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## A Study of Plant Extraction Effect on Experimental Infected Goats with *Haemonchus contortus*

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Nematodes are important cause of reducing the body weight and producing the disease, anemia in young or debilitated animals. The aim of this study is to perform experimental research to obtain the effects of plant extraction of fumatiaceae on control of *Haemonchus contortus* experimentally infected goats, and its effects on weight gain and hematological parameters changes due to this parasitic infection. In Ilam province Iran where this experiment had been conducted the farmers traditionally claims that this plant could be use as a anthelmintic medicine. Twenty four draft goats, 10-12 months of age and with the average weight of 15.550 kg divided in two groups (group 1 control and the group 2 as a experimental animal infected with *H. contortus* (5000 L3) orally) and after 10 weeks of parasitic infection the experimental group of animals were de wormed with 6 mL kg<sup>-1</sup> body weight fumatiaceae plant extraction. Plasma of blood sample was separated for determination of total protein, plasma total free amino acid and alkaline phosphates. At the results significant decrease in plasma total free amino acid, total plasma protein and significant increase in alkaline phosphates and acid phosphates were seen in infected group. Significant increases of body weight were observed in infected goats after 10 weeks de worming the animal with experimental fumatiaceae plant extraction. It can be concluded that fumatiaceae plant extraction could be use as a dewormer and need further investigation.

**Key words:** *Haemonchus contortus*, plant extraction, plasma protein, goat, hematological parameters, hematology

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## INTRODUCTION

Nature has served as a rich repository of medicinal plant 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 Kiderlen, 2001).

Iran is well-known for the exuberance and the variety of its mountain 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 disease 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 bovine breeding in all over the world. Parasites are harmful for 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 and also to an increased plasma protein loss 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 *N. dubius* showed increased whole body protein turnover (Symons and Jones, 1972). Changes in the rate of protein synthesis in sheep after *T. 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 to be due to the plasma protein loss into the intestine as a result of increased mucosal permeability caused by parasites. Change in the alkaline phosphates (ALP), glutamic oxaloacetic transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) levels in grazing Saanen goats from New Zealand 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 goats in India and New Zealand has been reported (McDougall *et al.*, 1991; Sharma *et al.*, 1990). The main objective of this study was to evaluate the effectiveness of fumatiaceae plant extraction as an antihelminthic medicine with the characteristic of low cost, absent of residues in meat and milk and its low environmental impact through the following parameters: faecal parasitological examination, hematological and serum biochemical analyses following by weight gain or loss in goats.

## MATERIALS AND METHODS

**Animals and experimental design:** Twenty four male and female healthy Kurdish breed goats 10-12 months of age, with the average weight of 15.550 kg, were collected randomly from Ilam province of Iran, situated in the western part of the national capital of Iran (Fig. 1) in 2009-2010 animal were divided in two groups (Each group contains 12 animals). All animals were dewormed with *Albendazole* (10 mg kg<sup>-1</sup>) and *Thiabendazole* 0.6%. The absence of parasites was conformed by examining the faecal samples from all animals by flotation, sedimentation and faecal culture techniques (McDougall *et al.*, 1991). The goats also were examined for blood protozoan infection by blood smear and inoculation in mice. All animals were free from any such infections. Experimental animal were managed and house in the hygienically environment and care was taken to avoid any contamination from outside. On day 0 the treatment group was received 5000 L3 (*H. contortus*) orally. Larvas were procured through faecal culture from female worm in the abomasums and duodenum of naturally infected laboratory raised goats (McDougall *et al.*, 1991). The viability motility of *H. contortus* L3 tested by McMaster slide. Based on the proportion of the active larvae the infective doses were prepared. The animals were weighed, in both groups and monitored twice a week and checked for the presence of parasitic eggs through faecal examination. Animals received food and water ad libitum. In both groups blood (10 mL) samples were collected from jugular vein after 9 weeks of infection and plasma was obtained. Ten micro milliliter of 1% sodium azide solution were added to plasma sample and kept in refrigerator for further procedure.

