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EFFECT OF NATURAL AND EXPERIMENTAL FASCIOLOSIS ON SOME BIOCHEMICAL PARAMETERS IN SHEEP

(With 2 Tables and 3 Figures)

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**تأثير الإصابة الطبيعية والتجريبية بمرض الفاشيولا على بعض القياسات
الكيميائية في الخراف**

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تهدف هذه الدراسة إلى استبيان تأثير الإصابة الطبيعية والتجريبية للديدان الكبدية في الخراف على بعض قياسات الدم مثل الضغوط المؤكسدة كحمض الثيوباربتوريك (TBARS) وبعض وظائف الكبد مثل إنزيم ألانين أمينو ترانسفيريز (ALT) وإنزيم أسبارتات أمينو ترانسفيريز (AST) وإنزيم جاما جلوتاميل ترانسفيريز (γ GT) وإنزيم لاكتيك ديهيدروجينيز (LDH) هذا بالإضافة إلى قياس مستوي البليروبين (bilirubin) وأخيرا أحد أنواع مضادات الأكسدة الغير إنزيمية مثل فيتامين (ج). ولهذا فقد تم إجراء التجربة على عدد ٥٠ خروف تم تقسيمها إلى ثلاث مجموعات ، تشمل المجموعة الأولى الخراف المعدي طبيعيا (٢٠ خروف) بينما شملت المجموعة الثانية الخراف المعدي تجريبيا (١٠ خراف) في حين استخدمت المجموعة الثالثة (٢٠ خروف) كمجموعة ضابطة. وقد تم تحليل كل القياسات السابق ذكرها ، وقد وجد من النتائج زيادة تركيز كل من حمض الثيوباربتوريك (TBARS) وإنزيم ألانين أمينو ترانسفيريز (ALT) وإنزيم أسبارتات أمينو ترانسفيريز (AST) وإنزيم جاما جلوتاميل ترانسفيريز (γ GT) وإنزيم لاكتيك ديهيدروجينيز (LDH) و البليروبين (bilirubin) في كل من المجموعتين المعديتين بالمقارنة بالمجموعة الضابطة ، في حين وجد أن تركيز فيتامين (ج) يقل في المجموعتين المعديتين بالمقارنة بالمجموعة الضابطة. ونستخلص من هذه الدراسة إلى أن تأثير الأكسدة في الخراف المصابة طبيعيا وتجريبيا بمرض الفاشيولا يكون مصاحبا بزيادة نسبة إنزيمات ALT ، AST ، LDH في السيرم، ومادة البليروبين وحمض الثيوباربتوريك (TBARS) كنتيجة لإصابة خلايا الكبد بالديدان الكبدية ، وإنزيم γ GT نتيجة تأثير الديدان على جدران القنوات المرارية ، في حين أن الإصابة أدت إلى نقص تركيز فيتامين (ج) لأن الجسم استخدمه كمادة مضادة للأكسدة.

SUMMARY

The aim of this study is to investigate the effect of natural and experimental fasciolosis in sheep on some blood parameters including oxidative stress as thiobarbituric-acid reactive substances (TBARs) and some liver function tests as alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (γ GT), lactate dehydrogenase (LDH) and blood bilirubin, as well as non-enzymatic antioxidants vitamin C. For this purpose, fifty sheep were divided into three groups, the 1st group (20 sheep) includes naturally infected sheep, the 2nd group (10 sheep) includes experimentally infected sheep and the 3rd group (20 sheep) includes the control non-infected ones. Serum liver function tests (S-ALT, S-AST, S- γ GT, S-LDH and bilirubin as well as oxidative stress indicators TBARs and vitamin C) were analyzed. The results revealed that both naturally and experimentally infected sheep showed highly significantly ($p < 0.01$) increased ALT, AST, γ GT and LDH enzyme activities in serum. Moreover, serum bilirubin level was elevated in comparison with control group. Data indicated that TBARs level was significantly higher ($p < 0.05$) in naturally and experimentally infected than control sheep, while serum vitamin C level was significantly ($p < 0.05$) lower in both naturally and experimentally infected sheep than control ones. It could be concluded that changes in the oxidative stress of infected sheep were accompanied with rising activities of S-ALT, S-AST, S- γ GT and S-LDH as markers of liver and gall bladder damage.

