EFFECT OF MIRAZID ON FASCIOLIASIS AND BLOOD CHEMISTRY OF SHEEP

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Abstract

Sheep and cattle can be seriously affected by liver dwelling trematodes *Fasciola hepatica* or *Fasciola gigantica.* The extensive damage and resultant hemorrhage of liver result in the clinical signs of Fasciolasis. Great efforts have been devoted to develop a safe and effective fasciolicidal agent.
Mirazid (Commiphora molmol extract) is a new drug used for treatment of schistosoma as well as fasciola in human.

In the present study, Mirazid was used for the treatment of fascioliasis in sheep as a trial for searching for a new and safe fasciolicidal drug. Two groups of naturally infected sheep with fasciola each of five sheep, diagnosed by detecting fasciola egg in the feacal samples and confirmed serologically by IHA test were treated with Mirazid at a dose of 10 mg/kg and 20 mg/kg orally for 6 consecutive days. Third group of five sheep was not treated as control group. The sheep were followed up for 4 months.

Mirazid proved to be a dose dependent effective for treatment of fascioliasis in sheep if given as oral drench at a dose of 10 mg/kg or 20 mg/kg for 6 successive days to naturally infected sheep, with pronounced improvement of the general condition, body weight and amelioration of all symptoms and signs of fascioliasis in treated sheep, fasciola antibody titres and elevated liver enzymes returned to normal levels. Hematological studies revealed significant increase in red blood cells, hemoglobin levels, and HCT% and significant decrease of the total white blood cells in treated sheep as compared to those values before treatment.

INTRODUCTION

Fascioliasis in sheep and cattle represents the most important infestation in economic terms. The losses caused by fascioliasis resulted in deaths, inefficient

conversion of feed, unthriftiness, reduced wool and milk production, condemnation of the infected liver, predisposition to other diseases as black disease and bacillary hemoglobin urea (Jensen and Swift, 1988). Both immature and mature flukes damage the host liver. The extensive damage and resultant hemorrhage of the liver host result in clinical signs of fascioliasis within 6-8 weeks after infection (Soulsby, 1982).

Outbreaks of the disease can be forecast and the problem can be suppressed with Judicious treatment but for the ordinary farmer, however, the best hopes of controlling fluke had depended on the discovery of drugs effective against all stages of the worm.

Corsulon proved to be effective against naturally and experimentally induced *Fasciola hepatica* infection in cattle (Molone *et al.*, 1984).

Mirazid (Commiphora molmol extract) which is a special formulation of myrrh was proved to be effective drug for treatment of fascioliasis in human (Massoud *et. al.,* 2001).

The present study was designed to investigate the efficacy of a new herbal compound (Mirazid) used as fasciolicidal and schistosomicidal in human and has not been tried in sheep, for treatment of fascioliasis in sheep.

MATERIALS AND METHODS

(1) Animals

The present investigation was carried out on (20) ewes aged 3-5 years old. These animals were selected from different localities at Dakahlia province during the period from October 2002 till may 2003. Five of these animals were apparently healthy and free from any internal and external parasites, while the remaining 15 ewes were clinically suffering from signs of chronic fascioliasis (emaciation, pallor mucosa, shedding of wool, semisolid to watery diarrhoea and developed sub-mandibular edema in most cases). Ten ewes only out of this number were positive to fasciola infestation by means of parasitological and serological examination using IHA Technique. The average weigh of diseased sheep was 52.5 kg.

(2) Drug used

Mirazid capsule, (each gelatinous capsule contains 300mg of purified commiphora molmol extract) kindly supplied from Pharco Pharmaceutical, Alexandria, Egypt.

(3) Experimental design

(a) Five healthy ewes were kept as control for determination of hematological and serum biochemical parameters.

(b) Ten positive fasciola ewes were divided into 2 groups:

The first group: treated with mirazid 10 mg/kg per os for six days.

The second group: treated with mirazid 20 mg/kg per os for six days.

(c) All diseased animals were weighed just before treatment and the hematological and some serum biochemical parameters were determined as well as fasciola antibody titre.

(d) After four months from treatment, the treated animals re-weighted again and the hematological and biochemical parameters re-examined in addition to determination of fasciola antibody titres.

(4) Parasitological examination

Two rectal faecal samples were collected individually from the rectum of each animal in clean plastic bags,the first sample just before treatment, while the second sample after four months post-treatment for detection of fasciola eggs in the faeces as described by Soulsby (1982). The positive cases were subjected to serological examination for determination the titre of fasciola antibodies using I.H.A. technique.

