

# In vivo evaluation of anti-parasitic effects of *Artemisia absinthium* extracts on *Syphacia* parasite

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## Citation

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## Abstract

Herbs are the common resources for drugs in the both forms of traditional or pure products. WHO prepared a comprehensive list of herbs as well as their extracts are currently using for experimental trials against a variety of diseases. Such herbal products could be used for providing novel effective drug targets. This study has been carried out in order to evaluate the effects of various native herbs are currently used for anti parasitic purposes in Iran as well as their application in traditional medicines. The authors examined direct effects of *Artemisia absinthium* extract in removing *Syphacia* parasite in the experimental mouse. Herbal extract was prepared by dried plant in absolute ethanol, mixed well, incubated and finally extracted by evaporator rotation device. Experimental mice were treated with *A. absinthium* extract 10 days after infection by orally inoculated with *Syphacia* ova. Mice were examined by observation of *Syphacia* ova in their feces. Three groups of mice were treated with *A. absinthium* extract at concentrations of 2.5%, 5% and 10% respectively, whereas the fifth group was administrated with Pyrantel pamoate. Microscopical examination of feces indicated no *Syphacia* ova in all experimental groups, which was verified later by hitopathological study of target organs at the terminal stage of experiment. It is concluded here that pharmacological application of *Artemisia absinthium* extract was able to decrease the number of *Syphacia* parasite ova in mice with least pathophysiological side effects. This will draw new insights in designing novel anti-parasitic drugs are extracted from plants are grown especially in the Middle East, South East and central Asia.

## INTRODUCTION

Using herbs for treatment of diseases returns to thousands years ago; and nowadays, one third of medicines has been estimated to contain natural herbs or their extracts (<sup>1</sup>). Herbs always account for one of the general medicine drugs because such products could be used for producing very important and effective medicines (<sup>2, 3</sup>).

*Artemisia* is a stable and very aromatic plant with 75 cm in length. Its stems covered by a soft fluff coloring grayish having needle leaves with coarse edges. This is the native of Mediterranean area and central Europe (<sup>4</sup>). In tropical regions, *Artemisia* grows in margins of roads and overt regions self-growingly with small, globular yellow- greenish flowers which appear in the tops of thin stems during middle summer to middle of the fall.

*Syphacia* parasite is a nematode worm including two genus *Syphacia obvolata* and *Syphacia murreli*, living mostly in cecum and colon of mice. This parasite is called mouse pinworm and its size ranging 1.1 to 1.5 mm with direct transmission. There have been reported only three *Syphacia*

infections in Iranian children (<sup>5</sup>). In regard to the investigations of various native herbs efficacy used for their anti parasitic purposes in Iran as well as using these herbs in traditional medicine; this study was designed to examine direct effects of *Artemisia* extract in removing *Syphacia* infection in mouse.

## MATERIALS AND METHODS

### PLANTS AND EXTRACT PREPARATION

The authors examined direct effects of *Artemisia absinthium* extract in removing *Syphacia* parasite in the experimental mouse. Following preparation of *Artemisia* from Agricultural Faculty of Shiraz University located in 12 km of Shiraz - Isfahan Road, in the Bajgah region, it transferred to the Medical Faculty of Babol. They were then air-dried in the sun shade, mixed using Blender device, drilled and powdered. Herbal extract was prepared using Reflux Condenser by adding 40 g of dried plant weight including leaves and floral top branch trimming of plant to 150 ml absolute ethanol (Merck), mixed well, incubated for 24 h at room temperature (22-25 C) and finally extracted by evaporator rotation device during 5 h.

## PARASITE

To prepare *Syphacia* nematode worm, young mice were trapped and spinal cord cut by vivisection from cecum and colon. After washing in normal sera, adult *Syphacia* was specified; female worms containing ova were identified and isolated from males. Mature female worms were selected and their ova were collected by removing uterus.

## ANIMALS AND GROUPS

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

## HERBAL TREATMENT

Experimental mice were treated with *A. absinthium* extract 10 days after infection by orally inoculated with *Syphacia* ova. First group was left as untreated control and received normal saline. Second, third and fourth groups were treated by 100  $\mu$ L of *Artemisia* extract in concentrations of 2.5%, 5% and 10% respectively every 12 h. The fifth group was received 5 mg/kg of Pyrantel pamoate at same condition. After 15 days of infection, fecal samples were collected at 12 h intervals and tested by flotation and Graham test procedure by observation of *Syphacia* ova in feces.

## RESULTS

Microscopical examination of feces indicated no *Syphacia* ova in all experimental groups, except control mice, which was verified later by histopathological study of target organs at the terminal stage of experiment. *Syphacia* ova were only observed in feces of control group starting 25 days after infection. Different concentrations of 2.5%, 5% and 10% of *Artemisia* extract represented high anti-parasitic effects on *Syphacia* parasite. The efficacy of extract was similar to choice treatment of nematode with Pyrantel-Pamoate. In extract-treated groups, some clinical symptoms were observed at third day of treatment, including confusion, imbalance and ventral fast respiration, which may be side effects of ethanol consumption.

In comparison of number of dead worms among control and test groups, a Statistical Mann-Whitney test was applied. It

was indicated a significant difference in control mice when compared with test groups ( $P<0.05$ ). In fourth group with the highest doses of extract treatment; two mice died at the middle stage of study. This may be a consequence of remaining toxic Comarrin substance (Thujone) in the extract.

## DISCUSSION

This study demonstrated that crude extracts of *A. absinthium* are effective at inhibiting the in vivo growth and development of the *Syphacia* parasite. Microscopic examination of the fecal samples showed a significant decline of parasite survival. There are some reports are supporting this study. Arab (2006), introduced *Artemisia sieberi* as one of the most effective candidates against Coccidiosis in the chickens infected with *Eimeria tenella* and *Eimeria acervolina*, but not on *Eimeria maxima* (6). In Dominican region located in the west of India, some worms became resistant against common medicines called Worm Bag; then various herbs or a combination of them used for curing infected people. *Artemisia* is one of the most common plants used for removing intestinal helminthiasis (7). The natural and synthetic *Artemisia* was able to act against malaria parasite, especially its drug resistant type of *Plasmodium falciparum* (8). Moreover, *Artemisia* can be used against micro organisms including *Helicobacter pylori* (9).

Therapies were well tolerated however; nausea, vomiting, dizziness, sleep disorders, and other neurological side effects were also reported (10). The clinical use and the toxicity and teratogenicity of *Artemisia* and its derivatives, however, raise some queries and require further studies. Nevertheless, the new compounds are of considerable promise and further intense collaboration has now been planned between the WHO and research laboratories (11). Although there have been considerable scientific advances over the past hundred years, the overall burden of plant chemistry is currently increasing. The WHO is recommending new therapies, based on the use of Artemisin derivatives or combination therapy (12). In addition to some of therapeutic effects of *Artemisia*, modern scientific studies are required to clarify the effective fraction sites of this herb (13).

## CORRESPONDENCE TO

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