

Full Length Research Paper

Pathology of worm infestation in ovine and its treatment with two different plants extraction

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The aim of this research was to obtain the effects of plant extraction of *Fumariaceae* on the control of experimental multiple nematodes infection as an antihelminthic and *Scrophularia striata* plant extraction as an anti-inflammatory of gastro intestinal tract (GIT) in lambs, and their effects on body weight gain. 24 lambs, 9 to 13 months of age with the average body weight of 16.175 kg, were divided in two groups. After eight weeks of parasitic infection, the experimental group of animals were treated with 3 ml/kg body weight *Fumariaceae* plant extract for one week. Confirmed infected animals were randomly selected and slaughtered for GIT tissues at the end of eight weeks, while the rest of lambs again treated with *Scrophularia striata* extraction orally were slaughtered at the end of the 11th week. Marked hypertrophy and hyperplasia was observed in all regions of GIT of infected animals. Villi were broad and appeared to be flattened in distal regions of small intestine of infected animal in comparison to treated group of the lambs after eight weeks with the plant extraction of *S. striata*. Significant changes were observed in protein fractions. Significant increases in body weight were also observed in infected lambs on 10th week treated animal with plant extraction. *Fumariaceae* and *S. striata* plant extraction could be use as an antihelminthic, and anti-inflammatory, although it needs further extensive study.

Keyword: Helminthes, plant extraction, total protein, hematology, histology.

INTRODUCTION

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin (Cowan, 1999; De Carvalho and Ferreira, 2001; Kayser and Klderien, 2001). Iran is well known for the exuberance and the variety of its mountainous plants. Many of these plants are used as traditional natural medicines without any scientific base. In recent years, several medicinal plants have been screened for the treatment of diseases caused by parasites (Kayser et al., 2003). Homeopathy drug may have a role in reducing the pathology in the host (Hektoen, 2005; Cabaret, 1996; Zacharias et al., 2008).

Infection with nematode represents the main cause of economic loss in ovine breeding all over the world. Parasites are harmful to their host and produce infection in several ways,

Changes in plasma protein levels in various infections are considered to be related to changes in protein metabolism. An increased plasma protein loss is as a result of increased permeability of the gut wall due to nematode infection (Symons et al., 1974; Jones and Symons, 1982). Young mice infected with *Nematospirides dubius* showed increased whole body protein turnover (Symons and Jones, 1972). Changes in the rate of protein synthesis in sheep after *Trichostrongylus colubriformis* infection and increase in liver protein synthesis and decrease in skeletal muscle and kidney protein synthesis were reported by several researchers (Jones and Symons, 1982; Symons, 1982). Liver proteins synthesis was found to be increased and was suggested

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to be due to the plasma protein loss into the intestine as a result of increased mucosal permeability caused by parasites.

More also, change in the alkaline phosphates (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT) levels in grazing Saanen goats from New Zealand was reported by researchers (McDougall et al., 1991). Enzymatic assays in sheep and goats infected with *Haemonchus contortus* and an intestinal parasitic infection has been reported by several researchers (Ahmad and Ansari, 1987; Siddiqua et al., 1990; Chakraborty and Lodh, 1994). The change due to parasitic infection on total serum protein levels in goats in India and New Zealand has also been reported (McDougall et al., 1991; Sharma et al., 1990). The main objective of this study was to evaluate the effectiveness of *Fumariaceae* plant extraction as an antihelminthic and *Scrophularia* as an anti-inflammatory medicine with the characteristic of low cost, absent of residues in meat and milk and its low environmental impact.

MATERIALS AND METHODS

Animals and experimental design

24 males and females healthy Sanjabi breed lamb 9 to 13 months of age, with the average weight of 16.175 kg, were collected randomly from Ilam province of Iran, situated in the western part of the national capital of Iran, in 2008 to 2010. These animals were divided in two groups (each group contained 12 animals). All animals were dewormed with *Albendazole* (10 mg/kg) and *Thiabendazole* (0.6%). The absence of parasites was confirmed by examining the fecal samples from all animals by flotation, sedimentation and fecal culture techniques (McDougall et al., 1991). The lambs also were examined for blood protozoan infection by blood smear and inoculation in mice. All animals were free from any such infections. Experimental animals were managed and housed in the hygienically environment, and care was taken to avoid any contamination from outside.

On day 0, the treatment group received 6000 L3 multiple nematodes [*H. contortus* (50%), *Ostertagia ostertagi* (25%), *Trichostrongylus axei* (12%), *Chabertia ovina* (8%), *Cooperia* (all most 5%)] orally. Larvae were procured through faecal culture from female worm in the abomasum and duodenum of naturally infected laboratory raised lamb (McDougall et al., 1991). The viability motility of nematodes L3 was tested by McMaster slide, and based on the proportion of the active larvae, the infective doses were prepared. The animals were weighed in both groups, monitored twice a week and checked for the presence of parasitic eggs through faecal examination. Animals received feeds and water *ad libitum*. In both groups, blood samples (10 ml) were collected from jugular vein after eight weeks of infection and plasma was obtained. 10 μ M of 1% sodium azide solution were added to plasma sample and kept in refrigerator for further procedure. Some lambs were selected randomly from this group at the end of the 8th week, slaughtered and then the other remaining lambs of this group were treated with *S. striata* plant extraction till the end of the 11th week. These lambs were then slaughtered and tissue sample of GIT including abomasums, duodenum, Jejunum, distal ileum and caecum were separated and preserved in 5% formalin. Tissues were prepared for microtomy, cut in 8 μ M and stained in hematoxylin and eosin. Slides were studied on Olympus camera attached microscope. Observations were recorded and microphotography was done for

projection slides and photographs.

