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Lipid Peroxidation and Antioxidant Potential of Sheep Liver Infected Naturally with Distomatosis

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SUMMARY: The aim of this study was to assess the effects of natural distomatosis infections on sheep liver malondialdehyde (MDA) concentration, activities of enzymatic antioxidants (glutathione peroxidase (GPx), superoxide dismutase (Cu, Zn-SOD), catalase (CAT)) and concentrations of non-enzymatic antioxidants (reduced glutathione (GSH), vitamin C, and β-carotene). Eighteen Akkaraman sheep naturally infected with *Fasciola* sp and *Dicrocoelium dentriticum* (*D. dentriticum*) and ten healthy Akkaraman sheep were included in the study Liver samples for the analysis of MDA, GPx, Cu, Zn-SOD, CAT, GSH, vitamin C, and β-carotene and blood samples for the measurement of alanine aminotransferase and aspartate aminotransferase were collected immediately after sheep in the two groups were slaughtered. The concentration of MDA and activity of GPx in the group with distomatosis were higher than in the control group ($P<0.001$). However, the Cu, Zn-SOD, CAT activities and the GSH, vitamin C concentrations in the infected group were significantly lower than in the control group ($P<0.001$). The serum β-carotene was not found to be statistically different in the two groups ($P>0.05$). ALT and AST serum activities of the group with distomatosis were significantly higher in comparison to the control group ($P<0.001$). In this study it was demonstrated that lipid peroxidation increased and activities or/and concentrations of antioxidant compounds were significantly changed in the liver of sheep with distomatosis.

Key Words: Lipid peroxidation, antioxidant, distomatosis, liver, sheep

Distomatosis ile Doğal Enfekte Koyun Karaciğerinin Lipid Peroksidasyon ve Antioksidan Potansiyeli

ÖZET: Bu çalışmanın amacı, koyun karaciğer malondialdehid (MDA) konsantrasyonu, enzimatik antioksidanların aktiviteleri (glutatyon peroksidaz (GPx), superoksid dismutaz (Cu, Zn-SOD), katalaz (CAT)) ve enzimatik olmayan antioksidanların konsantrasyonu (redükte glutatyon (GSH), vitamin C, β-karoten) üzerine doğal distomatosis'in etkilerini değerlendirmektir. *Fasciola hepatica, Fasciola gigantica* (*Fasciola* sp.) ve *Dicrocoelium dentriticum* (*D. dentriticum*) ile doğal enfekte onsekiz ve on sağlıklı Akkaraman koyun materyal olarak kullanıldı. İki gruptaki hayvanlardan, MDA, GPx, Cu, Zn-SOD, CAT, GSH, vitamin C, β-karoten analizi için karaciğer örnekleri ve alanin aminotransferaz (ALT) ve aspartat aminotansferaz (AST) ölçümleri için kan örnekleri kesimden sonra hemen alındı. Distomatosis'li grubun MDA konsantrasyonu ve GPx aktivitesi kontrol grubundan önemli derecede yüksekti ($P<0.001$). Bununla birlikte, enfekte grubun Cu, Zn-SOD, CAT aktiviteleri ve GSH, vitamin C konsantrasyonları kontrol grubundan önemli oranda düşüktü ($P<0.001$). β-karoten konsantrasyonu açısından gruplar arasında istatistiksel olarak fark bulunamadı ($p>0.05$). Kontrol grubu ile karşılaştırıldığında, distomatosisli grupta ALT ve AST serum aktiviteleri oldukça yüksekti. Bu çalışma distomatosisli koyunların karaciğerinde lipid peroksidasyonunda artış ve antioksidan aktiviteler ve/veya konsantrasyonlarda önemli değişiklikler olduğunu gösterdi.

Anahtar Sözcükler: Lipid peroksidasyon, antioksidan, distomatosis, karaciğer, koyun

INTRODUCTION

Distomatosis is an important animal and human disease caused by trematodes *(Fasciola hepatica, Fasciola gigantica and Dicrocelium dentriticum)*. These flukes specifically target the liver causing pathology and necrotic lesions such as fibrosis and cirrhosis, which result from the parasites' migration through the liver parenchyma. Further damage is caused when flukes enter the bile ducts causing haemorrhage. Acute and chronic distomatosis are observed primarily in sheep, goats, and cattle, causing important economic losses due to liver condemnation (26).

The generation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, hydroxyl radical, and singlet oxygen, in biological systems is dependent on oxygen consumption and can cause cellular damage by lipid peroxidation (20). Oxidative stress and enhanced lipid peroxidation have been associated with several models of liver

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injury (17). In a number of studies, it has been demonstrated that in the cells of hosts infected with different species of parasites, the amount of reactive oxygen radicals which cause lipid peroxidation are increased, thereby causing cell and tissue damage (22).

