes infiltration and edema surround the congested hepatic vein (Figure 5K). And at 14 dpi showed minimal lymphocytic reaction with no larva detection and nearly normal hepatic parenchyma (Figure 5L, Table 3).

Histopathological examination of rat brains at 7 dpi larvae detected only in one brain of *B. aegyptiaca* group histologically larvae noticed within ruptured superficial brain artery containing like clump of trapped larvae surrounded by hemorrhages (Figure 6A). Thrombus and hemorrhage of brain blood vessel appeared in the grey matter of the brain (Figure 6B). While, in 14 dpi most infected groups revealed signs of larval migratory lesions but larvae detected histologically in the brain of Piperazine group appeared as transverse sections of larvae around superficial vein and longitudinal larval sections (Figure 6C) and within gray matter of brain showed congested blood vessels, hemorrhagic tract and astrocytes reaction (Figure 6D). Histopathological score lesions for lung, liver and brain were shown in (Table 3).

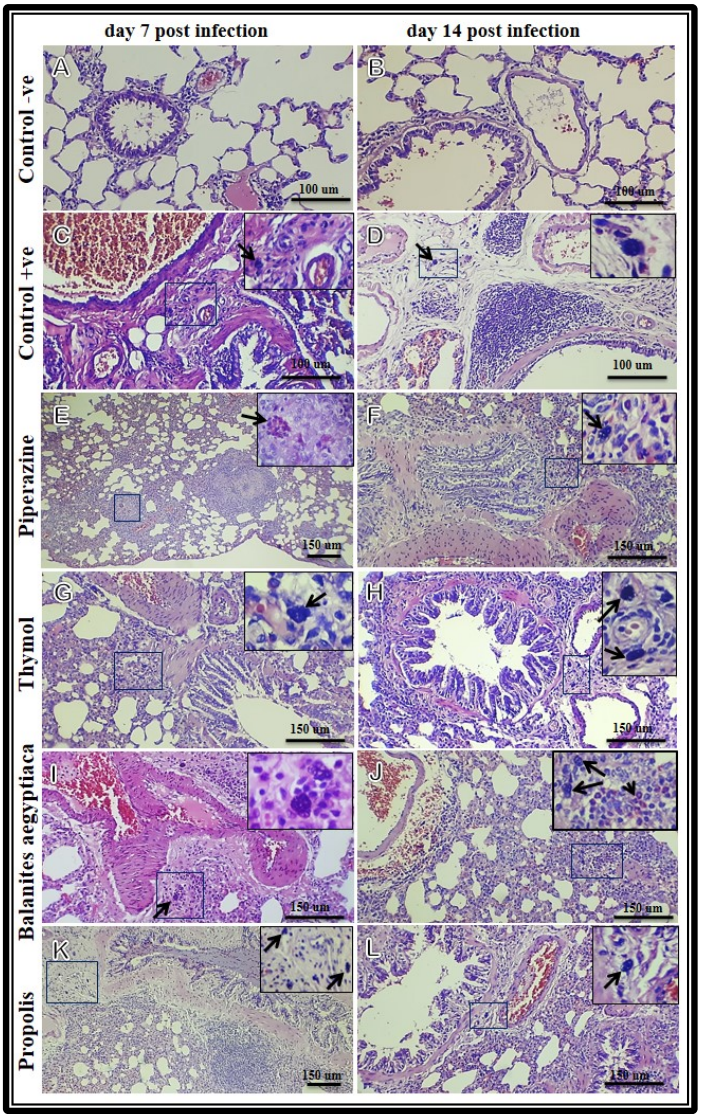

Figure 4 Photomicrographs of H&E stained rat's lungs: (A, B) Control negative group at 7 dpi and 14 dpi showing normal lung histology of alveoli and bronchioles. (C) Control positive group at 7 dpi larvae were recognized near congested blood vessel (square) associated with eosinophilic reaction, lymphocytes and macrophages. (D) Control +ve group at 14 dpi larval cross section surrounded by eosinophils (square), peribronchial granulomatous reaction and degenerative changes of bronchial epithelium. (E) Piperazine group at 7 dpi eosinophilic granuloma (square) but no recognizable larval remained. (F) Piperazine group at 14 dpi detection of larvae surrounded by eosinophilic reaction (square) and hyperplasia of bronchiolar epithelium. (G) Thymol group at 7 dpi larvae surrounded by eosinophil, lymphocytes infiltration (square) and hyperplasia of bronchiolar epithelium. (H) Thymol group at 14 dpi larvae were recognized with lymphocytes and periphery macrophage reaction (square) and mild bronchiolar epithelium hyperplasia. (I) *Balanites aegyptiaca* group at 7 dpi eosinophil and lymphocytic reaction around larval cross section (square) and congested blood artery. (J) *B. aegyptiaca* group at 14 dpi marked eosinophilic granuloma surround larvae (square), perivascular edema and hemorrhages. (K) Propolis group at 7 dpi larva with eosinophils and lymphocytes infiltration (square), peribronchial granuloma and increase bronchial goblet cells with intrabronchial sloughed cells and mucinous material. (L) Propolis group at 14 dpi larva detection between bronchiole and vein (square), congested vein and perivascular edema.