Plant extracts preparation

The plant material of *Fumariaceae* used in this study were collected from Zakros mountain area Southwest of Iran, and identified by Herbarium of the Institute of Medicinal plants –ACECR, Tehran, Iran. The whole part of the plant was dried and ground to powdery forms, then 200 g powder was separated, 500 ml of ethanol (96%) was added in sterile conical flasks and kept at +45°C in an oven overnight, after which the residue were obtained. The residue was diluted with deionized distilled water and 3 ml/kg body weight dilution were orally given to the animal daily for one week; to the experimental infected lambs with multiple nematode L3.

Treatment of experimental animal with the plant extraction

After eight weeks, infected lambs with the multiple nematodes L3 (group 2) were given daily orally 3 ml/body weight of *Fumariaceae* plant extraction for one week and then throughout the study period. Faecal samples from all lambs of this group were also collected and examined for eggs and larvae excretion (the dose of plant extraction has been selected according to preliminary experimental studies on several other groups of the lambs).

S. Striata plant extraction

The plant, *S. Striata*, used in this study were collected from Zakros mountain area Southwest of Iran, and identified by Herbarium of the Institute of Medicinal plants –ACECR, Tehran, Iran. The whole part of the dried plant was ground to powder, then 200 g powder was separated, 250 ml of ethanol (96%) was added in sterile conical flasks and kept at +45°C in an oven over night after which residue were obtain. The residue was diluted with deionized distilled water and daily 3 doses, (morning, evening and night 5 cc /dose), according to the preliminary study on lambs, were orally given as a anti-inflammatory medicine for repairing the tissue of GIT, then the animals' tissue were collected for histological comparison with tissue of infected period to see the effects of plant extraction.

Biochemical and hematological parameters

Total plasma proteins were measured by Biuret method as described by Dumas and Biggs (1972). Bovine serum albumin (Sigma Chemical Company, U.S.A.) was constituted in buffer of pH 7.00 to prepare standards. The most commonly used standards were 5 and 10 g/dl. Sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) was done for detecting the plasma proteins. Quantitative assay of alkaline phosphates were done using the method of Bessey et al. (1946). Plasma total free amino acids were measured using the method of Goodwin (1968); this procedure quantifies nitrogen of free amino acids. A standard of 20 amino acid was used, and the concentration of free amino acid in the sample was determined against the standard, indirectly, while estimating their nitrogen.

Plasma albumin was quantified by Bromocresol green method. The reagent was prepared and method was followed as described by Dumas and Biggs (1972). Ovine serum albumin was used for the preparation of standards. Total globulins in plasma were determined by subtracting the concentration of albumin from the concentration of total protein. In the first step, plasma globulins were separated from plasma by precipitating with ammonium sulphate and sodium chloride reagent (McDougall et al., 1991). SDS-PAGE based on the method of Laemmli (1970) was carried

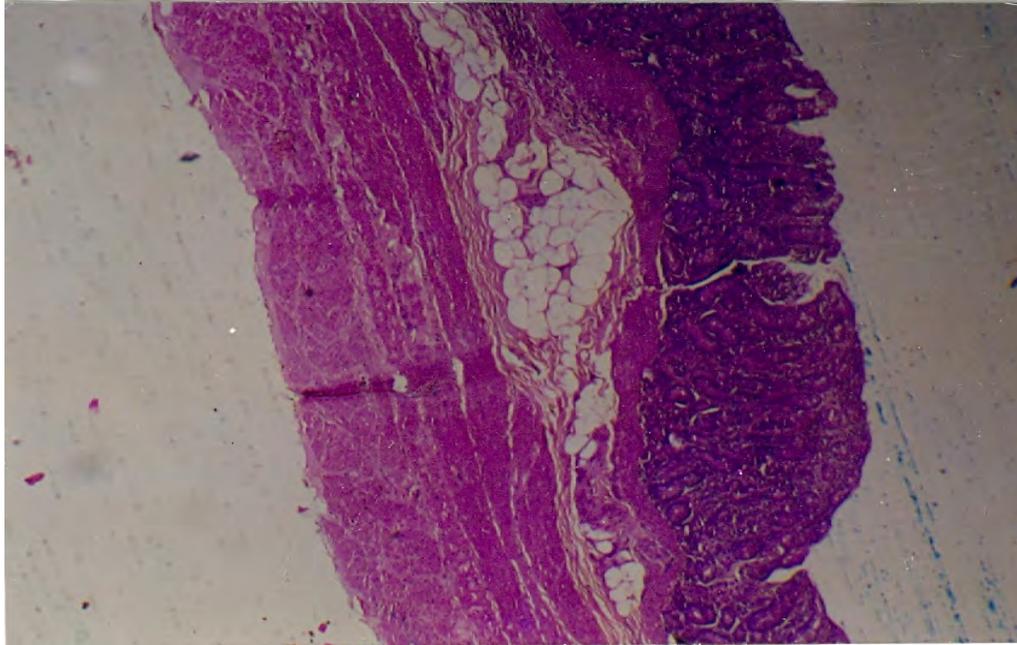


Figure 1. Section through a portion of abomasums (fundic region) of treated lamb with *S. striata* after multiple infections with nematodes L3.

