

## Evaluation of biochemical parameters and oxidative stress in sheep naturally infected with *Dicrocoelium dendriticum* and hydatid cysts

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**Abstract:** The purpose of the study was to investigate the lipid peroxidation and antioxidant status as well as biochemical profile in sheep naturally infected with *Dicrocoelium dendriticum* and hydatid cysts. Ten sheep naturally infected with *D. dendriticum*, 10 sheep naturally infected with hydatid cysts, and 10 healthy sheep were used. The blood and visceral organs of sheep brought to a slaughterhouse in the region of Kırıkkale were checked for *D. dendriticum* and hydatid cysts. Plasma total protein, albumin, total bilirubin, total cholesterol, Ca, Mg, inorganic P, Cu, Zn, vitamin A, vitamin C, vitamin E,  $\beta$ -carotene, AST, ALT, GGT, ALP, erythrocyte MDA levels, and SOD and CAT activities were detected. Erythrocyte MDA, plasma total bilirubin levels, and AST and ALP activities were increased while total protein and vitamin A levels were decreased in sheep infected with parasites. Erythrocyte SOD activities were decreased in *D. dendriticum* group, and CAT activities were decreased in the hydatid cyst group and *D. dendriticum* group. Total bilirubin levels were increased and total protein levels were decreased in sheep infected with parasites. These results suggest that endoparasitic infections such as *D. dendriticum* and hydatid cysts cause oxidative stress and change some biochemical parameters in sheep.

**Key words:** Antioxidant system, biochemical parameters, *Dicrocoelium dendriticum*, hydatid cyst, lipid peroxidation, sheep

### 1. Introduction

Parasitic diseases caused by liver trematodes have an important place among many parasitic infections causing reduced productivity in animals. Liver trematodes constitute an important parasitic group affecting domestic ruminants around the world and in Turkey. Animals infected by this parasite manifest symptoms such as deterioration of overall status, edema, anemia, and weakness. It has been reported that in infected animals the bile ducts are dilated, the gall bladder is filled with bile, and the liver becomes fibrotic and cirrhotic and thus stiffer and hard to dissect over time (1). Among liver trematodes, *D. dendriticum* settles in the liver of ruminants and causes weight loss and reduced milk yield (2). Another parasite causing hepatic injury in sheep is the hydatid cyst, which is the larval form of *Echinococcus granulosus*. After ingestion of food material contaminated by canine feces, hydatid cysts primarily infect ruminants, but also camels, rabbits, pigs, humans, and monkeys. It primarily involves the liver, but also the lungs, spleen, and heart, and rarely other organs. Since its larvae involve the liver and lungs of intermediary hosts, they do not pose many problems when

young. When they are fully grown, however, they cause jaundice if they reside in the liver. Hepatic dysfunction leads to digestive disorders, while cysts in other organ systems give rise to organ-specific disorders (3).

Biochemical changes in blood are used to diagnose many disorders (4). Important changes can occur in the serum biochemistry of animals during parasitic infections (5,6). These infections are held responsible for hypoproteinemia and reduced levels of trace elements due mainly to malabsorption and increase in liver enzymes due to resulting hepatic injury (5,7). Dağoğlu et al. (8) reported that tissue injury induced by parasites involving the liver, such as *D. dendriticum*, caused altered serum enzymatic activity, which is particularly important for early detection of toxic hepatic injury.

Minerals have an important role in maintaining normal physiological functions and protecting organisms against disease. These substances are found in the structure of cofactors that are necessary for enzymatic activity. Copper (Cu) is an element that must be supplied in the diet of domestic livestock. Its deficiency is characterized by oxidation disturbance, anemia, hair depigmentation,

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osteoporosis, and central nervous system demyelination. Zinc (Zn) deficiency causes disturbances in hepatic metabolism, visual functions, and spermatogenesis (4).

