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# Comparison of the effects of *Artemisia vulgaris* and *Artemisia absinthium* growing in western Anatolia against trichinellosis (*Trichinella spiralis*) in rats

Ayşe Caner [a], Mert Döşkaya [a,*], Aysu Değirmenci [a], Hüseyin Can [a,c], Şura Baykan [b], Ahmet Üner [a], Gülçin Başdemir [d], Ulvi Zeybek [b], Yüksel Gürüz [a]

[a] *Department of Parasitology, Ege University Medical School, Bornova, İzmir 35100, Turkey*
[b] *Department of Botanic Pharmaceuticals, Ege University Faculty of Pharmacy, Bornova, İzmir 35100, Turkey*
[c] *Department of Biology, Ege University Faculty of Sciences, Bornova, İzmir 35100, Turkey*
[d] *Mikropatoloji Laboratories, Alsancak, İzmir 35230, Turkey*

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**Abstract**

Trichinellosis often causing diarrhea and more rarely fever, periorbital edema and myositis in human, is commonly treated with benzimidazole derivatives. The *Artemisia* genus has been found to be effective against a variety of parasites. In the present study, the efficacy against trichinellosis (*Trichinella spiralis*) of *Artemisia vulgaris* and *Artemisia absinthium* was examined for the first time in rats. The results of trichinoscopy and artificial digestion, during the enteral (adult) phase of the illness show that 300 mg/kg doses of methanol extracts of the aerial parts of *A. vulgaris* and *A. absinthium* reduced the larval rate by 75.6% and 63.5% in tongue, 53.4% and 37.7% in diaphragm, 67.8% and 46.2% in quadriceps, and 66.7% and 60.5% in biceps–triceps muscles of rats, respectively. Furthermore, during the parenteral (encapsulated larvae) phase, 600 mg/kg doses of *A. vulgaris* and *A. absinthium* extracts decreased the larval rate by 66.4% and 59.9% in tongue, 57.4% and 50.0% in diaphragm, 47.6% and 43.7% in quadriceps, 60.2% and 46.4% in biceps–triceps muscles of rats, respectively. Analysis of antibody also showed that *A. vulgaris* significantly reduced the antibody response ($P < 0.05$) during the enteral and parenteral phases. Thus, the results of the present study revealed that *A. vulgaris* could be an alternative drug against trichinellosis.


*Index Descriptors and Abbreviations:* Trichinellosis; *Trichinella spiralis*; Nematode; *Artemisia vulgaris*; *Artemisia absinthium*

## 1. Introduction

Trichinellosis is a worldwide zoonosis caused by nematodes of the genus *Trichinella*. *Trichinella* species occur widely in wild carnivorous animals. However, pigs that consume uncooked trichinous scraps or carcasses of infected wild animals also become infected. Most human infections are accidental and caused by *Trichinella spiralis* after the ingestion of undercooked pork meat contaminated by infective larvae. The symptoms of the illness become serious as the size of the inoculum of viable larvae increases. Diarrhea is the most common symptom, whereas fever, periorbital edema and myositis may also be observed during the course of infection. Rarely, patients may experience fulminant illness due to myocarditis, pneumonia or encephalitis depending on the amount of larvae ingested (Grove, 2000). Approximately 11 million people are estimated to be infected with *Trichinella* species. (Dupouy-Camet, 2000). Trichinellosis can also occur in outbreaks which affect thousands of people (Laurichesse et al., 1997; Dupouy-Camet et al., 2002; Turk et al., 2006). Drugs such as mebendazole, albendazole, flubendazole, levamisole, and thiabendazole are used against trichinellosis, but their low water solubility limits their absorption resulting in reduced bioavailability (Levin, 1983; Fourestie et al., 1988; Cabie et al., 1996; Lopez-Garcia

* Corresponding author. Fax: +90 232 388 1347.
*E-mail address:* mert.doskaya@ege.edu.tr (M. Döşkaya).

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et al., 1998; Pozio et al., 2001; Derda et al., 2003; Garcia et al., 2003). There is considerable interest in developing new anthelminthic drugs including those from medicinal plants due to increasing evidence of resistance against present anthelminthic drugs and decreasing activity against encapsulated larval stages of parasites (Pozio et al., 2001; Iqbal et al., 2004; Gilleard and Beech, 2007; Prichard, 2007).

