EFFECT OF MIRAZID 'COMMIPHORA MOLMOL' IN TREATMENT OF EXPERIMENTAL GOAT FASCIOLIASIS

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ABSTRACT

A controlled test of the efficacy of mirazid against all stages immature and mature of Fasciola gigantica have performed in experimentally infected goats. Goats were orally infected with 100 viable metacercaria and treated post infection (PI) at 6 and 16 weeks with 10mg/Kg/daily mirazid for 6 days. The efficacy of mirazid in treatment of both immature and mature flukes was 86.46% and 96.64 % respectively. The influence of mirazid treatment on the pathophysiology of fascioliosis infected goats was also examined. Anemia was associated with the presence of immature and mature flukes in bile ducts. Treatment with mirazid largely induced hematological alterations as seen by reduced serum levels of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutathione transferase (GGT) levels associated with hepatic damage due to fasciolosis. Early treatment, 6 weeks PI, prevented the development of both parenchyma and bile ducts lesions. While, treatment at 16 weeks PI had no appreciable effect on the development of hepatic lesions. Enzyme-linked immunosorbent assay antibody response to F. gigantica excretory secretory product (ESP) was also affected by treatment with mirazid. In all treated goats, a peak in antibody levels was observed between weeks 12 and 14. The drug can be regarded as highly effective therapy against mature and immature flukes of goats but more investigations still needed to detect the effect of mirazid on immature stages of flukes in other animals.

INTRODUCTION

Liver fluke infection of cattle and sheep has important implications for animal health and welfare, farming economics, and food production in much of the world. It is considered to be among the main causes of reduced livestock productivity. In Egypt, fascioliosis is emerging as an important production and zoonotic disease.
According to the report of Central Organization of Mobilization and Computation, Cairo (COMC 2000), direct and indirect losses ascribed to fasciolosis were estimated at 484.5 millions LE per year. Ruminants are considered to be the reservoir of infection to man. In the last decade, fasciolosis had imposed itself as an important zoonotic disease in Egypt posing a clinical and epidemiological health problem. An estimated number of fasciolosis cases in Egypt were 830,000 individuals (Haseeb et al., 2002).

Fasciolosis was reported in sheep, goats, cattle, buffaloes and rabbits (Soliman, 1998) donkeys, horses, camels (Haridy and Morsy, 2000) and Rattus rattus (Haridy et al., 2003a). Examination of the animal feces for Fasciola eggs showed prevalence rates of 7.0% for both cattle and buffaloes and up to 78.0% for sheep in North Sinai (Soliman, 1998).

Economic losses are due to both the mortality arising from clinical processes and the decline in production caused by subclinical processes, in which migration of immature parasites through the liver gives rise to considerable liver damage.

Myrrh (Mirazid), which is a gum resin of Commiphora molmol, Family Burseraceae, has been licensed for medical use in Egypt as a trematocidal drug with high efficacy and safety. It is well tolerated remedy with high safety margin despite giving it repeated for relatively long period (Massoud et al., 2000). It is effective in treatment of dicrocoeliosis in man and animals in Egypt (Massoud et al., 2003).

Also, Myrrh has Heterophycidal activity (Massoud et al., 2001a), cestodicidal activity (Massoud et al., 2001b) and high efficacy against intestinal nematodes (Massoud et al., 2001c).

The aim of this study is to test the efficacy of mirazid against different stages (immature and mature) of *F. gigantica*, the influence of mirazid treatment on the pathophysiology of the disease, in terms of both hematological and serum biochemical parameters, and on the production of antibodies to ESP of *F. gigantica* in experimentally infected goats.

**MATERIALS & METHODS**

1. **Animals and experimental design**

Twelve male goats, aged from 6-12 months, free of *F. gigantica* infection at the start of the study were used for experimental infection and treatment. They were divided into four groups of three animals each (groups 1-4). All animals were housed in covered pens and fed with hay and commercial balanced ration for goats. Following two weeks of housing, each animal in three of four groups (group 1, 2, and 3) was orally infected with 100 viable metacercariae, group 4 served as nega-
tive control. Metacercaria of *F. gigantica* was kindly supplied by Tudor bilharz institute - Egypt.

2. **Treatment protocol**

   Mirazid capsules (300mg SGC) @ Pharco Pharmaceuticals Company were given 10mg/kg/day for six days (*Haridy et al., 2003a*), drugs given orally on an empty stomach early in the morning one hour before breakfast. Group 1 was treated 6 weeks post infection when the parasites were at the immature stage; group 2 was treated at 16 weeks post infection (mature stage); group 3 served as non treated, infected control.

3. **Pathophysiological parameters**

   Blood samples were collected from each animal in the four groups at two weeks intervals post infection. Hematological analysis were performed which included; red blood cell count (RBC), packed cell volume (PCV), and hemoglobin (Hb) according to *Schalm (1975)*, Gamma-glutathione transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum albumin (ALB) levels were determined from collected sera.

