Anthelmintic Effects of Herbal Mixture of Selected Plants of Apiaceae on Strongylus vulgaris and Fasciola hepatica

Nawal Al-Hoshani1*, Ruoa Almahallawi2, Eman A Al-Nabati3, Sarah A Althubyani3, Sally Negm4, Attala F El-Ikott5,6, Majed A Bajaber7, Soliman M. Soliman8, and Ahmed Ezzat Ahmed5,9

1Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
2Department of Biology, University College of Duba, University of Tabuk, Tabuk 71491, Saudi Arabia
3Department of Biology, College of Science, Taibah University, Madinah, P.O. 344, Saudi Arabia
4Department of Life Sciences, College of Science and Art Mahyel Aseer, King Khalid University, Abha 62529, Saudi Arabia
5Department of Biology, College of Science, King Khalid University, Abha 61413, Saudi Arabia
6Department of Zoology, Faculty of Science, Damahour University, Damahour 22511, Egypt
7Department of Chemistry, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia
8Department of Medicine & Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt.
9Department of Theriogenology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt
*Corresponding author: Nialhoshani@pnu.edu.sa

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Helminths are widespread infectious agents, causing severe economic and performance losses in all animals, especially donkeys. Strongylus (S.) vulgaris and Fasciola (F.) hepatica are abundantly present in the equines. Their control using herbal is being necessitated because of the resistance of parasites towards routinely used chemicals. In this study, 60 positive animals were divided equally into 4 groups, each having three replicates named A, B, C, and D. Donkeys in groups A, B, and C received 1200, 1000, and 800 mg/kg mixture of 4 plants of family Apiaceae while the group D was kept as non-medicated control. The medication of the animals was done for 4 weeks. fecal, hematological, and hepato-renal function-related serum samples were collected on days 0, 14, and 28 of the experiment to estimate the effects of herbal mixture on fecal egg count reduction, red blood cell counts, white blood cells, hemoglobin, packed cell volume, alanine aminotransferase, aspartate transferase, serum proteins, serum albumins, urea, and creatinine. The donkeys were weighed at the start and end of the trial to estimate the effect of the herbal mixture on weight gain. The results suggested that total helminth, S. vulgaris, and F. hepatica egg counts were significantly (p<0.05) less than the control group. Herbal mixture significantly (p<0.05) improved the red blood cells, packed cell volume, hemoglobin, total proteins, albumins, and weight gains of the donkeys treated with 1200 mg/kg herbal mixture. The results suggested that the herbal mixture from the plants of the family Apiaceae was effective in controlling the helminths and improving hematological and serological parameters.

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INTRODUCTION

Infectious diseases have been a great concern for humans since their existence (Saeed and Alsayegh, 2023). Helminths are among the most prominent infectious agents known to men and remain under consideration for the investigation of control (Rehman et al., 2023). Helminths in livestock reduce domesticated animals’ work efficiency, health span and economics (Mostafa et al., 2023). Donkeys are among the most prominent domestic animals, mainly beasts of burden. The donkeys are reared for the load and transportation in the areas where no vehicle can work (Kumaravel et al., 2023). Multiple factors may diminish their work efficiency among which helminths are the major factors leading to reduced work performance of donkeys (Clarence-Smith, 2020). Among the helminths infecting...
donkeys are Strongylus (S.) vulgaris and Fasciola (F.) hepatica (Minori, 2022). S. vulgaris is a nematode parasitizing the equines, causing severe economic and health losses (Jamil et al., 2022). F. hepatica is the trematode worm causing liver cirrhosis and ulceration leading to mortalities in multiple species of animals including donkeys (Sazmand et al., 2020). The researchers have reported that S. vulgaris is 60-70% prevalent in donkeys, while a 10-15% prevalence of F. hepatica is also stated. Focusing control of only S. vulgaris and F. hepatica parasites is crucial for the control of helminths in donkeys (Sazmand et al., 2020; Molento and Vilela, 2021). These trematodes and nematodes remain in high numbers and cause significant economic and health issues, reducing the efficiency of donkeys.

Researchers are trying to develop a comprehensive control strategy that could be safe and accurate for the control of the worms (Kandeel et al., 2022). Synthetic anthelmintic dewormers are among the most used drugs by practitioners and farmers because of their high accuracy and rapid action (Abbas et al., 2020). Chemical anthelmintic drugs are still being practiced, but the emergence of resistance and public health issues are among the major factors causing the limited use of these drugs. Chemical anthelmintic drugs are being resisted by multiple parasites and shortly, the use of these drugs will be useless (Ahuir-Baraja et al., 2021). There is a strong need for alternatives to chemical antiparasitic drugs (Abbas et al., 2023).

