IJMPES International Journal of
Medical Parasitology & Epidemiology Sciences

http://ijmpes.com
doi 10.34172/ijmpes.2023.06
Vol. 4, No. 1, 2023, 30-34
eISSN 2766-6492

Original Article

Evaluating the Effect of Methanolic Extract of *Astragalus effusus* Plant on the Hatching of Eggs of *Haemonchus* Species *In Vitro*

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Abstract

Introduction: Internal parasites, especially nematode infections of the digestive tract (e.g., different species of *Haemonchus*), have always been associated with economic losses in sheep production systems in tropical regions. Therefore, the implementation of environmentally benign treatment alternatives is essential for integrated parasite control programs. The current study aimed to evaluate the effect of the methanolic extract of *Astragalus effusus* plant on the hatching of eggs of *Haemonchus* species in laboratory conditions.

Methods: This study was conducted between 2021 and 2022 in Tabriz, Iran. *Haemonchus* nematode eggs were extracted from experimentally infected sheep and were exposed to different concentrations (10, 20, 40, 80, and 160 mg/mL) of the methanolic extract of the plant under laboratory conditions. The hatching percentage of *Haemonchus* eggs was recorded after repeating the experiments five times, and the 50% and 90% inhibitory concentrations of the methanolic extract of the plant (CL50 and CL90) were estimated through regression analysis.

Results: Our results demonstrated the effect of the methanolic extract of the *A. effusus* plant on the hatching of the eggs of *Haemonchus* species in laboratory conditions with satisfactory results. The effect of different concentrations of the methanolic extract of *A. effusus* plant from 1% to 8% (10-80 mg/mL) on the hatching of the eggs of *Haemonchus* species was significant, and 0.5% dimethyl sulfate was considered as the negative control of the group ($\chi^2 = 0.024$, $P < 0.05$). Likewise, the inhibitory effect on the hatching of the eggs of *Haemonchus* species in a concentration of 16% of the methanolic extract of the *A. effusus* plant (160 mg/mL) was similar to the effect of albendazole (positive control).

Conclusion: In laboratory conditions, the methanolic extract of the *A. effusus* plant was effective in preventing the hatching of the eggs of *Haemonchus* species, which is important to guide further studies on the other effective compounds of this plant in all stages of the life of Haemonchus because different species of the *A. effusus* plant have widely adapted to the geographical and climatic conditions of Iran.

Keywords: *Haemonchus*, Methanolic extract, *Astragalus effusus* plant, Sheep, Iran

Received: March 7, 2023, **Accepted**: March 20, 2023, **ePublished**: March 29, 2023

Introduction

Infection with parasites in the digestive system of ruminants, especially sheep and goats, is a serious problem for livestock farmers all over the world, but their effects are extremely greater in tropical areas due to climatic conditions. Today, high productivity in terms of meat, milk, wool, and other products has made sheep and goats in the first degree of importance both in terms of food and protein production, and with regard to economic matters (1,2). According to these variables, it is possible to understand the high importance of this animal in the countries. Unfortunately, in contrast to the high benefits of this animal, there are many factors that cause damage to sheep and goats. Among them are parasitic pathogens that cause significant economic losses to the country's livestock industry, including sheep and goats. The economic losses caused by the parasites of the digestive tract are due to reduced fertility, reduced work efficiency, reduced milk production, treatment costs, and mortality in severe infections (3,4).

Worm infestations, in addition to the direct problems they cause in livestock, create a favorable environment for viral and bacterial diseases and even lead to the death of livestock in many cases (5).

To prevent parasitic infections in most regions, only a few common drugs are used regardless of the type of parasite and the damage caused by it. Accordingly, knowing the type and amount of damage caused by different parasites can be effective in more accurate diagnosis and faster treatment of complications and thus reducing future costs (6).

Ruminants are the main hosts of helminth infections.

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Worm and parasitic infections occur mostly in chronic and clinical forms. On the other hand, climate and weather conditions, the way animals are fed, vegetation, and the like are among the factors affecting parasitic infections (7,8).

In ruminants, the abomasum is one of the most important tissues in which worms of the Trichostrongylidae family live, including different species of *Haemonchus*, *Teladorsagia*, *Ostertagia*, and *Trichostrongilus*. Most of the ruminant population in Iran is engaged in grazing in free areas and is constantly in direct contact with the third stage (L3) larvae of the digestive system parasitic nematodes (9,10).

