Etlik Vet Mikrobiyol Derg, 2024; **35 (1):** 34-43
doi: https://doi.org/10.35864/evmd.1374233

Original Article
Özgün Araştırma

In vivo anthelmintic effect of *Artemisia annua* L. on oxyurid nematodes of laboratory mice

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Geliş Tarihi / Received: 11.10.2023, **Kabul Tarihi** / Accepted: 28.05.2024

Abstract: Oxyurids, common intestinal parasites, pose a recurrent health risk, particularly among children. Seeking natural and repeatable cures, this study explored the antinematodal effects of *Artemisia annua* L. n-hexane extract on oxyurids in vivo. The extract was orally administered to mice naturally infected with *Syphacia obvelata* and *Aspiculuris tetraptera* at doses of 300, 600, and 1200 mg/kg for seven days. Changes in oxyurid egg numbers were assessed through fecal flotation. On the 8th day, nematodes were compared across groups in necropsy for number, gender, and species.

As a positive control, Albendazole (ABZ) was administered at 5 mg/kg for three days, with corn oil serving as the solvent control. Fecal flotation revealed a 43.51% decrease in oxyurid egg counts in the ABZ group and a 21.12% increase in the *A. annua* 1200 mg/kg group on the last day of application. In necropsy, compared to the corn oil group, ABZ 5 mg/kg, *A. annua* 300 mg/kg, and *A. annua* 600 mg/kg groups exhibited reductions of 35.76%, 68.50%, and 63.64%, respectively, in terms of nematode numbers. The likely contributors to these results are the non-polar compounds of *A. annua* L., the very low concentration of ABZ, and the dilution solvent corn oil.

Key words: Anthelmintic; *Artemisia annua* L.; laboratory mice; oxyurid; worm counting

Laboratuvar farelerinin oksiyür nematodları üzerine *Artemisia annua* L.'nin *in vivo* antihelmintik etkisi

Özet: Oksiyürler, yaygın olarak görülen bağırsak parazitleridir ve özellikle çocuklarda tekrarlayan sağlık riski taşırlar. Doğal ve tekrarlanabilir bir tedavi arayan bu çalışma, *Artemisia annua* L. n-hekzan ekstraktının oksiyürler üzerindeki antinematodal etkisini *in vivo* olarak araştırdı. *A. annua* L. n-hekzan ekstraktı *Syphacia obvelata* ve *Aspiculuris tetraptera* türleri ile doğal olarak enfekte olmuş farelere 300, 600 ve 1200 mg/kg dozlarında 7 gün süreyle oral olarak uygulandı. Oksiyür yumurta sayısında meydana gelen değişim fekal flotasyon yöntemi ile değerlendirildi. Nekropsideki nematodlar gruplardaki sayı, cinsiyet ve tür açısından 8. günde karşılaştırıldı. Çözücü kontrol olarak hizmet eden mısır yağı ile birlikte pozitif ilaç kontrolü olarak Albendazol (ABZ) 5 mg/kg dozunda üç gün süreyle uygulandı. Uygulamanın son gününde fekal flotasyonda oksiyür yumurtalarının sayısı ilaç kontrol ABZ grubunda %43,51 azalma ve *A. annua* 1200 mg/kg grubunda %21,12 artış gösterdi. Nekropside, Mısır yağı grubu ile karşılaştırıldığında ABZ 5 mg/kg, *A. annua* 300 mg/kg ve *A. annua* 600 mg/kg gruplarında nematod sayısı açısından sırasıyla %35,76, %68.50 ve %63.64 azalma saptandı. Muhtemelen bu sonuçlara katkı sağlayanlar polar olmayan *A. annua* L. bileşenleri, ABZ'nin çok düşük dozda olması ve çözücü solventi olarak mısır yağı kullanılmasıdır.

Anahtar kelimeler: Antihelmintik; *Artemisia annua* L.; laboratuvar fareleri; oksiyür; solucan sayımı

Introduction

The drug resistance problem is the essential cause of the discovery of new medicines. On the other hand, infections from soil-transmitted helminths are endemic specifically in some regions of Asia, and Africa, and they are repeated frequently. Therefore, it is necessary to refer to herbal medicines because constant or combined usage of anthelmintic drugs affects kidney and liver functions negatively (Arise and Malomo, 2009).

Syphacia obvelata (Rudolphi, 1802) and *Aspiculuris tetraptera* (Nitzch, 1821) are nematodes from Oxyurid groups commonly found in mice and some rodents (Soulsby, 1982). Both nematodes inhabit in the gastrointestinal tract of the host animals and can be transmitted to humans through the ingestion of

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eggs. *Aspiculuris tetraptera* is frequently used in anthelmintic research to determine the efficacy of various chemotherapeutic agents (Theodorides, 1976).

