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Erythrocyte Superoxide Dismutase, Catalase Activity and Malondialdehit Level in Hypodermosis

[1]Guzin Ozkurt-Borazan, [2]M. Sinan Aktas, [3]Ilker Camkerten,
[4]Ahmet Gokçen, [5]Hudai Ipek, [3]Tekin Sahin and [1]Nilgun Uren-Paksoy
[1]Department of Biochemistry, Faculty of Veterinary Medicine,
Harran University, Sanliurfa, Turkey
[2]Department of Internal Medicine, Faculty of Veterinary Medicine,
Ataturk University, Erzurum, Turkey
[3]Department of Internal Medicine,
[4]Department of Parasitology, [5]Department of Physiology,
Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey

Abstract: The aim of this study was to examine erythrocyte Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehit (MDA) level in bovine with hypodermosis. Study included 9 cattle diagnosed as hypodermosis (study group) and 10 healthy cattle (control group) making a total of 19 in the age group between 16 and 18 months montafon heifer. Parasitological examination in all larvae revealed *Hypoderma bovis*. No significant differences were observed in erythrocyte SOD, CAT activities and MDA levels between control and study groups. However, mean average of decreased SOD and CAT activities and increased MDA level associated with presence of oxidative stress and antioxidative imbalance in bovine with hypodermosis.

Key words: *Hypoderma bovis*, bovis, SOD, CAT, MDA, antioxidative

INTRODUCTION

Parasitic diseases of the skin are of major economic importance. Discomfort and pruritis interfere with the normal rest and feeding of the animal and the loss of protective function of the skin facilitates bacterial infection. In addition, the commercial value of the hides is often reduced.

Hypodermosis is a parasitic disease due to the development of the larval stages of insects inducing myiasis and belonging to the *Hypoderma* genus. Cattle hypodermosis is a myiasis caused by larvae of *Hypoderma bovis* and *Hypoderma lineatum* and is characterised by the presence of warbles under the skin of infested animals. Hypodermosis may also infect humans accidentally (Balkaya *et al*., 2010). Myiases impair animals' welfare and cause severe economic losses to the livestock industry, both in developing and developed countries through abortion, reduction of milk production and losses in terms of weight gain, fertility and hide quality (Treversa and Otranto, 2006).

Free radicals and other reactive oxygen species (ROS) in the form of the superoxide anion ($O2^-$), Hydrogen Peroxide (H_2O_2) and the hydroxyl radical (OH^*) cause damage to DNA, lipids and proteins. ROS are produced in metabolic and physiological processes and harmful oxidative reactions may occur in organisms which remove them via enzymatic and nonenzymatic antioxidative mechanisms. Under certain conditions such as human and canine leishmaniosis, Behcet's diseases, pneumonia, etc., the increase in oxidants and decrease in antioxidants cannot be prevented and the oxidative/antioxidative balance shifts towards the oxidative status (Camkerten *et al*., 2009).

There is compelling evidence that oxidative stress drives the production of oxidation products, such as 4-hydro-xy-2-nonenal or Malonaldehyde (MDA) which can denature proteins, alter apoptosis and influence the release of proinflammatory mediators, such as cytokines which may be critical for the induction of some inflammatory skin diseases. This is also based on the recognition that ROS can act as second messengers in the induction of several biological responses, such as the activation of NF-kB or AP-1, the generation of cytokines, the modulation of signalling pathways, etc. Antioxidant molecules, including Superoxide Dismutases (SODs) and Catalase (CAT), in the skin interact with ROS or their byproducts to either eliminate them or to minimize their deleterious effects (Bickers and Athar, 2006).

A possible roles of the highly ROS in the pathogenesis of parasitic infections has been an active area of research in recent years and we could not

Corresponding Author: Guzin Ozkurt-Borazan, Department of Biochemistry, Faculty of Veterinary Medicine, Harran University, 63100 Eyyübiye, Sanliurfa, Turkey

determine a study in the literature that examined oxidant and antioxidant status in bovine with *Hypoderma Bovis* infestations. Aim of this study was to examine erythrocyte Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehit (MDA) level in bovine with hypoderma bovis.

MATERIALS AND METHODS

Selection of animals: Study included 9 cattle diagnosed as hypodermosis by clinical and parasitological examination (study group) and 10 healthy cattle residing in special feedlot of Erzurum province in Turkey (control group) making a total of 19 in the age group between 16 and 18 months montafon heifer. Fecal samples were negative for eggs of internal parasites and no detectable sings of any other systemic disease were observed in study group. Healthy cattles had good body condition. Vital signs and feed intake were normal in control group. Cattles had 15-20 warbles on backs in study group. Larvae were identified according to the morphological and characteristics criteria described by Zumpt (1965).

