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Effect of *Matricaria chamomilla* L. Plant Extraction on Experimental Infected Lamb with *Ostertagia ostertagi* Parasits

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Abstract: This study was conducted in Ilam province Iran, to evaluate the effects of homeopathic treatment on control of *Ostertagia ostertagi* experimental infection in lamb and weight gain after treatment with the ethanol extraction of *Matricaria chamomilla* L. plant and haematological changes due to this parasitic infection. Herdsman do believed that feeding of *Matricaria chamomilla* L. plant in limited amounts could help to keep lamb more healthy and can act as an antihelminthic. The plasma protein (by SDS-PAGE) and enzyme levels were studied in Kordish breed lamb infected with (10000L3) *O. ostertagi* orally. Twenty four lambs age between 8-12 months, with the average weight 13.650 kg were divided in two groups 1 as control and the group 2 as an experimental animal infected with *O. ostertagi* and after 10 weeks of parasitic infection the experimental groups were de wormed with 5 mL kg⁻¹ b.wt. *Matricaria chamomilla* L. plant extraction. Plasma of blood sample was separated for determination of total protein, plasma total free amino and alkaline phosphates. At the results significant decrease in plasma total free amino acid and total plasma protein and significant increase in alkaline phosphates and acid phosphates were seen in infected group. Significant increase were observed in infected lamb after 10 weeks de worming the animal with experiment plant extraction. It can be concluded that *M. chamomilla* can be use as a de wormer, or can say that it is effective for increasing the body weight of animals, its need further investigation.

Key words: Alkaline phosphates, *Ostertagia ostertagia*, nematode, sheep, protein, hematology

INTRODUCTION

Plant extraction or plant-derived compounds are likely to provide a valuable source of new medicinal agents (De Carvalho and Ferreira, 2001; Kayser and Kiderien, 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 *O. ostertagi* represents the main cause of economic loss in ovine 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 Mc Dougall *et al.* (1991). Enzymatic assays in sheep and goats infected with *Haemonchus contortus* and intestinal parasitic infections have 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 *Matricaria chamomilla* L. plant extraction on the control of *O. ostertagi* in lamb through the following parameters: fecal parasitological examination, haematological and serum biochemical analyses following by weight gain or loss after deworming the animals.

MATERIALS AND METHODS

Animals and experimental design: In this experiment, 24 healthy Kurdish breed lambs with age of 8-12 months and with the average weight of 13.650 kg, were collected randomly from Ilam Province of Iran, in 2009-2010, situated in the Western part of the national capital of Iran (Fig. 1).

Samples were divided in two groups (each group contains 12 animals). All animals were dewormed with albendazole (10 mg kg⁻¹) and Malathion 0.6%. The absence of parasites was conformed by examining the fecal samples from all animals by flotation, sedimentation and fecal culture techniques (McDougall *et al.*, 1991). The sheep 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 a hygienic environment and care was taken to avoid any contamination from outside. On day 0 the treatment group was received 10000L3 (*O. ostertagi*) orally. Larvae were procured through fecal culture from female worms in the abomasums of naturally infected laboratory raised sheep (McDougall *et al.*, 1991). The viability motility of *O. ostertagi* 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 fecal examination. Animals received food and water *ad libitum*. In both groups blood (8 mL) samples were collected from jugular vein after 8 weeks of infection and plasma was obtained. Ten milliliters of 1% sodium azide solution per each mL of plasma was added to plasma samples.

Plant extracts preparation: The plant material *Matricaria chamomilla* L. 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 100 g powder separated, added with 250 mL of ethanol (96%) in sterile conical flasks and kept at +45°C in oven overnight and the residue was obtained. The residue was diluted with deionized distilled water and 5 mL kg⁻¹ b.wt. dilution were orally daily for one week given to experimental infected lambs.

Treatment of experimental animal with the plant extraction: After 10 weeks of infection with *O. ostertagi* the animals (group 2) were given orally daily 5 mL/b.wt. of *Matricaria chamomilla* L. plant extraction, for 1 week and throughout this study period fecal samples from all sheep of this group were also collected and examined for eggs and larvae excretion.



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

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⁻¹. SDS-PAGE was done for detecting the plasma proteins.