A. tetraptera has a direct life cyclus and infection occurs after ingesting the eggs by the host, with a prepatent period of 21-35 days (Pritchett-Corning and Clifford, 2012). In the first stage nematode larvae stay for a week in the submucosa of the colon and develop into third-stage larvae, then pass to the colonic lumen to develop into adult larvae (Pritchett-Corning and Clifford, 2012). The mature females settle down large intestine to lay, who spend 45 to 50 days before laying their eggs. The eggs that released at night can stay alive for weeks outside the host and become contagious in 6-7 days at 24°C (Stepek et al., 2006). Table 1 shows the some characteristic properties of *A. tetraptera* and *S. obvelata*.

Table 1. Some characteristic properties of *S. obvelata* and *A. tetraptera*

	S. obvelata	A. tetraptera
Characteristics		
Female length	3.5-6 mm	3-4 mm
Male length	1-1.5 mm	2-3 mm
Female tail	Long and pointed	Conical
Cervical alae	Subtle	Prominent
Egg	~ 134 × 36 µm, banana shape	~ 86 × 37 µm, ovoid and symmetrical
Location in host	Caecum and colon	Colon
Location of egg	Perianal skin	Feces
Prepatent period	11-15 days	21-25 days
Infection time of egg	5-20 hours	5-8 days

Table was obtained and changed from Pritchett-Corning and Clifford (2012)

Nematodes of the genus *Syphacia* Seurat, 1916 (family Oxyuridae) are parasitic pinworms possesing host-specificity in several rodents (Perec et al., 2006). Because there are not enough specific characters to differentiate between pinworms, molecular advanced tools are used to identify closely related oxyurid species (Omer et al., 2020). The nuclear small subunit of ribosomal DNA (18S) and the mitochondrial cytochrome c oxidase subunit 1 (cox1) (Stewart *et al*., 2018) are evaluated for molecular discrimination of *Syphacia* species. Both sequences of 18S rDNA and the internal transcribed spacer (ITS1, 5.8S, and ITS2) regions are used the identification of *S. obvelata* showing morphological characteristics (Mohammed et al., 2024).

Several techniques can be used for identification of oxyurid nematodes beside using molecular tools. Because the eggs of oxyurids have some characterictics, they can be seen and detected under optic microscope after application of flotation or perianal tape methods. Fecal flotation or McMaster techniques are used for *Aspicularis tetraptera*, while perianal tape method is prefered for detection of *Syphacia obvelata* eggs classically (Hedrich, 2012).

Although infection with these nematodes does not cause significant symptoms; they drive changes that will affect the results of the experiment (Theodorides, 1976; Fox et al., 1984; Pinto et al. 1994; Gonzalez 1996; Sueta et al. 2002). Therefore, it is crucial to ensure that laboratory animals are free from parasites. *Artemisia annua* L. known locally in Türkiye "Peygamber süpürgesi" is an annual plant. It has 50-150 cm length. It is used as an infusion (2-3%) internally against dysentery and tuberculosis (Baytop, 1999). *A. annua* L. (Chinese: "qing hao", namely "green herb") is native to China. Utilized in Traditional Chinese Medicine for over 2000 years to reduce fever, the plant gained global recognition after the discovery that the artemisinin substance isolated from *A. annua* L. was effective in treating malaria in 1970, leading to its widespread adoption (Willcox et al. 2004). *A. annua* L. has been used not only for malaria but also for headache, fever, infection and inflammation (Memorial Sloan Kettering Cancer Center, 2010). According to a 2010 investigation, *Artemisia annua* L. was recommended in the oldest Chinese prescriptions found in the Mawagndui tomb, dating back to BC 168, depicting its usage for hemorrhoids in females (McGovern et al., 2010). The plant, *Artemisia annua*, is from the Asteraceae family, it has fern-like leaves, yellow flowers and a particularly pleasant smell. Trichomes contain various active compounds, including flavonoids, chromens, and various terpenoids (sesquiterpenoids and triterpenoids) (Brown, 2010). One of the most significant components is artemisinin, used for treating malaria, which has demonstrated anticancer properties, effective against breast cancer and leukemia (Singh and Lai, 2001; Singh and Lai, 2005). Additionally, the anthelmintic effect of artemisinin was shown experimentally (Cala et al., 2014). Artemisinin-derived drugs have also proven effective against various parasites, including *Fasciola hepatica* and gastrointestinal nematodes in ruminants, as well as *Plasmodium* spp., *Coccidia* spp., *Babesia* spp., *Leishmania* spp., *Neospora caninum*, and *Schistosoma* spp. (Tariq et al., 2009).