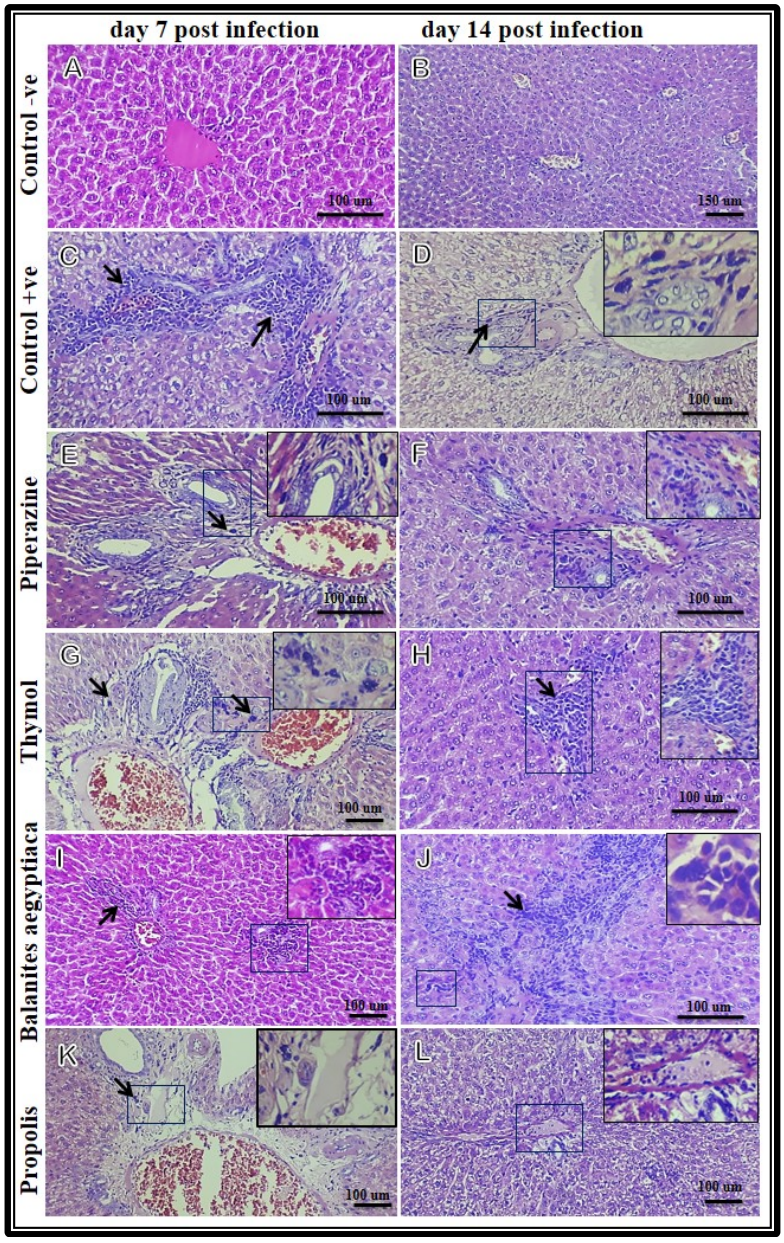

Figure 5 Photomicrographs of H&E stained rat's livers: (A, B) control negative group at 7 dpi and 14 dpi showing normal hepatic histology at central vein and portal area. (C) Control positive group at 7 dpi eosinophilic reaction with associated lymphocytes and macrophages but no recognizable larvae surrounding portal area (arrow) and vacuolar degeneration of hepatocytes. (D) Control +ve group at 14 dpi cross section of larvae (square) near portal area with mild lymphocytic reaction and degenerative changes of hepatocytes. (E) Piperazine group at 7 dpi larvae cross section near bile duct surrounded by lymphocytic reaction (square) and congested hepatic vein. (F) Piperazine group at 14 dpi larvae surrounded by lymphocytic reaction in portal area (square). (G) Thymol group at 7 dpi larvae surrounded by lymphocytic infiltration (square) and congested hepatic vein. (H) Thymol group at 14 dpi lymphocytes and periphery macrophage reaction but no larvae were recognized (square). (I) *Balanites aegyptiaca* group at 7 dpi, mild lymphocytic reaction around the portal area without larval detection (square). (J) *B. aegyptiaca* group at 14 dpi marked lymphocytes and macrophages infiltration surround larva (square) and mutable newly formed bile ductules. (K) Propolis group at 7 dpi larva with minimal lymphocytes infiltration (square) and edema surround congested hepatic vein. (L) Propolis group at 14 dpi minimal lymphocytic reaction, no larva detection (square) and nearly normal hepatic parenchyma.

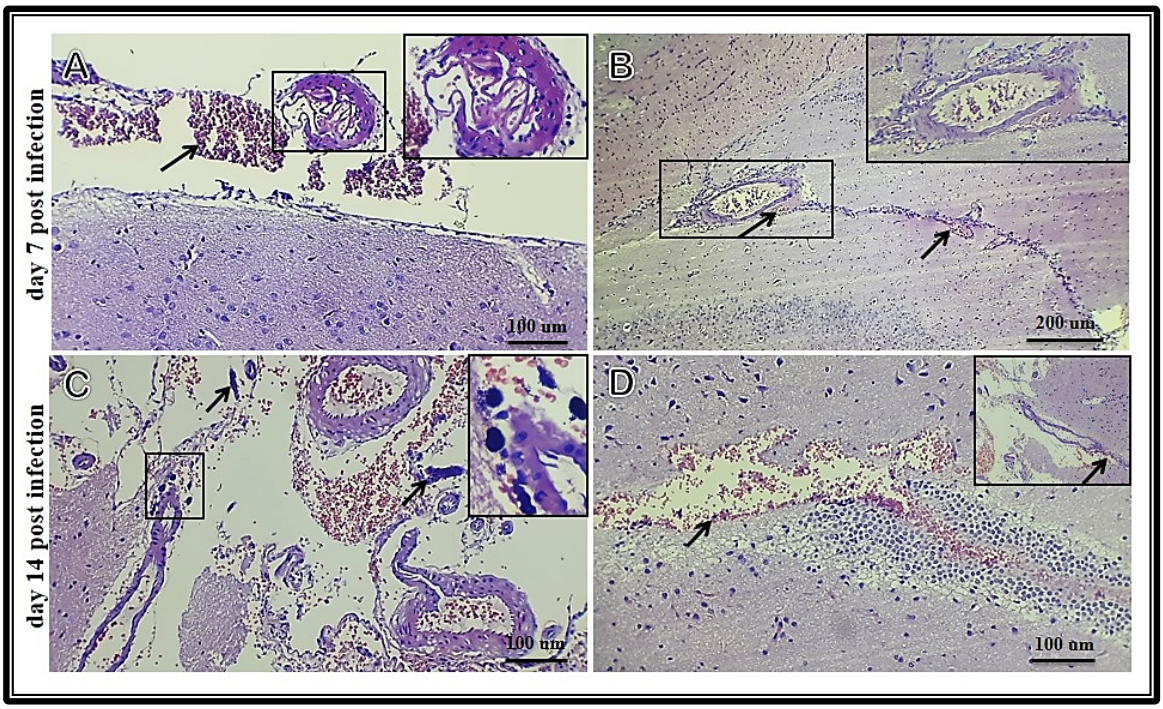

Figure 6 Photomicrographs of rats brains stained with H&E stain: (A) At 7 dpi: ruptured superficial brain artery containing like clump of trapped larvae (square) surrounded by hemorrhages (arrow). (B) At 7 dpi: thrombus and hemorrhage of brain blood vessel (arrows). (C) At 14 dpi: transverse sections of larvae around vein (square), longitudinal larval sections (arrows), congested blood vessels and hemorrhages. (D) At 14 dpi: hemorrhagic tract within the brain (arrow).