Sample collection: Blood samples of 9 mL volume were drawn from the jugular veins of the animals. Test tubes containing heparin were used to measure erythrocyte SOD, CAT and MDA. Samples were transferred to laboratory in a cold media and erythrocyte was prepared as previously described by Winterbourn *et al.* (1975). Briefly, the plasma and buffy coat were removed after centrifugation at 3000 rpm for 10 min at 4°C. The sedimented erythrocytes were washed 3 times with PBS. After each wash, the tubes were centrifuged at 3000 rpm for 20 min at 4°C. The erythrocyte sediments were stored at -70°C until analysis.

Estimations

Hemoglobin: Haemoglobin is oxidised to methaemoglobin by potassium ferricyanide. The methaemoglobin is then converted to the stable cyanmethaemoglobin by the addition of potassium cyanide. The formation of cyanmethaemoglobin is measured at 540 nm and is proportional to the haemoglobin concentration in the sample (Tietz, 1976).

Erythrocyte CAT: Erythrocyte CAT (EC 1.11.1.6) activity was determined by the method of Aebi (1974) using a UV spectrophotometer. The principle of the reaction is based on determination of the rate constant k of H_2O_2 decomposition. By measuring the absorbance change per min, the rate constant was determined. Activities were expressed as k g^{-1} Hb.

Erythrocyte SOD: Total SOD (EC 1.15.1.1) activity in erythrocytes was assayed by the method of Sun *et al.* (1988). The method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the sample after 1.0 mL of an ethanol/chloroform mixture (5:3, v/v) was added to an equal volume of erythrocyte sediment and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition of the NBT reduction rate. SOD activity was expressed as U g^{-1} Hb.

Erythrocyte MDA: Erythrocyte MDA levels were determined by the method of Buege and Aust (1978). Briefly, 250 μL erythrocyte; 500 μL of TBA reagent and 1.5 mL of 15% trichloroacetic acid (in 0.25 mol L μ HCl) were combined, mixed and heated for 30 min in boiling water. After cooling in an ice bath, centrifuged at 14000 rpm/5 min. The supernatant was determined spectro-photometrically at 535 nm against a blank.

Statistical analyses: Data were analyzed by non-parametric statistics (Mann-Whitney U test) using the Statistical Package for Social Sciences (SPSS, 11.0 Chicago, IL, USA) software for Windows. Results were expressed as mean±SD.

RESULTS AND DISCUSSION

Parasitological examination in all larvae revealed *Hypoderma bovis*. No significant differences were observed in erythrocyte SOD, CAT activities and MDA levels between control and study groups. Results of analyzed parameters are shown in Table 1.

In the present study, we found that the oxidative/antioxidative balance not altered significantly. To the best of the knowledge this is the first report showing that an association of SOD, CAT activity and MDA levels in hypodermosis.

Cattle hypodermosis is a myiasis caused by larvae of *Hypoderma* sp. and is characterised by the presence of warbles under the skin of infested animals (Balkaya *et al.*, 2010). *Hypoderma* sp. first instars regurgitate great amounts of three serine proteinases (i.e., hypodermins A, B and C) which allow their entry through the unbroken skin or follicular openings and subsequent migration within the host tissues. These proteins are also implicated in immunomodulatory processes in both the inflammatory and the specific immune responses that allow the survival of first instars (Dacal *et al.*, 2009). According to

Table 1: Erythrocyte SOD, CAT activities and MDA level in hypodermosis

Parameters	Control group	Study group	Insignificant
CAT (k g^{-1} Hb)	0.56±0.1700	0.49±0.2000	-
SOD (U g^{-1} Hb)	607.07±162.05	555.41±107.63	-
MDA (μmol L^{-1})	0.32±0.1900	0.47±0.4000	-

SOD: Superoxide Dismutase, CAT: Catalase MDA: Malondialdehit

Briganti and Picardo (2003) oxidative stress and antioxidant imbalance could play a pivotal role in the pathogenesis of skin diseases.

SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical ($^{\cdot}O_2$). Generated H_2O_2 in this reaction is restored to water in the presence of CAT and GPX. Alternatively CAT can catalyze the peroxidasis reaction. In normal conditions, CAT is of no great importance of most cell types but in the presence of oxidative stress it is the most adaptive antioxidant enzyme and plays a significant role in cell defense against oxidative damage. SOD is the most abundant antioxidant enzyme in animals (Todorova *et al*., 2005). MDA is the major and stable end product formed during the peroxidation of lipids. Serum/plasma level of MDA is regarded as a standard biochemical marker of lipid peroxidation and serves as a marker of cellular damage caused by free radicals (Abdel-Hafez *et al*., 2010).

In this study, there were no significant differences of SOD, CAT activities and MDA levels. However, mean average of decreased SOD and CAT activities and increased MDA level associated with presence of oxidative stress and antioxidative imbalance in bovine with hypodermosis.

CONCLUSION

As a conclusion, SOD, CAT activity and MDA levels determined in our study may contribute to the studies in the future as basic data in bovine with hypodermosis. The results associated with oxidative stress; however, in order to more definitively delineate the pathogenesis of hypodermosis, further studies are necessary.

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