When the anthelmintic activity of *Laurus nobilis* extract in mice naturally infected with *Aspiculuris tetraptera* was investigated, it was seen that the concentration of 400 ml/kg alcoholic extracts of *L. nobilis* showed the 96% and 100% lethal effects for worms on three and six days after giving the treatment (Mares et al., 2023).

It is a need to learn a new and accurate pharmaceutical procedure that contains new application styles, dilution agents and the effective lowest concentrations of a known marked drug used in control groups in order to discover novel herbal treatment methods against parasites of animals.

The objective of the present study was to demonstrate the anthelmintic activity of the extract, with different application way (by using corn oil for dilution) and with a different drug dose (being lower than normal according to literature), by assessing the reduction in both oxyurid egg count and worm numbers. It was aimed to demonstrate the anthelmintic activity of the extract by evaluating the reduction in oxyurid egg counts and worm numbers.

Materials and Methods

Plant Material and Extract Preparation: *Artemisia annua* L. leaves were collected in 2010 from the Acemler district of Bursa (40º12´ 106 47.12´´N, 29º 0´53.01´´ E),Türkiye. Leaves were dried under shade, pulverized with a fine grinder and extracted with n-hexane in the Soxhlet apparatus. The solvent was evaporated with a rotary evaporator. The yield of extract was 4.5% (w/w). The crude extract was kept at -20˚C.

Pharmacological Procedures

Animals: Balb-c mice of both sexes (28-35 g) were obtained from the animal breeding center of Bursa Uludag University, Bursa, Türkiye. The animals were kept in standard polypropylene cages, at 20-24ºC, with 55% relative humidity, they were fed standard pellets and water *ad libitum*. Naturally infected experimental animals for *S. obvelata* and/or *A. tetraptera* were selected from 150 mice by using perianal cellophane tape and fulleborn fecal flotation methods, respectively. Each group was made up of six animals, and each mouse was taken separate cage. All experiments were approved by the Experimental Animals Local Ethics Committee of Bursa Uludag University (no: 2017-10/07).

Infected mice were divided into six groups, four treated and two control groups by selection in the condition that approximately the same number of eggs would be in every group according to the results of fulleborn fecal flotation and perianal tape methods performed on two different days. Each group consisted of six animals three males and three females. Group I was 0.2 ml corn oil solvent control, Group II, III and IV of mice were treated with 300, 600 and 1200 mg/kg b.w. (body weight) doses of n-hexane extract of *A. annua* L. for seven days, respectively. Group V of mice was treated with a 5 mg/kg b.w. dose of reference drug Albendazole for three consecutive days. Group VI was the group drinking water. For oral application, except for Group VI, all groups were fed via steel esophageal gavage in the same manner and volume (by completing a total of 0.2 mL with corn oil). Corn oil was used as solvent for dilution of *A. annua* n-hexane extract because, according to literature (Harvey, 1996), corn oil could be used in the animal experiments in order to observe anthelmintic drug effectiveness.

Macroscopic Examination: Fecal materials were collected from cages of all mice by using pliers. Examples in the labeled plastic bags were weighed on electronic analytical balance. Fecal materials were transferred to petri dishes and physiological saline solution was added on it. It was waited for 10-15 minutes in order to be softened. Fecal materials were examined under stereo microscope by using plastic spatula to slightly spread the cumulative material. Founded worms were taken with a pin and transferred to optic microscope by microscopic slide preparation in order to determine the species and gender.

Fecal Egg Counting: After the acclimatization of mice, perianal tape and fecal flotation methods were used to be detected to be sure whether all mice had oxyurid infection.

The efficacy of plant extract on egg numbers was calculated by egg counting of fecal material collected from each mouse before the 7th and 1st days before the treatment and 1., 3., 5., and 7. days of treatment. Fecal materials were collected by searching through cages' shavings in which each mouse was held separate cage. Firstly, it was investigated whether adult forms of helminths and proglottids were in collected fecal samples. They were then passed to the flotation method (Kasım, 1994). To apply fecal flotation method, weighed feces was homogenized with approximately 10 mL of water, they were filtrated from a metal filter by spraying water on them, two lamels were put on this mixture and all the eggs on microscope slight were counted and the amount in grams of feces was found.

In the fecal flotation, decreasing % of oxyurid eggs was counted as follows:

Decreasing% = 100 × (C-T)/C

C is the arithmetic mean of oxyurid eggs of untreated control mice, and T is the arithmetic mean of oxyurid eggs in the treatment group obtained from fecal flotation.

