Evaluation of the effect of albendazole and *Nigella sativa* combination on Visceral Larvae Migrans (*Toxocara canis*) in mice

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ABSTRACT

Visceral Larvae Migrans (VLM) is a syndrome in humans, caused by *Toxocara canis* larvae. A current and completely successful treatment protocol against such a common infection has yet to be established. In this study, the effect of combination of albendazole and *N. sativa* oil for the treatment of VLM was investigated. Five experimental groups were constituted and a total of 125 *Swiss albino* (male, 6-8 weeks old) mice were used. All mice in each group were infected with 750 *T. canis* eggs with infective larvae, except the negative control group. 100 mg/kg albendazole and 0.15 ml *Nigella sativa* oil were applied orally to groups ALB and NSO separately and given orally to group COM in combination. The efficacy of the treatment was investigated parasitologically, histo-pathologically, and hematologically on the 7th, 14th, 28th, 45th, and 60th days post-infection with necropsies. The larval recovery analyses revealed that, the highest treatment efficacy was obtained in group of combination. The treatment efficacy was 72.46%, 48.81%, 36.25% in the groups of COM, ALB, and NSO, respectively. The most severe pathological changes were developed in Group ALB, and the inflammatory reactions and pathological changes in Groups of COM and NSO were mild. We conclude that *N. sativa* oil enhances the larvicidal effect of albendazole by having an anti-inflammatory effect and increasing tissue defense and immunity.

Introduction

Toxocara canis is a parasite frequently encountered in both puppies and adult dogs. In addition to being a common parasite in carnivore animals, *T. canis* is also an important parasite in humans, especially in play-age children, causing a zoonotic infection known as Visceral Larvae Migrans Syndrome (11).

Visceral Larvae Migrans (VLM) is characterized by hypereosinophilia (about 10.000 cells/mm3), hepatomegaly, fever, intermittent pulmonary infiltration, and hypergammaglobunemia, all caused by nematode larvae that have a non-human definitive host (5, 9).

The main purpose of the treatment of infection is to reduce the number of larvae migrating to tissues and also alleviate or eliminate clinical symptoms (23). It is recommended that patients be treated with long-term anthelmintic as well as an anti-inflammatory agent (29). Currently, albendazole can be used for VLM treatment in humans (31, 38). Since a fully effective anthelmintic has not been established yet, the researchers were encouraged to use microparticles, immunomodulatory agents, probiotics, immune system supporters and stimulants, tissue defense strengthening, mucosal integrity strengthening, and anti-inflammatory agents as alternative treatments to increase the treatment efficiency (3, 4, 14, 19, 32). There have also been several applications using molecules of plant origin for increasing the effectiveness of the treatments of VLM (34, 35). *Nigella sativa* has been considered as one of the medically important plants due to its immunomodulator effect on cellular and humoral immunity (13, 15, 27, 28) and anti-inflammatory effect on inflammation areas (6, 12, 24, 26). The main active agent showing this medicinal effect is thymoquinone (TQ) as a phytochemical agent (2-isopropyl-5-methyl-1,4-benzoquinone, $C_{10}H_{12}O_2$) (1, 36). Thymoquinone is found in 30-48% of the seeds (2, 16).

There have been a few studies investigating the effect of *N. sativa* on helminth infections (8, 25, 30). The aim of this study was to determine the effectiveness of a combination of albendazole and N. sativa oil against VLM in an experimentally infected mouse model. Albendazole is less absorbed from the digestive system, and it has been stated that this absorption increases in oily medium (16). Therefore, we hypothesized that whether a new drug formulation could be developed by enhancing the effect of albendazole with *N. sativa* oil.

Materials and Methods

Active substances and experimental groups: A total of 125 male *Swiss albino mice* (mean weight 30g and 6-8 weeks old) were used in this study, with 25 mice in experimental [albendazole (Vermiprazole oral suspension 10%), *N. sativa* oil, and combination] and control groups. The amount of albendazole active substance in the preparation was analyzed by a spectrophotometric method (37). The anthelmintic was administered orally to mice at a dose of 100 mg/kg in group of albendazole (ALB) and combination (COM) (39).

The oil obtained from the seeds of *N. sativa* was directly administered by oral gavage at a dose of 0.15 ml to mice in group of *N. sativa* oil (NSO) and COM (30). The modified method was used to determine the amount of TQ as the active ingredient in *N. sativa* oil, by High Performance Liquid Chromatography (HPLC) (10).