The total economic losses caused by these pollutions have caused different drugs to be used to treat them, and on the other hand, the technology of pharmaceutical science in the synthesis of chemical drugs and their wide application in treatment has been justified by the side effects and abnormality of these drugs (11). Sensitization, the phenomenon of drug resistance, the remains of drug compounds in nature, the high cash value of these compounds, and the like have caused more suitable alternatives to be considered by researchers. Although in the last few decades, the rapid progress in the production of synthetic drugs has caused the use of medicinal plants to lose their main and prominent role, the fact that these compounds are an ideal alternative to synthetic derivatives is inevitable (12,13).

In the last decades of the 20th century, it is associated with the approach of the world towards herbal medicines, and this process, which is called the green wave, is still going on (14).

This study sought to investigate the effect of the methanolic extract of the *Astragalus effusus* plant on the hatching of the eggs of *Haemonchus* species in laboratory conditions.

Materials and Methods

This study was conducted *in vitro* in four stages between 2021 and 2022 in Tabriz, Iran. In the first stage of this experiment, 10 kg of the leaves of the *A. effusus* plant were collected and the leaves were dried in a rotating air oven at a temperature of 40°C for 72 hours to prepare the methanolic extract of this plant.

Then, the dry and powdered materials were sieved homogenously. The permeated liquid was filtered and stored at 40°C. The obtained extract was diluted in 0.5% dimethyl sulfoxide solution (DMSO) and then collected in concentrations 10, 20, 40, 80, and 160 mg/mL.

The egg-hatch inhibition test was performed in the second stage. After experimentally infecting the sheep and preparing the reservoir animal, the stool sample was taken from the rectal ampoule of the sheep infected with *Haemonchus*.

Next, 20 g of feces were collected to determine the number of eggs per gram of feces and its culture. At this stage, the extract of the *A. effusus* plant in 5 different dilutions was added to the stool sample, along with the positive control group of 25 µg/mL albendazole and the negative control (0.5% DMSO). Each test was repeated five times, and all samples were kept at 27°C for 24 hours.

A drop of Lugol's iodine solution was used to stop the hatching process. At this stage, the first-stage larvae (L1) and eggs were counted using an inverted microscope, and the percentage of hatching inhibition (HI%) for each level of treatment was calculated using the following formula:

HI% = 100 (P- test/P- total)

where P-test and P-total represent the number of eggs and the total number of eggs plus the number of L1, respectively.

In the third stage, primary phytochemical screening was performed, and the primary phytochemical analysis of plant leaves was conducted following colorimetric and chromatographic methods. The fourth and last stages of statistical analysis were prepared and recorded as well. It should be noted that all ethical considerations were observed during and after the work.

Statistical Analysis

Chi-square statistical analysis, colorimetric and chromatographic methods, inverted microscope, and OpenStat statistical program were employed to analyze the data.

Results

Table 1 presents satisfactory results related to the effect of the methanolic extract of the *A. effusus* plant on the hatching of the eggs of *Haemonchus* species in laboratory conditions (Figure 1).

The effect of different concentrations of the methanolic extract of the *A. effusus* plant from 1% to 8% (10-80 mg/mL) on the hatching of the eggs of *Haemonchus* species was significant, and 0.5% DMSO was considered as the negative control of the group ($\chi^2 = 0.024$, $P < 0.05$).

Moreover, the inhibitory effect on the hatching of the eggs of *Haemonchus* species in a concentration of 16% of the methanolic extract of the *A. effusus* plant (160 mg/mL) was similar to the effect of albendazole (positive control).

Table 2 provides the secondary characteristics of metabolite groups in the methanolic extract of the *A. effusus* plant, which were obtained using the qualitative techniques of colorimetry and thin-layer chromatography.

These techniques allow us to identify the groups of alkaloids, and triterpenes, as well as anthracene and cardiogenic glucosides.

Discussion

Breeding of sheep and goats in Iran is of considerable importance considering the population of 70 million head of these two animals and as one of the sources of

Table 1. The Inhibitory Effect on the Hatching of Eggs of *Haemonchus* Species Exposed to the Methanolic Extract of the *Astragalus effusus* Plant Under Laboratory Conditions

***Astragalus effusus* Extract Concentration - Controls**	**% Inhibition of Hatching of *Haemonchus* Eggs**
10 mg/mL	26.2% [A]
20 mg/mL	31.3% [A]
40 mg/mL	33.7% [A]
80 mg/mL	51.8%[A]
160 mg/mL	82.9% [B]
Albendazole 25 µg/mL	100% [B]
DMSO 5 mg/mL	0%

Note. DMSO: Dimethyl sulfate.
Different superscript letters in a column of Table 1 indicate significant differences compared to the level of 0.5% DMSO negative control treatment based on the chi-square test result ([A] $P<0.05$, [B] $P<0.001$).