out on the sera of infected, and non-infected lambs sera were diluted in phosphate buffer (pH 7.2) and ultra-filtered to remove the ions and other low molecular weight component. Total protein contents of each ultra filtered sample were measured by Bradford reagent. 12% gels with the thickness of 1.0 mm were prepared for the separation of protein fractions. Serum samples were diluted finally after preparation with buffer and loading dye. A sample of 6 μ l was loaded onto the gel, lyophilized mixture of seven proteins as markers were reconstituted in buffer and loading dye to a concentration of 1 μ g/ml and loaded onto the gel. Gel was subjected for electrophoresis at a current supply of 12 mA and voltage of 150 in a cooling chamber maintained at 4°C. The gel was stained with coomassie blue R250 and bands were distinguished in fixative solution as required. Stained gels were photographed and its image was saved on a floppy disk with image store 5000 gel documentation system (UVP, U.K). The quantification of separated protein fractions was carried out by UVP gel based software program that provided the data of molecular weights and area covered by each fraction. The data was employed for finding the variations and the presence of different protein fraction for comparison. For ALP measurement, the method of Andersch and Scyzpinski (1947) was used. ALT and AST were determined according to the method of Jepsson et al. (1993). The data for enzyme and total protein were subjected to least squares analysis by applying model I (Laemmli, 1970).

RESULTS

Histological finding

Histological observations of abomasums revealed marked derangement of the mucosal tissues in infected animals. In infected animals, there were more gastric pits in lamina propria and tissue was highly vascularized as compared to the treated group of animal with the plant

extraction of *S. Striata* (Figures 1 and 2).

Duodenal epithelium in the control group of lambs was columnar with layer of muscular mucosa. Brunner's glands were also evident. Villi were thick and short and crypts of Lieberkuhn were seen at the base of villi (Figure 3). In the case of the infected animals, flattening and broadening of villous surface was evident along with exhausted secretory units of Brunner's glands (Figure 4). Jejunum of lambs had distinct separate villi; goblet cells were seen, whereas there were no Brunner's glands seen. In the case of infected tissue, derangement of jejuna mucosa was evident. Cellular infiltration was seen at mucosal surface and it was observed that in some cases, epithelial cells near border undergone autolysis and looked tattered (Figures 5 and 6) compared to the proximal regions of small intestine. The villi of distal ileum had Peyer's patches and more goblet cells in uninfected tissues. In the case of the infected lambs, the villi had more goblet cells and mucosal epithelium was rough, crypts were deep and mitotic figures were seen, suggesting the increased cell division (Figures 7 and 8). Series of slides from infected caecal tissue of lambs showed distorted, elongated villi in vascularized tissue where lots of goblet cells were found when compared with treated group of animal with plant extraction of *S. Striata*. Bands of extended muscles were distorted in infected tissue.

Fecal founding

After a week post infection, animals showed parasitic

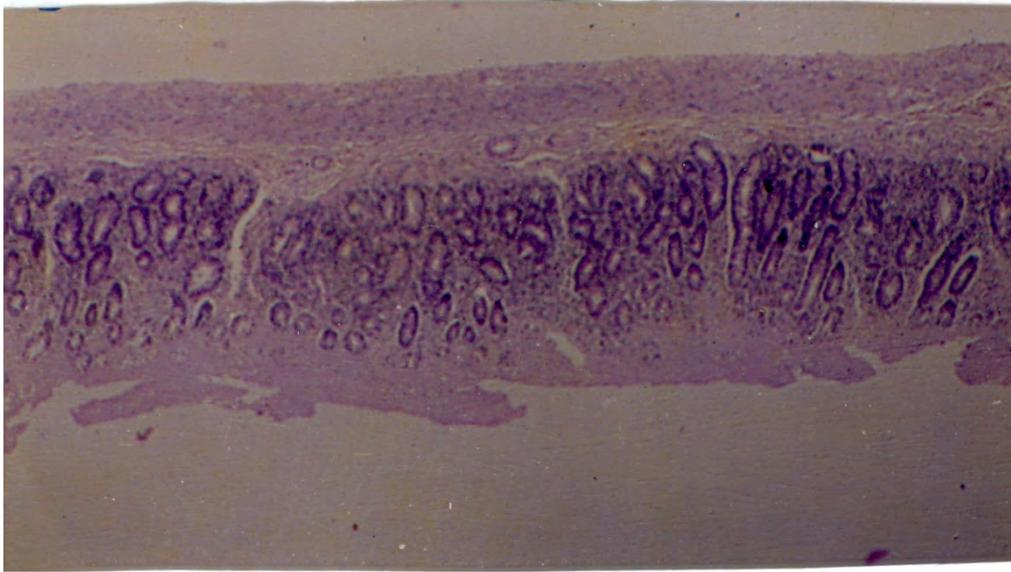


Figure 2. Section through a portion of abomasums (fundic region) of orally infected lamb with multiple nematode L3.