Key words: *Fasciola, fasciolosis, liver function, parasitology*

INTRODUCTION

Fasciolosis is a disease of grass-grazing animals and occasionally humans caused by the trematode flukes, *Fasciola* species which is one of the major liver parasitic infections in domestic animals and human beings. The worm lives in the bile ducts and cause liver cirrhosis (Schmidt and Roberts, 1985). Fasciolosis has an important impact on general health, weight gain and on the reproduction (Chick *et al.*, 1980). In animals, the adverse effect of fasciolosis includes decreased both meat and milk production, decreased fertility and increased veterinary expenses (Torgerson and Claxton, 1999). Also, Kithuka *et al.*, (2002) recorded that the total economic losses incurred by Kenya during the last

10 years of the 20th century as a result of condemnation of *Fasciola*-infected livers was approximately US\$ 2.6 million.

Fasciola gigantica infection in sheep produced liver lesions characterized by damage to blood vessels and parenchymal necrosis. The damage to the liver tissues was accompanied by increased activity in serum of AST after 2 weeks, and γ GT from 8 weeks. Bilirubin concentration was not affected (El-Samani *et al.*, 1985 and Yang *et al.*, 1998).

The routine parasitological examination of animals against fasciolosis alone is usually inadequate due to the late appearance of eggs in feces (Chen and Mott, 1990), so, they failed to detect the early infections. Therefore, the use of more advanced techniques such as detection of fluke-specific antibodies and the determination of liver function tests as well as other blood parameters will be much useful in this criterion.

Serum concentrations of the enzymes glutamate dehydrogenase, sorbitol dehydrogenase and aspartate amino transferase were elevated in the naturally infected sheep, indicating hepatic damage. A slight rise in the level of serum bilirubin was also observed (Haroun *et al.*, 1986). Serum activities of γ GT and glutamate dehydrogenase were used as markers of the different fluke stages during infection and associated liver damage in infection induced experimentally in sheep (Ferre *et al.*, 1997). They reported that γ GT is sensitive indicator of liver cell damage in chronic fasciolosis (Sykes *et al.*, 1980). Moreover, *Fasciola* infection of lambs increased the serum γ GT activity and the total serum bilirubin concentration (Prache and Galtier, 1990).

Increased AST activity reflected parenchymal liver damage, whereas increased γ GT as well as LDH reflected biliary damage as a result of enlargement and hyperplasia of the bile duct wall (Sandeman and Howell 1981).

Sheep serum ascorbic acid decreased after *Fasciola* infection due to its being act as non-enzymatic antioxidant (e.g. vitamins C, E and A) which were reduced simultaneously with enhancement of lipid peroxidation processes as evidenced by increased levels of TBARS. Changes in the antioxidant abilities of the liver and in the phospholipid structure of the cell membrane were accompanied by rising activities of ALT and AST (Kolodziejczyk *et al.*, 2005 and Gameel, 1982). Concentration of thiobarbituric acid-reactive substances (TBARS) is measured as markers of oxidative stress. In experimental infections,

inefficient elimination of reactive oxygen radicals takes place, resulting in oxidative liver damage (Sanchez *et al.*, 1999).

The aim of this paper is to assess the levels of vitamin C as an antioxidant and TBARs released from *Fasciola*-infected sheep, in addition to some liver function tests during the course of natural and experimental fasciolosis.

MATERIALS and METHODS

I. Parasitological investigations:

A number of 50 sheep under this investigation was divided into 3 groups as following:

1) Naturally infected group:

This group includes a number of 20 sheep showing distinct clinical signs of fasciolosis (such as icteric mucous membranes, greenish diarrhea, easily detached wool and emaciation); each was parasitologically positive for fasciolosis (indicated by the presence of *Fasciola sp.* eggs in feces) by using fluke finder technique (Welch *et al.*, 1987). Confirmly, serological techniques (ELISA) revealed presence of elevated levels of antibodies against *Fasciola sp.* indicating the presence of immunoglobulines against the fluke.

2) Experimentally infected group:

2.1. Preparation of animals:

This group included a number of 10 sheep, each of 5 – 6 months old, 30 – 35 kg. B. W. not exposed to a previous history of fasciolosis. This is achieved by a daily fecal examination for 3 weeks before experimentation and confirmed by the serological techniques. All these animals were kept indoors with concrete floors, under proper hygienic conditions, feeding on barely, tiben and hay and drinking on fresh water.

