Antiparasitic efficacy of worm wood (*Artemisia absinthium*) alcoholic extract on *syphacia obvolata*

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Abstract:

BACKGROUNDS: Occurrence of resistance against antiparasitic drugs has made it essential for researchers to find new sources for antiparasitic drugs. **OBJECTIVES:** This study was performed to determine the efficiency of alcoholic extract of worm wood (*Artemisia absinthium*) on Syphasia parasite. **METHODS:** *Artemisia absinthium* extract was examined on 3 groups of mice at 2.5%, 5% and 10% concentrations. A group of positive control received pyrantel pamoate, while negative control group was treated by a solution containing no extract. Mice were treated orally 28 days after infection by Syphacia eggs. The efficacy of treatment was determined by Syphacia eggs in the feces. **RESULTS:** In groups that received either 5% or 10% concentrations of *A. absinthium* extract or pyrantel pamoate, microscopic examinations of the feces demonstrated no Syphacia eggs. **CONCLUSIONS:** Data obtained from the present study showed that the alcoholic extract of *Artemisia absinthium* may lead to a decline in the number of Syphacia eggs in the feces with minimal side effects. The extract of this plant can probably be used as a suitable alternative in the treatment of some parasitic diseases.

Introduction

Over the years many drugs have been developed for the therapy of parasitic infections in animals. Nowadays, alternative therapy choices are increasingly tested for the treatment of parasitic diseases because of the development of the parasites' resistance to anti-parasitic drugs, residues in host tissues which were later consumed by humans, and harmful metabolites excreted by animal feces which free living arthropods into the environment (Anderson, 2000; Kader et al., 2011; Ayaz et al., 2001). Some plant extracts have been suggested to be successful agents in the treatment of parasitic diseases (Anderson, 2000; Githiori et al., 2005; Lans et al., 2007; Mueller, 2004). *Artemisia absinthium*, which is also known as wormwood has been used for therapeutic purpose since the times of the ancient Egyptians (Deans and Kennedy, 2002).

Artemisia is a stable and very aromatic plant growing to 75 cm in length. Its grayish stems are covered by soft fluff, and its needle leaves are coarse edged. This plant is a native of the Mediterranean area and central Europe (Blumenthol and Goldbery, 2000). In tropical regions, Artemisia grows on the sides of roads and overt regions growing, naturally, with small, globular yellow- greenish flowers that appear on the tops of thin stems during the middle of the summer to the middle of the fall.

The Syphacia parasite is a nematode worm including two genii, *Syphacia obvolata* and *Syphacia murris*, which lives, mostly, in the cecum and the colon of the mice. This parasite is called mouse pinworm and its size ranges from 1.1 to 1.5 mm when

it is directly transmitted (Eslami, 1997). Based on the existing evidence, this study was designed to examine the effect of worm wood alcoholic extract on the Syphacia in a murine model.

Materials and Methods

The Artemisia plant was collected from bajgah region in shiraz. following the preparation of botanist confirmed Artemisia plants located within 12 km from the Agricultural Faculty of Shiraz University on Shiraz' Isfahan Road, in the Bajgah Region. Samples of plant were transferred to the Medical Faculty of Babol. They were air dried in the sun and shade, mixed using a blender device, drilled and powdered. Herbal extract was prepared using a Reflux Condenser by adding 40 g of dried plant weight including leaves and the floral top branch trimming of the plant to 150 ml absolute ethanol (Merck), mixed well, incubated for 24 hours at room temperature (22-25 C) and, finally, extracted by an evaporator rotation device over a 5 hour period. To prepare the Syphacia nematode worm, young mice were trapped and euthanized by spinal cord cut, and the cecum and colon were removed. Then, by washing with normal saline, adult Syphacia were recovered and female worms containing ova were identified and isolated from males. Mature female worms were selected and their ova were collected by removing the uterus.

Male Balb/c mice weighing 20-25 g, and 4-6 weeks old, were purchased from Karaj Laboratory Animal Section of the Pasture Institute of Iran. All mice were initially examined for Syphacia infection by fecal examination of floated samples and all infected mice were exempted from the study. Twenty five animals were divided into five groups (n= 5 mice) and were kept at starvation for 12 hours before the study. All the groups were fed independently and orally by 200 µL of suspension containing Syphacia ova at the concentration of 10 ova in 20 µL of solution.

All mice in the study were treated with *A. absinthium* extract for 10 days, 28 days after infection by the orally inoculated Syphacia ova. The first group was left as an untreated control and received dosages of normal saline. The second, third and fourth groups were treated by 100 µL of Artemisia extract in concentrations of 2.5%, 5% and 10%, respectively, every 12 hours. The fifth group received 5 mg/kg of Pyrantel pamoate (Ridley et al., 1991), under the same conditions. 28 days after inoculation by the ova, before the start of treatment with Artemisia alcoholic extract, fecal samples were collected during a period of 24 hours and tested by the flotation and Graham test procedures to confirm the presence of Syphacia ova in feces and the occurrence of infection.

