Evaluation of the Anticestode and Antinematode Effects of the Methanol Extract of Ferula Asafoetida on Experimentally Infected Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Synthetic antiparasitic medications are often associated with drug resistance and adverse side effects. In traditional medicine, Ferula asafoetida has been widely used in the treatment of parasitic infections, and various studies have confirmed the anti-leishmanial, anti-Giardia, and antifungal properties of this medicinal herb. This study aimed to evaluate the antiparasitic effects of the methanol extract of asafoetida.

METHODS: This experimental study was conducted on 100 male rats equally divided into 10 groups. To induce infection, animals were fed the eggs of nematode Syphacia obvelata and cestode Hymenolepis nana via gavage. Animals in groups one and two were considered as control subjects for cestode and nematode, respectively and received no medications. The third group was administered with a standard anticestodal dose of praziquantel (25 mg/kg), and the fourth group was administered with a standard antinematodal dose of piperazine (20 mg/kg). Infected animals in experimental groups five, six and seven received treatment with 2.5%, 5% and 10% concentrations of asafoetida methanol extract, respectively. Moreover, induction of nematode infection was performed on the animals of experimental groups 8, 9 and 10, which were administered with 2.5%, 5% and 10% concentrations of asafoetida methanol extract, respectively. Treatment of the animals continued for two weeks. Number of parasite eggs in the fecal samples was determined at the end of the first and second week of treatment. After the intervention, total number of the intestinal parasites was calculated and assessed in the experimental groups.

FINDINGS: In the first week of treatment, no statistically significant difference was observed between the number of Syphacia obvelata eggs in rats administered with the methanol extract of asafoetida even at the highest concentration compared to control subjects (166.4±3.11 vs. 235±9.5). Similarly, treatment of nematode infection with the methanol extract of asafoetida could not decrease the number of eggs and parasites by the end of the second week of treatment (P>0.05). However, rats with cestode infection receiving different concentrations of asafoetida extract showed a significant reduction in the number of eggs and parasites compared to control subjects (p<0.05).

CONCLUSION: According to the results of this study, the methanol extract of Ferula asafoetida had no antinematode properties in vitro, while it exhibited anticestode effects on laboratory animals with induced parasitic infection.

KEY WORDS: Ferula asafoetida, Syphacia obvelata, Piperazine, Hymenolepis nana, Praziquantel, Rats.

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Introduction
Parasitic infections are associated with numerous health problems and may develop epidemically in a community. Although various pharmacological agents are effective in the treatment of these infections, some of these medicines often cause drug resistance, drug residues in tissues and other side effects. Throughout history, different medicinal herbs have been used in the treatment of parasitic infections across the world (1). Frequent references in relation to the antiparasitic effects of Iranian herbs in the sources of traditional medicine have urged researchers toward inclusive investigation in this regard in order to validate the data. Recently, studies have focused on the application of medicinal herbs owing to the approval of patients, remarkable efficiency of herbal remedies and low treatment costs (2). Ferula asafoetida is one of the therapeutic herbs commonly recommended in the Iranian traditional medicine for the treatment of parasitic diseases (3). This plant is grown in Iran and Afghanistan and belongs to the Apiaceae family (3).

Asafoetida has a strong odor due to the presence of sulfur compounds, such as disulfides, trisulfides and tetrasulfides. Moreover, this plant contains coumarin derivatives (4). In the traditional medicine, asafoetida is used as a mucokinetic, diuretic, sedative and antiparasitic agent. In traditional remedies, the most commonly administered dose of asafoetida was 0.2-0.5 grams daily (5, 6). In veterinary medicine, asafoetida was an essential ingredient in preparing antiparasitic baths (3). According to the literature, the aqueous extract of asafoetida is administered orally in Nepal as a remedy for intestinal worms (7). Previous studies have suggested that the resin extracted from the stems and roots of asafoetida contributes to the growth inhibition of Trichomonas vaginalis protozoan in vitro (8). Furthermore, researchers have claimed that oral administration of asafoetida essential oil in rats with Schistosoma mansoni infection could decrease the number of parasites in the treated samples (9).

With this background in mind, this study aimed to evaluate the anticestode and antinematode effects of the methanol extract of asafoetida on rats experimentally infected with nematode Syphacia obvelata and cestode Hymenolepis nana in vitro.

