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# Investigation of the anthelmintic effects of *Rosmarinus officinalis* L. (rosemary) and rosmarinic acid in mice naturally infected with *Aspiculuris tetraptera*

Buket EYLEK [1], Hikmet Sami YILDIRIMHAN [1],
Nurhan SÜMER [1,*], and Bayram ŞENLIK [2]

1. Department of Biology, Science and Literature Faculty, Uludag University, 16059 Bursa, Türkiye.
2. Department of Parasitology, Veterinary Medicine Faculty, Uludağ University, 16059 Bursa, Türkiye.
* Corresponding author: N. Sümer, E-mail: nsumer@uludag.edu.tr

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**Abstract.** In this study, the anthelmintic effects of *Rosmarinus officinalis* (rosemary) leaf extract and its polyphenolic constituent, rosmarinic acid, were investigated in mice naturally infected with *Aspiculuris tetraptera*. The feces of 90 Balb/c mice, naturally infected with A. tetraptera, were examined using the fecal flotation method to determine the infection status. Three doses of *R. officinalis* leaves extract were examined. Infected mice were divided into six groups. The first group received 250 mg/kg, the second group received 500 mg/kg, the third group was given 1000 mg/kg of *R. officinalis* leaves extract, the fourth group was treated with rosmarinic acid 5 mg/kg, the fifth group with albendazole 10 mg/kg and the sixth group served as the control, receiving physiological saline. The experimental and control groups were orally administered for 7 days. On the 8th day, all animals were euthanized, and the number and weight of parasites obtained from the gastrointestinal tract were counted and measured. Percentages of activity and p-values were calculated as follows: the efficacy values were 89%, 1399 of albendazole; 8%, 17 of rosmarinic acid; 25,44% of a 250 mg/kg rosemary dose; 46,54% of a 500 mg/kg rosemary dose and 7,16% of a 1000 mg/kg rosemary dose what do these numbers reflect. P-values were found to be significant in the second group, which received a 500 mg/kg dose of rosemary leaf extract ($p < 0.05$), and remained insignificant in the third, fourth, and fifth groups ($p > 0.05$).

**Keywords**: anthelmintic effect, *Aspiculuris tetraptera*, mouse, rosemary, *Rosmarinus officinalis* leaves extract, rosmarinic acid.

## Introduction

The family Lamiaceae takes its name from the ancient Latin or Lamium-like shape (İpek 2018). Members of this family are generally found in our country, especially in the Mediterranean region. Türkiye is a rich region in terms of the Lamiaceae family and endemic species. These endemic species are primarily seen in the Taurus Mountains and Amanos Mountains (Vatansever 2014). Members of this family have economic importance in the fields of perfumery, food, and medicine due to the essential oils, flavonoids, secondary metabolites, aromatic, and volatile oils they contain. Aromatic and essential oils are used in perfumery and pharmacology (Öz-Aslan 2017). Recently, some of the families have been used for medicinal purposes (İpek 2018). The rosemary plant, which belongs to the Lamiaceae family, is also known as kusdilli and hasalbal (Başkaya et al. 2016).

Rosemary is an annual plant that grows 50-100 cm tall and does not shed its leaves in winter; it can reach heights of up to 2 meters (Vatansever 2014). Various studies have been conducted on rosemary and its extract, both in vitro and in vivo, and dose determinations have also been made on animals.

It has been reported to have a wide dose range of 0.1–500 μg/ml in vitro and 1 mg/ml to 3333.3 mg/kg/day in vivo (Moore et al. 2016). Although rosmarinic acid is found in many plants, it is a compound first obtained from *R. officinalis* (Gonçalves et al. 2020). Rosmarinic acid is the primary active ingredient in the extract derived from rosemary leaves. It is a polyphenol compound obtained from rosemary at a rate of 8%. Rosmarinic acid has also been discovered in sage and stakewort (Çin 2018). Studies have reported that rosmarinic acid, a key compound in rosemary, exhibits anticancer properties (Moore et al. 2016). It is also frequently used in therapeutic and food applications due to its antioxidant, antidiabetic, and anti-inflammatory effects (Gonçalves et al. 2020). Rosmarinic acid is a highly absorbable component that has a significant impact on the gastrointestinal system and skin (Bulduk & Gökce 2017). Rosmarinic acid enters the circulation when taken orally. It enters the circulation by being hydrolyzed in the large intestine, and upon doing so, it affects the metabolic reactions by influencing carnosic acid and dihydroxyphenyl lactic acid. It is also stated that these phenolic acids ionize tissue pH (Çin 2018).