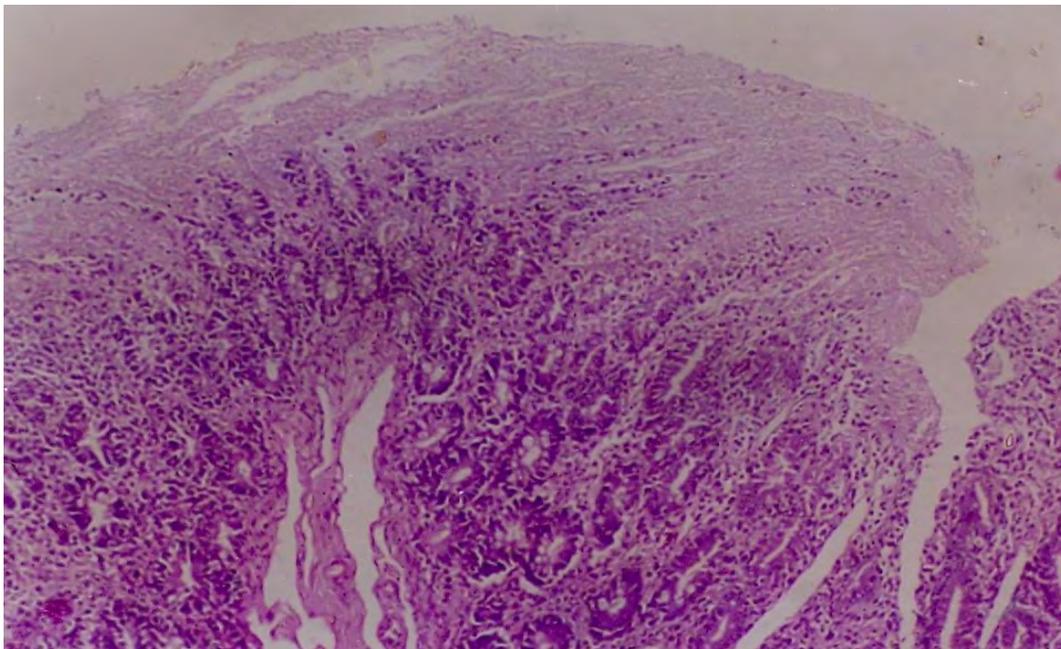


Figure 3. Section of the portion of duodenum of infected lamb with multiple nematodes L3 after treated with *S. striata*.

eggs in their feces. Mean egg count number per gram (EPG) on the 1st week was 1982 ± 145 and on 8th week was 9922 ± 653 . EPG started to decline to 3201 ± 432 on weeks 9 and then to 0 on weeks 11 after deworming the lamb with plant extraction (Figure 9). The average level of total plasma protein in the control group was 7.54 ± 0.67 g/dl and in the infected group, it was 6.04 ± 0.21 g/dl; therefore the level of total plasma protein was 12.50%

lower in the infected group. Hypoproteinaemia was statistically significant. Circulated level of total free amino acid was 0.93 ± 0.216 mg/dl in the control lambs and 0.46 ± 0.17 in the infected group. Infection caused a marked and significant reduction of 3.91% in free amino acid concentration. The alkaline phosphates' activity in the infected group was also significantly higher than the control group. This range was 26.41 ± 7.31 in infected