Methods
Preparation of asafoetida herbal extract: In this study, asafoetida was collected from the plains of Bojnurd city located in Khorasan province, Iran during the summer of 2014. Type and species of the plant were confirmed by Sari University of Agricultural Sciences & Natural Resources (Mazandaran province, Iran). Aerial parts of asafoetida were dried at room temperature and powdered afterwards. To provide the herbal extract, we used the percolation method and methanol. Using a distillation apparatus, the obtained methanol extract was condensed in vacuum and kept at the temperature of 4°C until the beginning of experiments.

Experimental animals: In total, 100 male rats (age: 60 days, weight: 25-30 grams) were purchased from Pasteur Institute of Iran (Amol Branch). The animals had been preserved in sterile conditions. Samples were transferred to the laboratory of the Faculty of Veterinary Medicine at Islamic Azad University of Babol Branch, Iran.

Induction of infection in rats: To induce infection in the experimental animals, eggs of nematode Syphacia obvelata and cestode Hymenolepis nana were separated from the fecal samples of infected rats in Pasteur Institute of Iran (Amol Branch). Samples were studied on wet mount slides using an optical microscope, and viability of the eggs was confirmed. To infect the healthy rats (as confirmed by fecal examination), they were each fed 200 cestode and nematode eggs (10).

Treatment groups and applied doses: Two weeks after the induction and confirmation of parasitic infection, the animals were randomly divided into 10 groups of 10. The first group was considered as cestode control, and the second group was determined as nematode control. None of the animals in these groups received treatment. Animals in the third study group received praziquantel at the standard anticestodal dose of 25 mg/kg, and rats in group four were administered with piperazine at the standard antinematodal dose of 20 mg/kg. Infected animals in experimental groups five, six and seven received 2.5%, 5% and 10% concentrations of the methanol extract of asafoetida, respectively. Similarly, nematode-infected rats in experimental groups 8, 9 and 10 received treatment with 2.5%, 5% and 10% concentrations of the methanol extract of asafoetida. All the mentioned dosages were administered via oral gavage (fig 1).

After the first week of treatment, animals in the experimental groups were evaluated in terms of the number of parasite eggs using the Willis method. At the end of the second week of treatment, the number of
intestinal parasites in all experimental groups was determined upon necropsy (10).

**Statistical analysis:** Data analysis was performed in SPSS V.13 using one-way analysis of variance (ANOVA) and the Bonferroni method, and p<0.05 was considered statistically significant.

Figure 1. Drug administration in rats via gavage

**Results**

After one week of treatment, a significant reduction was observed in the number of Syphacia obvelata eggs in animals administered with piperazine compared to the control subjects and other experimental groups (p<0.01). Moreover, animals receiving treatment with piperazine showed a significant difference in this regard compared to the other study groups (p<0.05).

On the other hand, treatment with the methanol extract of asafoetida was associated with no significant reduction in the number of parasite eggs in the fecal samples of the rats, even at the highest concentration (p<0.05). Nevertheless, after the first week of treatment, a statistically significant difference was observed in the number of Hymenolepis nana parasite eggs in the animals administered with praziquantel and 2.5%, 5% and 10% concentrations of the methanol extract of asafoetida compared to the control group (p<0.05) (table 1). After two weeks of treatment, a significant difference was observed in the number of Hymenolepis nana eggs in the fecal samples of all the experimental groups compared to control subjects (p<0.05). According to the results of this study, treatment of parasitic infections with the methanol extract of asafoetida at different concentrations significantly decreased the number of cestode eggs in the fecal samples of animals after two weeks. However, the anticestode effects of asafoetida methanol extract was found to be dose-dependent, so that treatment at higher doses caused a greater response in the animals. As such, there was a significant difference in the improvement of parasitic infection between the animals receiving 2.5% and 10% concentrations of the asafoetida extract (p<0.05).

As expected, piperazine treatment eliminated all the nematodes, while praziquantel destroyed all the cestodes. Consequently, the results of necropsy revealed no intestinal worms in the infected rats treated with the mentioned drugs. No statistically significant difference was observed between the animals in the Syphacia obvelata group treated with asafoetida extract and control subjects (p>0.05). In addition, treatment with the methanol extract of asafoetida had no significant effect on the number of intestinal nematodes even at high concentrations. On the other hand, asafoetida extract had a significant effect on the number of Hymenolepis cestodes (p<0.05).

It is noteworthy that all the concentrations of the herbal extract significantly reduced the number of intestinal cestodes in the samples obtained from the animals of the experimental groups compared to control subjects (p<0.05) (table 2).