Infecting mice and treatment procedure: Infected dogs were treated with Pyrantel Pamoate (Kontil®, oral suspension, 250 mg/5ml) at a dose of 0.1 cc/kg to collect mature *T. canis*. Eggs were collected from mature females and incubated in 0.5% formalin at 26-28°C in humidity for 21-23 days until infective larvae developed. Mice were infected with 750 eggs containing infective larvae by oral gavage. The day infected mice were determined as day 0 of the experiment. A physiological salt solution was given to the group of negative control by oral gavage to provide the same stress and ambient conditions. Albendazole (100 mg/kg p.o), *N. sativa* oil (0.15 ml p.o), and a combination of albendazole and *N. sativa* oil (100 mg/kg p.o albendazole + 0.15 ml p.o *N. sativa* oil) were applied to mice in treatment groups for 5 days post-infection. 0.2 ml dose of physiological salt solution was given orally to Groups of positive (PC) and negative control (NC).

Necropsies of mice: Necropsies were performed on 5 mice from each experimental group on the 7th, 14th, 28th, 45th, and 60th days post-infection. Three of 5 mice were examined parasitologically and two of them were examined pathologically.

Brain, eye, internal organs, muscle tissue, and mesenterial lymphatic nodules were examined for larvae (Figure 1). Also, the organs with lumen like stomach, intestine, urinary bladder, etc., and body cavities (abdominal and thoracic cavities) were examined parasitologically on stereo-microscope. The brain and eye were examined immediately after removal. The heart, lungs, diaphragm muscles, gastric mucosa, liver, spleen, kidneys, testes, mesenterial lymphatic nodules, forelimb, and hindlimb muscles, intestinal and urinary bladder mucosa were incubated in pepsin-HCl digestion solution (5 g pepsin + 7 ml HCl + 988 ml 0.9% isotonic solution) at 37°C for 24 hours. At the end of the period, the molten organs and tissue fragments were filtered and centrifuged in 15 ml conical falcon tubes at 2000 rpm for 10 minutes. After centrifugation, the supernatant was removed and the samples were kept at +4°C with 10% formalin until the larvae were counting.

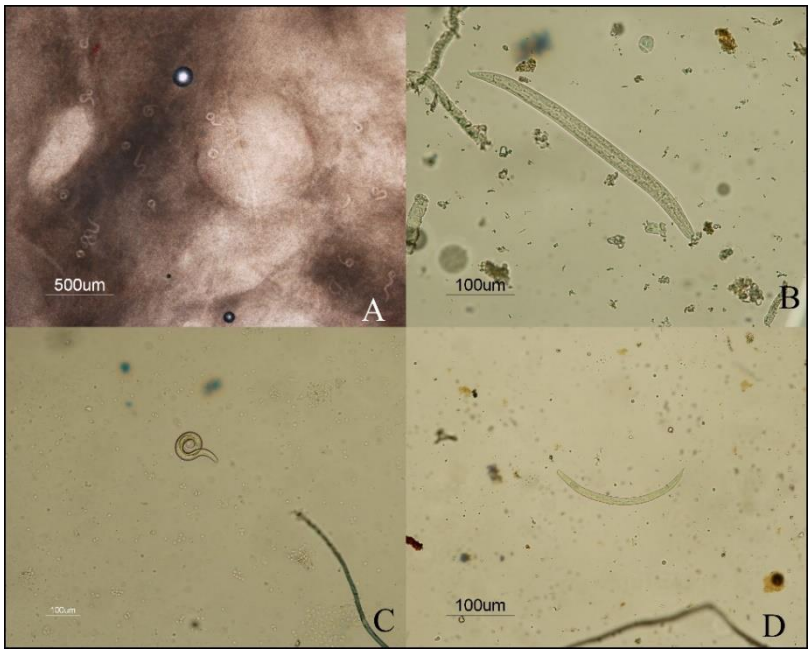

Figure 1. *Toxocara canis* larvae in different tissues. **A.** Brain, **B.** Hindlimb muscles, **C.** Eye, **D.** Diaphragm muscles.

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Haematological and histo-pathological examination: Blood taken during euthanasia was used to prepare smears for hematological analysis. Three preparations were made from each mouse to determine the effect of treatment on blood cells, especially eosinophil leukocytes. The May-Grünwald Giemsa staining technique was used to stain the smears (21). The type and rate of the first 100 blood cells encountered in the microscopic area were calculated with the formula-leucocitaria method (18).