Free radicals are unstable reactive molecules generated by normal cellular metabolism. These molecules can be formed in some pathological conditions (9). These radicals have also been shown to play a role in the pathogenesis of parasitic infections (10,11). Free radicals interact with macromolecules such as carbohydrates, lipids, proteins, and nucleic acids and cause dysfunction in cellular structures and organelles, collectively referred to as oxidative stress. Reactive oxygen species act on unsaturated fatty acids on cell membranes and induce lipid peroxidation, ultimately giving rise to cellular structural and functional disturbances. Malondialdehyde (MDA) measurement is used as an indicator of lipid peroxidation (9). Oxidative damage and hence lipid peroxidation take place when parasites inflict injury to organs, tissues, and organelles of a host. Various vitamins (A, C, E) and antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are responsible for the protection of cells from lipid peroxidation and the harmful effects of reactive oxygen species caused by parasites (12). These antioxidant substances help maintain the oxidant/antioxidant balance using at least one of the mechanisms of clearing free radicals from their site of action, halting chemical reactions producing radicals, limiting reaction rates, repairing molecular injury, and increasing endogenous antioxidant capacity (9,13).

Many studies have reported increased blood levels of lipid peroxidation products in parasitic infections of animals (14–19). Although there are a limited number of studies reporting that *D. dendriticum* (16,19) and hydatid cyst endoparasites augment serum lipid peroxidation in sheep, no study has yet investigated the antioxidant status in these infections. The present study aimed to find out if the parasites *D. dendriticum* and hydatid cyst cause oxidative stress and hence lipid peroxidation, and to determine antioxidant status under these condition. In the area of clinical biochemistry, determination of blood parameters is important for clinicians to make accurate diagnoses. Additionally, this study determined biochemical levels in the plasma of healthy and diseased sheep to aid clinicians in making diagnoses.

## 2. Materials and Methods

### 2.1. Animals

This study was performed on 30 Akkaraman sheep that were clinically healthy or naturally infected with *D. dendriticum* or hydatid cysts. The sheep were assigned to 3 groups, consisting of 10 animals per group: Group 1 was the control group, Group 2 was the *D. dendriticum*-infected group, and Group 3 was the hydatid cyst-infected

group. All sheep were about 1 year old. After slaughtering, blood samples and internal organs were collected from all sheep. Other helminths were examined, especially in the liver bile ducts and gall bladder. Blood samples were taken from the sheep infected with *D. dendriticum* and hydatid cysts after examining them. Sheep were selected from a slaughterhouse in Kirikkale Province.

### 2.2. Preparation for analysis of blood samples

Blood was taken from the jugular vein into heparinized tubes for the assessment of some biochemical parameters and MDA, enzymatic, and some nonenzymatic antioxidants. Blood samples were centrifuged at  $1600 \times g$  for 10 min at 4 °C. After centrifugation, plasma samples are gathered into Eppendorf tubes and the buffy coat was rejected. The erythrocytes were washed 3 times in phosphate-buffered solution (20). All samples were stored at -80 °C until use.

### 2.3. Biochemical analysis

Plasma aspartate aminotransferase (AST) (EC 2.6.1.1), alkaline phosphatase (ALP) (EC 3.1.3.1) (BIOLABO, France), alanine aminotransferase (ALT) (EC 2.6.1.2), and gamma glutamyl transpeptidase (GGT) (EC 2.3.2.2) (TML, Turkey) activities, as well as the plasma total cholesterol, total protein, albumin, total bilirubin (BIOLABO), calcium (Ca), inorganic phosphorus (Pi) (TML, Turkey), and magnesium (Mg) concentrations (Spinreact, Spain), were analyzed spectrophotometrically (Shimadzu UV-1700, Japan) using diagnostic kits. Plasma Cu and Zn concentrations were determined by atomic absorption spectrophotometer by the method of Tiftik (21).

### 2.4. Oxidative stress markers

After washing, erythrocytes were lysed with 5 volumes of ice-cold distilled water. Hemoglobin (Hb), MDA levels, and SOD and CAT activities were analyzed in the lysed erythrocytes spectrophotometrically according to the methods of Fairbanks and Klee (22), Buege and Aust (23), Sun et al. (24), and Aebi (25), respectively.