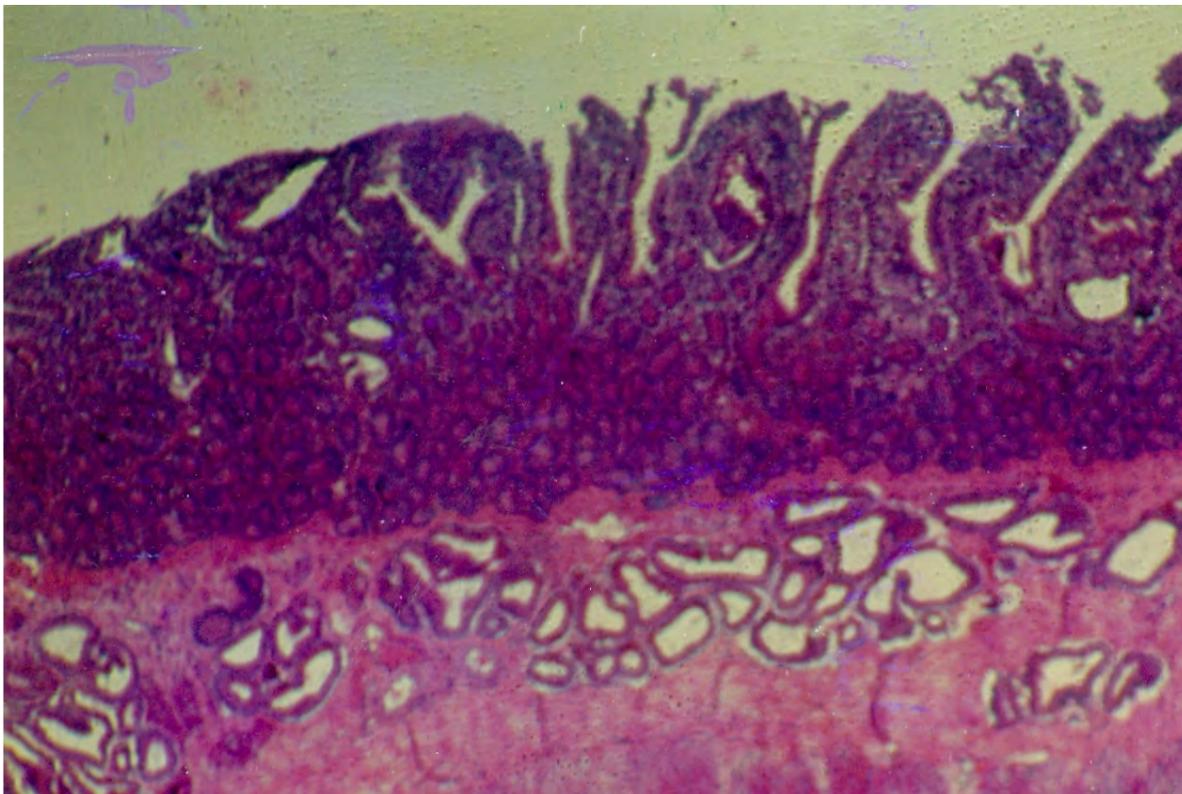


Figure 4. Portion of the duodenum of infected lamb with multiple nematode infections showing flattened mucosal surface.

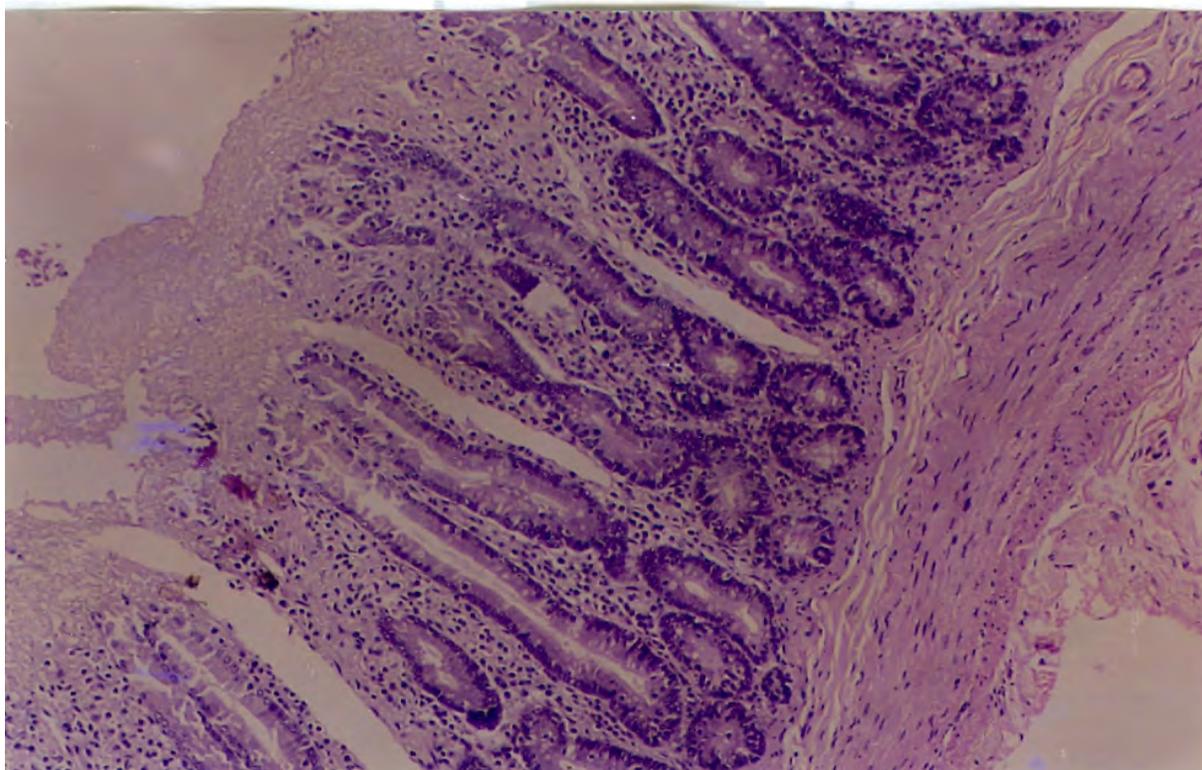


Figure 5. Section through the portion of Jejunum of treated lamb with *S. striata*

