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RESEARCH ARTICLE

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

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ABSTRACT

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 Alsayeqh, 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 herbals (Saeed and Alkheraije, 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 anisun*) 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 anisun* belonging to the family *Apiacea* 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 parasite 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:

% Fecal egg count reduction= ((Initial egg counts-Final egg counts)/Initial egg counts) ×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:

Percent weight gain= ((Final Weight-Initial Weight)/ Final Weight) ×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 Tuckey's post-hoc test was used for the statistical comparison of the values. A 95% confidence interval was maintained for the determination of significance.

Table 1: Eggs per gram (EPG) values of *Strongylus vulgaris, Fasciola hepatica,* and overall helminths (mean±SD) in the donkeys

Groups	*S. vulgaris*			*F. hepatica*			Total helminths		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
A	666.66±125.83^{a}	250±50^{cd}	83.33±28.86^{d}	133.33±28.86^{ab}	250±50^{cd}	83.33±28.86^{d}	983.33±251.66^{a}	300±132.28^{cd}	83.33±28.86^{d}
B	683.33±76.37^{a}	400±50^{bc}	233.33±28.86^{cd}	116.66±76.37^{ab}	400±50^{bc}	233.33±28.86^{cd}	966.66±104.08^{a}	450±50^{bc}	333.33±104.08^{cd}
C	666.66±76.37^{a}	483.33±76.37^{ab}	383.33±76.37^{bc}	183.33±57.73^{a}	483.33±76.37^{ab}	383.33±76.37^{bc}	983.33±104.08^{a}	666.66±28.86^{ab}	516.66±28.86b^{c}
D	650±100^{a}	666.66±115.47^{a}	666.66±76.37^{a}	100±50^{ab}	666.66±115.47^{a}	666.66±76.37^{a}	916.66±125.83^{a}	933.33±76.37^{a}	933.33±57.73^{a}

All the values with similar superscripts within a row of a single factor or a column are statistically comparable (p>0.05).

Table 2: Percent fecal egg count reduction (%) values of *Strongylus vulgaris, Fasciola hepatica,* and overall helminths in the donkeys

Groups	*S. vulgaris*		*F. hepatica*		Total helminths	
	Day 14	Day 28	Day 14	Day 28	Day 14	Day 28
A	62.55±1.05	86.72±6.24	83.33±28.86	100±0	69.87±7.97	91.6±1.96
B	41.34±5.48	65.47±6.62	33.33±28.86	66.66±28.86	52.69±10.55	64.49±15.2
C	27.35±10.36	42.47±10.27	17.77±16.77	57.77±15.39	31.66±8.03	47.03±6.61
D	-2.68±9.07	-3.49±13.28	-16.66±28.86	27.77±25.45	-2.34±6.16	-2.57±8.91

A: Herbal dewormer @ 1200 mg/kg receiving donkeys; B: Donkeys receiving herbal dewormer@ 1000 mg/kg herbal dewormer; C: Donkeys receiving 800 mg/kg; D: non-medicated control

Table 3: Hematological values of *Strongylus vulgaris, Fasciola hepatica,* and overall helminths in the donkeys

Red Blood Cells (x10^{12}/L)			White Blood Cells (x10^{9}/L)			Packed cell volume (%)			Haemoglobin (g/dL)		
Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
4.3±0.26c	4.83±0.41^{bc}	6.33±0.58a	8.66±2.12^{a}	9±1.57^{a}	9±1.57^{a}	26.66±4.72^{b}	34.33±2.08^{a}	39.66±1.52^{a}	8.56±0.5^{c}	10.86±1.26^{bc}	14.2±0.36^{a}
4.13±0.15c	4.5±0.09^{bc}	5.3±0.4b	8.9±2.1^{a}	8.9±2.1^{a}	8.9±2.1^{a}	27±3^{b}	29.66±2.08^{ab}	33.33±2.51^{ab}	8.81±0.57^{c}	9.46±1.53^{bc}	11.4±1.1^{b}
4.43±0.35bc	4.4±0.1^{c}	4.8±0.1bc	8.43±1.1^{a}	9.03±1.55^{a}	9.03±1.55^{a}	26.66±4.04^{b}	28.33±3.21^{b}	29.33±2.08^{b}	8.86±0.6^{c}	9.53±0.95b^{c}	10.43±0.7^{bc}
4.33±0.25c	4.3±0.17^{c}	4.26±0.2c	8.66±1.43^{a}	9.4±1.55^{a}	9.4±1.55^{a}	28±5.29^{b}	27.66±4.04^{b}	28±3.6^{b}	8.93±0.2^{c}	9.06±0.32^{c}	9.06±0.32^{c}