Worm Counting: On the last day of the application, the mice were euthanized under anesthesia with sevoflurane. After the internal organs were taken into the petri dish, all organs were separated and taken into labeled vials containing 70% alcohol. All organs in the body cavity were investigated by stereomicroscope for the presence of helminths. They were opened seperately in petri dishes by pouring 70% alcohol on them. Organs were cut very carefully with a small metal scissors by avoiding from any damage on helminths. Intestines firstly were seperated according to regions, then all regions (jejunum, duedonum, cecum, and large intestine) opened in different petri dishes. Parts of the intestine were put on one side by pliers and were cut with small scissors by above side through all part. Contents of intestine were investigated by spraying 70% alcohol or physiological saline solution on them in order to diluate. A pin was used to disperse the content and to take the helminths. The collected helminths were identified under the light microscope, were separated, and counted according to their species, genders.

The percentage reduction, indicative of efficacy against oxyurid nematodes, was calculated as follows:

(%)E = 100 × (C-T)/C

In this formula, E is Efficacy, C is the geometric mean of worm numbers obtained from the necropsy of the corn oil solvent control group, and T is the geometric mean of worm numbers obtained from the necropsy of the treatment group. (%)Efficacy is calculated with this formula according to Kozan et al. (2011) and Turel et al. (2013).

The pictures were taken with Olympus CX23 microscope. The samples were sealed with Entellan® and preserved as permanent preparations.

Hematocrit Counting: The hematocrit counting was made in three groups; *A. annua* 600 mg/kg b.w., corn oil 2 ml and ABZ 5 mg/kg b.w. The blood taken from the hearts of mice were collected in two Eppendorf tubes one of them was added EDTA. Hematocrit counting was made using Wintrobe tubes.

Statistical Analysis

The post hoc test was chosen by making the normality analysis. Shapiro Wilk test was used to detect normality. The results of Levene's statistics were evaluated to detect the homogeneity of variance. For comparing the means of parasite egg numbers Kruskal Wallis non-parametric one-way ANOVA analysis method, comparing the means of helminths in the treatment groups, the Tamhane-T2 test and also Kruskal Wallis tests were used. $P<0.05$ was admitted as significant.

Results

Macroscopically, the first finding was that the adults of *S. obvelata* in the fecal examinations were found in the mice group of *A. annua* L. extract at 600 mg/kg b.w. dose (Group III), whereas they were not in groups of ABZ (Group V) and corn oil (Group I). *S. obvelata* adults began to be seen three days after the starting of application and no more than 2-3 worms. In the extract groups (Group II, III and IV), while the decreases in the number of oxyurid eggs were observed in some of the mice, there was no significant difference between the first and last days of the application in terms of the group of mice.

Considering the average number of helminths per group of mice according to the necropsy results, the anthelmintic activity in the *A. annua* 600 and the *A. annua* 300 group was found to be at the similar level as shown in Table 2. The anthelmintic activity, which was detected as approximately 35.76% efficacy, shows the application of ABZ at a dose of 5 mg/kg b.w.for three days have a mild effect. *A. annua* 600 mg/kg b.w. dose also shows a very similar effect (63.64%) in terms of worm count reduction comparing a 300 mg/kg b.w.dose of *A. annua* L. n-hexane extract for seven days (Table 2).

Table 2. Worm count at necropsy

	Worm count at necropsy			
Treatment	**Total number**	**Min-Max**	**Geometric Mean**	**% Efficacy**
Corn oil 2 ml	233	4-107	20.16	-
ABZ 5 mg/kg b.w.	152	2-78	12.95	35.76
A. annua 300 mg/kg b.w.	128	2-115	6.35	68.50
A. annua 600 mg/kg b.w.	196	0-125	7.33	63.64
A. annua 1200 mg/kg b.w.	98	3-38	17.43	13.54

ABZ: Albendazole, *A. annua*: *Artemisia annua*

Although *A. annua* 1200 mg/kg b.w. dose was expected to be at these values, only a slight decrease was observed (13.54%). Based on the fecal flotation results, there was a 68.56% increase observed in the corn oil group on the 7th day of the application compared to the initial count (the arithmetic mean of the counts on the -7th and -1st days). As indicated in Table 3 and Figure 1, the ABZ group exhibited the most significant decrease, although this decrease of 43.51% suggests a moderate effect.