Products of lipid peroxidation formed in various biochemical reactions are normally scavenged by antioxidants. Antioxidants are compounds that are involved in effective scavenging of free radicals and in suppressing the actions of reactive oxygen substances. Antioxidant defences are widely distributed and include both enzymatic and nonenzymatic systems. The major enzymatic antioxidants are superoxide dismutase, glutathione peroxidase and catalase. Reduced glutathione, vitamin C, vitamin E, , β-carotene, ceruloplasmin and bilirubin are some of the nonenzymatic factors that may function as antioxidants (14).

Even though there is a large body of literature regarding the antioxidants status of host liver in dicroceliosis and fasciolosis, there is, to our knowledge, no study which evaluates the antioxidants status in the distomatosis. Therefore we have decided to examine the effects of distomatosis on antioxidative properties in the liver of sheep.

MATERIALS AND METHOD

Animal treatment

Twentyeight Akkaraman sheep, weighing 20–25 kg and 6–12 months old were used for the experiment. The animals were maintained in a controlled environment (paddock) that mimicked their natural habitat. Faecal samples obtained from animals under study were analysed for helminth eggs and larvae by sedimentation method and Baermanns technique (3). According to the analysis of the faeces, eighteen sheep were found to be natural infected with a mixture of *Fasciola sp.* and *D. dentriticum,* other ten sheep were free from parasites. This ten sheep were treated with anti-helminthic agent (Rafoxanide +Albendozale) (Pfizer, Inc., NY, USA) twice , at one-week long interval against any parasitic contamination. Fifteen days following the last treatment, the sheep were examined by using methods above. These animals were used as controls after it was demonstrated that they were free of parasites.

Tissue and serum preparation

Sheep in the two groups were slaughtered. After weighed and cutting of the liver tissues into small pieces with a scissors, liver tissues were divided into four unequal parts randomly; 1 part for malondialdehyde, glutathione peroxidase and superoxide dismutase, catalase, 1 part for reduced glutathione, 1 part for vitamin C, and the last 1 for β-carotene. Serum was obtained from blood samples by centrifugation and used for the determinaton of the serum activities alanine aminotransferase and aspartate aminotransferase.

Biochemical procedures

Liver tissues were homogenized for MDA, CAT, Cu, Zn-SOD and GPx (16). MDA concentrations were determined by Sushil et al. (27). CAT activity was determined according to Aebi's method (1). Cu, Zn-SOD and GPx activities were determined by the use of commercially available kits (Randox Laboratory, Crumlin, Ireland). tissue preparation and GSH concentration measurements were done according to Değer *et al*. (5). After liver tissues were homogenized to vitamin C (9) and β-carotene (23)**,** analysis of vitamin C was made by employing the methods of Omaye *et al*. (15) and β-carotene by Suzuki and Katoh (28). The activities of serum ALT and AST were determined by using automated analysis (Roche Diagnostic Kits, Modular PP+ISE 900).

Parasitological procedures

Parasite species obtained from 18 livers were identified and counted (3, 26). Liver trematodosis idendification and counts were carried out by opening the gall bladders and making crossed sections from liver and bile ducts. The data were expressed as means±standard deviation (SD) and compared statistically by using Duncan's tests.

RESULTS

Parasitological findings

The maximal and minimal numbers of the *F. hepatica, F.gigantica* and *D. dentriticum* in an animal liver were, 6–35, 1–7 and 300–5156 respectively (26).

Biochemical findings

Activities of antioxidant enzymes and concentrations of nonenzymatic antioxidants in the liver of infected and control groups are shown in Table 1. Concentration of lipid peroxidation product in the liver of infected and control groups are shown Table 2.

Table 1. Activities of antioxidant enzymes and concentrations of non-enzymatic antioxidants in the liver of infected and control groups.

Parameters	Control group (n: 10)	Infected group (n: 18)
Cu-Zn SOD (U/mg protein)	5,00±0,21	2,34±0,16*
GPx (U/mg protein)	18,71±1,11	34,63± 4,20*
CAT (k/g)	849,24±23,83	463,91±17,94*
GSH(μmol/g)	10,88±0,35	6,41±0,23*
Vitamin C (100mg tissue/ml)	37,80±0,92	26,79±0,75*
β-carotene (μg/100 gr)	*19,00±0,31*	18,50±0,36**

Table 2. Concentration of lipid peroxidation product in the liver of infected and control groups.

Parameter	Control group (n: 10)	Infected group (n: 18)
MDA (nmol/g)	45,26±1,15	66,29±1,09*

ALT and AST activities in the serum of infected and control groups are shown in Table 3.