In order to examine the histopathological changes, 5µm thick sections were taken from tissue samples after 10% formalin fixation and paraffin blocking. The preparations were stained with Hematoxylin & Eosin (21). All treatment groups were compared to Groups PC and NC.

Statistical analysis: IBM SPSS Statistics Version 23 was used to evaluate the data. The statistical difference between the groups was analyzed by the Kruskal Wallis Test and the statistical difference was taken as P<0.10.

Results

Pharmacological results: In spectrophotometric analysis, the amount of albendazole in the oral suspension was 98%. Thymoquinone in *N. sativa* oil was found to be 1.39% using High Performance Liquid Chromatography method. According to the TQ percentage taken, 0.17% TQ was detected in 0.15 ml (0.13 g) *N. sativa* oil dosed to each mouse.

Parasitological results: On the 7th day, the total number of migrated larvae were 343.65. The number of migrating larvae decreased in each treatment group. The fewest larvae was found in the Group COM. Larvae were 31.00 in Group COM, and 36.33; 61.66 and 214.66 in Group ALB, NSO, and Group PC, respectively. They were especially concentrated in the liver and lungs, especially in Group PC. Numbers of larvae in liver and lungs were statistically different compared to the other necropsy days (P<0.05). It was also observed that the larvae began to migrate to brain on 7th day in all experimental groups. The decrease in the number of larvae in the extremity muscles of Group ALB was statistically different compared to Group PC (P<0.10). The decrease in the number of larvae of brain and diaphragm muscles in Group COM were statistically different compared to the Group PC and Group ALB (P<0.10) (Table 1).

Table 1. Average number of larvae on 7th, and 14th days.

	7th day					14th day				
	PC n=3 $\bar{x} \mp S_{\bar{x}}$	ALB n=3 $\bar{x} \mp S_{\bar{x}}$	NSO n=3 $\bar{x} \mp S_{\bar{x}}$	COM n=3 $\bar{x} \mp S_{\bar{x}}$	P	PC n=3 $\bar{x} \mp S_{\bar{x}}$	ALB n=3 $\bar{x} \mp S_{\bar{x}}$	NSO n=3 $\bar{x} \mp S_{\bar{x}}$	COM n=3 $\bar{x} \mp S_{\bar{x}}$	P
Brain	22.00∓1.528[3]	10.67∓1.667	22.00∓2.517	6.33∓3.1803[3]	0.033**	40.33∓2.603[3]	24.67∓6.173	32.33∓5.207	13.00∓3.786[3]	0.063*
Eye	-	-	0.33∓0.333	-	-	0.67∓0.333	-	-	-	-
Heart	1.33∓1.333	1.00∓1.000	0.33∓0.333	0.33∓0.333	0.983	1.67∓0.667	0.67∓0.333	1.33∓0.882	-	0.139
Lungs	36.33∓15.857	8.67∓3.180	6.00∓1.732	4.33∓1.667	0.107	2.00∓0.577	1.67∓1.202	0.33∓0.333	1.33∓0.882	0.391
Diaphragm Muscles	1.67∓0.333	0.67∓0.333[5]	1.33∓0.667	4.00∓1.000[5]	0.083*	1.67∓0.667	1.00∓1.000	0.67∓0.333	0.67∓0.667	0.585
Stomach	-	-	-	-	-	-	-	-	-	-
Liver	122.33∓65.804	10.33∓4.978	8.67∓5.175	6.67∓4.702	0.281	26.67∓5.925[3]	8.00∓4.041	11.33∓1.333	5.00∓0.577[3]	0.051*
Spleen	-	-	-	0.67∓0.667	-	1.00∓0.577	0.67∓0.667	0.67∓0.333	-	0.415
Kidneys	8.67∓1.667	2.00∓2.00	11.67∓3.667	4.67∓2.186	0.101	2.00∓0.000	2.00∓0.577	0.67∓0.333	0.67∓0.333	0.249
Testes	-	-	0.33∓0.333	-	-	0.33∓0.333	-	-	0.33∓0.333	0.532
M.Lymph Nodes	1.33∓0.333[3]	0.33∓0.333	1.00∓0.000	0.00∓0.000[3]	0.040**	1.00∓0.000	0.33∓0.333	0.33∓0.333	0.67∓0.333	0.326
Forelimb Muscles	11.67∓2.848[1]	1.33∓0.882[1]	7.67∓3.667	2.33∓1.202	0.043**	5.67∓3.667	4.33∓0.333	4.67∓1.764	1.33∓0.333	0.137
Hindlimb Muscles	9.00∓1.155[1]	1.00∓1.000[1]	2.33∓0.882	1.67∓0.882	0.072*	10.00∓3.464[3]	3.33∓0.333	7.00∓2.309	1.00∓0.577[3]	0.041**
Intestines	0.33∓0.333	0.33∓0.333	-	-	0.532	-	-	-	-	-
Urinary Bladder	-	-	-	-	-	-	-	-	-	-