### 2.5. Assay of antioxidant vitamins

The plasma vitamin A,  $\beta$ -carotene, vitamin C, and vitamin E concentrations were analyzed spectrophotometrically by the methods of Suzuki and Katoh (26), Haag (27), and Martinek (28), respectively.

### 2.6. Statistical analysis

The statistical analysis was performed with SPSS 15.0 for Windows. The results are shown as means  $\pm$  standard error. For comparison of three groups of continuous variables, one-way ANOVA testing was used. Differences among means were determined with Duncan's multiple range test. Significance were considered at a probability of  $P < 0.05$ , and additionally high significance ( $P < 0.001$ ) is reported in some places to show the power of the test.

### 3. Results

In the present study, the groups with parasitic infections had significantly increased plasma AST ( $P < 0.001$ ) and ALP ( $P < 0.05$ ) activities compared to the control group. The *Dicrocoelium dendriticum* group had significantly increased plasma AST activity ( $P < 0.001$ ) compared to the hydatid cyst group. Gamma glutamyl transpeptidase and ALT activities showed numeric increases in parasite groups ( $P > 0.05$ ) (Table 1). Plasma total bilirubin levels were higher in the parasitic groups than in the control group ( $P < 0.001$ ). The highest total bilirubin level was determined in the hydatid cyst group. Plasma total protein values showed a significant decrease in parasitic groups compared to the control group ( $P < 0.001$ ). The lowest total protein level was determined in the hydatid cyst group. The groups with parasitic infection showed numeric increases in plasma total cholesterol level compared to the control group ( $P > 0.05$ ). The hydatid cyst group had decreased albumin levels compared to the *D. dendriticum* and control groups ( $P > 0.05$ ) (Table 1).

The *Dicrocoelium dendriticum* group had significantly increased plasma Mg levels compared to the hydatid cyst group ( $P < 0.05$ ). Plasma Cu level was numerically decreased in the groups with parasitic infection compared to the control group ( $P > 0.05$ ). Plasma Ca, Pi, and Zn levels were not affected by the parasitic infections ( $P > 0.05$ ) (Table 2).

In the current study, the groups infected by parasites had a significantly increased erythrocyte MDA level as compared to the control group ( $P < 0.05$ ). As compared to the control, the group with hydatid cysts infection showed reduced erythrocyte CAT ( $P < 0.01$ ) activity, while the group with *D. dendriticum* infection had both SOD ( $P < 0.05$ ) and CAT ( $P \leq 0.001$ ) activities reduced. As compared to hydatid cyst infections, the group with *D.*

*dendriticum* infection showed reduced erythrocyte SOD ( $P < 0.05$ ) activity. As compared to the control group, the groups with parasitic infection showed reduced vitamin A concentration ( $P < 0.001$ ), whereas vitamin C, vitamin E, and  $\beta$ -carotene concentrations were not affected by infection ( $P > 0.05$ ) (Table 3).

### 4. Discussion

It has been reported that host biochemistry shows some alterations from normal physiology in animals with parasitic infections (5,29). Serum ALT and AST activities were reported to increase in mice experimentally infected by *D. dendriticum* (30). Yüksek et al. (31) indicated that serum ALP and AST activities were higher in sheep infected by endoparasites compared to a control group, and they attributed this increase to necrosis and/or cholestasis of hepatic cells. Parallel to what Yüksek et al. (31) reported, this study found that sheep infected by endoparasitic *D. dendriticum* and hydatid cysts had increased AST and ALP activities.