(5) Serological examination for fasciola titre

The serum samples were used for determination of fasciola antibodies titre just before treatment and four months post-treatment for all infested animals by Indirect Haemagglutination test (I.H.A) technique using kits supplied by Laboratories Fumouze–Fumouze Dignostics: (26, rue des Frereis Chauss on 92600 Asnieres, France).

(6) Hematological and biochemical examination

Two blood samples were collected from each animal just before and four months post treatment. The first sample (5ml) placed into tubes containing EDTA for hematological studies according to standard techniques described by Jain (1993). The second sample was collected into a plain centrifuge tube and serum was separated and clear supernatant serum was used for serological and biochemical examination. The values of serum transaminases (AST, ALT), serum total protein, serum albumin, serum bilirubin, blood urea, creatinine and glucose were determined using reagent kits supplied by Boehringer, Mannheim, Germany. Serum globulin was calculated mathematically by substracting the albumin value from total protein value.

(7) Statistical analysis

The obtained data were statistically analysed according to Snedecor and Cochran *(1982)* by using a computer program (costate) one way completely randomized, analysis of variance test "f test" treatment means were then compared by the least significant difference test "LSD" at 0.05 level of probability.

RESULTS AND DISCUSSION

Fecal examination of diseased sheep revealed that fasciola eggs were detected in 10 out of the examined sheep which were naturally infested with fasciola. The detailed morphological characters of the investigated eggs were similar to the principal picture described by Soulsby (1982).

The serological examination of the diseased sheep showed titre of antibodies ranging from (1:16 - 1:64).

On studying the blood picture of diseased sheep, the results shown in Table 1 revealed significant decrease in erythrocytic count, Hb concentration and HCT value indicating anaemia. The ertyhocytic indices (MCV, MCH and MCHC) showed macrocytic hypochromic type of anaemia, which may be attributed to the acute loss of the blood be sucking activity of the flukes and the continuous drain of iron reserves. Similar explanation was mentioned by Holmes *et al.* (1968).

The leucogram of the diseased sheep showed leucocytosis contributed by neutrophilia and eosinophilia with lymphopenia and monocytopenia. The neutrophilia and eosinophilia might be due to inflammation and infection resulting from the activity of adult flukes in the bile ducts, as mentioned by Radostits *et al.,* (2000). Eosinophilia has been linked to antigen antibody interaction which occurred when the sensitivity to the protein of the parasites has developed or when the secretory products of the parasites were released within the blood (Jain, 1993).

Lowering of lymphocyte and monoctye numbers in the infested animals might be caused by increased chemotaxis to the inflammatory process in the bile ducts or by decrease lymphopoiesis (Coles, 1980).

The results of blood serum constituents (Table 2) revealed that the activities of AST and ALT were elevated in the affected sheep in comparison to healthy ones. This significant elevation may be attributed to the liver cell damage resulting from the sudden invasion of the liver by young fluke or by the destructive effect of the adult fluke on the liver parenchyma (Thomas, 1982).

The proteinogram of the infested sheep revealed a significant reduction in the serum albumin, while the serum globulin showed a significant elevation in their values, but the serum total proteins were within normal. These results coincided with those previously mentioned by Holmes *et al.,* (1968), and Radostits *et al.,* (2000). The recorded hypoalbuminaemia could be due to reduced albumin synthesis and plasma volume expansion caused by liver damage (Thomas, 1982).

Regarding the serum total and direct bilirubin in the diseased sheep, the obtained results showed a significant elevation in their levels when compared with healthy ones.

Significant increase in the blood urea was observed in the diseased sheep, while the serum creatinine was within normal range. The increased levels of blood urea could be attributed to the failure of detoxification of ammonia and other nitrogenous substances by the damaged liver.

Hypoglycaemia was marked in the diseased sheep than healthy ones. Similar results were obtained by Pinsent (1982). The low blood glucose level in the diseased animals possibly resulted from decreased hepatic glycogenesis and decreased hepatic blood flow secondary to hepatic cell damage by the invasion of the fluke to the liver (Duncan and Prasse, 1979).

The effect of treatment with mirazid at a dose of 10mg/kg B.W. and 20mg/kg. B.W. per day orally for 6 consecutive days and after 4 months from treatment showed that this drug was effective with pronounced improvement of the general health condition, amelioration of all symptoms of fascioliasis in treated sheep and increase in their body weight to 63 and 67 kg on average for the first and second groups, respectively.