Drugs used in the treatment of diseases are called anthelminthic drugs. Ivermectin, albendazole, mebendazole, and nitazoxanide are commonly used antiparasitic chemical drugs (Korkmaz 2012). There are also many effective drugs, such as niclozamide and piperazine. Albendazole derivatives are used mainly against nematodes and are particularly effective against nematodes such as *Aspiculuris tetrapreta* (Öztop 1996), a parasite of house mice (Şenlik et al. 2005).

Medicines are often used to treat these diseases, but these drugs cause many problems, such as damage to the immune system, economic issues, increased drug use, and environmental pollution. The use of these medicinal plants has also been demonstrated in scientific studies (Çeliktaş 2010). As a result, with the emergence of natural and less toxic alternatives, the use of synthetic medicine is becoming less common. The importance of medicinal plants, which have been used for centuries, has increased (Malayoğlu-Basmacıoğlu 2010). As medicinal plants are increasingly recognized for their therapeutic potential, their usage in teas, foods, and herbal medicines has also expanded.

However, the active compounds of these plants must be known and used in treatment accordingly (Gökçen-Özçay 2017). One of the plants rich in phytochemical compounds is rosemary. Rosemary has been used in the treatment of diseases since ancient times and has broader practical applications in areas such as pharmaceuticals, culinary, and cosmetics (Cerit 2008). Studies have reported the antibacterial, antioxidant, antiviral, and immune-strengthening effects of rosemary extract (Malayoğlu-Basmacıoğlu 2010).

Compounds such as carnosol, carnosic acid, rosmarinic acid, and rosmanol, obtained from rosemary leaf extract, are reported to have antioxidant effects (Öz-Aslan 2017). While rosmarinic acid has shown antioxidant, antibacterial, antiviral, and antiallergic effects in in vitro studies, antiallergic and anticarcinogenic effects have been observed in vivo (Bulduk & Gökce 2017).

In addition to the numerous beneficial effects of medicinal plant extracts, several studies have also reported the parasite-reducing properties of various plants. In addition to these effects, a study also observed that rosemary extract has an anthelmintic impact on fish (Zoral et al. 2017). Another study showed that rosemary leaf extract was effective against *Echinococcus granulosus*, a cestode that causes hydatid cyst disease (Albani et al. 2014).

*Artemisia absinthium* is a medicinal plant with antihelminthic properties. Its effect on *Syphacia obvelata* was examined (Youssefi et al. 2012). Different doses and extracts were given in these other plants that were studied. However, the egg counting methods used and the tests employed to interpret the results are similar, and the common feature among all of them is the investigation of their anthelmintic properties.

For this reason, in our study, *R. officinalis* was selected because rosemary is rich in many aspects, and few studies have been conducted.

Herbal medicines often have several broad, synergistic actions on physiological systems simultaneously, which usually have non-specific therapeutic effects. It is stated that rosemary is widely recognized for its ability to alleviate several health issues, including the reduction of parasites. For this reason, we decided to explore the anthelmintic potential of the rosemary plant and rosmarinic acid, a primary polyphenolic compound known for its antioxidant and anti-inflammatory effects. The primary objective is to extract and dissolve the active ingredients from rosemary in preparation for further analysis.

This study was undertaken to determine the anthelmintic properties of rosemary leaf extract and rosmarinic acid. Rosmarinic acid will be given special attention because of its well-recognized effects and extensive research supporting its presence in rosemary.

## Materials and methods

*Rosmarinus officinalis* plant extract was received from Kale Naturel Herbal Product Food Cosmetic and Agricultural Products, Balıkesir, Türkiye. Rosmarinic acid was obtained from the pharmacology department of Eskişehir Anadolu University. The mice used in this study were obtained from Uludağ University Experimental Animal Research Center. Ethics committee permission for this study was received from the university with the decision 2019-9/2. Forty-seven females and 43 male Balb/c mice (25-30 g) were used for the study.

The Fecal Flotation Method was used to select mice naturally infected with *Aspiculuris tetraptera*, and the flotation fluid used was a saturated sodium chloride (NaCl) solution. The feces samples were kept in saturated (NaCl) solution until they became soft. Then, feces were crushed with the help of a baguette until a homogeneous mixture was obtained. The mixture was filtered into a collection tube.