Table 3. The arithmetic mean of EPG (Egg per gram) counted at fecal flotation

	-7th day	-1st day	1st day	3rd day	5th day	7th day
Drinking water	416.3	434.5	415.7	445.3	245.1	466.4
Corn oil	230.4	166.5	327.5	575.8	469.5	631.2
ABZ 5 mg/kg b.w.	70.4	78.5	59.0	77.7	20.7	42.0
A. annua L. 300 mg/kg b.w.	165.1	59.8	76.2	270.8	64.5	112.6
A. annua L. 600 mg/kg b.w.	325.1	163.0	83.5	133.6	165.1	233.8
A. annua L. 1200 mg/kg b.w.	52.3	76.0	76.2	133.0	213.9	81.3

ABZ: Albendazole

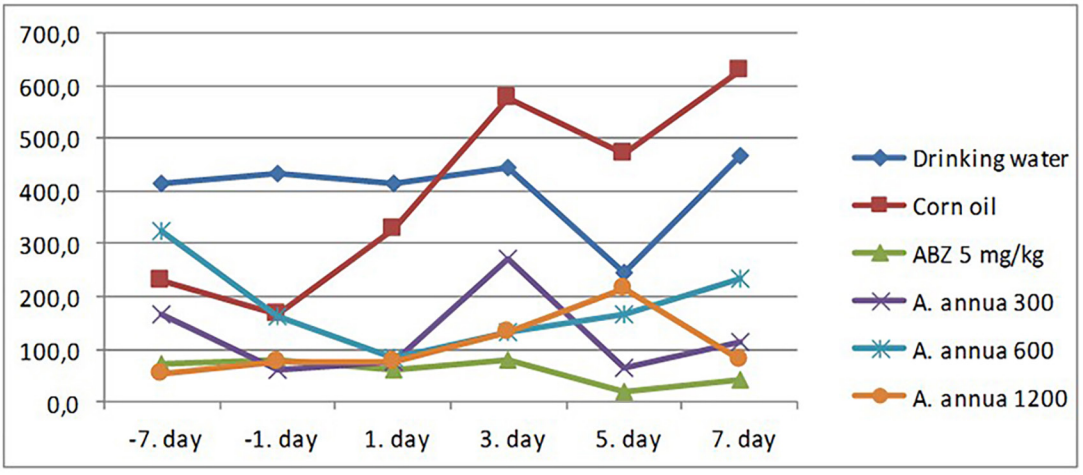

Figure 1. Arithmetic mean of *A. tetraptera* EPG for each mice group at fecal flotation

As shown in Figure 2, a slight decrease (4.22%) in the *A. annua* 600 group and an increase of 21.12% in the *A. annua* 1200 group indicated that the antinematodal effect of the extract could not be detected by fecal flotation or it would take a little longer to see for the result.

The oxyurid eggs seen in the fecal flotation have belonged to *Aspicularis tetraptera*, *Syphacia obvelata* and *Syphacia muris*. Their photographs are given in Figure 3.

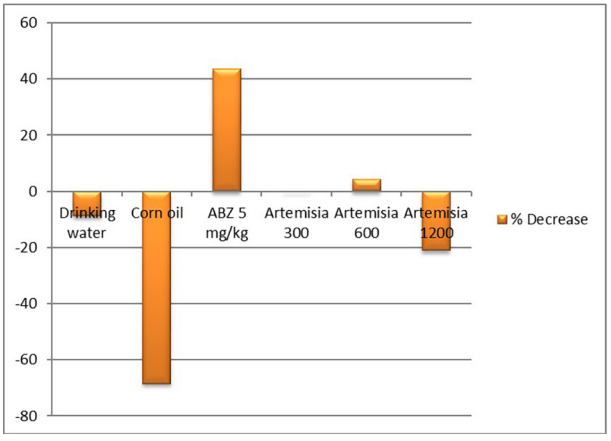

Figure 2. Decrease rate of EPG counted at fecal flotation on the last day comparing the arithmetic mean of -7th and -1st days. *Artemisia*: *Artemisia annua* L. n-hexane extract