Fasciola eggs were not detected in the faeces, and the fasciola antibody titres were declined to a range from 1:8 – 1:32 in sheep treated with a dose of 10mg/kg. B.W. and to a range from 1 : 4 –1 : 8 in those treated with a dose of 20 mg/kg B.W.

Massoud *et al.,* (2001) reported that myrrh (mirazid) at a dose of 12 mg/kg B.W. per day for 6 consecutive days given for patients suffering from fascioliasis and after 3 months follow up, the therapy proved to be effective with pronounced improvement of the general condition of the patients.

On studying the effect of mirazid at a does of 10 or 20 mg/kg B.W. daily for 6 days on the disturbed hematological and serum biochemical parameter and after 4 months from treatment of naturally infested sheep, RBCs count, Hb concentration, HCT value, total leucocytic count, neutrophil%, lymphocyte% eosinophil%, monocyte % as well as liver enzymes, total protein, albumin, globulin, total bilirubin, direct bilirubin, blood urea, creatinine and glucose returned to nearly normal values.

From the all results, it could be concluded that mirazid has good fasciolicidal activity and helped to bring the blood picture and some blood serum biochemical constituents in treated sheep to the normal physiological ranges resulting from the improvement in the general condition of treated sheep. Moreover, the results indicated that the use of mirazid at a dose 20mg/kg B.W. per day orally for 6 days gave the best effect.

Table 1. Hematological values in diseased animals before and after treatment as compared with control healthy (Mean ± S.E.).

Parameters		*Healthy animals*	*Diseased animals before treatment*	TREATED ANIMALS	
				10 mg dose	20 mg dose
Erythrocytes	(x $10^6/\mu L$)	11.36 ± 0.64^A	6.66 ± 0.61^C	7.59 ± 0.45^B	10.96 ± 0.50^A
Hb	(g/dL)	12.95 ± 0.57^A	9.08 ± 0.15^B	10 ± 0.42^B	12.40 ± 0.86^A
HCT	(%)	37.42 ± 1.43^A	29.04 ± 0.56^B	32.39 ± 1.30^B	35.51 ± 2.20^A
MCV	(H)	32.94 ± 1.09^B	43.60 ± 3.88^A	42.67 ± 1.57^A	32.40 ± 1.32^B
MCH	(pg)	11.40 ± 0.30^B	13.63 ± 1.18^A	13.18 ± 0.59^A	11.31 ± 0.61^B
MCHC	(g/dL)	34.61 ± 0.56^A	31.27 ± 0.55^B	30.87 ± 0.28^B	34.92 ± 0.68^A
TLC	(x $10^3/\mu L$)	8.99 ± 0.52^C	11.92 ± 2.28^A	10.97 ± 2.03^B	9.32 ± 1.74^C
Neutrophil	(%)	34.40 ± 1.81^A	42 ± 0.84^B	33.8 ± 2.01^A	35.4 ± 1.08^A
Lymphocytes	(%)	57.8 ± 1.53^A	47.6 ± 0.81^B	56 ± 1.70^A	56.4 ± 1.12^A
Eosinophil	(%)	3.4 ± 0.68^B	8.2 ± 0.58^A	6.6 ± 0.67^A	4.4 ± 0.51^B
Monocyte	(%)	3.6 ± 0.63^A	1.4 ± 0.24^B	2.4 ± 0.37^A	2.7 ± 0.20^A
Basophil	(%)	0.8 ± 0.2^A	0.8 ± 0.37^A	1.2 ± 0.40^A	1.1 ± 0.24^A

*Different litters A,B,C within the raws means significant at (P = 0.05)

* Means with the same letters are not significantly different

Table 2. Serum biochemical parameters of diseased animals before and after treatment as compared with control healthy

(Mean ± S.E.)