The tube was later filled with saturated NaCl solution until it reached the top of the tube. Coverslips were placed on top of the tube surface and were kept for 20 minutes. Then, the coverslips were taken off with the help of forceps, placed on the slide, and examined under a light microscope for the detection of infected animals. Those with *A. tetraptera* eggs were reserved for use in the experiment. After obtaining the desired number of mice, experimental groups were created (Tınar et al. 2006).

The McMaster method was used to group the selected infected mice. The experiment consists of 6 groups. 250 mg/kg, 500 mg/kg, and 1000 mg/kg of *R. officinalis* leaves extract were administered to the first, second, and third experimental groups, respectively. The fourth experimental group received a 5mg/kg dose of rosmarinic acid. Albendazole was administered at a dose of 10 mg/kg to the five groups, while the 6th group received 0.9% physiological saline (Mahdy et al. 2017).

*R. officinalis* leaves and rosmarinic acid were dissolved in 0.9% NaCl saline (Fetoni et al. 2018). 10 g of leaves were dissolved in 100 ml NaCl saline. The solution started to dissolve after stirring, and it completely dissolved within 1 hour. *R. officinalis* leaves extracts were prepared in various doses to be given to mice after thawing. The extracts were administered to the animals orally via gavage for 7 days, as described by Ayaz et al. (2015). Following the 7 days of administering the plant extracts, rosmarinic acid, and albendazole, euthanasia was performed on the 8th day.

The intestines of the dissected animal were opened. *A. tetraptera* were examined under a stereoscopic microscope and later preserved in containers containing 70% ethanol (Mahdy et al. 2017). Differences between all groups were examined within a 5% confidence interval using the Mann–Whitney test, focusing on egg numbers and intestinal parasite numbers at autopsy. Analyses were performed using the

Minitab 17.1.0 statistical program.

## Results

Concerning the results, the eggs examined for 7 days are presented in Table 1. Considering the egg numbers, the highest count was observed on the 3rd day, at 2150, and the lowest was on the 4th day, at the 250 mg/kg dose of *R. officinalis*. There was an increase on day 1 compared to day 0; a decrease was observed on days 4 and 5, but an increase was seen again on days 6 and 7. At the 500 mg/kg dose of *R. officinalis*, the highest increase was observed on the 3rd and last day, while the lowest increase was noted on days 0 and 4. Although a decrease was seen on the 4th day, the number of eggs increased again on the remaining days. At the dose of *R. officinalis* 1000 mg/kg, the lowest egg count was observed on day 0 and the highest on day 3. An increase was observed until the 3rd day, followed by a decrease thereafter. The egg numbers in the rosmarinic acid group have remained remarkably consistent. The highest counts were recorded on the 3rd and 7th days, while the lowest count was observed on the 6th day. A decrease in the number of eggs was observed on the 7th day when albendazole was used. The egg numbers on other days were close to each other, but the highest effect was noted on the 2nd day. In the control group, the number of eggs on the 7th day was the highest among all other egg numbers. It is also quite a high count, observed on the 4th day. The lowest effect was seen on the 2nd day.

On the 8th day, the average number of *A. tetraptera* in the groups was taken (Table 2).

Table 1. Seven-day egg numbers of *R. officinalis*, rosmarinic acid, albendazole and control group in mice naturally infected with *A. tetraptera.*

| | 0.day | 1.day | 2.day | 3.day | 4.day | 5.day | 6.day | 7.day |
|---|---|---|---|---|---|---|---|---|
| Rosemary 250 mg/kg | 900 | 1500 | 1350 | 2150 | 400 | 800 | 1200 | 1100 |
| Rosemary 500 mg/kg | 900 | 950 | 1400 | 2450 | 900 | 1300 | 1850 | 2400 |
| Rosemary 1000 mg/kg | 200 | 1350 | 2100 | 2400 | 650 | 1500 | 1200 | 1050 |
| Rosmarinic acid 5 mg/kg | 300 | 400 | 400 | 500 | 400 | 450 | 50 | 500 |
| Albendazole 10 mg/kg | 250 | 400 | 500 | 350 | 450 | 300 | 500 | 50 |
| Control Group | 1050 | 650 | 400 | 750 | 1950 | 900 | 650 | 2950 |