*: P<0.10; **: P<0.05; There are statistically differences between groups [1]: PC and ALB; [2]: PC and NSO; [3]: PC and COM; [4]: ALB and NSO; [5]: ALB and COM; [6]: NSO and COM.

On the 14th day, the total number of migrated larvae was 222.99. The number of migrated larvae decreased, especially in Group COM compared with Group PC. The prevalences of larvae were 24.00; 46.00; 59.33; 93.00 in Groups COM, ALB, NSO, and Group PC, respectively. The number of larvae that passed into the neurotropic-myotropic phase from 14th day was determined more intensively. In all experimental groups, the count of larvae in the internal organs such as the liver, lungs, and kidneys decreased and increased in the brain. The larvae in brain were found to be closer in Group NSO than in Group PC. The liver was the second most common larvae in all groups. The extremity and diaphragm muscles were the most common tissues in this period after brain and liver. Compared to all groups, the minimum number of larvae in the brain extremity muscles, and liver were determined in Group COM. The number of larvae in the brain, liver ($P<0.10$), and hindlimb muscles were statistically different compared to Group PC ($P<0.05$) (Table 1).

The total number of migrated larvae on day 28th was 587.65. The numbers of larvae were 91.00 in Group COM; 102.66 in Group ALB; 179.66 in Group NSO; and 214.33 in Group PC. In all groups, the maximum number of larvae was detected in the brain and decreased in the liver and lungs. The decrease in the number of larvae in hindlimb muscle in Group COM was statistically different compared to Group PC ($P<0.05$) (Table 2).

The total number of migrated larvae on the 45th day was 603.99. The numbers of larvae were 42.00 in Group COM; 164.33 in Group NSO; 196.33 in Group ALB and 201.33 in Group PC. On 60th day, a total of 319.65 larvae migrated. Numbers of larvae were 48.00 in Group COM; 56.66 in Group ALB, 81.33 in Group NSO and 133.66 in Group PC. On the 28th, 45th, and 60th days, larvae in the brain were found to be increasing compared to the 7th and 14th days and this increase was statistically different ($P<0.05$) (Table 2).

Histopathological results: All histopathological results were evaluated by comparison with Groups NC and PC on each necropsy day. On the 7th day, Group ALB showed severe inflammatory cell infiltration in the lungs, liver, and diaphragm muscles. Severe neutrophil leukocyte cell infiltration around alveoli in the lungs, emphysema, edema, and eosinophil leukocytes were noted (Figure 2). Neutrophil leukocytes, mononuclear cell infiltration, and perivasculitis were detected with numerous Kupffer cells in the liver between the remark cords and around the *Vena centralis*. In Group NSO, severe inflammation replaced by mild cellular infiltration, and changed from polymorphonuclear to mononuclear (Figure 2). Also, it was found that the level of inflammation decreased and changed from polymorphonuclear to mononuclear in Group COM as Group NSO.

On the 14th day, it was observed that the inflammation in Group ALB was severe and that many organs and tissues (lungs, liver, kidneys, etc.) were affected. There was mononuclear cell infiltration around the alveoli in the lungs and *V. centralis* and trias hepatis in the liver, as well as emphysema and focal granulomas. Mononuclear cell infiltrations and eosinophil leukocytes were noted in extremity muscles. There were no serious pathological observations in Group NSO, unlike in Group ALB. In Group COM, mild mononuclear cell infiltration in the lungs, liver, heart, extremity and diaphragm muscles, and kidneys, emphysema, edema, and eosinophil leukocytes and granuloma structures, were observed in part of the mice (Figure 3).

On the 28th, 45th, and 60th days, similar organs were affected by infection. The inflammation became chronic, and the mononuclear cells dominated the lesions. Tissue repair was started on the 28th day. Also, granuloma formation was observed in the liver, heart, and lungs on the 45th and 60th days (Figure 4).