DISCUSSION

In tropical countries and nations, toxocariasis is one of the most grave parasitic diseases, resulting in high morbidity and mortality in buffalo calves (Srivastava and Sharma 1981). In addition, Dewair and Bessat (2020) identified *T. vitulorum* larvae in the infected buffalo cows colostrum/milk samples which mainly detected through the first two weeks (3–12 days) after parturition. The transmammary route considered the main mode of infection in bovine toxocariasis. That establish a challenge to the keepers of livestock, especially when toxocariasis is widely spread with non-routinely applied of anthelmintic treatment (Jones et al., 2009; Borgsteede et al., 2012; Chelladurai et al., 2015; Dewair and Bessat, 2020). Conventional microscopic inspection with tissue digestion considered one of the most essential diagnostic methods to decide migration routes of parasite and the lesions that induced through the experimental infection by *Toxocara species* in paratenic hosts (Cardillo et al., 2009; Zibaei et al., 2010; Naderbandi et al., 2022).

Table 3 Histopathological score lesions for lung, liver and brain in all 6 groups

Organs \ Groups	Control -ve		Control +ve		Piperazine		Thymol		*Balanites aegyptiaca*		Propolis	
	7dpi	14dpi	7dpi	14dpi	7dpi	14dpi	7dpi	14dpi	7dpi	14dpi	7dpi	14dpi
Lung												
Larval histological detection	-	-	+++	+++	+	+	+	+	+++	+++	++	++
Inflammatory cell reaction	-	-	+++	+++	+++	++	++	++	++	+++	+++	++
Thickening of alveolar septa	-	-	+++	+++	++	++	+	+	+++	++	++	+
Peribronchial granuloma	-	-	+++	+++	++	++	+	+	+++	+++	++	++
Degeneration of bronchial epithelium	-	-	+++	+++	++	++	+	+	++	++	++	+
Liver												
Larval CS detection near portal	-	-	+++	++	++	+	++	+	+++	+	++	++
Periportal inflammatory infiltration	-	-	+++	++	++	++	++	+	++	+++	++	+
Inflammatory cells aggregation	-	-	+++	+++	++	++	+	+	++	+++	+	+
Hepatocytes vacuolar degeneration	-	-	+++	+++	+	++	+	+	+	++	+	-
Periportal hepatocellular necrosis	-	-	+++	++	++	++	++	-	+++	++	+	+
Congested vasculature	-	-	+++	+++	+++	++	+	-	++	++	++	+
Brain												
Larval section detection	-	-	-	-	-	+	-	-	+	-	-	-
Congestion of superficial B.V	-	-	++	++	+	++	-	-	++	++	+	+
Hemorrhages	-	-	++	++	+	++	-	-	++	++	-	-

N.B: (-) means 1-3 slides showing lesions, (+) means 4-7 slides showing lesion, (++) means 8-11 slides showing lesion and (+++) means 12-15 slides showing lesion.

Our findings identified the adult worms of *T. vitulorum* based on its morphological characters and recognized into males (17 – 21 cm length) and females (27 - 31 cm length). The extracted eggs from adult females were (ovoid to spherical) with average measured 75 × 81.4 µm (70.2×80.6 µm to 83×84.8 µm) and showed successful embryonic development in the vitro, where eggs developed from "unembryonated stage to eggs containing fully developed larvae" passing through various embryonic developmental stages which identified inside the eggs by microscopic inspection. While some larvae were naturally emerging from a small hole in the eggshell. Similar to results recorded by Mehlhorn (2015) who found males and females adult worms of *T. vitulorum* in the ruminant's small intestine. The females length measure (20–30 cm) and the males length measure (15–25 cm). The eggs were typically with rough surface which measure (70–95 × 60–75 µm) and excreted unembryonated in the feces.

Our observation has shown many developmental stages of *T. vitulorum* eggs. Similar developmental stages reported previously by Cruz et al. (2012) and Abou-El-Naga (2018) who mentioned the developmental stages of *Ascaris lumbricoides* and *Toxocara canis* (*T. canis*) eggs. In the current study, larvae were found in liver, lung and brain of the infected rats. This indicates that *T. vitulorum* had undertaken body migration in rats. The observed gross and histopathological tissues alterations attributed to the migration of these larvae and established that the rats might act as *T. vitulorum* paratenic host and could be used as toxocariasis animal model as mentioned by (Llanes et al., 2019). This clarified by Cardillo et al. (2009) who recorded hatching of larvae and its migration out of the intestine to be lodged in tissues and organs.

In the current work, the control positive group revealed microscopically 41 larvae in lung and liver at 7 dpi. While, the maximum number of *T. vitulorum* larvae were 52 that observed in the lung and liver and brain of control positive group at 14 dpi. This agree with Llanes et al. (2019) who mentioned that the neurotrophic and hepato-pulmonary phases of migration of *T. vitulorum* larvae in rats have been detected and larvae were observed in the infected rats lungs, liver and brains. Also, Naderbandi et al. (2022) who stated that the tissues digestion with microscopic examination revealed occurrence of larvae in the lungs and liver. This explained by Zibaei et al. (2010) who suggested that the transmission of larvae was starting from the intestine toward the lungs and liver then to the kidneys, heart, muscles and brain.

In our findings, gross examination of rats at 7 dpi showed congested lung and liver in control positive group. Which matching with Cardillo et al. (2009) and Janecek et al. (2014) who recorded grossly hemorrhages widely spread in the infected mice lungs with *Toxocara cati* (*T. cati*). Histopathological inspection of control positive rats lungs showed eosinophilic cellular granuloma which embedded in pulmonary tissues with degenerative changes of bronchial epithelium with collapsed alveoli and thickening of inter alveolar septa. Our results matching with Bowman et al. (1987) and Llanes et al. (2019) who detected the existence of eosinophilia, persistent pulmonary inflammation, and increased IgE production without presence of *Toxocara* larvae as a result of presence of high levels of circulating parasite antigens which still in the blood circulation even after infection. Also, Cardillo et al. (2009), Resende et al. (2015) and Llanes et al. (2019) added that the immunological aberrations, especially the existence of inflammatory cells and airways destruction coincided in different animals including mice and rats.