Table 3. ALT and AST activities in the serum of control and infected groups.

Parameters	Control group (n: 10)	Infected group (n: 18)
AST (U/L)	166,27±6,5	359,31±14,6*
ALT (U/L)	32,62±1,5	98,24±4,71*

* (P<0.001), **(P>0.05) compared to control; Values are expressed as mean±SD;

When the infected group was compared with control group, despite activities of Cu-Zn SOD and CAT and levels of GSH and vitamin C were lower, activity of GPx was higher (P<0.001). There was no significant change in the level of β -carotene between two groups. Liver MDA concentration was significantly higher in infected group than that of the other group (P<0.001). Both ALT and AST serum activities of the group with distomatosis were significantly higher in comparison with the control group (P<0.001).

DISCUSSION

In the present study, 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 as markers of liver damage. Liver trematods cause the release reactive oxygen species producing a damage to the cell membrane and components and thus leading to cell death. The development of lipid peroxidation has been described in livers of rats infected by *F. hepatica* (7, 11) and of hamsters infected by *D. dentriticum* (20). The results of the present study showed that natural distomatosis also courses with oxidative stress and lipid peroxidation as indicated by the significant increase in liver MDA concentration, as a marker of lipid peroxidation. This result was similar to those reports mentioned above.

Antioxidant systems comprising enzymes and vitamins have a cellular protective action against oxidative stress resulting in cell, organ and tissue damage as a result of parasitic invasion (4). The elevation in the antioxidant enzyme GPx could represent an adaptative change against potential liver injury, reflecting the ability of the liver to scavenge excess ROS. This compensatory increase in GPx has previously been reported in different situations that course with oxidative stress, such as acute exercise or liver diseases, (10, 18). Several authors have investigated liver GPx activity in host with fasciolasis. The decreased liver GPx activity has been reported in the *F. hepatica* infection (11). However, Benzer and Temizer Ozan (2) showed increased liver GPx activity in this infection. In addition to, a significant elevation in liver GPx activity of hamster infected with *D. dentriticum* has been found by Sanches et al. (20). In our study, the liver GPx activity in the infected group was significantly higher than the control group.

The liver Cu-Zn SOD activity in the infected group was found to be significantly lower in our study (11, 20). The drop in Cu, Zn-SOD activity could be explained by the superoxide anion dismutation to hydrogen peroxide caused by the overproduc-tion of the superoxide anion linked to oxidative stress (21). A similar phenomenon has previously been reported to occur in rats receiving chronic ethanol administration (24). Depression of the protective capability against oxidative stress by Cu-Zn SOD may lead to greater tissue damage and initiate a vicious cycle by increasing free radical production, thereby exceeding the antioxidant liver capacity and resulting in further oxidative damage.

The data of the present study show that activity of CAT was significantly decreased in the liver tissue of infected group (2). This result was also consistent with that of Kolodziejczyk et al. (11) which showed a increase in liver CAT activity of the rats with fasciolasis. However, Sanches et al. (20) found that liver CAT activity did not change in hamsters with dicroceliosis.

GSH and its redox enzymes are the most important cellular antioxidants and play a major role in protecting cells against oxidative stress caused by ROS (25). It has been postulated that loss of GSH may impair cellular antioxidant defences and lead to the accumulation of reactive oxygen species (11). In this study the concentration of liver GSH was found to be significantly lower in the infected group than in the control group. It was reported that in hosts infected with *F. hepatica, D. dentriticum, Schistosoma mansoni* the levels of GSH fell in comparison to healty controls (6, 11, 12, 20), thus supporting the findings of this study.

Vitamins have a cellular protective action against oxidative stress resulting in cell, organ and tissue damage as a result of parasitic invasion. Vitamins A, C, E, thiamin, riboflavin, pantothenic acid, biotin, and folic acid have a protective role on the liver (4). Vitamin C is an important water-soluble free radical scavenging compound and plays a role in synthesis of collagen. Loss of vitamin C in rats with fasciolosis (8, 11) and in camel with trypanosomiasis, helminthiasis (13) were previously reported.

In addition, a decrease in the concentration of vitamin A in animals infected with parasites has been reported (11, 19). In the present study, vitamin C level was lower in the infected group compared with control group. Vitamin C deficiency is associated with disorders of collagen synthesis which result hepatic fibrogenesis (11). However, no significant difference was found in the level of β-carotene, is the most important precursor of vitamin A. These results show that while the oxidative processes occurred at the site of parasitic invasion, at the same time activities or/and levels antioxidant capacity of the liver decreased, leading to the generation of lipid peroxides. The resulting imbalance between oxidant and antioxidant processes may play a central role in the pathology associated with distomatosis.

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