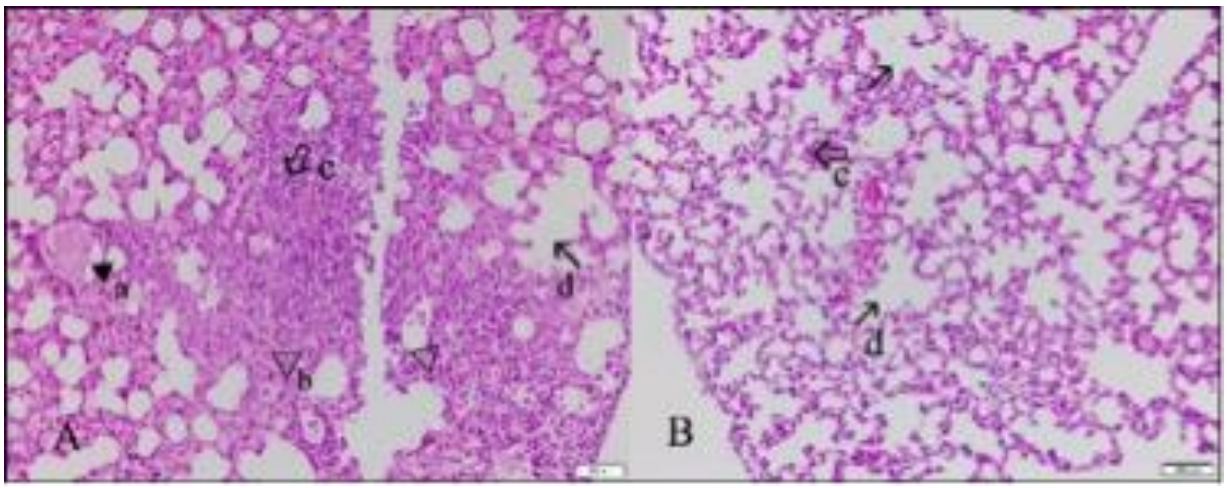

Figure 2. A. 7th day, Group ALB; edema in lung tissue (a), eosinophil leukocytes (b), multiple neutrophil leukocyte cell infiltration around alveoli (c) and emphysema (d). (H&E, scale bar: 50 µm). **B.** 7th day, Group NSO; mild cell infiltration around the alveoli in lung tissue (c) and areas of emphysema (d) (H&E, scale bar: 100µm).

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Table 2. Average number of larvae on 28th, 45th, and 60th days.

