

## Fenbenzyl and fenbendazole impact on the dog's liver protein synthesizing function during experimental infestation with the pathogen toxocariasis

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The study aimed to investigate the fenbenzyl and fenbendazole impact on the indicators of the dog's liver protein synthesizing function during experimental infestation with the pathogen toxocariasis. The investigation was performed on 18 dogs, two to four months of age, and three groups of six animals in each were formed: control (C) and two experimental (E<sub>1</sub>, E<sub>2</sub>). Puppies were experimentally infected with the pathogen toxocariasis at a dose of 5,000 invasive *T. canis* eggs per kg of body weight. The control dogs were as untreated. Puppies of the (E<sub>1</sub>) group were fed the drug "Fenbendazole" at a dose of 150 mg per 3 kg of body weight once a day for three days. Dogs of the (E<sub>2</sub>) group have applied the "Fenbenzyl" at a dose of 350 mg per 3 kg of body weight once a day for three days. Toxocariasis invasion in canines suppressed the liver protein synthesizing function, indicated by a decrease in total blood protein, albumin fraction, and globulin levels. During fenbendazole treatment for 25 and 30 days, the liver protein synthesizing function was not completely restored, confirmed by the low level of total protein and its albumin fraction. After studying the impact of fenbenzyl on the protein synthesizing function of the liver of dogs infected with *Toxocara*, a gradual normalization of serum levels of total protein and its fractions was established. The progress of total protein content in the serum of canines of the (E<sub>2</sub>) group in this period of the trial shows the stimulating effect of milk thistle in the drug "Fenbenzyl" on protein synthesis, compared with the (C) and (E<sub>1</sub>) groups.

**Keywords:** invasion, toxocariasis, dogs, proteins, albumins, globulins, blood.

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### Introduction

A significant number of works by parasitologists represented in the literature are devoted to spreading toxocariasis in Europe and Ukraine (Rubinsky-Elefant et al., 2011; Bodnia, 2016; Ozlati et al., 2016; Dralo et al., 2017; Noor et al., 2019). Thus, the average intensity of soil contamination by nematodes eggs of the genus *Toxocara* in different regions of Ukraine is 1.6-10.7% (Pryima, 2010; Said et al., 2018). Toxocariasis is a helminthic disease caused by the nematode *Toxocara canis* in dogs (Noor et al., 2019). Mature helminths parasitize in the small intestine (Said et al., 2018). In the case of the significant intensity of invasion, adult parasites cause inflammation of the mucous membrane of the small intestine, stomach, and bile ducts of the liver and pancreas. *Toxocara* secretes toxins that cause general intoxication of the body (Dralova et al., 2017).

The occurrence of environmental contamination by *Toxocara* eggs is associated with violations of sanitary and epidemiological surveillance, inadequate housing conditions, and an increased number of stray animals, which are a source of environmental pollution and pose a potential risk of human infection. (Usachova & Dralova, 2012; Lovytskaia et al., 2013). It was found that the maximum incidence of toxocariasis was registered in puppies under six months of age - 85%. The extent of infestation was slightly lower in animals 6-9 months of age (61.5%) and at the age of 9-12 months, which is 45.4%. Subsequently, their infestation

rates decreased (25% at the age of 1 to 2 years). The lowest level of infestation was registered in adult dogs, mainly in pregnant females over three years of age (11%) (Stybel & Pryima, 2010; Said et al., 2020).

The pathogenic effect of helminths on the host is mechanical, toxic, trophic, allergic, and inoculative. It is also reflected in physiological processes, morphofunctional characteristics of organs and tissues. Local organ damage, loss of nutrients, development of stress, cytogenetic disorders, and changes in immune status - as far from the complete list of consequences of toxocariasis (Svirzhevskaya, 2011; Svirzhevskaya, 2013; Moisieieva et al., 2017; Zakharchuk & Harazdiuk, 2014; Holovakha et al., 2018). The mechanisms of toxic action of *Toxocara* play a notable role in stimulating free radical oxidation. Moreover, the imbalance between the content of oxidants and antioxidants with the subsequent development in animals of so-called oxidative stress includes blood leukocytes, which are among the first to respond to changes in the internal environment action of *Toxocara* metabolites. An essential role in developing oxidative stress is the balance of prooxidant synthesis and antioxidant protection. This equilibrium shift towards prooxidants causes compensatory activation of the antioxidant regularity from the damaging effects of free radicals and peroxide compounds.