Results

A light microscopic examination of the feces indicated the presence of *Syphacia obvolata* eggs in the control group mice during the 10 days treatment. In group two, which received Artemisia extract at 2.5% concentration, *Syphacia obvolata* eggs were observed during treatment with the total count being less than that of the control group, but, statistically, insignificant ($p>0.05$). Concentrations of 5% and 10% of Artemisia alcoholic extract represented high anti-parasitic effects on the Syphacia parasite, and generally decreased fecal egg counts in all the examined mice ($p<0.05$). The efficacy of extract with 5% and 10% concentration was similar to the choice treatment of the nematode with Pyrantel pamoate. In comparing the number of dead worms among the control and test groups, the statistical Mann-Whithney test was applied. This test indicated a significant difference in control mice when compared with test groups ($p= 0.036$). In the fourth group (10% alcoholic extract), two mice died at the middle stage of the study. This probably indicated that the dose of 10% alcoholic extract was too high and it may contain some ingredients which were toxic for mice at that dosage.

Discussion

This study demonstrated that crude extracts of *Artemisia absinthium* are effective in the in vivo growth and development of the Syphacia parasite. Microscopic examination of fecal samples showed a significant decline of parasite survival. There are some reports supporting this study. *Artemisia sieberi* was introduced as one of the most effective candidates against coccidiosis in the chickens infected with *Eimeria tenella* and *Eimeria acervolina*, but not on *Eimeria maxima* (Arab et al., 2006). In the Dominican region of the West Indies, some worms became

resistant against common medicines, called Worm Bag; then, various herbs or a combination of them, were used for curing infected people. Artemisia is one of the most common plants used for treatment of intestinal helminthiasis (Quinlan et al., 2002). It was reported that alcoholic extract of Artemisia was effective against *Toxocara cati*, an intestinal nematodal infection found in cats (Yildiz et al., 2011).

The natural and synthetic Artemisia was able to act against the malaria parasite, especially its drug resistant type *Plasmodium falciparum* (Paradise et al., 2006). Moreover, Artemisia can be used against micro-organisms including *Helicobacter pylori* (Shirazi, 2003). Therapies were well tolerated. However, nausea, vomiting, dizziness, sleep disorders, and other neurological side effects were reported (Vugt et al, 1998). The clinical use, the toxicity, teratogenicity of Artemisia and its derivatives, however, raise some questions and require further study. Nevertheless, the new compounds hold considerable promise and further intense collaboration has been planned between the WHO and research laboratories (Bruce, 1982). Although there have been considerable scientific advances over the past hundred years, there is an overall increased opportunity found in exploring plant chemistry. The WHO is recommending new therapies, based on the use of Artemisin derivatives, or a combination therapy (Afonso et al, 2006). In addition to some of the therapeutic effects of Artemisia, modern scientific studies are required to clarify the effective fraction sites of this herb (Zargari, 1997).

The data from this study suggest that *Artemisia absinthium* alcoholic extract, in a dosage of 5%, may decrease the number of Syphacia parasite ova in mice and cause minimal side effects. This plant extract may be useful as an alternative choice in the treatment of parasitic diseases, but it is suggested that it needs further investigations on other animal models.

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بررسی کارایی ضد انگلی عصاره الکلی افسنطین بر انگل سیفاسیا ابو لاتا

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چکیده

زمینه مطالعه: بروز مقاومت نسبت به داروهای ضدانگل موجود، لزوم بررسی برای یافتن منابع جدید از داروهای ضدانگلی را ضروری نموده است. **هدف:** هدف از مطالعه حاضر تعیین اثر بخشی عصاره الکلی گیاه افسنطین بر انگل سیفاسیا می باشد. **روش کار:** اثرات عصاره گیاه افسنطین (با غلظت های ۲/۵٪، ۵٪ و ۱۰٪) بر پاکسازی انگل سیفاسیا در ۳ گروه موش سوری مورد مطالعه قرار گرفت. گروهی مجزا به عنوان کنترل مثبت پیرانتل پاموات دریافت نمودند در حالی که گروه کنترل منفی محلول فاقد عصاره افسنطین را دریافت نمودند. حیوانات ۲۸ روز پس از القای عفونت توسط خوراندن تخم سیفاسیا، با عصاره افسنطین تحت درمان قرار گرفتند. کارایی درمان در موش ها با مشاهده میکروسکوپی تخم انگل سیفاسیا در مدفوعشان مورد ارزیابی قرار گرفت. **نتایج:** در گروه های دریافت کننده عصاره افسنطین ۵٪ و ۱۰٪ و همچنین گروه دریافت کننده پیرانتل پاموات پس از طی ۱۰ روز دوره درمان، ارزیابی میکروسکوپی مدفوع وجود تخم انگل سیفاسیا را نشان نداد. **نتیجه گیری نهایی:** داده های بدست آمده از مطالعه حاضر پیشنهاد می کند که عصاره الکلی افسنطین احتمالاً موجب کاهش تعداد تخم انگل سیفاسیا با حداقل عوارض جانبی می شود. عصاره این گیاه احتمالاً می تواند به عنوان جایگزینی مناسب برای درمان بیماری های انگلی مطرح شود.

واژه های کلیدی: افسنطین (*Artemisia absinthium*)، سیفاسیا، عصاره الکلی، موش سوری.

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