Histopathological findings of control positive rats livers showed characteristic vacuolar degenerative alterations in hepatocytes, congestion of portal blood vessel with peripheral blood eosinophilia, cholangitis, edema with eosinophilic cellular granuloma that embedded inside hepatic tissues. Similar results recorded by Shehata et al. (2022) in the liver of rats infected by *T. vitulorum*. These findings coincide with those of Auliyah et al. (2021) who described that liver damage particularly around the portal venous region as a result of *Toxocara* spp. migration through blood stream which caused hemorrhage, inflammatory cells infiltration, multifocal necrosis and epithelial proliferation of the bile duct. Also, Azizi et al. (2007) added that infected larvae of *T. vitulorum* during parenchymal migration in the liver leading to secrete toxic metabolites damage the integrity of hepatocytes leading to its degenerative cloudy swelling. Furthermore, these toxic larval secretions stimulate the production of eosinophils as an immune response (Auliyah et al., 2021). While, Musa et al. (2011) and Amin and El-Kabany (2013) explained that these lesions which caused by *T. vitulorum* larvae could be attributed to the proteolytic activity of enzymes which secreted by the larvae and caused moderate to severe histopathological changes in the liver parenchyma.

The effectiveness of toxocariasis control should be assessed by retention of larvae in the lung and liver, the reduction of eosinophilic granuloma formation in the liver and finally the resistance of the lungs to infection (Shehata et al., 2022).

Piperazine used to control ascariasis in birds and mammals since a long period. The piperazine has effect on adult worms more than younger stages. However, the drug has little effect on the larval worms found in the host's tissues (Courtney and Roberson, 1995).

In the present study, no reduction in larvae in piperazine citrate group at day 7 post infection with no significant difference between control positive group and piperazine citrate group. While, the reduction percent of larval number in piperazine citrate group was (84.6%) at 14 dpi with significant difference amongst larval recovery in "lung, liver and brain" between control positive group and piperazine citrate group. These results agreed with Islam et al. (2006) who confirmed that exposure to piperazine instantly resulting in partial relaxation of "*Ascaris suum* lung third stage larvae", which could lead to partial paralysis of the larvae. Furthermore, it inhibit a wide range of functional enzymes/proteins of the third stage larvae of *Ascaris suum* which invasing lung due to the strong anti moulting effect of piperazine.

Current research revealed that the lung tissue of piperazine group showed remarkable eosinophilic granulomas with no recognizable larvae at 7 dpi. But, at 14 dpi, the larvae were detected and surrounded by eosinophilic reaction with peribronchial lymphocytic infiltrates and hyperplasia of bronchiolar epithelium. While, in liver tissue showed cross sections of larvae near the bile duct surrounded by lymphocytic reaction, congested hepatic vein, and degenerative changes of hepatocytes around the portal area at 7 dpi. However, at 14 dpi, the larvae persist detected in portal area associated with mild lymphocytic reaction with minimal cytoplasmic fat vacuoles of periportal hepatocytes. These results were supported by other studies which refered to that piperazine caused side effects on *Toxocara* infected young calves (Rajkhowa et al., 2004). These side effects included diarrhea and restlessness with resistance of parasite in some treated animals with it as antitoxocariasis drug (Avcioğlu and Balkaya, 2011).

The using of the medicinal herbs increased over the last few years (Shalaby et al., 2012). Medicinal plants are broadly utilized for the treatment of numerous diseases, involving parasitic infections. It is imperative to conduct thorough scientific investigations to encourage the utilization of herbal remedies and plant-derived medications owning antiparasitic properties which are less toxic, less expensive and more readily available compared to commercial anthelmintics. Furthermore, the hazards associated with the veterinary drugs residues in animal by-products particularly milk and underscore the significance of complementary medicines (Shehata et al., 2022).

Duru et al. (2023) and Chroho et al. (2024) stated that Thyme have phyto-therapeutic potentials as antioxidant, antiparasitic, antiviral, antibacterial and antifungal. The thyme essential oils contain compounds like carvacrol, along with a significant amount of thymol. In the our investigation, the reduction percent of larvae in Thymol group was (61% at day 7 and 75% at day 14 post infection) with significant difference between Thymol and control positive groups. This in agree with Shehata et al. (2022) who stated that recovered larvae numbers of *T. vitulorum* at day 7 post-infection from different organs was significantly lower in thymol treated group than in control positive infected rats.

The anti-helmintic effect of thymol attributed to the paralysing of the parasite, similarly to the macrocyclic lactones mechanism of action and the inhibition of feeding functions and movement (Duru et al., 2023). Besides, Ferreira et al. (2016) discovered that thymol, which constitutes about 50% of the active components of thyme extract, mediates its antiparasitic activity.

Our findings matched with Amin and El-Kabany (2013) who mentioned that the treatment with oil of Thyme was significantly reduced the number of *T. vitulorum* larvae (38.2%) in the infected rat's livers on 7 dpi. Likewise, Amin et al. (2016) and Hafez et al. (2019) added that the treatment of experimentally *T. canis* infected rats by thyme oil was effective in comparison with the control groups. Also, Arafa et al.

(2020) recorded that many researchers reported therapeutic use of thymol as treatment for a variety of parasitic infections.

Histopathological examination of liver and lung of thymol treated group at 7 dpi showed larvae in the lung and the liver that surrounded by lymphocytic reaction with nearly normal adjacent parenchyma. Also, at 14 dpi, the larvae still observed in the lung and surrounded by macrophages and lymphocytes, while the liver contained mild lymphocytic reaction without recognizable larvae with quite normal surrounding tissues. These findings agreed with Shehata et al. (2022) who confirmed that the treatment with thymol had a substantial improvement in lung and hepatic tissues with reduction in the inflammation degree (reduced the damage of liver and severity of its inflammation) as a result of thymol anti-inflammatory and antioxidative properties with reduction in the numbers of *Toxocara* larvae in lungs and livers of rats. Also, Sahoo et al. (2021) explained the powerful antioxidant properties of Thymol due to the existence of a phenolic hydroxyl group in it which given it its capablity in reducing the production of reactive oxygen species. Moreover, Amin and El-Kabany (2013) added that thyme administration pre or after *T. vitulorum* infections improve the histopathological changes resulted from infection. These results revealed that thymol had influences on the development of larvae and *T. vulgaris* has a powerful effect in protection of organs against its damage induced by *T. vitulorum* infection.