The current study also agrees with the report of Yüksek et al. (31) in that it detected increased total bilirubin levels in sheep with endoparasite infections compared to the controls. This increase is thought to result from intrahepatic cholestasis or extrahepatic bile duct obstruction due to destruction and excretion of parasites from the liver (31). On the contrary, Samadieh et al. (19) suggested that serum total bilirubin levels were significantly lower in *D. dendriticum*-infected sheep. Although reduced serum total protein and albumin levels were reported by Yüksek et al. (31) in sheep with endoparasitic infections and by Tanritanir et al. (6) in goats with endoparasitic infections, Ayaz et al. (32) reported increased total plasma protein levels but reduced albumin levels in sheep infected by hydatid cysts. In the current study, total plasma

**Table 1.** Some plasma enzyme activities and metabolite levels in the control and groups infected with parasites (n = 10).

Parameters	Control	<i>D. dendriticum</i>	Hydatid cysts	P
AST (U/L)	91.32 ± 6.20 <sup>c</sup>	128.65 ± 6.03 <sup>a</sup>	110.06 ± 2.84 <sup>b</sup>	<0.001
ALT (U/L)	13.00 ± 1.17	17.70 ± 2.07	18.34 ± 1.18	>0.05
GGT (U/L)	40.10 ± 2.63	44.95 ± 2.22	46.32 ± 3.63	>0.05
ALP (U/L)	182.90 ± 10.79 <sup>b</sup>	255.03 ± 54.99 <sup>a</sup>	266.31 ± 23.31 <sup>a</sup>	<0.05
T. cholesterol (mg/dL)	56.62 ± 4.46	64.90 ± 5.52	66.35 ± 3.69	>0.05
T. bilirubin (mg/dL)	0.08 ± 0.008 <sup>c</sup>	0.12 ± 0.014 <sup>b</sup>	0.16 ± 0.013 <sup>a</sup>	<0.001
T. protein (g/dL)	12.31 ± 0.42 <sup>a</sup>	10.44 ± 0.96 <sup>b</sup>	7.01 ± 0.21 <sup>c</sup>	<0.001
Albumin (g/dL)	3.39 ± 0.14	3.21 ± 0.23	2.91 ± 0.13	>0.05

<sup>a-c</sup>: Values within each row with different superscripts differ significantly.

**Table 2.** Some mineral levels in the control and groups infected with parasites (n = 10).

Parameters	Control	<i>D. dendriticum</i>	Hydatid cysts	P
Ca (mg/dL)	13.25 ± 0.79	13.21 ± 0.69	13.68 ± 0.71	>0.05
Pi (mg/dL)	10.09 ± 0.57	10.41 ± 0.41	9.88 ± 0.45	>0.05
Mg (mg/dL)	3.28 ± 0.16 <sup>ab</sup>	3.89 ± 0.39 <sup>a</sup>	2.90 ± 0.23 <sup>b</sup>	<0.05
Zn (ppm)	0.93 ± 0.057	0.96 ± 0.05 (n = 8)	0.91 ± 0.05	>0.05
Cu (ppm)	1.06 ± 0.12	0.95 ± 0.06 (n = 8)	0.74 ± 0.09	>0.05

<sup>a,b</sup>: Values within each row with different superscripts differ significantly.

**Table 3.** Erythrocyte MDA levels, SOD and CAT activities, and plasma vitamin A, C, E, and β- carotene concentrations in the control and groups infected with parasites (n = 10).

Parameters	Control	<i>D.dendriticum</i>	Hydatid cyst	P
MDA (nmol/g Hb)	52.06 ± 3.92 <sup>b</sup>	70.42 ± 3.47 <sup>a</sup>	76.77 ± 5.32 <sup>a</sup>	<0.05
SOD (U/g Hb)	432.28 ± 32.98 <sup>b</sup>	338.61 ± 28.16 <sup>a</sup>	425.91 ± 15.82 <sup>b</sup>	<0.05
CAT (k/g Hb)	64.16 ± 7.25 <sup>a</sup>	43.17 ± 7.10 <sup>b</sup>	30.78 ± 5.15 <sup>b</sup>	<0.001
Vitamin A (µg/dL)	63.75 ± 4.48 <sup>a</sup>	37.93 ± 2.82 <sup>b</sup>	33.88 ± 2.12 <sup>b</sup>	<0.001
β-Carotene (µg/dL)	6.76 ± 0.73	5.89 ± 0.73	6.32 ± 0.64	>0.05
Vitamin C (µg/mL)	5.46 ± 0.40	4.44 ± 0.38	4.63 ± 0.26	>0.05
Vitamin E (mg/dL)	0.13 ± 0.02	0.11 ± 0.03	0.12 ± 0.02	>0.05

<sup>a,b</sup>: Values within each row with different superscripts differ significantly.

protein level was reduced in the group infected with *D. dendriticum* and hydatid cysts compared to the control group. Decreased total protein level has been speculated to result from disturbed hepatic protein synthesis (31).