	28th day					45 th day					60th day				
	PC n=3 $\bar{x} \mp S_{\bar{x}}$	ALB n=3 $\bar{x} \mp S_{\bar{x}}$	NSO n=3 $\bar{x} \mp S_{\bar{x}}$	COM n=3 $\bar{x} \mp S_{\bar{x}}$	P	PC n=3 $\bar{x} \mp S_{\bar{x}}$	ALB n=3 $\bar{x} \mp S_{\bar{x}}$	NSO n=3 $\bar{x} \mp S_{\bar{x}}$	COM n=3 $\bar{x} \mp S_{\bar{x}}$	P	PC n=3 $\bar{x} \mp S_{\bar{x}}$	ALB n=3 $\bar{x} \mp S_{\bar{x}}$	NSO n=3 $\bar{x} \mp S_{\bar{x}}$	COM n=3 $\bar{x} \mp S_{\bar{x}}$	P
Brain	163.33∓50.466	70.67∓11.096	140.00∓43.405	69.00∓21.733	0.270	162.00∓90.279	140.67∓39.074	124.67∓17.072	36.67∓11.260	0.282	70.67∓6.009	30.33∓4.055	48.67∓10.975	30.67∓7.126	0.189
Eye	0.33∓0.333	0.33∓0.333	0.33∓0.333	0.33∓0.333	1.000	0.67∓0.667	-	-	-	-	0.67∓0.333	-	-	-	-
Heart	3.67∓0.882	1.33∓0.333	1.00∓0.00	1.00∓0.577	0.145	-	1.00∓0.577	0.33∓0.333	0.33∓0.333	0.367	2.33∓0.882	1.00∓1.000	1.67∓1.202	0.33∓0.333	0.328
Lungs	4.67∓0.882	2.67∓1.202	4.67∓1.202	1.67∓0.882	0.169	1.00∓1.000	-	0.33∓0.333	0.33∓0.333	0.737	2.67∓0.882	0.33∓0.333	0.33∓0.333	0.33∓0.333	0.120
Diaphragm Muscles	1.33∓0.882	1.00∓0.000	1.67∓0.882	1.33∓1.333	0.951	1.00∓0.577	0.67∓0.667	0.33∓0.333	-	0.438	1.33∓0.33[3]	0.33∓0.333	0.33∓0.333	0.00∓0.000[3]	0.088*
Stomach	-	0.67∓0.333	-	-	-	0.00∓0.000[1]	1.33∓0.333[1, 5]	0.33∓0.333	0.00∓0.000[5]	0.037**	-	-	-	-	-
Liver	11.00∓1.528	8.00∓2.517	8.33∓2.333	8.33∓5.364	0.852	4.00∓2.517	12.00∓5.196[5]	5.00∓1.732	0.33∓0.333[5]	0.058*	17.67∓6.692	9.33∓6.360	10.00∓3.786	7.33∓1.856	0.532
Spleen	-	-	-	-	-	0.33∓0.333	-	-	-	-	-	-	-	-	-
Kidneys	8.00∓3.055	10.33∓2.963	10.33∓4.333	4.67∓1.453	0.410	4.00∓1.732[3]	1.67∓0.333	2.67∓1.202	0.00∓0.000[3]	0.072*	3.67∓1.764	1.67∓1.202	1.00∓0.577	0.67∓0.667	0.377
Testes	-	-	0.33∓0.333	0.33∓0.333	0.532	-	-	0.33∓0.333	-	-	0.67∓0.667	0.33∓0.333	0.33∓0.333	-	0.737
M.Lymph Nodes	-	-	0.67∓0.667	0.33∓0.333	0.530	-	-	-	-	-	-	-	-	-	-
Forelimb Muscles	3.00∓1.000	3.67∓0.333	7.00∓1.528	2.67∓1.333	0.104	14.00∓8.327	20.33∓4.055	22.00∓7.550	3.00∓2.082	0.115	14.67∓2.028	6.00∓5.000	10.33∓1.764	2.33∓1.333	0.137
Hindlimb Muscles	19.00∓4.933[3]	4.00∓1.000	5.33∓0.882	1.33∓0.333[3]	0.025**	13.67∓7.265	18.67∓2.603[5]	8.33∓0.882	1.33∓0.882[5]	0.066*	19.33∓4.910	7.33∓0.882	8.67∓2.848	6.33∓2.906	0.138
Intestines	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urinary Bladder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*: P<0.10; **: P<0.05; There are statistically differences between groups [1]: PC and ALB, [2]: PC and NSO, [3]: PC and COM, [4]: ALB and NSO, [5]: ALB and COM; [6]: NSO and COM.

Table 3. Average of blood cell rates on 7th, and 45th days.

	7th day					45th day				
	PC n=3 $\bar{x} \mp S_{\bar{x}}$	ALB n=3 $\bar{x} \mp S_{\bar{x}}$	NSO n=3 $\bar{x} \mp S_{\bar{x}}$	COM n=3 $\bar{x} \mp S_{\bar{x}}$	P	PC n=3 $\bar{x} \mp S_{\bar{x}}$	ALB n=3 $\bar{x} \mp S_{\bar{x}}$	NSO n=3 $\bar{x} \mp S_{\bar{x}}$	COM n=3 $\bar{x} \mp S_{\bar{x}}$	P
Lymphocyte	60.3100∓4.06955	37.7867∓10.02937	52.9400∓3.34936	52.1333∓23.19438	0.468	68.8867∓4.94431	70.6667∓4.16356	73.3700∓8.41900	89.5533∓3.25677	0.144
Neutrophil Leukocyte	24.9733∓5.70535	9.1567∓3.54941	33.4567∓7.06373	24.7900∓15.39505	0.282	26.9967∓4.87826	23.5233∓1.91068	23.7733∓7.22509	9.5533∓2.80586	0.141
Monocyte	13.0400∓3.44730	52.6000∓13.35886[1]	5.3733∓2.52171[1]	23.0700∓9.71321	0.075*	2.5533∓0.29356	5.6833∓4.51934	2.7233∓1.42995	0.8867∓0.48254	0.354
Eosinophil Leukocyte	1.6600∓1.49323	0.4433∓0.44333	-	-	0.224	1.4400∓0.67486[2]	0.1133∓0.11333	0.1200∓0.12000	0.0000∓0.00000[2]	0.099*
Basophil Leukocyte	-	-	-	-	-	0.1100∓0.110000	-	-	-	-

*: P<0.10; **: P<0.05; There are differences between groups [1]: ALB and NSO; [2]: PC and COM.