2.2. Collection of encysted metacercariae:

Eggs were collected from gall bladders of naturally infected cattle in abattoirs, thoroughly washed and incubated at 27–29°C for 13 days for embryonation (Boray, 1966) and examined daily till hatching and liberation of viable miracidia as indicated by continuous jerky movement. Then, *Lymnaea* snails are exposed to be infected with these viable miracidia at a dose of 3 – 5 miracidia/snail, in plastic plates, and then kept for 6 hours till complete penetration of snail tissues. Furthermore, the infected snails were transferred into plastic aquaria containing dechlorinated water at room temperature away from the direct sunlight. Daily examination of the snails was done to detect the presence

of cercariae or even rediae. Then, cellophane sheets were put in the aquaria for collection of the encysted metacercariae produced, which appeared as whitish pin-point spots. Finally, these sheets were contained in saline and kept in refrigerator till the time of infection (El-Bahy, 1984).

2.3. Challenge of infection:

Each animal was administered orally with about 125 encysted metacercariae, given mixed with the green fodder (Bautista-Garfias *et al.*, 1989; Mousa, 1994 and Dumenigo *et al.*, 2000).

3) Control group:

This groups includes a number of 20 sheep of the same conditions. They were parasitologically negative for fasciolosis by using the fluke finder technique. Serological assays also confirmed this finding.

II. Biochemical investigations:

*** Blood sampling and Biochemical analysis:**

Blood samples from groups were collected at morning by jugular vein puncture from overnight fasted animals. Samples were collected in plain tubes for serum preparation. Serum samples were aliquated and stored at -20 C° until analysis. Serum used for determination of TBARS (Sato, 1978), vitamin C (Jagota and Dani, 1982) by using spectrophotometer (Hitachi 2000). Biochemical tests were applied for evaluation of ALT and AST (Reitman and Frankel, 1975), γ GT according to (Tietz, 1994), LDH according to (Kachmar and Moss, 1976) and bilirubin (Sherlock, 1951) by using spectrophotometer (Hitachi 2000).

III. Statistical analysis:

The obtained data were statistically analyzed by t-test using Graphpad Primstate Analysis of Software Computer Program. IOWA, state USA, 2002 (Snedecor and Cochran, 1967).

RESULTS

Table 1: Mean levels of serum ALT, AST, γ GT, LDH enzymes activities as well as direct bilirubin (mg/dl) in control, naturally and experimentally infected sheep.

Parameters \ Groups	1 st group (Naturally infected)	2 nd group (Experimentally infected)	3 rd group (Control)
ALT (U/L)	125.75 \pm 8.37**	78.04 \pm 5.08**	53.50 \pm 2.73
AST (U/L)	116.33 \pm 10.57 **	141.78 \pm 9.41**	62.20 \pm 4.90
γ GT (U/L)	30.34 \pm 1.84**	24.22 \pm 1.52**	10.15 \pm 0.53
LDH (U/L)	227.04 \pm 17.34**	262.71 \pm 10.74**	100.74 \pm 6.36
Direct bilirubin (mg/dl)	1.53 \pm 0.27**	1.68 \pm 0.26**	0.52 \pm 0.04

* Significant at $P < 0.05$

** Highly significant at $P < 0.01$

Table 2: Mean levels of serum TBARs (nmol/ml) and vitamin C (mg/dl) in control, naturally and experimentally infected sheep.

Parameters \ Groups	1 st group (Naturally infected)	2 nd group (Experimentally infected)	3 rd group (Control)
TBARs (nmol/ml)	10.39 \pm 0.56 *	13.9 \pm 0.98 *	8.74 \pm 0.53
Vitamin C (mg/dl)	0.69 \pm 0.061*	0.79 \pm 0.03*	1.15 \pm 0.06

* Significant at $P < 0.05$

** Highly significant at $P < 0.01$

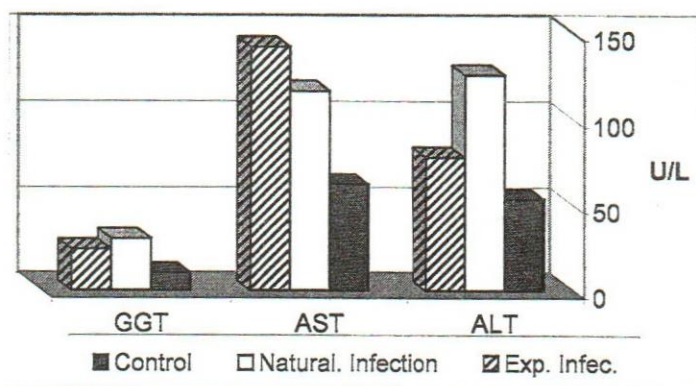


Fig. 1: Concentrations of ALT, AST and γ GT in the 3 groups of sheep.