4. **Serological examinations**

   According to *Mousa, (1992)* ESP antigen against *F. gigantica* were prepared for detection of specific antibodies titer using an enzyme-linked immunosorbent assay. *F. gigantica* ESP were obtained from adult liver flukes collected from control positive goats at the end of the experiment. The flukes were washed repeatedly in 0.01M Phosphate Buffer Saline (PBS), pH 7.4. The living worms were then incubated for 3 hours at 37°C (one worm/ 5ml in 0.01M PBS, pH 7.4). after incubation, the worms were removed and the supernatant fluid (PBS + E/S) was collected and subjected to a high-speed centrifugation (12000rpm) for one hour at 4°C. the supernatant was concentrated by using PM 10-membrane filter (Amicon corp. Lexington, Massachusetts). The protein content was measured according to the method described by *Lowry et al., (1951)*. The antigen was aliquoted and stored at -70°C until use. The ELISA technique was done according to *Martinez et al., (1997)*.

5. **Worm counts**

   At 20 weeks PI all animals were slaughtered. The liver and the gall bladder were dissected and all flukes were recovered and counted.

6. **Statistical analysis**

   All obtained data were expressed as mean ±SE. Groups means were compared with an analysis of variance (ANOVA) using a computer program (Costate).
RESULTS

1. Drug efficacy
The effects of mirazid treatments against *F. gigantica* in goats were shown in Table (1). Overall the fluke recovery rate after experimental infection in untreated control G3 was (100 %), while in G1, the efficacy of mirazid was 86.46% and 96.64 % for immature and mature *F. gigantica* respectively (G2).

Table (1): Efficacy of mirazid against immature and mature *F. gigantica* in goats.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Metacercaria dose</th>
<th>Time of treatment (Weeks PI)</th>
<th>No. of recovered flukes (mean ±SE)</th>
<th>Efficacy ( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3</td>
<td>100</td>
<td>6</td>
<td>2.66±0.3</td>
<td>86.46</td>
</tr>
<tr>
<td>G2</td>
<td>3</td>
<td>100</td>
<td>16</td>
<td>0.66±0.3</td>
<td>96.64</td>
</tr>
<tr>
<td>G3</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>19.66±0.8</td>
<td>100</td>
</tr>
<tr>
<td>G4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2. Pathophysiological changes
As shown in Fig. (1, 2 and 3), hemato logical changes in G3 showed a significant decrease in RBC, Hb and PCV from 12 week till end of experiment (20weeks PI). While animals of G2 showed significant decreases only in RBC, between 12 and 18 weeks PI and animals of G1 did not show any changes as compared to G4. Serum levels of ALB (Fig. 4) showed a significant elevation in all three infected groups from week 4 - 12 PI, while serum AST levels (Fig. 6) showed a significant elevation in groups 2 and 3 from week 6 - 12 PI and in the G1 from 6 to 8 weeks PI. GGT serum levels (Fig. 7) was elevated in groups 2 and 3 from 8 to 16 weeks PI and in G1 only in weeks 8 PI.

3. Antibody response
Evaluation of antibody levels in all groups was shown in Fig. (8). All groups 1,2 and 3, showed increase in antibody
levels that began to rise 2 week PI, till, reaching a peak at week 14 PI, after which antibody levels began to decline until the end of the experiment, although still remaining clearly positive with levels significantly higher in both G2 and G3 (P < 0.05) compared to G4. While in G1, the levels at 20 weeks PI were negative, compared to G4 (P < 0.05).

Fig. (1): Changes in RBC count during experimental fascioliasis and treatment

Fig. (2): Changes in Hb content during experimental fascioliasis and treatment
Fig. (3): Changes in PCV % during experimental fascioliasis and treatment.

Fig. (4): Changes in serum ALB during experimental fascioliasis and treatment.
Fig. (5): Changes in Serum activity of ALT during experimental fascioliasis and treatment.

Fig. (6): Changes in Serum activity of AST during experimental fascioliasis and treatment.
Fig. (7): Changes in Serum activity of GGT during experimental fascioliasis and treatment.

Fig. (8): Specific *F. gigantica* ESP antibodies and during experimental fascioliasis and treatment
DISCUSSION

Wood et al., (1995) concluded that a controlled test calculated by comparing parasite populations in groups of treated and untreated animals was considered the most reliable method for determining the efficacy of anthelmintic activity of a drug. Moreover, Rapic et al. (1988) and Richards et al. (1990) mentioned that worm counts were more accurate than fecal egg counts to evaluate the efficacy of fasciolicides drugs. Similarly, in this study, the efficacy of mirazid was calculated depend up on the flukes count in the liver. In the present study, the efficacy of mirazid against immature and mature stages of *F. gigantica* was conducted in goats. Mirazid at a dose of 10 mg/ kg/ 6 days was highly effective against mature and immature stages of *F. gigantica* (96.64% and 86.46% respectively). These results confirm the findings of Massoud et al., (2001c), who reported that mirazid was highly effective against mature flukes in sheep, and in accordance with the data obtained by Haridy et al. (2003a) and Haridy et al. (2006) in sheep and cattle respectively.