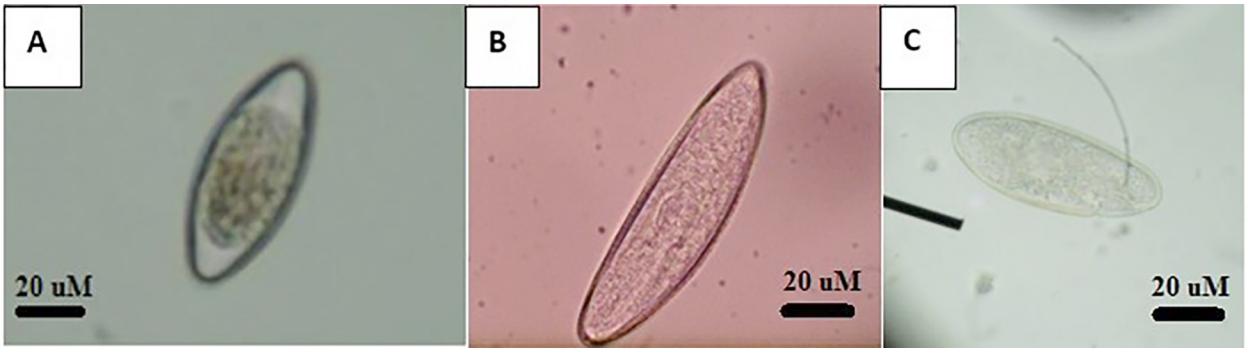

Figure 3. Oxyurid eggs in fecal flotation A) *A. tetraptera* B) *S. obvelata* C) *S. muris*

When comparing the numbers of helminths collected from the internal organs of each mouse during necropsy, the homogeneity of variance is found to be unacceptable according to Levene's statistics. While ANOVA is robust against violations of the assumption of normality and homogeneity of group variance when the group sizes are equal, in this study, some treated mice died before the eighth day, resulting in unequal group sizes in the final assessment.

Based on the results of the Tamhane 2 test, no significant difference was observed between the groups in terms of the number of helminths collected during necropsy. However, according to Figure 4, designed for the comparison of independent samples, there is a considerable disparity between group means and standard deviations, with the *A. annua* 600 group being notably prominent. This disparity in group means and standard deviations can be attributed to the substantial variation in the distribution of infection within the groups from the beginning. Contrary to expectations, the necropsy results did not reveal the anticipated effects from the extracts.

Because the normality could not be provided, non-parametric tests were used. According to the Kruskal Wallis test, there was a statistically significant difference (P=0.019) only between ABZ and corn oil in terms of eggs counted in fecal flotation. This result showed that *A. annua* n-hexane extracts applied at three different doses did not have a significant effect on *A. tetraptera* egg numbers. The statistical correlation between the treatment groups is shown in Figure 5.

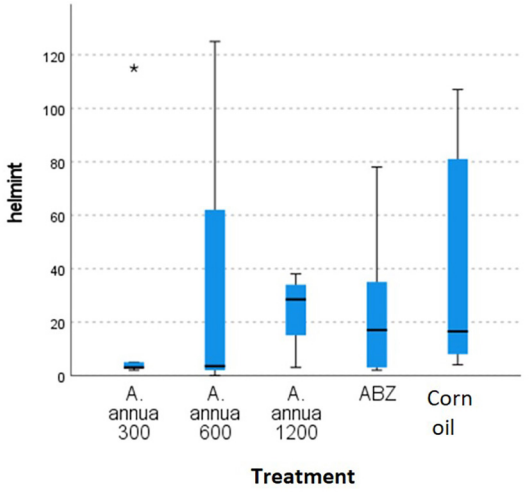

Figure 4. The comparison of the helminths found at necropsy via Kruskal Wallis

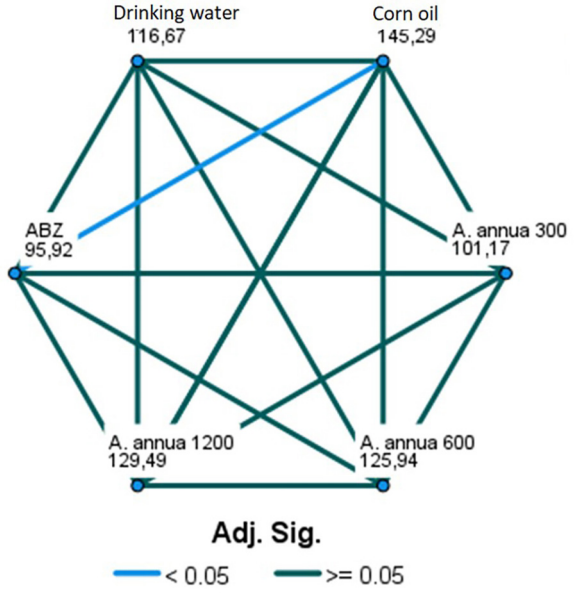

Figure 5. Relationships for adjusted significance (Adj. Sig.) in administration groups in fecal flotation

The results of the blood values of the mice showed that the mice in the ABZ-treated group had higher hematocrit levels than the other mice. This result is in line with the expected. A comparison of the arithmetic mean of hemoglobin and hematocrit count in groups of mice is shown in Figure 6.