All the values with similar superscripts within a row of single factor or a column are statistically comparable (p>0.05): A: Herbal dewormer @ 1200 mg/kg receiving donkeys; B: Donkeys receiving herbal dewormer@ 1000 mg/kg herbal dewormer; C: Donkeys receiving 800 mg/kg; D: non-medicated control

Table 4: Weight gain and percent weight donkeys during the trial

Groups	Initial weight (Kg)	Final weight (Kg)	Weight gain (Kg)	Weight gain (%)
A	112.66±8.02	129.33±5.13	16.66±3.05^{a}	12.95±2.84
B	102.66±5.5	111.33±6.5	8.66±1.15^{b}	7.76±0.64
C	109.66±5.5	114.33±5.85	4.66±0.57^{bc}	4.07±0.41
D	102.33±4.04	104±3.6	1.66±0.57^{c}	1.61±0.58

All the values with similar superscripts within a column are statistically comparable (p>0.05): A: Herbal dewormer @ 1200 mg/kg receiving donkeys; B: Donkeys receiving herbal dewormer@ 1000 mg/kg herbal dewormer; C: Donkeys receiving 800 mg/kg; D: non-medicated control

RESULTS

Egg per gram and fecal egg count reduction: Egg per gram values were measured on days 0, 14, and 28 of the experiment. The values were subjected to estimation of percent fecal egg count reduction. There was a trend of reduction in the egg counts overall on each calculation and group A (herbal mixture @ 1200 mg/kg) had a significant difference ($p<0.05$) from the control group at the first count while all the treatments differed significantly ($p<0.05$) from the control group (Table 1 and 2).

Hematological values: Hematological values were observed on days 0, 14, and 28 and significant differences ($p<0.05$) were observed in animals of group A group A (herbal mixture @ 1200 mg/kg) for the red blood cells, packed cell volumes, and hemoglobin concentrations while there were no significant differences ($p>0.05$) for the leukocytes count throughout the experiment (Table 3).

Serum chemistry: ALT, AST, total protein, albumins, globulins, urea, and creatinine values remained nonsignificant ($p<0.05$) throughout the trial. All the values remained in normal reference ranges (Fig. 1).

Weight gain: The percentage weight gain was calculated, and it was found that there was a significant increase ($p<0.05$) in weight in the group A (herbal mixture @ 1200 mg/kg) donkeys (Table 3).

DISCUSSION

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 donkey farming (Saeed and Alsayeqh, 2023). Multiple researchers have reported that herbal preparations can be effective for the control of nematodes and trematodes (Abbas *et al.*, 2020; Azizova and Uslu, 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