**Plant extracts preparation:** The plant material fumatiaceae used in this study were collected from Zakros mountain area southwest of Iran and identified by Herbarium of Institute of Medicinal plants-ACECR Tehran, Iran. The whole part of the plant dried, grinded to powder, then 200 g powder separated, added with 500 mL of ethanol (96%) in sterile conical flasks and kept at +45°C in oven overnight and the residue were obtain. The residue was diluted with de ionize distilled water and 6 mL kg<sup>-1</sup> body weight dilution were orally daily for one weeks given to experimental infected goats.

**Treatment of experimental animal with the plant extraction:** After 10 weeks of infection with *H. contortus* L3 the animals (group 2) were given orally daily 6 mL/body weight of fumatiaceae plant extraction, for one week, and than throughout this study period faecal samples from all goats of this and

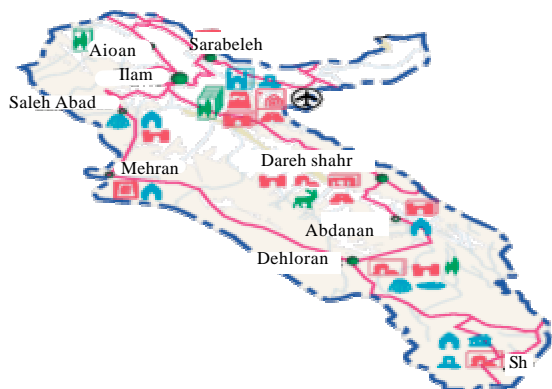


Fig. 1: Map of study setting in bordering regions between Iran and Iraq

group were also collected and examined for eggs larvae excretion.

**Biochemical and hematological parameters:** Total plasma proteins were measured by Biuret method. Copper reagent was prepared and method was followed as described by Doumas and Biggs (1972) Bovine serum albumin (Sigma Chemical Company, USA) was constituted in buffer of pH 7.00 to prepare standards. The most commonly used standards were 5 and 10 g dL<sup>-1</sup>. 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 method of Goodwin (1968). This procedure quantifies nitrogen of free amino acids. A standard of 20 amino acid was used; therefore the concentration of free amino acid in 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 Doumas and Biggs (1972). Ovine serum albumin was used for the preparation of standards. Plasma Total Globulins in plasma were determined by subtraction the concentration of albumin by 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). 2.4 mL of the ammonium sulphate sodium chloride reagent was measured in a glass test tube. One hundred microliter of plasma was layered on the top and mixed for 30 sec on an electric vibrating mixer until the turbidity reached a maximum. It was centrifuged for half an hour at 3000 rpm. If supernatant was not clear, it was allowed to cool in running cold water and was centrifuged again. For

accuracy of results, the supernatant should be clear. The supernatant was carefully poured off without disturbing the precipitates. The tubes were centrifuged again for 5 min and carefully inverted and left on a filter paper to drain supernatant as completely as possible. To the precipitates, 2.5 mL of biuret and 1.0 mL of distilled water. These were shaken vigorously and were placed in a water bath at 37°C for 10 min. Optical density was read at 450 nm using biuret reagent as blank. Ovine serum albumin was used for standard preparations. Sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) based on the method of ADEWOYE and FAWIBE (1978) was carried out on the sera of infected and non-infected goats. 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. Twelve percent 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 µL was loaded onto the gel. Lyophilized mixture of 7 proteins (Sigma chemical company, USA) as markers were reconstituted in buffer and loading dye to a concentration of 1 µg mL<sup>-1</sup> 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 acid phosphatase measurement, method of Andersch and Scyzpinski (1947) was used. The data for enzyme and total protein were subjected to least squares analysis by applying model I (Laemmli, 1970).

## RESULTS

**Hematological founding:** After 9 days post infection, animals showed parasitic eggs in their feces. Mean egg number per gram (EPG) on day 14 was 701±74 and on day 42 was 90050±582. EPG started to decline to 7764±461 on day 49 and then 3641±369 on day 77 (Fig. 2).

The average level of total plasma protein in control group was 7.99± 0.70 g dL<sup>-1</sup> and in treatment group was 6.57±0.34 g dL<sup>-1</sup>; therefore the level of total plasma protein was 15% lower in treatment group.