Controlled tests have been carried out in cattle and sheep using triclabendazole, albendazole, clorsulon, nitrooxynil, oxyclozanide and rafoxanide, testing their efficacy against immature and adult *F. hepatica* (Martinez et al., 1997). In goats, albendazole was tested against mature flukes (Foyret, 1988), triclabendazole against late immature flukes (Kinabo and Bogan, 1988) and triclabendazole against early, late immature and adult flukes (Martinez et al., 1997). The present study examined the influence of mirazid treatment on the clinical course of goat fasciolosis. The results obtained for G3 (infected and untreated) indicated the development of a moderate anemic process which was evident from 8th week PI due to the migration of immature flukes through the hepatic parenchyma and the presence of flukes in bile ducts.

These results were in the line with those of El-Haroun et al. (1989) and Ganga et al. (2007). On the other hand, treatment with mirazid in G1 and G2, eliminated most of immature and mature flukes that reduced hematological parametars especially in animals of G1 while animals of G2 showed a significant drop in RBC, Hb and PCV from week 10 then rapid return to normal values.

The elevation of liver enzymes that reflected liver damage during goat fasciolosis showed early increase in ALT and AST levels in G2 (trea-
tment at 16 weeks PI) and G3 (control positive) was referred to the migration of young flukes, while late increase of GGT (8 weeks PI) was referred to penetration of flukes into bile ducts then rapid return to normal values in G2 due to removal of flukes and liver regeneration. These results were in accordance with Symonds et al. (1983); Jemli et al. (1993); Ferre et al. (1994) and Mbuh and Mbwaye, (2005). Early treatment at 6 weeks, in Group1, eliminated the parasites and consequently decreased liver damage resulted in steady level in serum hepatic enzymes, ALT and AST after 6 week PI, as well as a in GGT levels that reflect reduction of the bile duct lesions.

Groups (1, 2 and 3), showed gradual increase in antibody levels at 2nd week PI, till, reaching a peak at week 14 PI due to primary response to immature \textit{F. gigantica}, after which antibody levels began to decline until the end of the experiment (20 weeks P I) due to of treatment, these results were coincided with Fetterer et al. (1985); Martinez et al. (1997) and Phiri et al. (2006). On the other hand, Zhang et al. (2006) mentioned that anti \textit{F. gigantica} antibody levels were increased significantly from 3rd weeks PI and displayed a peak at 13 weeks PI in experimentally infected buffaloes. In these animals in which mirazid totally eliminated the parasite burden, antibody levels were decreased to negative values as in G1 which means that the antibody response to \textit{F. gigantica} ESP was also affected by mirazid treatment, while, animals in G2, showed positive titer till 20 weeks PI. This means that the initial antibody response is directed against antigen determinants which are present in both the juvenile tegument and in excretory/secretory products in the adult but subsequent maintenance of antibody levels appear to be related to the sequential release of ESP by migrating flukes and surviving adult flukes in bile ducts (Martinez et al., 1997). It could be concluded from the above results that mirazid was highly effective treatment against mature and immature stages of \textit{F. gigantica} in goats but more investigations still needed to detect the effect of mirazid on immature stages of fasciola in other animals.

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الملخص العربي

تأثير دواء الميرازيد لعلاج مرض الفاشيولا في الماعز

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لقد تم تجربة تأثير دواء الميرازيد لعلاج مرض الفاشيولا (الطور الناضج والطور الغير ناضج) في الماعز حيث تم عمل عدوى تجريبية لها بعد 60 طور معدي لكل ذكر ماعز وقد تم إعطاء الدواء بعد العدوى ب 6 أسابيع (المجموعة 1) و 12 أسبوع (المجموعة 2) وقد أثبتت النتائج كفاءة الدواء بجرعة 0 1ملجم / كجم وزن حي على الطور الناضج بنسبة 87,66% و على الطور الناضج بنسبة 46,96% وقد تم دراسة تأثير الدواء على ميكانيكية حدوث المرض حيث أثبتت النتائج أن الدواء قد تخلص من الديدان المسببة للمرض و عليه قد كانت التغيرات الدموية و مستوي أنزيمات وظائف الكبد قليلة.
في الماعز التي تم علاجها مبكراً عند 6 أسابيع بعد العدوى مقارنة بالماعز التي تم علاجها عند 16 أسبوع بعد العدوى والتي تم عمل عدوى لها بدون علاج وقد كانت مستوى الأجسام المناعية عند أعلى معدل لها عند الأسبوع 12 حتى الأسبوع 14 في جميع الماعز التي تلقت العلاج ثم بدأ المستوى في الانخفاض وخاصة في الماعز التي تم علاجها مبكراً عند 6 أسابيع بعد العدوى لمستوى مشابه للماعز والتي لم يتم عدويها وبناءً عليه ينصح باستخدام الميرازيد لعلاج فاشيولا جيجانتكا في الماعز كدواء فعال للقضاء على الطور الناضج والطور الغير ناضج.