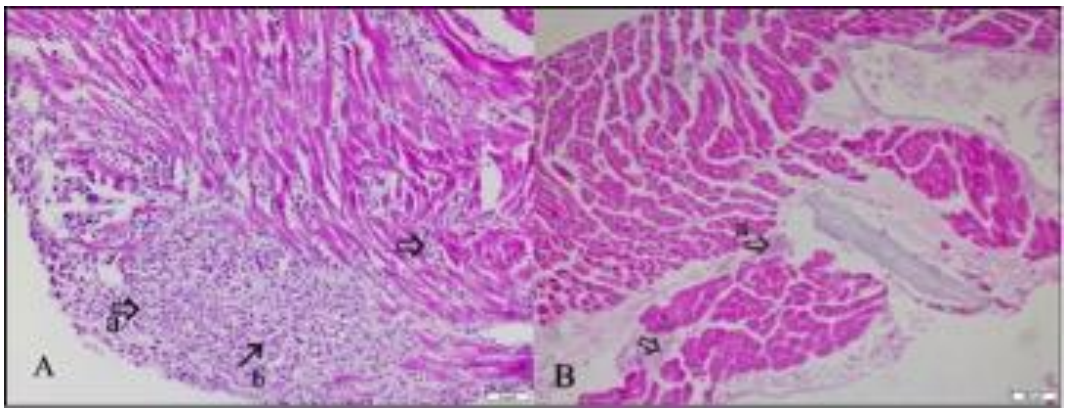

Figure 3. A. 14th day, Group ALB; heart muscle tissue severe mononuclear cell infiltration between muscles (a) and eosinophil leukocytes (b) (H&E, scale bar: 50 µm). **B.** 14th day, Group COM; mild mononuclear cell infiltration between muscles (a) (H&E, scale bar: 100µm).

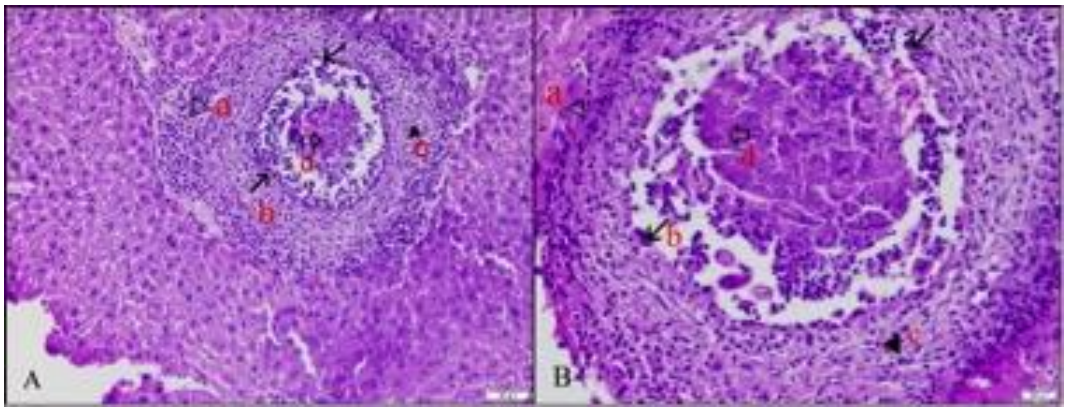

Figure 4. 60th day, Group NSO; coagulation necrosis in the center of liver tissue (d), around multinucleated giant cells (b), mononuclear cell infiltration (c) and granuloma structure surrounded by connective tissue (a) (**A.** H&E, scale bar: 50 µm), (**B.** H&E, scale bar: 20 µm).

Hematologic results: Blood cell ratios for all necropsies are given in Table 3. On the 7th day, the treatment groups were compared with Group PC; the ratio was decreased in all groups, and the values were similar in Groups NSO and COM. There was a statistical difference (P<0.10) between Group ALB and NSO. No statistical difference was found between the groups in terms of cell availability on the 14th and 28th day (P>0.10).