There are about 300 species in the *Artemisia* genus, one of the most widely distributed genera of the Compositae family (Kordali et al., 2005). The genus contains old and popular medicinal species and some species have been found to be effective against a variety of parasites including *Plasmodium* species (Dhingra et al., 2000; Borstnik et al., 2002; Wright, 2005; Liu et al., 2006), *Leishmania* species (Sen et al., 2007), *Haemonchus* species, *Pheritima posthuma*, *Taenia* species, *Ascaris* species, *Strongyloides*, *Nematodirus* species, *Trichostrongylus* species, *Dipylidium caninum*, *Oesophagostomum columbianum*, *Strongyloides papillosu*, *Trichuris ovis*, *Trichinella spiralis* (Iqbal et al., 2004; Sukul et al., 2005), and *Shistosoma mansoni* (Shuhua et al., 2000; Utzinger et al., 2002). There are 22 species of the genus *Artemisia* in the Turkish flora, distributed throughout the country (Kordali et al., 2005). *Artemisia absinthium* and *Artemisia vulgaris* grow wild in large areas of Anatolia and are called locally "Pelin otu" and "Ayvadana", respectively. They are used as antipyretic, analgesic, antiseptic, antihelminthic, diuretic, and appetite enhancing agents in Turkish folk medicine (Baytop, 1999; Kordali et al., 2005).

There are no reports on the antihelminthic effects of *A. vulgaris* and *A. absinthium*. Therefore, the objective of the present study was to determine the antihelminthic effects of *A. absinthium* and *A. vulgaris*, growing in western Anatolia, during enteral (adult) and parenteral (encapsulated larvae) phases of trichinellosis (*T. spiralis*) in rats. The amount of larvae in the muscles of rats was investigated by trichinoscopy, artificial digestion and histopathological examination. Anti-*T. spiralis* antibodies were also studied in the serum samples collected from the rats during the course of the infection.

## 2. Materials and methods

### 2.1. Animal, parasite and infection

Wistar albino rats (8- to 10-week old) weighing 100–150 g were used during the experiments. Rats were housed and fed under standard, suitable conditions. The experimental plan was performed under the instructions and approval of the Institutional Animal Care and Use Committee of Ege University for animal ethical norms. *T. spiralis* GM-1 strain (ISS048) was used during the study. The rats were administered 900 larvae orally to initiate the infection.

### 2.2. Preparation of extracts and administration

Methanol extracts of the plant samples were tested against trichinellosis. The plant samples were harvested from Antalya (*A. absinthium*) and Denizli (*A. vulgaris*) in western Anatolia during their flowering period. Voucher specimens were deposited in Herbarium of Ege University, Faculty of Pharmacy (Table 1). Aerial parts of the plants were dried in the shade and powdered. Crude material was extracted with methanol (Merck) in a Soxhlet extractor at 64 °C for 3 days. Methanol was evaporated to dryness under reduced pressure and temperature on a rotary evaporator (Buchi Re 111, Switzerland). The extracts were dissolved in distilled water and the lipophilic phase was removed by filtration. Then, they were lyophilized (Christ, Germany) to provide complete dryness and to increase stability. Two groups of rats (each of 5 rats) were orally administered 300 mg/kg of *A. vulgaris* or *A. absinthium* extract, diluted in 2 ml of distilled water, 5 days after the inoculation of *T. spiralis* (enteral phase of trichinellosis) for 20 consecutive days. Two further groups (each of 5 rats) were orally administered 600 mg/kg of *A. vulgaris* or *A. absinthium* extract, diluted in 2 ml of distilled water, 45 days after the inoculation of *T. spiralis* (parenteral phase of trichinellosis) for 5 consecutive days. The extracts were prepared fresh before each administration. Control groups of 5 rats were only administered distilled water. All groups of rats were observed daily during the course of the study.

### 2.3. Trichinoscopy and artificial digestion

All of the rats were sacrificed 60 days after they were inoculated with *T. spiralis*. Subsequently, the diaphragm, tongue, triceps, and biceps brachialis, and quadriceps femoralis muscles were removed. For trichinoscopy, muscle samples were reduced to 5× 1 mm sub-samples. The samples were then pressed between two glass slides and the amount of encysted larvae was identified under light microscopy with 4× magnification. Artificial digestion was performed in a solution as previously described (Gamble et al., 2000; Kociecka et al., 2004). The solution contains 0.5% HCl (37% HCl; v/v) (Merck) and 1.5% pepsin (70FIP-U/; w/v) (Merck) diluted in distilled water heated up to 50 °C. During artificial digestion, same amounts of muscle as used in trichinoscopy were mixed with the solution in a proportion of 7.5 ml/g and incubated at 39 °C for 6 h. Larvae in each digested sample were counted using light microscopy with 4× magnification.

### 2.4. Histopathological examination

Conventional methods were used to prepare the specimens for histopathological examination. The rats in all

Table 1
Voucher No., collection date, and locality of *A. absinthium* and *A. vulgaris*

| | *A. absinthium* | *A. vulgaris* |
|---|---|---|
| Voucher No. | 5657 | 5663 |
| Collection date | 29.07.2003 | 30.09.2003 |
| Locality | Antalya, Alanya, Gündoğmuş village | Denizli, Başkarcı village |