Balanites aegyptiaca fruits is edible, produce valuable oil and contains saponins that are lethal to some invertebrates (Chapagain and Wiesman, 2005; Shalaby et al., 2012). Based on the results presented in our study, the number of larvae reduced in *B. aegyptiaca* group 9.8% at day 7 and 53.8% at day 14 post infection with significant difference of the total larval recovery between *B. aegyptiaca* and control positive groups. Our findings compatible with Shalaby et al. (2010) who documented significant reductions in the number of encysted and migrating *Trichinella spiralis* (*T. spiralis*) larvae in rats after treatment with *B. aegyptiaca* and Shalaby et al. (2012) who found that the *B. aegyptiaca* fruits methanolic extract could provide a cheaper and suitable alternative treatment for *T. vitulorum* worms. It exhibited a strong and progressive effect on the cuticle of adult *T. vitulorum* in vitro and had a noticeable inhibitory effect on the development of *T. vitulorum* eggs. Consequently, it could potentially aid in decreasing the presence of infective eggs in the host environment post-treatment. This was concurred with that previously reported by Chapagain and Wiesman (2008) who stated that the saponin is one of the major *B. aegyptiaca* secondary metabolites. It has a strong relationship between the saponin content of the methanolic extracts of *B. aegyptiaca* which had effect on *T. spiralis* adult worms (Shalaby et al., 2010). In addition to Gnoula et al. (2006) who insulated a steroidal saponin, balanitin 7 from *B. aegyptiaca* fruits and verified its significant potential for anthelmintic activity. Therefore, it might be suggested that the influence of *B. aegyptiaca* on adult worms of *T. vitulorum* could be attributed to the presence of secondary metabolites with one or multiple biological activities as saponins (Shalaby et al., 2012).

In *B. aegyptiaca* treated group at 7 dpi there were mild lymphocytic reaction without larval detection in the liver. But in the lung larvae were observed surrounded by eosinophilic reaction. At 14 dpi both liver and lung larvae were found surrounded by characteristic eosinophilic infiltration and the adjacent parenchymal tissue appeared nearly normal. The reduction of inflammation besides pathological lesions could be attributed to the antimicrobial, anti-inflammatory properties of *B. aegyptiaca* extract which was in agreement with Murthy et al. (2020) who documented that extracts isolated from *B. aegyptiaca* had anti-inflammatory, antioxidant, hepatoprotective and antimicrobial activities in numerous previous studies.

The potential utilize of natural products such as a source of the novel anthelmintic drug remains limited. There has been an increasing interest on natural

products such as propolis which act as a significant source of biologically active composites (Hassan et al., 2016; Hossain et al., 2022). Propolis is primarily composed of "resin (50%), wax (30%), essential oils (10%), pollen (5%) and another organic compounds (5%)" which include phenolic compounds, flavonoids, esters, beta-steroids, terpenes, aromatic aldehydes, sesquiterpenes, alcohols and stilbene terpenes (Hassan et al., 2016; Hossain et al., 2022).

In the current study, the larval number declined in Propolis group 24.4% at day 7 and 55.8% at day 14 post infection with significant difference of the total larval recovery between Propolis and control positive groups. Our findings indicated that ethanolic extract of propolis has anthelmintic effect against *T. vitulorum* larvae. This was coincided with Hassan et al. (2016) who discovered that propolis ethanolic extract had anthelmintic activity against adult worms of *T. vitulorum* resulting in significant damage and subsequent death of the worms. Also, Hegazi et al. (2007) reported anthelminthic potential of an ethanolic extract of Egyptian propolis, in vitro it had effect on the adult worms of *Fasciola gigantica* and an ovicidal activity on eggs of *Fasciola gigantica*, resulting in complete inhibition of egg development and eventually death.

Histopathological picture of lungs and livers in Propolis group revealed improvement in lung and hepatic histology even though larvae with lymphocytes and eosinophils infiltration. This improvement in tissue may be due to the biological properties of Propolis such as anti-inflammatory, antioxidant, anti-proliferative, neuroprotective effects, stimulating the immune system and activating the parasite death mechanisms (Hossain et al., 2022). Which is in agreement with Soares (2006) who mentioned that propolis can act on microorganisms directly in vitro. While, activating the microorganisms death mechanisms as a result of stimulation of the immune system in vivo.

There are no larvae observed in the brain tissues of examined rats at 7 dpi of all groups except group of *B. aegyptiaca*. While, larvae were detected in the brain tissues examined at 14 dpi in all groups except Propolis group. This in agreement with Cardillo et al. (2009) who reported that *T. cati* causes significant brain lesions in mice on 4 to 28 days post inoculation and Santos et al. (2009) reported an increase in the recovery of larvae in the brain of rats starting at 15 dpi. This explained by Samanta and Ansari (1990) and El-Nahas et al. (2020) who established that the larvae can escape from the lethal response of immune started with treatment and so, this larvae that reach the brain are no longer susceptible to antihelminthic agents. In contrast with Naderbandi et al. (2022) who mentioned microscopic examination revealed the absence of *T. cati* larvae through migration into brain tissue and Zibaei et al. (2010) recorded an increase in *T. cati* larval infection after day 70 dpi in the brain of rats.