On the 45th day, when the ratios of all the experimental groups were compared with Group PC, it was observed that the lymphocyte ratio increased and the neutrophil leukocyte ratio decreased. Monocytes were increased in Group ALB, decreased in Group COM, and were similar in Group NSO. The absence of eosinophil leukocytes in Group COM showed statistical difference (P<0.10) with Group PC. No statistical difference was found between groups on 60th day (P>0.10).

Discussion and Conclusion

Numbers of migrated larvae were found at 856.98 in Group PC; 236.00 and 436.64 in Group COM and ALB respectively. It was found at 546.31 in Group NSO. The maximum reduction in the number of larvae was seen in Group COM compared to Group PC. The second group was ALB, followed by Group NSO. The most effective group was Group COM (72.46%), the second group was ALB (48.81%), and Group NSO was found to be minimal (36.25%) in terms of parasitological effect level.

Parasitological effect of treatment was 85.55% on the 7th day, and found to be 74.19%; 57.69%; 79.13%, and 64.08% on the 14th, 28th, 45th, and 60th, respectively. Musa et al. (30) reported that the effect in the combination group was 87.00% on the 7th day, showing a similarity with this study (85.55%) and *Nigella sativa* oil in two different doses for 7 days was given to mice, and the effect level was determined as 31.0% and 39.3% on the 7th day. But, in this study, the effect level of Group NSO 71.27% were found on the 7th day and a much higher effect level was obtained. It was thought that using the oil extract obtained directly from seed, the difference in the amount of *N. sativa* and TQ in the extract used and the geographic differences in plant structure/components could be effective on an effect level. The geography of the plant, the way it is obtained, the döşe, and the application time can affect the percentage of activity (20). It is reported that most of the TQ found in the composition of *Nigella sativa* is found in the seeds (30-48%) (2). In addition, it is reported that the fatty extract of the plant is more easily

absorbed from intestinal cells than alcohol and aqueous extract (33). In the current study, the oil extract obtained from the seeds of *N. sativa* was applied directly, and it's considered that a higher level of effect was achieved, unlike in the previous study (30).

The partial decrease in the number of larvae in Group NSO arises from the immunomodulatory and anti-inflammatory effect of the oil by increasing the host's tissue defense and regulating the immune response to affect the reduction of parasite settlement and tissue damage as noted by some researchers (7, 13, 15, 28). The changes in the tissues of mice in Group NSO and COM were milder than Group ALB and PC. Musa et al. (30) reported that the destruction of lungs, liver, and brain was decreased in *N. sativa* oil and albendazole group compared to the positive control group, whereas in the combination group. In this study, no antiparasitic effect was observed in Group NSO, as in some studies (17, 22, 25). In both Group NSO and COM, inflammation severity and degree of inflammatory changes were less than Group ALB. It has been observed that the application of oil with albendazole both regulates the body and tissue defense mechanisms of the host and prevents the localization of the parasite as well as mitigating the damage caused by the parasite.

It was determined that the blood values during the first two weeks of migration of the infection were consistent with the pathological findings. When evaluated together with pathological findings, the predominance of lymphocytes and macrophages in the inflammatory cells supports the decrease in the amount of blood as a result of migration to the tissue. Some researchers reported that *N. sativa* oil can increase and decrease the neutrophil leukocytes and interleukins in the blood and regulate the severity of the immune response (13, 15, 28).

In conclusion, the larvicidal-migration inhibitory effect of albendazole and the immunomodulatory, anti-inflammatory effect of *N. sativa* oil were found in Group COM with the highest parasitological and pathological effects. It's thought that *N. sativa* oil enhanced the effect of albendazole with its immunomodulatory and anti-inflammatory properties, so that infection was observed to be milder both parasitologically and pathologically in Group COM. The effect of *Nigella sativa* oil, when used alone, is lower than the other two treatment groups and being close to the Group PC. It's showed that plant oil does not directly affect larvae when applied alone. Pathological findings supported that the oil exhibits an anti-inflammatory effect in tissues and blood preparations. It is thought that such studies will open a new perspective on the development of the phytotherapy field in parasitic diseases. With this study, *Nigella sativa* oil has no direct lethal effect on the larvae, but may help in the development of supportive/complementary treatment procedures to reduce the side effects of immune system supplementation and/or host infection.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

CA, and HÖ conceived and planned the experiments. CA prepared all samples, carried out all experiments and interpreted the results. HÖ contributed to interpretation of the results. CA took the lead in writing the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Gazi University Animal Experiments Local Ethics Committee (207-22055/ G.U.ET-11-104).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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