Minerals are important molecules that take part in sustaining physiological functions and protecting organisms against disease (4). It has been reported that endoparasites lead to a reduction in some trace elements (5). Heiderpour et al. (33) showed that serum Cu, Zn, and Fe levels were significantly decreased in sheep with liver cystic echinococcosis. Yüksek et al. (31) reported significantly lower plasma Cu, Mg, and Zn levels in sheep with endoparasitic infection than the control group. The present study found a significant decrease in plasma Mg levels in the group with *D. dendriticum* infection compared to hydatid cyst infections (P < 0.05).

Parasites are known to generate free radicals and induce lipid peroxidation in organs, tissues, and cells (34). Activated phagocytes release free radicals onto a parasite's surface. The generation of free radicals follows the macrophage activation via cytokines from stimulated lymphocytes, although they act nonspecifically as effectors of resistance (35). Hydrogen peroxide, superoxide anion,

and hydroxyl radicals cause oxidative damage. Oxidative damage results in structural alteration in membrane release of cell and organelle contents and essential fatty acids loss, which is seen with cytosolic aldehyde and peroxide product formation. The end product of free radical reactions on membrane fatty acids is called MDA (36). Free radicals have been reported to play a role in the pathogenesis of parasitic infections (10,11,36). Many studies to date have reported increased blood concentrations of lipid peroxidation products during parasitic infections (14–19). The results of this study were in agreement with those reported by Şimşek et al. (16) and Samadieh et al. (19), who showed increased serum MDA concentration in sheep infected with *D. dendriticum*. An increase of MDA concentration showed that *D. dendriticum* infection evokes significant changes in the oxidative status of infected hosts (36). Some studies showed that serum MDA levels were increased in sheep (33) and cattle (37) with liver cystic echinococcosis. These studies are in agreement with the present study. The increases in the MDA of sheep with *D. dendriticum* or hydatid cyst infection were due to reduced activity of the antioxidant defense system, protecting from free radical injury (16,37).

Antioxidant systems such as SOD, CAT, and GPx protect cells from detrimental effects of free radicals against lipid peroxidation induced by parasites (12). SOD is the major enzyme that plays an important role in the first level of antioxidant defense in the cell. This enzyme dismutates the superoxide radical (38). CAT detoxifies the hydrogen peroxide produced from SOD action. CAT also converts hydrogen peroxide into water (13,38). Özkurt et al. (39) reported that SOD activity is reduced but CAT activity is insignificantly increased in the erythrocytes of nematode-infected Kilis goats. This study, however, demonstrated significant reductions in only CAT activities in sheep infected by hydatid cysts, while the sheep infected by *D. dendriticum* had a significant reduction in both SOD and CAT activities. A significantly increased MDA level and significantly reduced SOD and CAT activities strongly suggest that parasitic infections increase free radicals and thereby induce membrane lipid peroxidation and cellular injury

in cells of the host (40). Tanritanir et al. (6) reported that serum vitamin A concentration was reduced in goats infected by *Linognathus africanus* and Trichostrongylidae sp. The present study also documented reduced plasma vitamin A concentration in groups infected by other parasites compared to the control group. It is considered that vitamin A concentration is reduced because it is affected by the type of parasite and host (6).

In conclusion, endoparasitic infections such as *D. dendriticum* and hydatid cysts caused oxidative stress and changed some biochemical parameters (AST, ALP, total bilirubin, and total protein) in sheep.

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