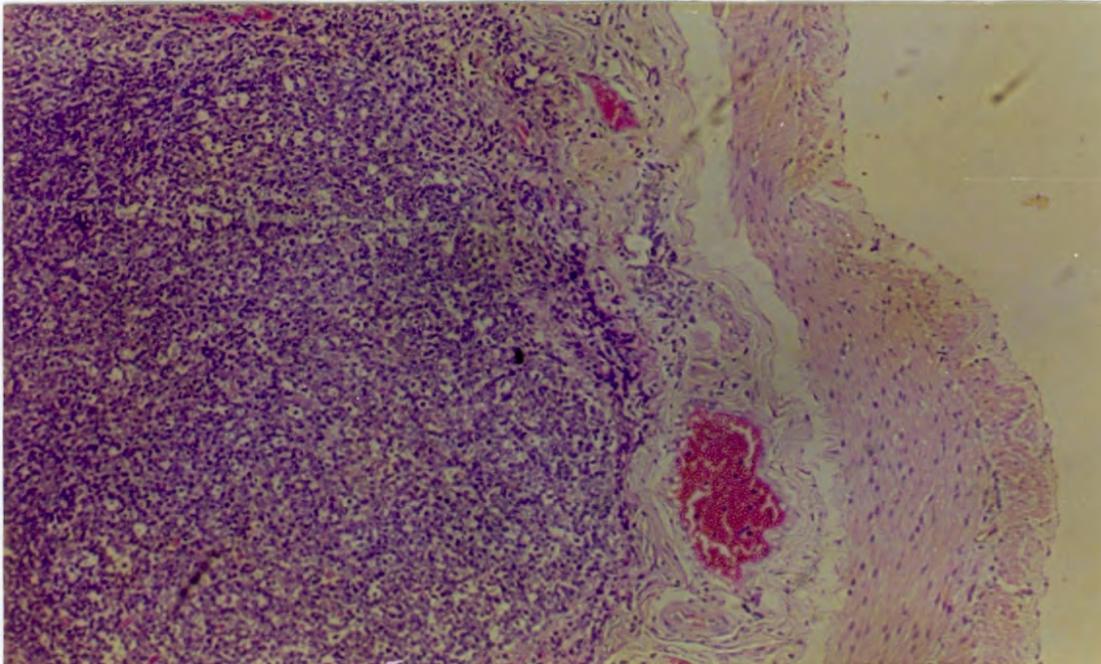


Figure 6. Section of the portion of Jejunum of infected lamb with multiple nematode infections showing cellular infiltration at the mucosal surface.

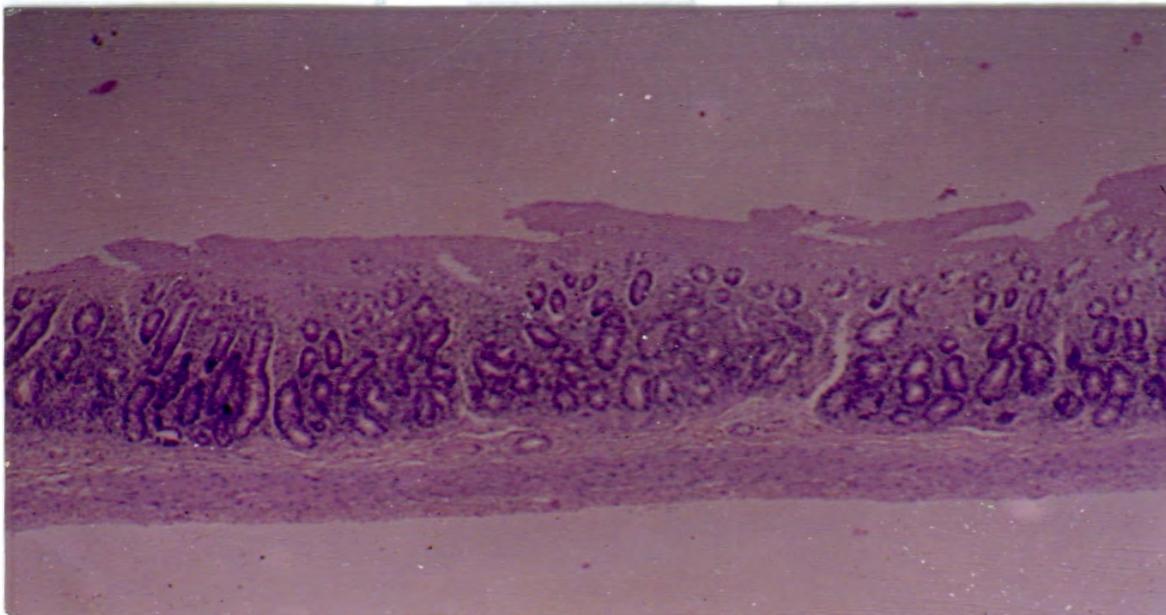


Figure 7. Section through the portion of distal ileum of infected lamb after infection with multiple nematodes L3 and then treated with *S. striata*.

goats and 17.86 ± 7.34 U/L in the control group. The alkaline phosphatase activity was 6.53 ± 0.76 U/L in the infected group and 3.67 ± 0.86 U/L in the control group. Alkaline phosphatase activity in the infected group was significantly high.

Plasma albumin level in the control and infected group was 4.56 ± 0.23 and 3.64 ± 0.83 dl, respectively. Total plasma globulin concentration was 4.12 ± 0.57 in the control group and 3.21 ± 0.43 g/dl in the infected group. Decrease of 7.58% in total plasma globulin in the infected