Table 2. Characteristics of Secondary Metabolite Groups in the Methanolic Extract of *Astragalus effusus* Plant

Secondary Metabolites Group	**Colorimetric Test/Result**	**Thin-Layer Chromatography - Pure Sample/Result**
Alkaloids	Mayer's reagent (-) Ammonium reineckate (-) Dragendorf's reagent (+) Valser's reagent (-)	Quinidine (+)
Flavonoids	Shinoda's reagent (-)	Flavone (-)
Triterpenes	NA	(+)
Anthracene glycoside	NaOH 5% NH4 2% (+)	Sacred Cascara (-)
Cardiogenic glycosides	Kedde's reaction (-) Keller-Kilian's reaction (-)	Digitalina (-)

Note. (-): Not present; (+): Present, NA: Not applicable.

animal protein. All these animals have free grazing and are exposed to various parasitic infections. Especially worm infestations are located in the digestive tract. The infection of ruminants with gastrointestinal nematodes leads to loss of weight, wool, and milk. Further, the total economic losses caused by these infections have caused different drugs to be used to treat them. On the other hand, pharmaceutical science technology in the synthesis of chemical drugs and wide application have been justified in the treatment, with the side effects and abnormality of these drugs (15).

The researchers have considered more suitable alternatives due to sensitization, the phenomenon of drug resistance, the remains of drug compounds in nature, the high cash value of these compounds, and the like (16). Although in the last few decades, the rapid progress in the production of synthetic drugs has caused the use of medicinal plants to lose their main and prominent role, it is inevitable that these compounds are an ideal alternative to synthetic derivatives (17,18).

Considering the importance of parasitic diseases, especially helminth infections in livestock and the indiscriminate use of artificial drugs, which are certainly associated with adverse effects, the present study was conducted, which is the first experience of its kind in the field of using herbal compounds against helminth infections in veterinary medicine. In addition, this study investigated the effect of the methanolic extract of the *A. effusus* plant on the egg-hatching stages of *Haemonchus contortus*, as the most important and pathogenic nematode of sheep abomasum in laboratory conditions (19).

In the present investigation, the effect of the methanolic extract of *A. effusus* plant on the hatching of the eggs of *Haemonchus* species in laboratory conditions represented satisfactory results. Additionally, the effect of different concentrations of the methanolic extract of the *A. effusus*