Many endogenous compounds represent the regularity of antioxidant protection. The activity in the cells is not constant and changes under certain conditions, especially under prolonged and severe pressure (Gutyj et al., 2016; 2017; 2018; 2019; Shcherbatyy et al., 2019; Martyshuk et al., 2019; 2020; Bashchenko et al., 2020; Lesyk et al., 2020; Zakharskyi et al., 2020; Lieshchova et al., 2020; Grymak et al., 2020; Vasylyev et al., 2021; Roman et al., 2021; Brezvyin et al., 2021). It is known that parasitic diseases, including toxocariasis, also contribute to the development of oxidative stress (Khariv et al., 2016; Yevstafieva et al., 2019; Said et al., 2020). The available literature, which reflects numerous studies on the effects of *Toxocara* and their metabolites on animals, does not fully reflect their mechanism of action. Studies have been performed to reveal disorders of the cardiovascular and central nervous systems and digestive tract with the development of toxocariasis invasion]. Some scientists have established the complexity of the pathogenesis of this invasion in mammals: metabolic disorders, physiological functions of the body, and scientists have proposed various treatments (Pryima, 2010; Moisieieva et al., 2017).

Therefore, the research aimed to study the fenbenzyl and fenbendazole impact on the indicators of the dog's liver protein-synthesizing function during experimental infestation with the pathogen toxocariasis.

## Materials and methods

The work was performed during 2017-2020 at the Department of Parasitology and Ichthyopathology Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. The investigation was performed on 18 dogs, two to four months of age, and three groups of six animals in each were formed: control (C) and two experimental (E<sub>1</sub>)(E<sub>2</sub>). Puppies were experimentally infected with the pathogen toxocariasis at a dose of 5,000 invasive *T. canis* eggs per kg of body weight. The control dogs were as untreated. Puppies of the (E<sub>1</sub>) group were fed the drug "Fenbendazole" at a dose of 150 mg per 3 kg of body weight once a day for three days. Dogs of the (E<sub>2</sub>) group have applied the remedy "Fenbenzyl" (TU U 00492990-027: 2020 at a dose of 350 mg per 3 kg of animal weight once a day for three days.

The drug "Fenbenzyl", which contains fenbendazole and milk thistle, was developed at the Department of Pharmacology and Toxicology and the Department of Parasitology and Ichthyopathology of Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. The total protein concentration, its fraction, was studied according to the method (Vlizlo et al., 2012). All animal manipulations were performed following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986). The analysis of research results was conducted using Statistica 6.0 software. The Student's t-test assessed the probability of differences. The results of the mean values were considered statistically significant at \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 (ANOVA).

## Results and discussion

It was found that after the invasion of dogs with the pathogen toxocariasis, the level of total protein in the blood of the control group of animals decreased throughout the test, where, respectively, on the 25th day, it was 56.9±1.47 g/l (Table 1).

**Table 1.** Fenbenzyl and fenbendazole impact on the total protein content in the dog's blood infected with the pathogen toxocariasis (M±m, n=6)

Blood test time (days)	Total protein, g/l Groups of animals		
	Control	Experimental 1	Experimental 2
Before treatment	63.3±2.95	63.5±3.01	63.3±2.84
5th day	62.7±1.92	62.9±2.15	63.0±3.04
10th day	61.9±2.86	62.4±2.18	62.8±2.54
15th day	60.2±1.56	62.1±2.24	63.0±2.46
20th day	59.6±2.05	61.9±1.89	63.4±2.15
25th day	56.9±1.47	60.2±2.05	63.3±1.35
30th day	57.2±1.99	60.4±2.10	63.7±2.57

After Fenbendazole administration to infected dogs, a slight increase in the total protein level in the blood of animals of the E<sub>1</sub> group was found throughout the research. On the 20th, 25th, and 30th days, the total protein level grew but did not reach physiological values. Insufficient recovery of this indicator is due to low albumin levels in the serum of dogs during experimental infestation with the pathogen toxocariasis. Their levels on the 20th and 25th day ranged from 42.6±1.16 to 41.2±1.08 g/l (Table 2).

**Table 2.** Fenbenzyl and fenbendazole impact on the albumin content in the dog's blood infected with the pathogen toxocariasis (M±m, n=6)

Blood test time (days)	Albumins, g / l		
	Control	Experimental 1	Experimental 2
Before treatment	45.2±1.20	45.4±1.17	45.3±1.21
5th day	43.4±0.98	44.9±1.05	45.0±1.17
10th day	42.9±0.86	44.1±1.11	44.9±1.30
15th day	39.8±1.02	43.5±1.20	44.8±1.25
20th day	37.2±0.91	42.6±1.16	45.0±0.98
25th day	35.9±0.89	41.2±1.08	45.2±1.26
30th day	34.4±0.87	41.6±1.14	45.5±1.32

A more probable increase in total protein content was found in the E<sub>2</sub> group. In particular, we found the growth in the level of this indicator on the 20th and 25th days; by 6.4 and 11.2% compared to the (C) group. On the 30th day, the highest protein level in the blood was in the E<sub>2</sub> group of dogs.

Similar changes we observed in the albumin fraction in the blood of dogs of the E<sub>2</sub> group. We found that the albumin level on the 25th day probably grew by 25.9% compared with the (C) group. On the 30th day, the albumin level of the E<sub>2</sub> group was 45.5±1.32 g/l, while in the control group, this figure was much lower - 34.4±0.87 g/l.