Toxocara vitulorum larvae could be detected histopathologically in one brain of *B. aegyptiaca* group at 7 dpi and appeared as a clump of trapped larvae within ruptured superficial cerebrum arteriole. While, at 14 dpi, the larval cross sections were observed around the venule without any evidence of inflammatory cell infiltration or granulomatous reaction. On the other hand, in piperazine group, hemorrhagic tract that surrounded by astrocytes reaction were detected inside the brain gray matter with no larval detection in brain of propolis group at 7 dpi or 14 dpi. Similarly, Llanes et al. (2019) recorded that active migration of larvae in the brain lead to larval sections without cellular reaction around them and areas of hemorrhages. Also, Cardillo et al. (2009) and Janecek et al. (2014) reported numerous superficial hemorrhagic foci which seemed on the brain of mice through the first week of infection due to the bleeding and injuries caused by larvae which penetrated out of arteries on the surface of the brain. In addition, larvae of *T. canis* do not accumulate purposely in the brain and be incapable to leave and trapped in the brain because of their size that near those of the brain arteries (Bisseru, 1969). Remarkably, the brain is deemed as a site of ''immunological privilege'' where inflammatory cells do not or rarely encircle the larvae compared to other organs

(Llanes et al., 2019). Our findings were in disagreement with Resende et al. (2015) who observed in the mice brain, very mild inflammatory reaction which can explain the neuroaffinity of *T. canis*. Additionally, the brain functions as a reservoir for the parasite, and the regular presence of the larvae in the host's brain acts as a significant source of infection for both humans and other animals, with the rat serving as a paratenic host (Dunsmore et al., 1983).

CONCLUSIONS

The natural alternative remedies (Thymol, *B. aegyptiaca*, and Propolis) reduced *T. vitulorum* larvae count and improved the pathological lesions induced by it in experimentally infected rats, so it is advised to include them as a possible treatment for *Toxocara* infection in calves. This is no published data available about use of *B. aegyptiaca* and propolis as natural therapies of *T. vitulorum* in vivo. So, we encourages further studies of using combination of these natural products for the remedy of *T. vitulorum* in vivo.

AUTHOR CONTRIBUTIONS

Kuraa, H.M., Malek, S.S. and EL Hendy, A.H.M. designed the study and helped in experimental procedure. Malek, S.S. provided thymol. Malek, S.S. and Kuraa, H.M. collection of worms and preparation of eggs for infection. Kuraa, H.M. microscopic examination of worm eggs and prepared inoculum, data analysis, and interpretation. Kuraa, H.M. wrote the manuscript. EL Hendy, A.H.M. performed the histopathological examination and interpretation of samples and Abdel-Rahman, I.A.M. prepared plant extract.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

REFERENCES

Abou-El-Naga, I.F., 2018. Developmental stages and viability of *Toxocara canis* eggs outside the host. Biomédica. 38,189-197.

Amin, M.M., El-Kabany, H., 2013. Evaluation of protective and treatment of Thyme (Thymus vulgaris) oil on *Toxocara vitulorum* infected rats. J. Radiat. Res. Appl. Sci. 6(1), 209-232.

Amin, M.M., Hafez, E.N., Abd Raboo, M.A., 2016. Assesment of radiation-attenuated vaccine or thyme oil treatment on controlling DNA damage and nitric oxide synthesis in brain of rat infected with *Toxocara canis*. Arab. J. Nucl. Sci. Appl. 49(2), 199-210.

Arafa, W.M., Abolhadid, S.M., Moawad, A., Abdelaty, A.S., Moawad, U.K., Shokier, K.A., Shehata, O., Gadelhaq, S.M., 2020. Thymol efficacy against coccidiosis in pigeon (Columba livia domestica). Prev. Vet. Med. 176, 104914.

Auliyah, R., Kusnoto, Hamid, I.S., 2021. Histopathology Description of Chicken Liver Infected by L2 Toxocara vitulorum. J. World. Poult. Res. 11(1), 123-128.

Avcioğlu, H., Balkaya, İ., 2011. Prevalence of *Toxocara vitulorum* in Calves in Erzurum, Turkey. Kafkas Univ. Vet. Fak. Derg. 17(3), 345-347.

Aydin, A., Goz, Y., Yuksek, N., Ayaz, E., 2006. Prevalence of *Toxocara vitulorum* in Hakkari eastern region of Turkey. Bull. Vet. Inst. Pulawy. 50(1), 51-54.

Azizi, S., Oryan, A., Sadjjadi, S.M., Zibaei, M., 2007. Histopathologic changes and larval recovery of *Toxocara cati* in experimentally infected chickens. Parasitol. Res. 102(1), 47-52.

Bancroft, J.D., Gambl, M., 2008. Theory and practice of histological techniques, 6th edition. Churchill Livingstone, New York, London, Philadelphia, pp. 126-134.

Bisseru, B., 1969. Studies on the liver, lung, brain and blood of experimental animals infected with *Toxocara canis*. J. Helminthol. 43(3), 267-272.

Borgsteede, F.H., Holzhauer, M., Herder, F.L., Veldhuis-Wolterbeek, E.G., Hegeman, C., 2012. *Toxocara vitulorum* in suckling calves in The Netherlands. Res. Vet. Sci. 92(2), 254-256.

Bowman, D.D., Mika-Grieve, M., Grieve, R.B., 1987. Circulating excretory-secretory antigen levels and specific antibody responses in mice infected with *Toxocara canis*. Am. J. Trop. Med. Hyg. 36(1), 75-82.

Cardillo, N., Rosa, A., Ribicich, M., López, C., Sommerfelt, I., 2009. Experimental infection with *Toxocara cati* in BALB/c mice, migratory behaviour and pathological changes. Zoonoses. Public. Health. 56(4), 198-205.

Carvalho, E.A., Rocha, R.L., 2014. Visceral larva migrans syndromes associated with toxocariasis: epidemiology, clinical and laboratory aspects of human toxocariasis. Curr. Trop. Med. Rep. 1, 74–79.

Chapagain, B.P., Wiesman, Z., 2005. Larvicidal effects of aqueous extracts of *Balanites aegyptiaca* (desert date) against the larvae of Culex pipiens mosquitoes. Afr. J. Biotechnol. 4(11), 1351–1354.

Chapagain, B.P., Wiesman, Z., 2008. Metabolite profiling of saponins in *Balanites aegyptiaca* plant tissues using LC (RI)-ESI/MS and MALDI-TOF/MS. Metabolomics. 4, 357–366.

Chelladurai, J.J., Bader, C., Snobl, T., Magstadt, D., Cooper, V., Brewer, M.T., 2015. *Toxocara vitulorum* infection in a cohort of beef calves in Iowa. Vet. Parasitol. 214(1-2), 96-99.

Chroho, M., Rouphael, Y., Petropoulos, S.A., Bouissane, L., 2024. Carvacrol and thymol content affects the antioxidant and antibacterial activity of origanum compactum and Thymus zygis Essential Oils. Antibiotics. 13, 139.