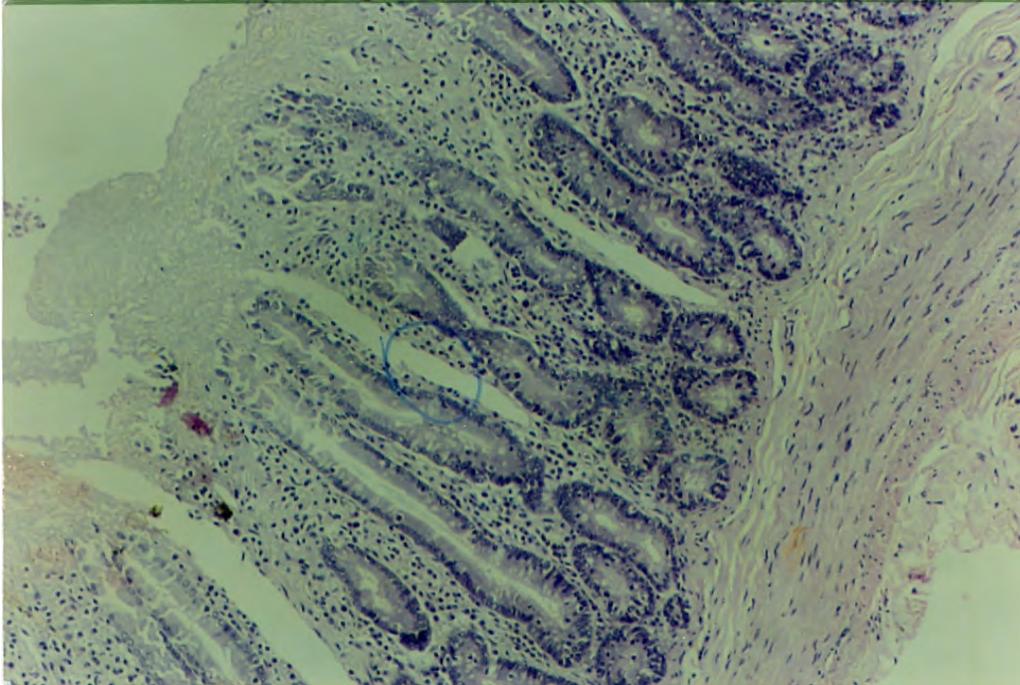


Figure 8. Section of portion of distal ileum of infected lamb after infected with multiple Nematodes.

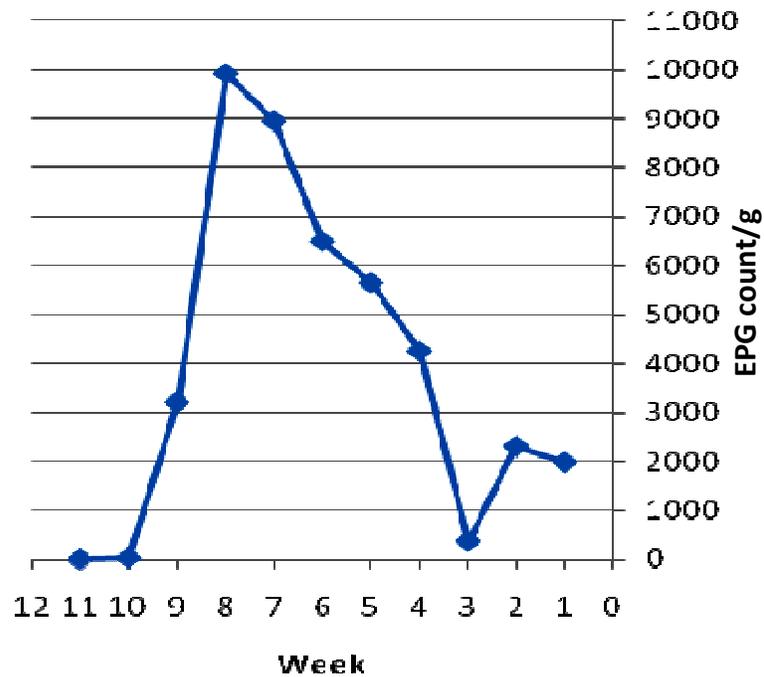


Figure 9. Average fecal egg per germ (EPG) count of multiple nematodes L3 in lambs per week.

group was observed. Circulating level of gamma globulin was 2.65 ± 0.23 in the infected group and 1.53 ± 0.20 g/dl in the control group, which showed 9.33% decrease. More also, non gamma globulin in infected group was

0.56 ± 0.09 and value for the control group was 1.87 ± 0.65 g/dl. The ratio of non gamma globulin to total globulin was 20 and 24.83% for infected and control group, respectively.

In addition, 29 protein fractions were identified on SDS-PAGE in the sera of both groups and ranged 14 to 134 KDa. 21 fractions were expressed in the control group but only 19 fractions were expressed in the treatment group. A few fractions were identified only in one goat in each group. Those fractions that appeared in both groups had different concentration. The fractions of 61, 53, 45, 42, 38, 26 and 22 KDa were identified in the infected goats, while fractions of 59, 52, 47, 41, 36, 27, 25 and 21 KDa were identified in the control group. Fraction of significant measurement with the size of 38 and 26 KDa and of noticeable amounts of 53, 45, and 21 KDa were identified in the treatment group. The fractions of larger size (61, 53, 45 and 42 KDa) also appeared in the treatment group. Among the smaller fractions, 53 and 26 KDa were increased in infected animals. It is noteworthy that the fractions peculiar to the infected goats were of larger size in a series when compared with the fractions of none infected goats.

Effect of plant extract

Average body weight of experimental lambs at the start was 16.175 kg and started to decline after the 4th day post infection with multiple nematodes L3. This significant decrease of body weight was 12.765 at the end of the 8th week post infection (Table 1). Body weight level of animal started to increase in the 9th week after using the extraction of *Fumariaceae* plant as an antihelminthic. Later, this increased on 13th week and showed significant increase in body weight of experimental animal (Table 2). Three days after given daily oral dose of *Fumariaceae* plant extraction, experimental infected animal excreted larvae and eggs of nematodes, which were seen in faecal examination. Faecal examination 10 days later however, showed that faeces were free of any larvae or egg of the parasites.