As can be seen from Table 3, in dogs with experimental toxocariasis, the use of fenbenzyl and fenbendazole helped reduce the globulin level of both groups. Thus, in the blood of the E<sub>1</sub> group, the globulin level on the 15th day was 56.5±2.00 g/l, while in the (C) group, this figure was 60.2±1.85 g/l. At 25 and 30 days of research, the globulin level of the E<sub>1</sub> group was the lowest but did not reach the limits of physiological values (Table 3).

**Table 3.** Fenbenzyl and fenbendazole impact on the globulin content in the dog's blood infected with the pathogen toxocariasis (M ± m, n = 6)

Blood test time (days)	Globulins, g/l		
	Control	Experimental 1	Experimental 2
Before treatment	54.8±2.50	54.6±2.34	54.7±2.47
5th day	56.6±1.96	55.1±2.09	55.0±2.52
10th day	57.1±2.54	55.9±2.18	55.1±2.63
15th day	60.2±1.85	56.5±2.00	55.2±2.31
20th day	62.8±2.01	57.4±2.23	55.0±3.10
25th day	64.1±1.99	58.8±2.08	54.8±2.43
30th day	65.6±2.07	58.4±2.14	54.5±2.55

We observed a decrease in the globulin level in the blood of the (E<sub>1</sub>) group throughout the test. The studied indicators ranged from 55.2±2.31 to 54.5±2.55 g/l during our research. Starting from the 20th day, the globulin level in the blood of the (E<sub>2</sub>) group was within physiological values. On day 30, the globulin level of animals treated with fenbenzyl was lower by 16.9% than the control group of dogs.

Along with the investigation of albumin and globulin fractions in the blood of dogs during experimental infestation with the pathogen, toxocariasis is the determination of albumin-globulin ratio (A/G ratio), which is an essential indicator of the functional state of the liver of animals. The smaller it is than optimal, the more reduced the liver protein synthesizing function of animals. Based on the studies, it was found that the albumin-globulin ratio of the blood during the experiment gradually decreased. Thus, on the 15th day of the experiment, this (C) group indicator was 0.66 ± 0.05, and on the 25th day - 0.56±0.05. The lowest A/G ratio was on the 30th day, and compared with the initial values, it decreased by 36.6% (Table 4).

As shown in Table 3, in dogs treated with fenbendazole, the A/G ratio value gradually declined compared to the initial data. However, on the 20th and 25th days, it was an increase in the A/G coefficient by 25.4 and 25.0% compared with the (C) group.

The use of fenbenzyl in experimental animals increased the value of the A/G ratio throughout the experiment. On the 15th and 20th day, it was higher than the (C) group of dogs by 22.7 and 38.9%. On the 25th day of the trial, the albumin-globulin ratio in dogs of the (E<sub>2</sub>) group was 0.82±0.04, while in the control group, it was 0.56±0.05. On the 30th day of the investigation, the A/G ratio in the animals of the second experimental group raised slightly compared to the previous day.

**Table 4.** The fenbenzyl and fenbendazole impact on the albumin-globulin ratio in the dog's blood infected with the pathogen toxocariasis (M±m, n=6)

Blood test time (days)	Albumin-globulin ratio		
	Groups of animals		
	Control	Experimental 1	Experimental 2
Before treatment	0.82±0.02	0.83±0.02	0.83±0.03
5th day	0.77±0.04	0.81±0.03	0.82±0.03
10th day	0.75±0.05	0.79±0.03	0.81±0.04
15th day	0.66±0.05	0.77±0.06	0.81±0.03
20th day	0.59±0.03	0.74±0.04	0.82±0.05
25th day	0.56±0.05	0.70±0.03	0.82±0.04
30th day	0.52±0.04	0.71±0.05	0.83±0.04

Therefore, in dogs with experimental toxocariasis, after treatment with fenbendazole for 25 and 30 days, the liver protein synthesizing function was not completely restored, indicated by the low level of total protein and its albumin fraction. Elevated levels of globulins indicate the existing inflammatory processes that occur with toxocariasis in dogs. After studying the effect of fenbenzyl on the liver protein synthesizing function of dogs infected with *Toxocara*, a gradual normalization of serum levels of total protein and its fractions was established.

The increase in the total protein content in the serum of dogs of the second experimental group in these periods of the experiment compared with the control and first experimental groups indicates the stimulating effect of milk thistle with the drug "Fenbenzyl" on protein synthesis.

## Conclusions

With the development of toxocariasis invasion in dogs, the liver protein synthesizing function was suppressed, as indicated by decreased total blood protein, albumin fraction, and globulin levels. Thus, there was a change in the percentage of serum protein fraction of dogs experimentally infected with the pathogen toxocariasis, which may be caused by toxic lesions and reduced liver function as the main organ of synthesis of many proteins.

Application of the drug "Fenbenzyl" under oxidative stress in the serum of dogs leads to normalization of liver protein synthesizing function: on the 15th day, it returns to physiological levels in terms of total protein, albumin, and globulin.

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