Table 2. Averages of *A. tetraptera* numbers determined by looking at *R. officinalis*, rosmarinic acid, albendazole, and the control group at the end of the 8th day.

| | Average of *A. tetraptera* numbers at the end of the 8th day |
|---|---|
| *Rosmarinus officinalis* 250 mg/kg (Group 1) | 185 |
| *Rosmarinus officinalis* 500 mg/kg (Group 2) | 133 |
| *Rosmarinus officinalis* 1000 mg/kg (Group 3) | 231 |
| Rosmarinic Acid (Group 4) | 270 |
| Albendazole (Group 5) | 27 |
| Control Group (Group 6) | 250 |

After examining *A. tetraptera* averages, effect rates, and p-values were calculated. Looking at the impact values, albendazole at 89%, rosmarinic acid at -8%, and *R. officinalis* at 25% of the 250 mg/kg dose, *R. officinalis* at 500 mg/kg dose was 46%, and *R. officinalis* at 1000 mg/kg dose was 7%. Looking at the p-values, albendazole is 0.0144, rosmarinic acid is 0.4601, *R. officinalis* 250 mg/kg dose is 0.3246, *R. officinalis* 500 mg/kg dose is 1.0000, the dose of *R. officinalis* 1000 mg/kg is 0.5635. The effect rate and p-value are given in Table 3.

Table 3. Efficacy percentages of active ingredients, which are the active ingredients and the controls

| Group | Impact Rate | P-value |
|---|---|---|
| Control group | - | - |
| Albendazole | 89.13% | 0.0144 |
| Rosmarinic Acid | -8.17% | 0.4601 |
| *Rosmarinus officinalis* 250mg/kg | 25.44% | 0.3246 |
| *Rosmarinus officinalis* 500mg/kg | 46.54% | 1,0000 |
| *Rosmarinus officinalis* 1000mg/kg | 7.16% | 0.5635 |

**Discussion**

The importance and use of medicinal plants have increased recently, but studies have shown various effects of rosemary leaf extract. Upon reviewing previous studies, it is evident that there are few studies on *R. officinalis* with anthelmintic effects on *Dactylogyrus minutus* (Zoral et al. 2017). Studies on various plants have demonstrated that they exhibit anthelmintic and healing effects against gastrointestinal parasitic infections. *Nigella sativa, Cucurbita maxima, Urtica dioica*, and *Artemisia absinthium* have been studied, and it has been stated that these plants reduce *A. tetraptera* eggs. The anthelmintic effect of *Nigella sativa* oil on *A. tetraptera* in mice at a dose of 250 μL/kg was examined, and it was observed that it reduced the number of parasite eggs. Two control and two treatment groups were also studied (Ayaz et al. 2007).

The anthelmintic effect of the *Cucurbita maxima* plant on *A. tetraptera* was also examined, and *A. tetraptera* eggs were detected in Swiss albino mice using cellophane tape and the centrifugal flotation method. The control group received ivermectin, while the experimental groups received the aqueous and methanolic extracts of *Cucurbita maxima* for 7 days. It was observed that *Cucurbita maxima* had exhibited a high anthelmintic effect (Ayaz et al. 2015). The methanol extract of the *Urtica dioica* plant was studied in naturally infected mice at a dose of 250 μl/kg, and it was reported that the plant leaves exhibited an anthelmintic effect (Turel et al. 2008).

When the averages were taken and compared with the control group, albendazole was found to be more effective in reducing *A. tetraptera* parasites. When *R. officinalis* extracts and rosmarinic acid were compared with the control group, a 500 mg/kg dose of *R. officinalis* was more effective than the doses of 250 and 1000 mg/kg, and the number of *A. tetraptera* worms treated with rosmarinic acid was higher than the *R. officinalis* doses. Albendazole was reported to be significant ($p<0.05$), while the 3 doses of rosmarinic acid and *R. officinalis* were insignificant ($p>0.05$).

In conclusion, the highest efficacy percentage was demonstrated by albendazole and the lowest by rosmarinic acid. However, in future studies, the effects of these extracts should be

investigated by examining higher doses and utilizing several other active compounds responsible for imparting pharmacological characteristics to rosemary. Additionally, the extraction methods used to obtain plant extracts vary depending on both the plant species and the solvent employed. In this case, the effect of the extracts may change.

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