DISCUSSION

Marked differences in histology of the various tissues of gastrointestinal tract of infected lambs with the multiple oral doses of nematodes L3 were observed. An evidence increase in crypt length and mitotic rate of epithelial cells in small intestine was observed. Villi were broader and appeared to be flattened in proximal as well as in distal regions of the small intestine changes in the structure of small intestine of the host with nematode infection; have been reported by many workers. Short and fused villi, longer crypt of Lieberkuhn, increased crypt cells proliferation and villous atrophy are common. Nematodirus battus in lamb caused changes in duodenum (Martin and Lee, 1980). Heavy infection with *T. colubriformis* in rabbit caused lesions of villi and marked hyperplasia, whereas changes were less marked with small number of worms

and suggested that pathogenicity of the intestine with nematodes infections could be dose-dependent (Hoste and Mallet, 1990). *Strongyloides papillosus* infection in goats resulted in defused ulceration of intestinal mucosa (Bahia et al., 1994).

The reduction of hematological parameter was seen in the experimental infection of nematodes, anemia and changes in plasma protein are common clinical symptoms of gastrointestinal parasitism (Harvey, 1975). In this study, plasma protein level and the concentration of amino acid was studied and the results agree with the explanations given earlier. Plasma protein loss was also associated with *T. colubriformis* infection in sheep and goats (Yacob and Basazinev, 2008) and *Trichinella spiralis* infection in mice (Barker, 1973). Symons (1982) found that in sheep infected with *T. colubriformis*, synthesis of skeletal muscle protein was reduced, while that of liver protein increased. Various studies in relation to gastrointestinal infection have been performed and have provided evidence in support of the result of this study and occurrence of hypoproteinaemia as a result of infection. Parkins et al. (1973) found that ovine ostertagiasis resulted in change in nitrogen balance and digestibility. Abomasal damage caused by daily feeding of *Ostertagia circumcincta* larvae was been reported by Sykes and Coop (1976) and Sykes and Coop (1977). Gastrointestinal nematodes cause such changes, which may seriously altered the amount of amino acid and ammonia absorbed by the parasitized ruminant (Steel, 1974). Lower nitrogen balance and lower contents of protein was observed as a result of gastrointestinal parasites in sheep (Sykes and Coop, 1976; Sykes and Coop, 1977) and this could be due to the greater losses of fecal nitrogen or urinary nitrogen or both. It is proposed that a diversion of amino nitrogen from productive synthesis in *T. colubriformis* infected sheep and guinea-pigs after single as well as multiple infections could occur (Symons, 1982). It was further suggested that although gastrointestinal nematode infection reduces the availability of nitrogen and energy, it is not the sole factor.

Increased activity of ALP in this study is in agreement with Sharma et al. (2001). A significant increase of ALP have been reported and has been supported by research in *H. contortus* infection in goats, which reported significant rise in serum ALP in goats infected by haemonchosis in natural condition (Siddiqua et al., 1990). In contrast to our results that *O. ostertagi* infection increased the level of ALP, Siddiqua et al. (1990) reported a decline of acid phosphatase in sheep and goats infected with haemonchosis. The higher alkaline phosphatase in the middle of the first week may be due to damage to gastric mucosa by developing larvae. Elevated ALP level also indicated haemolysis, though not known to occur in haemonchosis (Siddiqua et al., 1990). The results obtained in this study regarding plant extraction shows that they can act as dewormer. Also, effects on body weight gain of animal are same to the results

Table1. Average body weights of animal in the experimental animal infected orally with multiple nematode L3 and control group from start up to end of the 8th week.

Week	Group II (experiment) average body weight/kg	Group I (control) average body weight/kg
0	16/100	16/250
1	15/902	16/610
2	15/423	16/965
3	15/00	17/325
4	14/540	17/723
5	14/115	18/105
6	13/685	18/502
7	13/185	18/900
8	12/765	19/280

Table2. Average body weigh of animal in experimental and control group of lambs after de worming with plant extraction of *Fumariaceae* from 9th to 13th week.

Week	Group II (experiment) average body weight/kg	Group I (control) average body weight/kg
9	13/103	19/655
10	13/520	20/044
11	13/980	20/435
12	14/365	20/825
13	14/710	21/215

reported by other researchers that worked on homeopathic medicine (Zacharias et al., 2008). Body weight gain is an important parameter for evaluating the body condition of the animals when infected by helminthes (Zacharias et al., 2008). Economic losses are related to productivity indexes, in particular to decrease in body weight that can range from 20 to 60% (Zacharias et al., 2008; Sykes and Coop, 1997; Kawano et al., 2001). Our results shows that antihelmintic treated goats gained body weight compared to the control groups of the animals; the results would have a considerable impact in ovine flocks bred on a commercial scale.

Conclusion

Fumariaceae plant extract showed favorable results in terms of antihelmintic in goats. Additional studies with more animals are therefore required in order to confirm the results. Infection with *H. contortus* in Kurdish goats resulted in changes in hematological factors. Hypo-proteinaemia, decrease in serum amino acid and increase in enzymatic activity in infected goats could be helpful in a better understanding of pathogenesis of anemia, especially in the absence of other possible factors that may influence these changes. Our results could pave the way for studying the effects of parasites on their host.

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