Multiple alternatives to anthelmintics are being suggested, including probiotics, postbiotics, organic acids, and plant-based medicines. Plants are among the greatest natural sources of diverse groups of chemicals, which provide several drugs. Because of the safety, accuracy, and availability of plants, scientists are showing great interest in herbs (Saeed and Alkheraïje, 2023). Multiple researchers have reported that plant and plant-based preparations can control parasitic infectious agents (Al et al., 2023). Research reports that making combinations of more than one plant for the control of the parasites shows a boosted effect (Mravčáková et al., 2020; Ukwa et al., 2024).

Apiaceae is the 16th largest family of flowering plants. The members of the family Apiaceae are well-known for their nutritional and medicinal properties (Wang et al., 2022). Several plants of Apiaceae have been used for the treatment of various diseases of infectious agents (GabAllah et al., 2020). Members of Apiaceae have been proven to be anthelmintic in experiments because of the active compounds in them (Abbas et al., 2020).

In this study, a combination of various parts of plants belonging to the family Apiaceae (Cuminum cyminum, Trachyspermum ammi, Foeniculum vulgare, and Pimpella anisum) was used for the control of helminths especially S. vulgaris and F. hepatica in naturally infected donkeys. Moreover, the effects on hematological parameters, serum hepatic and renal function parameters, and weight gain were also evaluated.

**MATERIALS AND METHODS**

**Plant material:** Plant material of the *Cuminum cyminum, Trachyspermum ammi, Foeniculum vulgare* and *Pimpella anisum* belonging to the family Apiaceae was collected from authenticated sources and verified by the botanist. The collected material was ground, mixed and dried, as described by Abbas et al. (2020).

**Experimental animals:** Adult non-lactating, non-pregnant females and male donkeys were selected randomly. The animals were kept with no anthelmintic for three months. The animals were reared in semi-intensive systems and were not exposed to any pack or work-related stress 7 days before the start of the study and until the completion of the trial. Animals were given basal diets and uniform environmental conditions.

**Experimental design:** Fecal, blood, serum samples, and weights were taken at the start of the trial from multiple animals. The 60 animals with similar parasite/indices were equally divided into 4 groups A, B, C, and D, each having three replicates in a way they have similar mean values of parasitic count first and other parameters on second importance, at the start of the trial. Donkeys in groups A, B, and C received 1200, 1000, and 800 mg/kg mixture of 4 plants of family Apiaceae while group D was kept as non-medicated control. Animals were given an herbal mixture every week. The egg counts, serum, and hematological samples were taken on days 0, 14 and 28 of the trial. Weights were recorded at the start and end of the trial. The values were recorded in Microsoft excel® for the analysis.

**Parameters**

- **Egg count and Fecal egg count reduction:** Fresh fecal samples were collected from the rectum of animals, kept in a cold chain, and transported to the laboratory for analysis of the samples. Concentration techniques were applied for the isolation of eggs and the collected eggs were counted in the McMaster counting chamber (Vadlejch et al., 2011). The fecal egg count reduction was determined using the following formula:

\[
\text{Fecal egg count reduction} = \left( \frac{\text{Initial egg counts} - \text{Final egg counts}}{\text{Initial egg counts}} \right) \times 100
\]

- **Hematological and serological parameters:** Blood samples were collected from the jugular veins of the animals and collected in EDTA and Gel-clot vacutainers for the blood and serum samples collection, respectively. These samples were then transported to the lab. Auto-hemocytometer (TC20® Bio-Rad®) was used for the calculation of erythrocytes and leukocytes (Abbas et al., 2020). Serum values were measured using spectrophotometric kits (Elbascience®). ALT, AST, total protein, albumins, globulins, urea and creatinine were estimated using kits, as described by Mostafa et al. (2023).