Parameters		*Healthy animals*	*Diseased animals before treatment*	TREATED ANIMALS	
				10 mg dose	20 mg dose
AST	(µ/L)	59.86 ± 1.20^{C}	88.22 ± 2.51^{A}	72.5 ± 4.35^{B}	61.92 ± 0.73^{C}
ALT	(µ/L)	16 ± 0.49^{B}	18.99 ± 0.85^{A}	18.24 ± 0.72^{A}	16.59 ± 0.45^{B}
Total protein	(g/dL)	7.15 ± 0.36^{A}	6.54 ± 0.37^{A}	6.59 ± 0.26^{A}	7.05 ± 0.30^{A}
Albumin	(g/dL)	3.18 ± 0.27^{A}	2.31 ± 0.16^{B}	2.84 ± 0.09^{B}	3.11 ± 0.21^{A}
Globulin	(g/dL)	3.97 ± 0.36^{B}	4.23 ± 0.22^{A}	3.75 ± 0.38^{B}	3.94 ± 0.36^{B}
A/G ratio		0.8 ± 0.05^{A}	0.55 ± 0.09^{B}	0.76 ± 0.13^{A}	0.79 ± 0.05^{A}
Total bilirubin	(mg/dL)	0.18 ± 0.03^{B}	0.35 ± 0.01^{A}	0.35 ± 0.02^{A}	0.23 ± 0.03^{B}
Direct bilirubin	(mg/dL)	0.15 ± 0.02^{C}	0.33 ± 0.2^{A}	0.30 ± 0.02^{A}	0.21 ± 0.01^{B}
Blood urea	(mg/dL)	10.46 ± 1.15^{B}	28.68 ± 4.79^{A}	17.73 ± 1.14^{B}	11.8 ± 1.08 ^{B}
Creatinine	(mg/dL)	1.31 ± 0.09^{A}	1.57 ± 0.29^{A}	1.46 ± 0.32^{A}	1.86 ± 0.2^{A}
Glucose	(mg/dL)	65.86 ± 1.62^{A}	39.32 ± 1.8^{C}	50.65 ± 3.56^{B}	62 ± 3.15^{A}

*Different litters A,B,C within the raws means significant at (P = 0.05)

* Means with the same letters are not significantly different

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تأثير عقار الميرازيد على ديدان الكبد وكيمياء الدم فى الأغنام

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تشكل الإصابة بالديدان الكبدية فى الأغنام أهمية كبيرة وخطيرة حيث إنها قد تؤدى إلى نفوق الحيوان فى الحالات الحادة أو الإصابة بالهزال فى الحالات المزمنة مما يؤثر على إنتاجيته.

وتوجد أدوية تستخدم فى معالجة تلك الديدان الكبدية فى الأغنام والأبقار ولكن قد يختلف تأثيرها بين عقار وآخر فمنها ما يؤثر على الديدان البالغة وقليل منها يؤثر على الأطوار الأولى لتلك الديدان . وعقار الميرازيد من العقارات المستخلصة من نبات الكامفورامولمول والذى ينمو فى مصر وبلدان أخرى وقد استخدم حديثا فى علاج ديدان البلهارسيا والديدان الكبدية فى الإنسان بكفاءة عالية مما شجع الباحثين على تجربة هذا العقار فلا علاج الديدان الكبدية فى الأغنام . وقد قام الباحثون بفحص البراز فى الأغنام الظاهر عليهما أعراض الهزال للكشف عن وجود بويضات الديدان الكبدية وقد عزلت الأغنام التى كان برازها إيجابياً للبويضات وأخذ منها عينات الدم قبل المعالجة لعمل صورة دم وفحص الدم كيميائيا وقياس الأجسام المناعية المضادة للديدان الكبدية فى بلازما الدم وأثبتت النتائج التأثير المعنوى للديدان الكبدية على صورة الدم وإنزيمات الكبد.

كما أن الأجسام المناعية المضادة للديدان الكبدية كانت مرتفعة بصورة ملحوظة فى تلك الأغنام المصابة بالديدان الكبدية.

ثم قسمت تلك الأغنام المريضة إلى مجموعتين واحدة منها عولجت بالميرازيد عن طريق الفم بجرعة ١٠ ملليجرام/ ك يوميا ولمدة ٦ أيام متتالية ايضا وتم متابعة الأغنام على مدى ٤ شهور وبعد ٤ شهور تم فحص البراز لتلك الأغنام ولم يستدل نهائيا على وجود أى بويضات للديدان الكبدية كما تم أخذ عينات دم منها لعمل صورة الدم وقياس المكونات الكيميائية وقياس الأجسام المضادة للديدان الكبدية فى بلازما الدم باختبار التلازن الدموى الغير مباشر. وقد كانت النتيجة هى التحسن الواضح على الأغنام المعالجة بجرعة ٢٠ ملليجرام /ك كما أنه قد تلاحظ تناقصاً واضحاً فى الاجسام المناعية المضادة للديدان الكبدية بعد المعالجة بأربعة شهور. وقد أوصت النتائج بإمكانية استخدام عقار الميرازيد لمعالجة الديدان الكبدية فى الأغنام بجرعة ٢٠ ملليجرام /ك لمدة ٦ أيام متتالية عن طريق الفم .