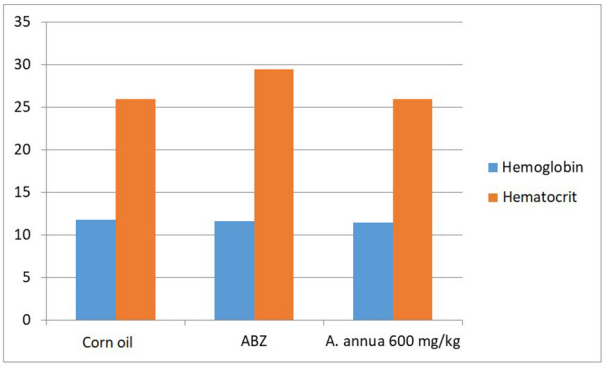

Figure 6. The arithmetic mean of the blood values of the mice in each group on the 8th day of the administration

Both the hematocrit and hemoglobin values of the mice in the other two groups (the mice in the group treated with corn oil as solvent control and the group treated with *A. annua* extract at a dose of 600 mg/kg b.w.) were found to have more intense infections, which were determined by the results of both fecal flotation and necropsy, were quite close to each other. This showed that this dose of the extract did not affect the hemoglobin and hematocrit counts of the mice.

Discussion and Conclusion

Various model organisms are employed in laboratory experimental animals to study soil-transmitted helminths, with *Trichuris muris* being a perfect model for trichuriosis in mice (Keeling, 1961), and *Strongyloides ratti* utilized for strongyloidiasis in rats (Wertheim and Lengy, 1965). Infected rodents with *Ascaris suum* serve as models for the onset of *Ascaris lumbricoides* infection (Tritten et al., 2011).

In our study, *Aspicularis tetraptera* and *Syphacia obvelata*, both belonging to Oxyurid nematodes, were selected as relevant organisms to demonstrate the antinematodal effect of plant extract in the gastrointestinal system. The significance of these two nematodes lies not only in their prevalent existence in laboratory mice and rats but also in their zoonotic characteristics. Given that most commonly used antinematodal drugs are generally broad-spectrum, the present study provides evidence that *A. annua* L. affects nematodes in the gastrointestinal system, suggesting its potential efficacy against soil-transmitted helminths.

Syphacia spp. and *A. tetraptera* were the common parasites of mice. In a parasitology research in Istanbul, Türkiye, the laboratory and pet mice (n=75) had been detected by Çetinkaya et al. (2017) with flotation technique in terms of the species of nematodes. According to distribution of them, *Syphacia* spp. and *Aspicularis tetraptera* were found as %20 and %40 in mice, respectively. If it was used the perianal tape method to detect the *S. obvelata* eggs, it could be found higher infection rate for *S. obvelata*.

According to a study by Turel et al. (2008), giving *Urtica dioica* L. leaves and seeds methanolic extracts orally for seven days to mice naturally infected with *A. tetraptera*, *Urtica dioica* L. seed methanolic extract had not a significant effect but leaves methanolic extract showed a potent anthelmintic effect. A study in sheep infected with *Haemonchus contortus* showed that oral administration of *A. annua* L. water extract and artemisinin had a low-level anthelmintic effect (Cala et al., 2014). In sheep infected with *Fasciola hepatica*, a single dose of 40 mg/kg b.w. (i.v.) of artesunate from *A. annua* L. reduced the egg count by 69% and the worm count by 77%. The same application of artemether obtained from the same plant reduced the number of eggs by 97.6% and the number of worms by 91.9% (Keiser et al., 2010). Extracts of *A. annua* L. were found to be 81.6–83.2% suppressive against *Cryptosporidium parvum* in mice (Youn and Noh, 2001).

In a recent study (Taljaard et al., 2022) that infusions of *Artemisia afra* and *Artemisia annua* were submitted to liquid-liquid partitioning with n-hexane and dichloromethane to provide samples for *in vitro* bioassays using newly transformed schistosomulas (NTS) and adult *Schistosoma mansoni* worms obtained from infected mice. *A. afra* and *A. annua* infusions and extracts were found that they possessed potent *in vitro* antischistosomal activities against NTS, at 100 µg/ml.