Courtney, C.H., Roberson, E.L., 1995. Chemotherapy of parasitic diseases, In: Adams, H.R. (Ed.), Veterinary pharmacology and therapeutics, (7th edition). Iowa State University Press, Ames, pp. 886–888.

Cruz, L.M., Allanson, M., Kwa, B., Azizan, A., Izurieta, R., 2012. Morphological changes of Ascaris spp. eggs during their development outside the host. J. Parasitol. 98(1), 63-68.

Dewair, A., Bessat M., 2020. Molecular and microscopic detection of natural and experimental infections of *Toxocara vitulorum* in bovine milk. PLoS ONE. 15(5), e0233453.

Dunsmore, J.D., Thompson, R.C., Bates, I.A., 1983. The accumulation of *Toxocara canis* larvae in the brains of mice. Int. J. Parasitol. 13(5), 517-521.

Duru, Ö., Gökpınar, S., Duru, S.Y., Akanbong, E.A., Kara, E., 2023. The therapeutic effectiveness of thyme extract in naturally infected puppies with ascariasis. T.J.V.R. 7(1), 39-45.

El-Nahas, N.S., Abd-Elatty, A.F., Rady, A.A., El-Aswad, B.E., Attallah, A.M., Hemida, A.S., 2020. Anti-Toxocara effects of Cassia nodosa plant extract in experimentally infected mice. Menoufia Med. J. 33, 191-195.

Fan, C., Su, K., 2004. Cross-reactions with Ascaris suum antigens of sera from mice infected with *A. suum*, *Toxocara canis*, and *Angiostrongylus cantonensis*. Parasitol. Int. 53(3), 263-271.

Ferreira, L.E., Benincasa, B.I., Fachin, A.L., França, S.C., Contini, S.S.H.T., Chagas, A.C.S., Beleboni, R.O., 2016. Thymus vulgaris L. essential oil and its main component thymol: Anthelmintic effects against *Haemonchus contortus* from sheep. Vet. Parasitol. 228, 70-76.

Gasser, R.B., 2013. A perfect time to harness advanced molecular technologies to explore the fundamental biology of Toxocara species. Vet. Parasitol. 193(4), 353-364.

Gibson-Corley, K.N., Olivier, A.K., Meyerholz, D.K., 2013. Principles for valid histopathologic scoring in research. Vet. Pathol. 50(6), 1007-1015.

Glickman, L.T., Magnaval, J.F., Domanski, L.M., Shofer, F.S., Lauria, S.S., Gottstein, B., Brochier, B., 1987. Visceral larva migrans in French adults: a new disease syndrome? Am. J. Epidemiol. 125(6), 1019–1034.

Gnoula, C., Guissou, P., Dubois, J., Frédérich, M., Duez, P., 2006. Nematocidal compounds from the seeds of *Balanites aegyptiaca* isolation and structure elucidation. Planta. Med. 72, 33.

Hassan, S.E., Abou-El-Dobal, S.K., Hegazi, A.G., 2016. Bioassay of Egyptian Propolis on *Toxocara vitulorum* adult worms. World. Appl. Sci. J. 34(3), 283-289.

Hafez, E.N., Hafez, M.N., Amin, M.M., 2019. Effect of vaccination with irradiated *Toxocara canis* larvae or thyme oil treatment on testicular histochemical and immunohistochemical changes of rats. Trop. Biomed. 36(2), 430-442.

Hegazi, A.G., Abd El Hady, F.K., Shalaby, H.A., 2007. An in vitro effect of propolis on adult worms of *Fasciola gigantica*. Vet. Parasitol. 144 (3-4), 279-286.

Holland, C.V., 2017. Knowledge gaps in the epidemiology of Toxocara: the enigma remains. Parasitology. 144(1), 81-94.

Hossain, R., Quispe, C., Khan, R.A., Saikat, A.M., Ray, P., Ongalbek, D., Yeskaliyeva, B., Jain, D., Smeriglio, A., Trombetta, D., Kiani, R., Kobarfard, F., Mojgani, N., Saffarian, P., Ayatollahi, S.A., Sarkar, C., Islam, M.T., Keriman, D., Ucar, A., Martorell, M., Sureda, A., Pintus, G., Butnariu, M., Sharifi-Rad, J., Cho, W.C., 2022. Propolis: an update on its chemistry and pharmacological applications. Chinese. Med. 17, 100.

Iqbal, Z., Akhtar, M.S., Sindhu, Z., Khan, M.N., Jabbar, A., 2003. Herbal dewormers in livestock - a traditional therapy. Int. J. Agri. Biol. 5(2), 199-206.

Islam, M.K., Miyoshi, T., Yamada, M., Alim, M.A., Huang, X., Motobu, M., Tsuji, N., 2006. Effect of piperazine (diethylenediamine) on the moulting, proteome expression and pyrophosphatase activity of Ascaris suum lung-stage larvae. Acta Trop. 99(2-3), 208-217.

Janecek, E., Beineke, A., Schnieder, T., Strube, C., 2014. Neurotoxocarosis: marked preference of *Toxocara canis* for the cerebrum and *T. cati* for the cerebellum in the paratenic model host mouse. Parasit. Vectors. 7(1), 194.

Jones, J.R., Mitchell, E.S., Redman, E., Gilleard, J.S. 2009. *Toxocara vitulorum* infection in a cattle herd in the UK. Vet. Rec. 164(6), 171-172.

Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A., Fecka, I., 2020. Thymol and Thyme essential oil—new insights into selected therapeutic applications. Molecules. 25(18), 4125.

Llanes, K.K., Besana, C.M., Paller, V.G., 2019. Larval distribution, migratory pattern and histological effects of *Toxocara canis* in *Rattus norvegicus*. J. Parasit. Dis. 43(4), 679–685.

Ma, G., Holland, C.V., Wang, T., Hofmann, A., Fan, C., Maizels, R.M., Hotez, P.J., Gasser, R.B., 2018. Human toxocariasis. Lancet Infect. Dis. 18(1), e14-e24.

Macpherson, C.N., 2013. Dog zoonoses and human health: a global perspective - mini review. CABI Reviews. 8(002), 1-2.

Mehlhorn, H., 2015. Toxocara (syn. Neoascaris) vitulorum. In: Mehlhorn, H. (Ed.), Encyclopedia of parasitology. Springer, Berlin, Heidelberg.