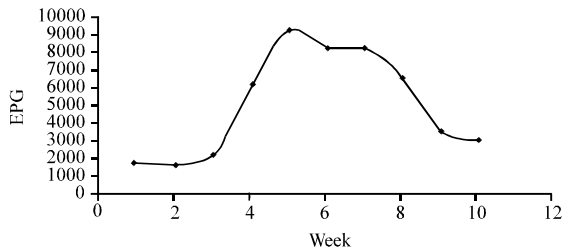


Fig. 2: Average faecal Egg Per Germ (EPG) count of *Haemancus contortus* in goat per week

Hypoproteinaemia was statistically significant. Circulating level of total free amino acid was  $0.69 \pm 0.16 \text{ mg dL}^{-1}$  in infected goats and  $0.98 \pm 0.27 \text{ mg dL}^{-1}$  in control group. Infection caused a marked and significant reduction of 21% in free amino acid concentration. The alkaline phosphates' activity in infected group was significantly higher than control group. This range was  $24.36 \pm 6.12$  in infected goats and  $18.89 \pm 7.99 \mu\text{L}^{-1}$  in control group.

The average acid phosphates' activity was  $2.18 \pm 0.023$  to  $6.71 \pm 0.59 \mu\text{L}^{-1}$  in treatment group and  $2.48 \pm 0.99$  to  $4.27 \pm 0.41 \mu\text{L}^{-1}$  in control group. Acid phosphates' activity in treatment group was significant high (Table 1).

Plasma albumin level in control and treatment group was  $4.00 \pm 0.18 \text{ g dL}^{-1}$  and  $3.91 \pm 0.1 \text{ g dL}^{-1}$  respectively. Total plasma globulin concentration was  $3.44 \pm 0.41 \text{ g dL}^{-1}$  in control group and  $3.12 \pm 0.33 \text{ g dL}^{-1}$  in treatment group. Decrease of 10% in total plasma globulin in treatment group was observed. Circulating level of gamma globulin was  $2.03 \pm 0.18 \text{ g dL}^{-1}$  in treatment group and  $1.61 \pm 0.19 \text{ g dL}^{-1}$  in control group which showed 24% decrease.

Non gamma globulin in treatment group showed a 25% decrease (compared with control group) and was  $1.67 \pm 0.010 \text{ g dL}^{-1}$ . This value for infected group was  $1.25 \pm 0.44 \text{ g dL}^{-1}$ . The ratio of non gamma globulin to total globulin was 37.65 and 45.87% for treatment and control group, respectively.

Twenty-nine protein fractions were identified on SDS-PAGE in the sera of both groups and ranged 14 to 134 kDa. Twenty one fractions expressed in control group and only 19 fractions in treatment group.

A few fractions were identified only in one goat in each group. Those fractions that appeared in both groups have different concentration.

The fractions of 61, 53, 45, 42, 38, 26 and 22 kDa were identified in infected goats. Fractions of 59, 52, 47, 41, 36, 27, 25 and 21 kDa were identified in control group. Fraction of significant measurement with the size of 38 and 26 kDa and of noticeable amounts of 53, 45 and 21 kDa identified in treatment group.

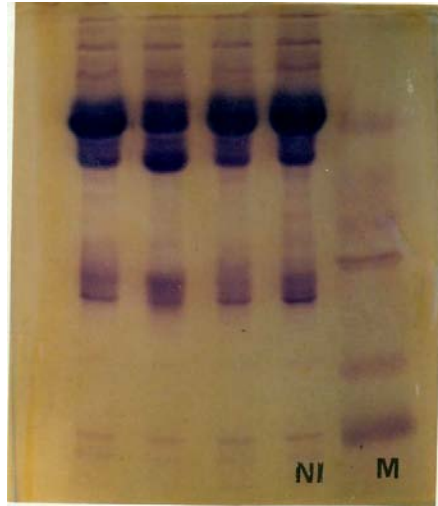


Fig. 3: Photograph and electrophoretically separated serum protein fraction in noninfected and infected male goat. (NI: Non-infected, 1: Infected, M: Markers)

Table 1: Average level of acid phosphates' in infected and none infected group of animal

Week	Group A	Group B
I	2.31±0.88	3.71±0.51
II	2.92±0.60	4.34±0.72
III	4.26±0.67	6.91±0.55
IV	4.00±0.17	5.32±0.54
V	3.70±0.39	5.45±0.61
VI	2.90±0.51	5.13±0.52
VII	3.29±0.65	3.32±0.84
VIII	3.74±0.90	3.29±0.80

The fractions of larger size (61, 53, 45 and 42 kDa) were appeared in treatment group. Among the smaller fractions, 53 and 26 kDa were increased in infected animals. It is noteworthy that the fractions peculiar to infected are of larger size in a series when compared with the fractions of none infected goats (Fig. 3).