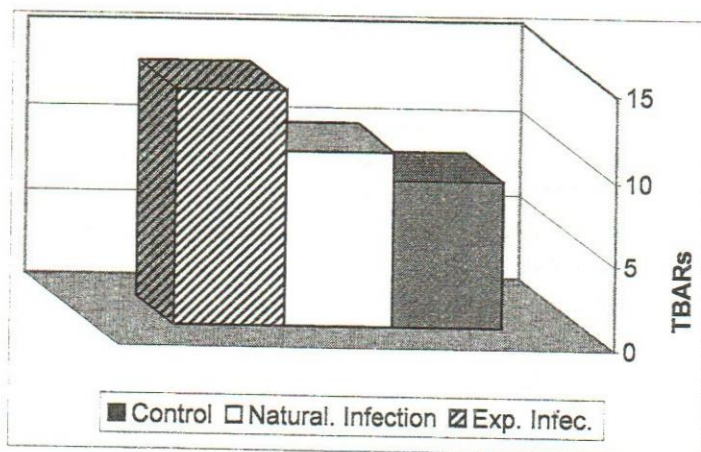


Fig. 2: Concentrations of TBARs in the 3 groups of sheep

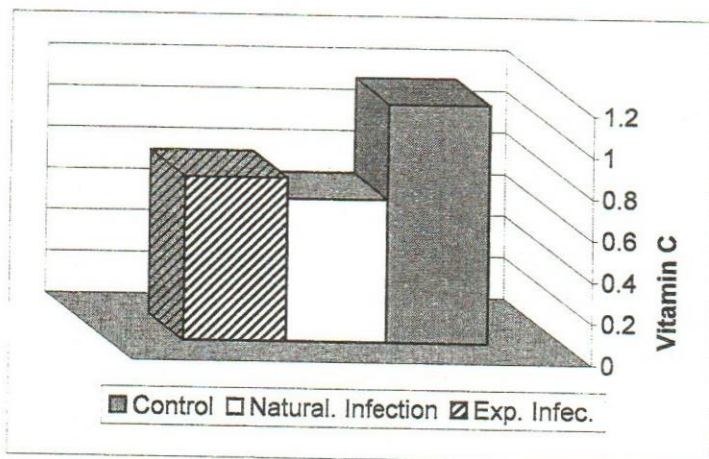


Fig. 3: Concentrations of vitamin C in the 3 groups of sheep

The data were analyzed and demonstrated in Tables (1 & 2) and Figs. (1,2 &3) as follow:

Table (1) Shows that mean levels of serum ALT, AST, γ GT, LDH enzymes activities increased significantly ($P < 0.01$) in naturally and experimentally infected sheep in comparison with control one respectively.

Table (2) Displays that naturally and experimentally infected sheep had significantly higher ($P < 0.05$) TBARs in serum, while mean serum vitamin C levels were significantly lower in the naturally and experimentally infected sheep compared to control.

DISCUSSION

The current data showed a high significant increasing ($P < 0.01$) in serum ALT and AST activities (as shown in Table 1) in both naturally and experimentally infected sheep compared to control group. These findings were in accordance with those obtained by Yang *et al.*, (1998) and Mbuh and Mbwaye (2005). In addition Wyckoff and Bradley, (1985) found that there was a significant increasing in AST and γ GT activities occurred as early as post-infection as compared with those in control non-infected animals. Increasing in AST activity reflected parenchymal liver damage which occurred due to continuous migration of the flukes. Rising activities of ALT and AST, as markers of liver damage in experimental infection with *Fasciola*, was also reported by Kolodziejczyk *et al.*, (2005). Moreover, it is an important criterion in studying fluke-induced liver damage.