- **Weight gain:** Weights of animals were taken at the start and end of the trial. The percent weight gain was estimated using the following formula:

\[
\text{Percent weight gain} = \left( \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \right) \times 100
\]

**Statistical analysis:** All the data was organized and analyzed in Minitab® 26.0 software. A generalized linear model was applied for the analysis of variance and Tukey’s post-hoc test was used for the statistical comparison of the values. A 95% confidence interval was maintained for the determination of significance.
RESULTS

Parasitic infections are among the major threats to domesticated livestock. Helminths, especially nematodes and trematodes are among the major threatening factors to the livestock because of their abundant presence in the environment (Raza et al., 2022). S. vulgaris is a nematode belonging to the Strongyle group. Strongyles are the major parasites that affect the equines, including donkeys (Jürgenschellert et al., 2022). It causes gastrointestinal problems, including gastric colic, diarrhea, anemia, etc. F. hepatica is a trematode that comes into the body because of the consumption of unhygienic water or feed (Patil et al., 2021). It causes hepatic and gastric issues in animals, including donkeys (Mas-Coma et al., 2020). The donkeys are among the least considered among the equines (Geiger et al., 2021). They are used for packing and agricultural purposes, especially in villages of deserts, marshy and hilly areas. They remain infected with multiple parasites, which decreases their work efficiency (Geiger et al., 2021). Management of worm burdens in donkeys using herbal preparations is key for safe, economical, and convenient consumption of unhygienic water or feed (Geiger et al., 2022).

In this study, we evaluated the effects of the herbal mixture on the fecal egg count reduction of the helminths. S. vulgaris and F. hepatica were observed in this study for the determination of specific effects of herbal mixture.
against nematodes and trematodes. We observed that the herbal mixture reduced the eggs per gram of feces significantly leading to a great fecal egg count reduction percentage. Overall egg counts were reduced significantly during the first week and a more declining trend was observed in the second week. These results were in line with the Abbas et al. (2020). They worked on a complex herbal mixture for the treatment of helminths similarly focusing especially on a nematode (Haemonchus contortus) and trematode (F. hepatica). They achieved similar results and attributed these activities to the phenolic compounds present in the plants. Likewise, Saeed and Alsayeqh (2023) worked on the parasites of cattle with similar results. This study is also in line with the results of Kļaviņa et al. (2023) where the researchers have also justified the same findings. These results have been justified because of the presence of phytochemicals in the plants. The diversity of biologically active phytochemicals has been proven anthelmintic with direct and indirect anthelmintic effects (Akram et al., 2021).

The effects of the herbal mixture on the hematological values were also observed in the donkeys. The findings of our research showed that there was a significant increase in red blood cell counts, packed cell volume, and hemoglobin concentration. Our results were in line with the results of Saeed and Alsayeqh (2023). They stated that the mixture of various parts of plants was effective in elevating the blood cell counts in the helminth-infected animals. Similar findings have been reported by Papini et al. (2020) mentioning that herbal dewormers are efficient at improving the blood cell counts in the herbal mixture of treated animals. The increase in red blood cells, packed cell volume, and hemoglobin concentration can be linked to the reduction of parasitic burden (Flay et al., 2022). S. vulgaris feeds on blood, while fascioliasis is also a source of blood and protein loss in animals. Reduction in parasite burden results in an increase in blood cell values (Flay et al., 2022). However, no significant shift (p>0.05) in white blood cells was seen. This can be related to the fact that other infectious factors were not counted in this study and along with it there may be no direct association between the parasite burden and the white blood cell counts.

In this study, the effects of herbal mixture on renal and hepatic function-related serum parameters, i.e. ALT, AST
total proteins, Albumins, globulins, Urea, and creatinine were estimated. Whenever a new drug is being tested, its first major concern is that it should have safe metabolic pathways and excrete with no toxic effects. In this study, no significant shift was found between the quantity of herbal mixture used and the serum parameters, except for the total proteins and albumins levels. All the parameters remained in the normal range in all the groups. A significant increase in total protein and albumins was observed. Similar results have been reported by Abbas et al. (2020) associated with the safe metabolism of herbal compounds in the body, leading to normal renal and hepatic functions. The increase in protein and albumin levels is directly correlated with the reduction in worm burden (Abosse et al., 2022). These worms cause protein loss because of their blood-feeding activities (Martin-Martin et al., 2023). Because of the herbal mixture, these serum values were improved, indicating that the herbal mixture helps reduce protein loss because of parasitic infections.

Conclusion: This study concludes that the herbal mixture of the family Apiaceae is effective in controlling gastrointestinal worms of donkeys especially *S. vulgaris* (Nematode) and *F. hepatica* (Trematode) along with showing improvement in the red blood cell counts, packed cell volume, hemoglobin, total proteins, and albumin levels in donkeys. The herbal mixture showed the maximum results at a 1200 mg dose rate. The herbal mixture is also a safe preparation for being administered for four weeks for renal and hepatic functions.

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Authors contribution: NA-I-H, RA, EAI-N, SAA designed the research idea and protocol. SN, AFE-I-l, MAB conducted the research and collected data. SMS and AEA were actively involved in the supervision of research. All authors were involved in the data analysis and final write up of the manuscript.

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