Moyo, D.Z., 2002. The Migratory behaviour of *Toxocara canis* and *Toxocara vitulorum* in Balb/c Mice. Zimb. Vet. J. 33(1), 7-13.

Murthy, H.N., Yadav, G.G., Dewir, Y.H., Ibrahim, A., 2020. Phytochemicals and biological activity of desert date (*Balanites aegyptiaca* (L.) Delile). Plants (Basel). 10(1), 32.

Musa, D., Senocak, G., Borazan, G., Altas, M., Ozgonul, A., Sogut, O., Güldür, M.E., 2011. Effects of Nigella sativa and albendazole alone and in combination in *Toxocara canis* infected mice. J. Pak. Med. Assoc. 61(9), 866-870.

Naderbandi, M., Zibaei, M., Haniloo, A., Firoozeh, F., Hatami, Z., Shokri, E., Taira K., 2022. Larva migrans in BALB/c mice experimentally infected with *Toxocara cati* ensured by PCR assay. BMC Vet. Res. 18, 257.

Rajkhowa, S., Bujarbaruah, K.M., Rajkhowa, C., 2004 . Haematobiochemical changes in ascarosis of Mithun calves. Indian. Vet. J. 81, 571–572.

Raza, M.A., Murtaza, S., Ayaz, M.M., Akhtar, S., Arshad, H.M., Basit, A., Bachaya, H.A., Ali, M., Khan M.I., 2013. *Toxocara vitulorum* infestation and associated risk factors in cattle and buffalo at Multan district. Pakistan. Sci. Int (Lahore). 25(2), 291-294.

Resende, N.M., Gazzinelli-Guimarães, P.H., Barbosa, F.S., Oliveira, L.M., Nogueira, D.S., Gazzinelli-Guimarães, A.C., Gonçalves, M.T., Amorim, C.C., Oliveira, F.M., Caliari, M.V., Rachid, M.A., Volpato, G.T., Bueno, L.L., Geiger, S.M., Fujiwara, R.T., 2015. New insights into the immunopathology of early *Toxocara canis* infection in mice. Parasit. Vectors. 8, 354.

Rizk, M.A., Osman, S.A., AL-Gaabary, M.H., EL-Khodery, S.A., 2018. Comparative clinical and parasitological efficacy of moxidectin pour-on, ivermectin, and piperazine citrate on *Toxocara vitulorum* infection in buffalo calves (*Bubalus bubalis*): a randomized clinical trial. Turk. J. Vet. Anim. Sci. 42(1), 29-33.

Roberts, J.A., 1990. The life cycle of *Toxocara vitulorum* in Asian buffalo (*Bubalus bubalis*). Int. J. Parasitol. 20(7), 833-840.

Roberts, J.A., Fernando, S.T., Sivanathan, S., 1990. *Toxocara vitulorum* in the milk of buffalo (*Bubalus bubalis*) cows. Res. Vet. Sci. 49(3), 289-291.

Sahoo, C.R., Paidesetty, S.K., Padhy, R.N., 2021. The recent development of thymol derivative as a promising pharmacological scaffold. Drug. Dev. Res. 82, 1079–1095.

Samanta, S., Ansari, M., 1990. Embryonation of ova of *Toxocara canis* (Werner, 1782) and migration of the larvae in mice. Indian J. Anim. Sci. 60, 1391–1393.

Santos, S.V., Lescano, S.Z., Castro, J.M., Chieffi, P.P., 2009. Larval recovery of *Toxocara cati* in experimentally infected *Rattus norvegicus* and analysis of the rat as potential reservoir for this ascarid. Mem. Inst. Oswaldo Cruz. 104(6), 933-934.

Shalaby, M.A., Moghazy, F.M., Shalaby, H.A., Nasr, S.M., 2010. Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. Parasitol. Res. 107(1), 17-25.

Shalaby, H.A., El Namaky, A.H., Khalil, F.A., Kandil, O.M., 2012. Efficacy of methanolic extract of *Balanites aegyptiaca* fruits on *Toxocara vitulorum*. Vet. Parasitol. (183), 386–392.

Shehata, O., Aboelhadid, S.M., Arafa, W.M., Moawad, U.K., Hussien, K.H., Ali, M.I., El-Ashram, S., Abdel Gawad, S.S., Abdel-Aziz, S.A., 2022. Assessment of the efficacy of thymol against *Toxocara vitulorum* in experimentally infected rats. J. Parasit. Dis. 46(2), 454-465.

Singh, K., Mishra, S.K., Pruthi, A.K., 2008. Pathology of parasitic infestations in gastrointestinal tract in buffalo calves. J. Vet. Parasitol. 22(1), 17-20.

Soares, M.J., 2006. Endocytic portals in *Trypanosoma cruzi* epimastigote forms. Parasitol. Res. 99, 321–322.

Soulsby, E.J.L., Mönnig, H.O., 1968. Helminths, arthropods & protozoa of domesticated animals, 6th edition. Williams and Wilkins, Baltimore, pp. 824.

Srivastava, A.K., Sharma, D.N., 1981. Studies on the occurrence, clinical features and pathomorphological aspects of ascariasis in buffalo calves. Vet. Res. J. 4, 160–162.

Sultan, K., Omar, M., Desouky, A.Y., El-Seify, M.A., 2015. Molecular and phylogenetic study on *Toxocara vitulorum* from cattle in the mid-Delta of Egypt. J. Parasit. Dis. 39(3), 584-587.

Venjakob, P.L., Thiele, G., Clausen, P.H., Nijhof, A.M., 2017. *Toxocara vitulorum* infection in German beef cattle. Parasitol. Res. 116(3), 1085-1088.

Zibaei, M., Sadjjadi, S.M., Uga, S., 2010. Experimental *Toxocara cati* Infection in Gerbils and Rats. Korean. J. Parasitol. 48(4), 331-333.

How to cite this article;
Huda Mohammed Kuraa, Safaa Sayed Malek, Iman Abdel-Raheem Mahmoud Abdel-Rahman and Abeer H.M. El Hendy. Therapeutic role of Thymol, Propolis and *Balanites aegyptiaca* against experimental *Toxocara vitulorum* infection. Veterinary Integrative Sciences. 2025; 23(1): 1-23.