The present study indicated significant increase in γ GT in naturally and experimentally infected sheep compared to normal control group. This elevated level of the enzyme reflected biliary damage which occurred due to enlargement and hyperplasia of bile duct wall as a result of secretion of large quantities of amino acid proline from the liver flukes. The later is responsible for collagen synthesis by epithelial and subepithelial cells (Modavi and Isseroff, 1984). These findings were matched with those obtained by Sykes *et al.*, (1980) and Ferre *et al.*, (1997) who reported that liver damage could be detected in sheep post infection with liver flukes. The authors added that, γ GT activity was more sensitive indicator of liver cell damage in chronic subclinical fascioliasis than AST activity, and thus it might be act more suitable as a useful diagnostic aid on account of its greater stability.

The present data showed a significant increase ($P < 0.01$) in serum LDH in both natural and experimental infected sheep. It is suggested that the presence of adult flukes in the bile ducts causes tissue damage, which is shown by fluctuations in LDH and γ GT levels after adult flukes have become established in the bile ducts. It is considered to be due to the feeding activity of adult flukes and the deposition of immune complexes in the liver parenchyma to challenge infection. This coincided with those obtained by Sandeman and Howell, (1981) who found that sheep infected with *Fasciola* sp. showed increased LDH level. The authors added that after the challenge infection, levels of both LDH and γ GT rose substantially. In addition, Ali *et al.*, (1999) recorded

elevated liver ALT and γ GT enzyme activities in sheep infected with *Fasciola* sp. associated with clear anemia and fever.

The present results illustrate a significant increasing in the serum bilirubin concentration in both natural and experimental infection with *Fasciola* sp. It is suggested that increased concentration of direct bilirubin might be occurred in both parenchymal and biliary stages of fasciolosis where the flukes began to enter the bile duct and commence blood feeding. Thus, a combination of bile duct hyperplasia and an effective contribution of blood to the bile could be the sharing factors of increased bilirubin concentrations. Consequently, a risk of induced yellow fat might be increased (Prache and Galtier, 1990).

All these findings indicated that, abnormal results of liver function tests were common in sheep with fasciolosis and changes of serum γ GT activity go parallel with those of ALT and AST activities.

The level of lipid peroxidation, which expressed as thiobarbituric acid reactive substances (TBARs) was significantly elevated ($p < 0.05$) in both naturally and experimentally infected sheep compared to control ones (Table 2). This occurred as *Fasciola* sp. invasion, migration and growth resulted in intensive destruction of phagocytic cells (Kupffer cells) and liberation of H_2O_2 , OH radicals and other free radicals as a consequence to peroxidative destruction of the cell membrane lipids leading to oxidative stress. These results are in agreement with those stated by Kolodziejczyk *et al.*, (2005) who observed an increasing in TBARs level in the course of acute and chronic fasciolosis.

Also, the results of the current study revealed that both naturally and experimentally infected sheep had significantly lower ($p < 0.05$) mean serum antioxidant levels of vitamin C (Table 2). This occurred due to utilization of its major amount as an antioxidant against liberation of free radicals that obtained due to *Fasciola* infection. These findings agreed with that of Kolodziejczyk *et al.*, (2005). The authors reported lower concentrations of the non-enzymatic antioxidant vitamins C, simultaneously with enhancement of lipid peroxidation processes as evidenced by increased levels of TBARs. Rehim *et al.*, (2003) found that TBARs levels were significantly increased, in addition to decreased plasma ascorbic acid after *Fasciola* sp. infection, indicating that inefficient elimination of the reactive oxygen radicals resulting in oxidative liver damage (Sanchez *et al.*, 1999).

Data indicated that experimentally infected sheep showed biochemical changes more or less similar to those of naturally infected ones.

Current results might be used to issue advice on the need for follow up liver function in the treatment course of fasciolosis. The indicators of the infection alone were considered useless, and it is concluded that herd diagnosis of fasciolosis may rely on the rise of specific antibody levels, possibly associated with increased hepatic enzyme activities.

It could be concluded that free radical-initiated lipid peroxidation may play a role in hepatic fibrogenesis through an effect of peroxidative products on Kupffer cells and lipocytes. This hypothesis is supported by the observation that dietary supplementation with vitamin E and C has a protective effect on *Fasciola*-induced hepatic fibrosis (Britton and Baco, 1994). Changes in the antioxidant abilities of infected sheep were accompanied by rising activities of ALT, AST, LDH and γ GT as markers of liver damage.

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