**Table 1. Mean number of cestode and nematode parasitic eggs in fecal samples of study groups during treatment**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Syphacia obvelata Mean±SD</th>
<th>Hymenolepis nana Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week One</td>
<td>Week Two</td>
</tr>
<tr>
<td>Control</td>
<td>235±9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>214.2±12.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Asafoetida Methanol Extract</td>
<td>166.4±3.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>121.2±11.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Asafoetida Methanol Extract</td>
<td>176.3±3.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>147.9±20.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5% Asafoetida Methanol Extract</td>
<td>210.9±9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>195.7±15.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Table 2. Mean of intestinal parasites after necropsy of studied rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Syphacia obvelata Mean±SD</th>
<th>Hymenolepis nana Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week One</td>
<td>Week Two</td>
</tr>
<tr>
<td>Control</td>
<td>221.3±40.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.5±10.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Piperazine</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>...</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Asafoetida Methanol Extract</td>
<td>177.4±13.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Asafoetida Methanol Extract</td>
<td>209.7±22.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.6±1.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5% Asafoetida Methanol Extract</td>
<td>214.6±34.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.7±0.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Dissimilar letters in each column indicate a significant difference based on Bonferroni correction method (p<0.05).
Discussion

According to the results of the present study, treatment with the methanol extract of asafoetida could effectively reduce Hymenolepis nana parasites in experimentally infected rats. In another study, Maraghi et al. performed the laboratory and clinical evaluation of the antiparasitic effects of asafoetida against Hymenolepis nana in comparison with niclosamide. According to the findings, administration of 17.7 mg/ml of asafoetida extract for two weeks improved the health of 90% of the infected rats (11).

This was in congruence with the results of the current study, which marked a significant reduction in the number of mature parasites in the experimental animals receiving treatment with the methanol extract of asafoetida compared to control subjects. This finding confirms the anticestode properties of asafoetida plant, which has been reported by Nazemi et al. as well (12). In a study in this regard, Barati assessed the anti-Leishmania effects of asafoetida herbal extract against Leishmania major promastigotes, and the obtained results were indicative of the anti-leishmanial effects of this medicinal herb (13). In this regard, findings of Ramdan et al. revealed that oral administration of asafoetida essential oil could significantly decrease the number of eggs and worms in rats infected with Schistosoma mansoni (9). Another study by the same researchers was performed to evaluate the antiparasitic effects of asafoetida against Trichomonas vaginalis in vitro. According to the findings, the resin extracted from the stems and roots of asafoetida could significantly inhibit the growth of Trichomonas vaginalis in vitro (8).

According to the results obtained by Sarkari et al., treatment with the herbal extract of asafoetida at the concentration of 2 mg/ml for one hour after exposure to Trichomonas vaginalis reduced the number of parasites by 90% (14). Furthermore, Rezaeimanesh et al. reported that the aqueous and alcoholic extracts of asafoetida showed lethal effects against Giardia cysts; however, this effect was found to be more significant with the alcoholic extract (15). Artemisia absinthium is another medicinal herb grown in Iran widely used in the traditional medicine for the treatment of parasitic infections. In a study by Youssefi et al. conducted to evaluate the antiparasitic effects of Artemisia absinthium, it was reported that the alcoholic extract of this herb demonstrated therapeutic effects against experimental infection with Syphacia obvelata. Nevertheless, the herbal extract of asafoetida was observed to have no significant effect against nematode Syphacia obvelata in the present study, which rules out the antinematode potential of this medicinal plant (16). Medicinal herbs are healthy, abundant and sustainable sources of nature, which have been prominently considered in the traditional medicine. Due to the increasing resistance to available antiparasitic medications and subsequent side effects of these agents, discovering new and low-risk therapeutic sources in this regard is of paramount importance (8).

One of the most beneficial medicinal herbs in the treatment of parasitic diseases is Ferula asafoetida, which is grown in Iran and has long been used in the Iranian traditional medicine. In conclusion, results of the present research indicated that the methanol extract of asafoetida had no antinematode effect on the studied samples, while the anticestode effects of this medicinal plant were confirmed.

Therefore, it is recommended that different compounds of asafoetida with possible anticestode properties be identified and extracted in future studies. Furthermore, complementary research in this regard could deliver this medicinal herb or its compounds as useful anticestode medications. Adequate knowledge of medicinal herbs and their associated therapeutic effects could promote public health in society, prevent the complications caused by chemical medications and reduce healthcare costs.

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References