Quantitative assay of Alkaline phosphatase were done using the method of Cheema and Scofield (1985). 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). Bovine serum albumin was used for the preparation of standards. Plasma Total Globulins 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. Bovine serum albumin was used for standard preparations. Sodium dodecyl sulphat polyacrylamide electrophoresis (SDS-PAGE) based on the method of Maff (1986), Wolfson *et al.* (1948) was carried out on the sera of infected and non-infected lamb. 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 ultrafiltered samples 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⁻¹ 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 coomasie 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, UK). 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 (Cheema and Scofield, 1985) was used. The data for enzyme and total protein were subjected to least squares analysis by applying model I (Laemmli, 1970; Harvey, 1975).

RESULTS

Hematological founding: After 2 weeks of infection, animals showed parasitic eggs in their feces. Mean egg number per gram (EPG) on day 14 was 750±80 and on day 42 was 10000± 628. The EPG started to decline to 8070±511 on day 49 and then 3188± 399 on day 77 (Fig. 2).

Total plasma protein in control group was 7.41±0.17 g dL⁻¹ and in treatment group was 6.32±0.20 g dL⁻¹ therefore, the level of total plasma protein was 15% lower in treatment group. Hypoproteinaemia was statistically significant (Fig. 3).

Circulating level of total free amino acid was 0.58±0.04 mg dL⁻¹ in infected sheep and 0.74±0.07 mg dL⁻¹ in control group. Infection caused a marked and significant reduction of 21% in free amino acid concentration (Fig. 4).

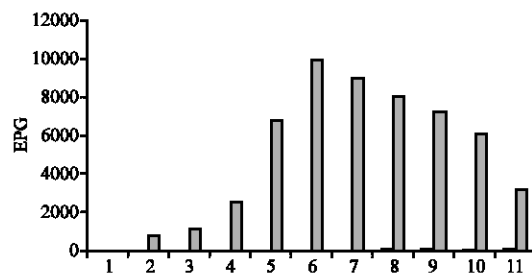


Fig. 2: Average faecal egg per (EPG) count of *O. ostertagi* in kurdisch sheep

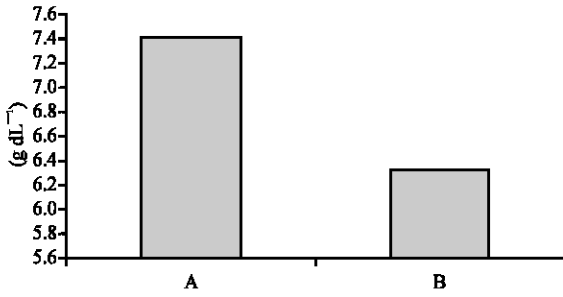


Fig. 3: Average level of total plasma protein (m dL⁻¹) in circulatory blood of infected and non-infected kurdish sheep with *O. ostertagi*

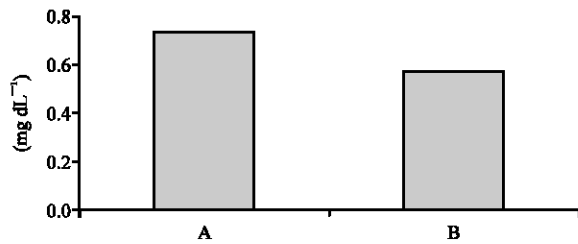


Fig. 4: Average level of total plasma protein (g dL⁻¹) in circulatory blood of infected and non-infected kurdish sheep with *O. ostertagi*

Table 1: Average level of acid phosphatase in infected and non infected group of animal

Week	Groups	
	A	B
IST	2.41±0.98	3.62±0.42
II	3.01±0.69	4.23±0.61
III	4.38±0.79	6.98±0.62
IV	4.11±0.28	5.21±0.43
V	3.82±0.51	5.32±0.48
VI	3.01±0.62	5.02±0.41
VII	3.42±0.78	3.21±0.73
VII	3.83±0.99	2.11±0.20

The alkaline phosphatase activity in infected group was significantly higher than control group. This range was 30.41±8.01 in infected sheep and 17.89±8.11 U L⁻¹ in control group.

The average acid phosphatase activity was 2.11±0.020 to 6.98±0.62 U L⁻¹ in treatment group and 2.41±0.98 to 4.38±0.49 U L⁻¹ in control group. Acid phosphatase activity in treatment group was significant high (Table 1).