According to the results of the present study, since the *A. annua* L. n-hexane extract contains the non-polar active components of the plant, these components may have a mild anthelmintic effect. In the macroscopic examination, only a few helminths were found in the feces of mice in the *A. annua* 600 groups. These worms may have fallen due to the extract or may have been discarded randomly due to the intensity of the infection. As indicated in Table 3, corn oil increased the number of oxyurid

eggs. Despite the expected reduction in the number of eggs and helminths due to the laxative effect of corn oil, the observed increase on the last day of the application in fecal flotation could be related to the stress induced by gavage application, leading to a weakened immune system. The *A. annua* n-hexane extract being diluted in corn oil might have made it challenging to observe the anthelmintic effect, contributing to the 21.12% increase in the average number of eggs at a dose of 1200 mg/kg b.w. This effect may be attributed to the use of corn oil. According to some studies (Harling et al. 1988; Harvey, 1996; National Toxicology Program, 1998) corn oil was preferred as solvent controls. Harvey (1996) used to corn oil as solvent in a patent study to show anthelmintic properties of some macrocyclic lactones. However, it is recommended to add an alcohol type with at least four carbon atoms to the mixture as a carrier when delivering macrocyclic anthelmintic drugs (Harvey, 1996). In our study, alcohol was not added to the mixture to avoid potential interference with the anthelmintic effect. The absence of alcohol might have affected the solubility of the extract, potentially causing it to remain relatively undissolved, despite observations indicating homogenization in corn oil. Therefore, in investigating the anthelmintic activity of *A. annua* L., it may be worthwhile to explore extracts prepared with polar solvents via *in vitro* experiments or consider adding the extract to the foods of experimental animals instead of employing gavage for *in vivo* experiments.

It was stated by Brisibe et al. (2009) that *Artemisia annua* L. has nutritious ingredients and they can be a good food supplement. In our study, the observed increase in the number of oxyurid eggs with the *A. annua* extract at a dose of 1200 mg/kg b.w. may be attributed to this nutritive feature. While this plant is known for its anthelmintic properties, it is also recognized for its nutritional richness. Consequently, one could expect a simultaneous reduction in the number of worms and an increase in ovulation, as indicated by the results of our study. In addition, as a reaction to the extract, the number of eggs in helminths may be increased. Many comments can be made on the fact that *A. annua* L. n-hexane extract increases ovulation.

In this study, both perianal tape and fecal flotation methods were used to select the infected mice. It is advised that the perianal tape method be used for counting *Syphacia obvelata* eggs and fecal flotation for *Aspiculuris tetraptera* eggs (Hedrich, 2012). According to the literature, the combined use of these two methods is more effective in determining *S. obvelata* and *A. tetraptera* eggs. While *A. tetraptera* eggs are rarely found with the perianal tape test, the fecal flotation method is considered less successful for detecting *S. obvelata* eggs (Hedrich, 2012). However, in our study, the fecal flotation method was found to be as effective as the perianal tape method in detecting the presence of *S. obvelata* eggs, even in some cases where there were no eggs on the cellophane band, there were many *S. obvelata* eggs seen in flotation. The recommended usage of Albendazole in oxyurid treatment is 10 mg/kg b.w. a single dose. This study was administered at a dose of 5 mg/kg b.w. for three days with a different application, and its effect in this situation was investigated. In the literature, an *in vivo* anthelmintic effect of ABZ at a dose of 5 mg/kg b.w. was found to be %32.6 against lungworm larvae *Metastrongylus apri* (Ferguson, 1981). In our study, ABZ was suspended in corn oil and given by gavage. Although ABZ was given with gavage, and there was its dissolution problem, it was observed that the average number of eggs per gram in fecal flotation decreased by 43.51% on the seventh day compared to the pre-application. This relatively low effect of ABZ may be attributed to its administration after suspension in corn oil. Another contributing factor could be the administration of ABZ for three days, whereas extracts and corn oil were given for seven days. In necropsy, the number of worms in the ABZ group (Group V) decreased by 35.76% compared to the corn oil group (Group I).

A. annua L. n-hexane extract showed a mild anthelmintic effect when it was given to infected mice by dilution in corn oil. It is an interesting result that the 1200 mg/kg b.w.dose of the extract on the seventh day caused an increase of 21.12% in the number of eggs, compared to the average of the first days. ABZ caused a 43.51% reduction of eggs at the dose of 5 mg/kg. This study has shown the slight anthelmintic activity of the n-hexane extract of *A. annua* L. against oxyurid nematodes of laboratory mice. However, further investigations are required to isolate the active compounds responsible for the anthelmintic activity and to predict the mechanisms of action via *in silico* modeling.

Ethics committee for the use of experimental animals and other ethical committee decisions and permissions: All experiments were approved by the Experimental Animals Local Ethics Committee of Bursa Uludag University (no: 2017-10/07).

Conflict of interest: All authors declare that there is no conflict of interest.

Acknowledgement : We would like to dedicate this study to our esteemed Professor, deceased Prof. Dr. Hulusi Malyer, for his support and encouragement.

Contribution of authors: D K made in vivo experiments and wrote the article. A O G read and corrected the article. O G collected the plants and prepared the n-hexane extract. H M contributed to the collecting of *A. annua* from field.

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