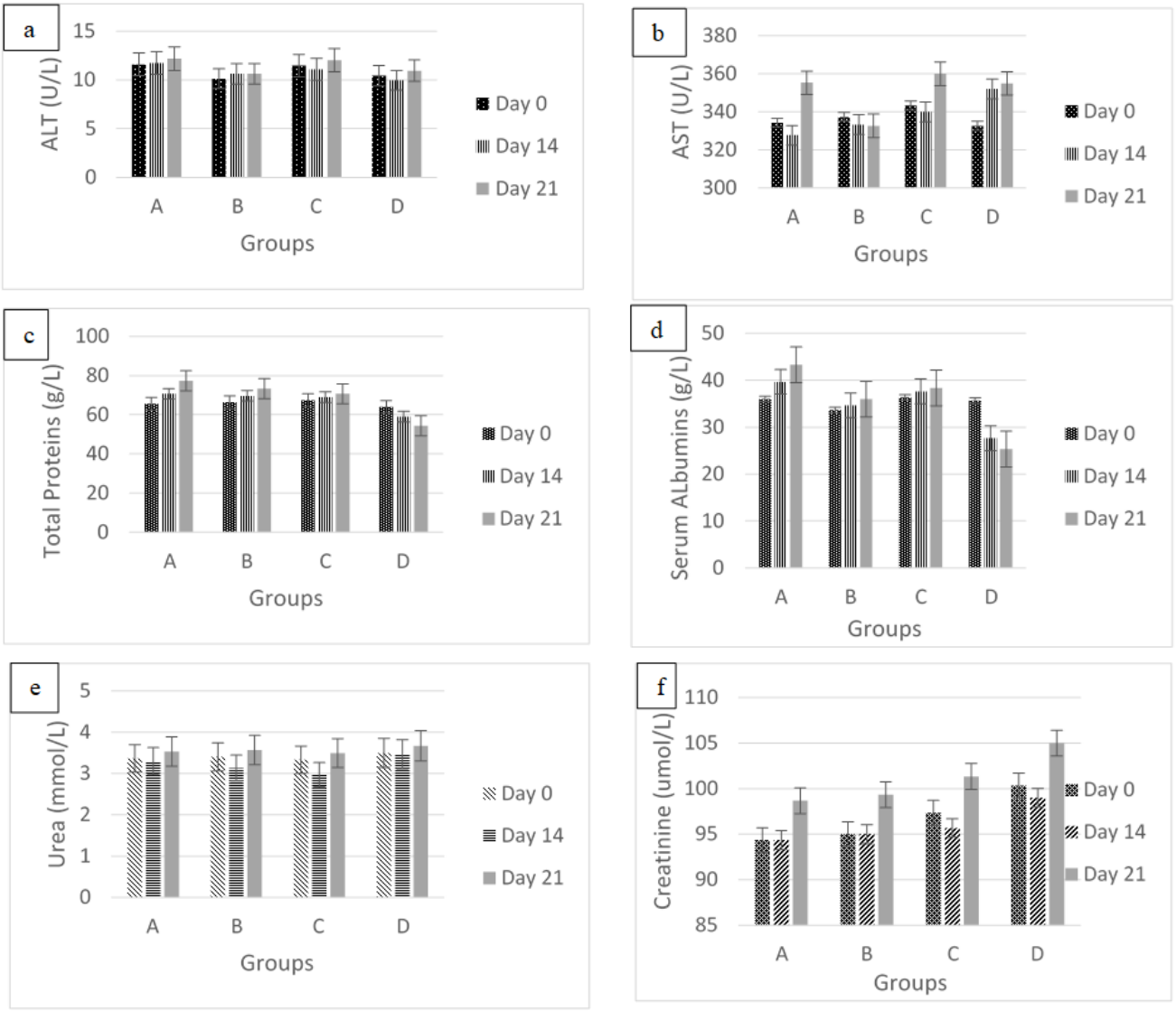

Fig. 1: Effect of different doses of the herbal mixture from the plants of *Apiaceae* family on different serum parameters (a) Alanine aminotransferase (ALT); (b) Aspartate transferase (AST); (c) Total proteins; (d) Serum albumins (e) urea and (d) creatinine in donkeys: A: Herbal dewormer @ 1200 mg/kg receiving donkeys; B: Donkeys receiving herbal dewormer@ 1000 mg/kg herbal dewormer; C: Donkeys receiving 800 mg/kg; D: non-medicated control

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: NAl-H, RA, EAl-N, SAA designed the research idea and protocol. SN , AFEl-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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