Plasma albumin level in control and treatment group was 3.87±0.09 g dL⁻¹ and 3.41±0.26 g dL⁻¹, respectively. Total plasma globulin concentration was 3.35±0.36 g dL⁻¹ in control group and 3.0±0.27 g dL⁻¹ in treatment group. Decrease of 10% in total plasma globulin in treatment group was observed. Circulating level of gamma

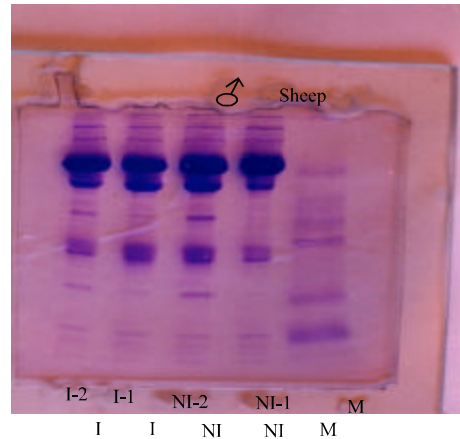


Fig. 5: Photograph and electrophoretically separated serum protein fraction in non-infected and infected male sheep. NI1: Non-infected, 1: Infected, M: Markers

globulin was 2.32±0.19 g dL⁻¹ in treatment group and 1.88±0.21 g dL⁻¹ in control group which showed 24% decrease.

Non gamma globulin in treatment group showed a 54% decrease (compared with control group) and was 0.68±0.095 g dL⁻¹. This value for control group was 1.47±0.17 g dL⁻¹. The ratio of non gamma globulin to total globulin was 19.72 and 43.40% 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 130 KDa. 21 fractions expressed in control group and only 19 fractions in treatment group.

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

The fractions of 130, 116, 106, 98, 69, 52, 36 and 21 KDa were identified in infected sheep. Fractions of 118, 99, 68, 51, 29, 22 and 19 KDa were identified in control group.

Fraction of significant measurement with the size of 69 and 52 KDa and of noticeable amounts of 116, 106 and 36 KDa identified in treatment group.

The fractions of larger size (116, 106, 98 and 69 KDa) were appeared in treatment group. Among the smaller fractions, 52 and 36 KDa were increased in infected animals. Fraction 71 KDa was expressed in both groups but markedly enhanced due to infection (Fig. 5).

Founding the plant extraction on parasites: The average body weight of experimental animals in comparison to

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

Week	Groups	
	I (control) average body weight (kg)	II (experiment) average body weight (kg)
0	13/350	13/650
1	13/500	13/254
2	13/560	13/050
3	14/027	12/853
4	14/487	12/200
5	14/502	12/000
6	15/954	12/000
7	16/454	11/825
8	16/803	11/500
9	17/205	11/155
10	17/597	10/725

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	Groups	
	I (control) average body weight (kg)	II (experiment) average body weight (kg)
11	17/980	11/000
12	18/412	11/381
13	18/805	11/650
14	19/212	12/050
15	19/605	12/410
16	20/056	12/803
17	20/495	13/204
18	20/908	13/632
19	21/339	14/074
20	21/775	14/506

control group were decrease after 3rd weeks post infection and this decrease were significantly on weeks 10th (Table 2).

On weeks 11th of oral dose of plant extraction to experimental groups the body wight of animal start to increase. This increase found to be significant on weeks 20th pot de worming animals (Table 3).

Two days after given oral dose of plant extraction to experimental infected animal excreted larvae and eggs of *O. ostertagi* were seen in fecal examination and follow 20 weeks later fecal examination were free of any larve or egg of parasites.

DISCUSSION

The reduction of hematological parameter was seen in experimental infection of nematodes in Anemia (Harvey, 1975) and changes in plasma protein are common clinical symptoms of gastrointestinal parasitism; in present study plasma protein level and the concentration of amino acid was studied and results was agreed with the explanations given earlier (Gill *et al.*, 2001). Plasma protein loss was also associated with *T. Colubriformis* infection in sheep (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 fecal 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. conturtus* 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 antihelminthic treated lamb gained of body weight compared to the control group lambs. The results would have a considerable impact in lambs flocks bred on a commercial scale.

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

Infection with *O. ostertagi* in kordish sheep results in changes in hematological factors. hypoproteinaemia, decreased in serum amino acid and increased in enzymatic activity in infected sheep 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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