**Founding the plant extraction on parasites:** Averages body weight of experimental goats at the start was 15.350 kg and decline after 4 th day's infection with *H. contotus* L3, this significant decrease of body weight were 11.622 at the end of the 10th week post infection (Table 2). Body weight level of animal started too increased in day 11th after using the extraction of fumatiaceae plant as de wormer, but this increase was not significant up to day 17 post de worming, later on this increase on weeks 20 shows significantly increased in body weight of experimental animal (Table 3). Three days after given oral dose of plant extraction to experimental infected animal excreted larvae and eggs of

Table 2: Average body weights of animal in control and experimental group per week up to weeks 10th

Week	Average body weight (kg)	
	Group I	Group II
0	15/750	15/350
1	16/000	15/105
2	16/345	14/725
3	16/895	14/359
4	17/301	14/911
5	17/711	13/425
6	18/105	13/010
7	18/458	12/735
8	18/906	12/345
9	19/375	12/000
10	19/735	11/622

Table 3: Average body weight of animal in control and experimental group after de worming with extraction of *Matricaria chamomilla* L. plant from weeks 10th to twenty

Week	Average body weight (kg)	
	Group I	Group II
11	20/011	11/135
12	20/358	11/182
13	20/712	11/512
14	21/012	11/923
15	21/401	12/313
16	21/759	12/748
17	22/085	13/101
18	22/531	13/459
19	22/837	13/810
20	23/159	14/201

*H. contortus* were seen in faecal examination and follow 13 days later faecal examination were free of any larvae or egg of parasites.

### DISCUSSION

The reduction of hematological parameter was seen in experimental infection of nematodes, anemia and changes in plasma protein are common clinical symptoms of gastrointestinal parasitism (Harvey, 1975). In present study plasma protein level and the concentration of amino acid was studied and results was agreed with the explanations given earlier. Plasma protein loss was also associated with *T. colubriformis* infection in sheep and goats (Yacob and Basazinew, 2008) and *T. 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 the present 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. Abomasol damage caused by daily feeding of *O. circumcincta* larvae has been reported by Sykes

and Coop (1976, 1977). Gastrointestinal nematodes cause such changes which may seriously alter 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, 1977) and this could be due to the greater losses of faecal nitrogen or urinary nitrogen or both. There 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 will happen (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 alkaline phosphatase (ALP) in this study is in agreement with Sharma (Sharma *et al.*, 2001). Significant increases of ALP have been reported by this worker has been supported by other that in *H. contortus* infection in goats and reported significant rise in serum alkaline phosphatase 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 acid phosphatase (Siddiqua *et al.*, 1990) reported a decline of acid phosphatase in sheep and goats infected with Haemonchosis. The higher acid phosphatase in the middle of first week may be due to damage to gastric mucosa by developing larvae. Elevated acid phosphatase level also indicated haemolysis, though not known to occur in haemonchosis (Siddiqua *et al.*, 1990). The results obtained in this study regarding plant extraction can act as de wormer and effect on body weight gain of animal are resemble to the results reported by other researchers that they work 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, 1977; Kawano *et al.*, 2001). Present results showed 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.

*Matricaria chamomilla* L. plant extraction showed favorable results in terms of antihelmintic in goats, Additional studies with more animals are required in order to confirm the results.

Infection with *H. contortus* in kordish goats results in changes in hematological factors. hypoproteinaemia, decreased in serum amino acid and increased in enzymatic activity in infected goats could be helpful in better understanding of pathogenesis of anemia especially in the absence of other possible factors which may influence these changes. Present results could pave the way for studying the effects of parasites on their host.

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