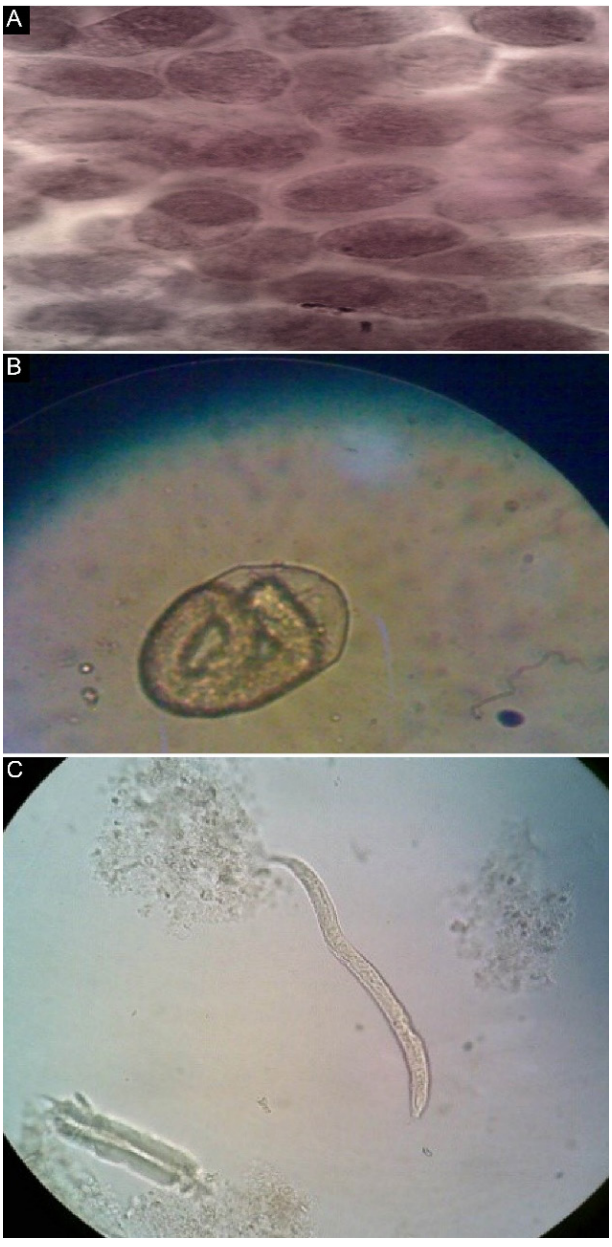

Figure 1. Different Stages of Development and Hatching of Eggs of the Haemonchus Worm in Laboratory Conditions: (A) Eggs Containing Embryo Mass. (B) Egg Containing in Vitro Developed Neonate (L1) Inside the Egg, and (C) Hatchling From Inside the Egg. *Note*. The division and development of the early embryo can also be observed in some eggs (A).

plant from 1% to 8% (10 to 80 mg/mL) on the hatching of the eggs of *Haemonchus* species was significant, as the inhibition of the hatching of the eggs of *Haemonchus contortus* increased with an increase in the concentration of the plant extract and reached the highest level.

Similar research has been performed in this field, but it has been about the effect of thyme, cumin, and geranium plant extracts on different stages of the growth of *H. contortus* in laboratory conditions (20,21).

In a study conducted by experimentally infecting 10 sheep in controlled laboratory conditions at different stages, it was found that when the thyme extract is added to the culture medium, there is a clear decrease in hatching, This sentence is completely correct because the scientific terms are plant extracts (22), which is consistent with the findings of our current research conducted on the effect of the methanolic extract of the *A. effusus* plant on the hatching of the eggs of *H. contortus*.

The anthelmintic effect of plant extracts can be related to the tannin present in different parts of the plant, although the amount of this compound varies in different parts of the plant; for example, there is a type of tannin called geranin in the geranium plant, the amount of which is up to 45% in the root and about 3.5% in the other parts of the plant.

Min et al (15) confirmed the effect of tannin on reducing the digestive parasites of Angourah rabbits, and Hoste et al (11) showed the effect of condensed tannin on decreasing the digestive nematodes of goats.

Jaramillo-Hernández et al (12) evaluated the effect of the extracts of *Momordica charantia* plant on the egg stage of the *H. contortus* worm in sheep and observed that this plant significantly decreases the hatching percentage of *Haemonchus* eggs. This is in good agreement with our current research, which was conducted on the effect of the methanolic extract of the *A. effusus* plant on the hatching of the eggs of *H. contortus*, which reduced the hatching of *Haemonchus* eggs.

Likewise, Squires et al investigated the effect of the arrhythmias plant and extract on the parasite *H. contortus* in rats, but the results were not satisfactory (18).

In another study, Minho et al examined the effect of the extract of a group of specific medicinal plants on the parasite *H. contortus* in sheep. Based on their findings, the 5 selected plants were *Ananas comosus, Aloe ferox, Allium sativum, Lespedeza cuneata*, and *Warburgia salutaris*. They further reported that the number of larvae produced by the worm *H. contortus* decreased by increasing the concentration of the extracts of these five plants (23), which also corroborates the results of our research.

Similarly, Jaramillo-Hernández et al determined the effect of condensed tannin on reducing the worm load of different nematodes, especially *Haemonchus*, *Cooperia*, *Nematodirus*, and *Trichostrongylus*.

Astragalus effusus plant also has vegetable tannin, and this plant is widely used in traditional pharmaceutical industries.

In the last decades of the 20th century, it is associated with the approach of the world towards herbal medicines, and this process, which is called the green wave, is still going on (24).

The vast climatic diversity and multiplicity of plant species in Iran have opened up a wide horizontal field for the researchers and practitioners of herbal medicines to continue the path started by great people such as Razi, Bu-Ali Sina, Ahwazi Jurjani, and others. Furthermore, it presents a positive future for the communities to fight against various pollutions (25).

It is hoped that in the future, many studies will be conducted on the effects of the *A. effusus* plant extract on the parasitic infections of abomasum nematodes, as well as the effects of the methanolic extract of this plant on the other helminth infections of ruminants so that to complement our current research.

Ethical Approval

This study was approved by Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran. (Ethical code: IR.IAU.TABRIZ.REC.1401.315).

Funding

The described results in this paper are part of an MSc thesis submitted by Elnaz Sagheb. The study was financially supported by the office of the Vice-chancellor for the Research of Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran.

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