

## **Assessment of serum antioxidant enzymes activity in cattle suffering from Theileriosis**

**Ali Hassanpour<sup>1\*</sup>, Yousef Ghader Sabegh<sup>1</sup> and Ali Sadeghi-nasab<sup>2</sup>**

<sup>1</sup>*Department of Clinical Science, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz- Iran*

<sup>2</sup>*Department of Clinical Sciences, Faculty of Para Veterinary Sciences, Bu-Ali Sina University, Hamedan, I.R. Iran*

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### **ABSTRACT**

*In this research, serum activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase and total antioxidant capacity (TAC) of 37 cattle suffering from theileriosis were compared with 35 healthy ones. Mean± SD of all variables in patients and control animals were significantly different [SOD 7.07±1.26 vs. 10.70±1.98 u/mgHb (P=0.001), GSH-Px 2.33±0.89 vs. 26.67±0.497 u/mgHb and catalase activity 0.96±0.497 vs. 2.33±0.89 u/mgHb (P=0.0001), respectively. Serum TAC of cases (0.71±0.21 mmol/l) were significantly (P=0.001) lower than healthy group (1.62±0.35 mmol/l). There was only a significant correlation coefficient relationship between serum catalase activity and TAC levels of affected cows (P=0.049, r=0.389). According to these results, oxidative enzymes activities and TAC could be dramatically decreased in this protozoan infection and it is concluded that improving this condition might accelerate treatment procedure.*

**Key words:** total antioxidant capacity, theileriosis, cattle.

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### **INTRODUCTION**

Tropical theileriosis is a progressive bovine lympho-proliferative disease caused by the intracellular protozoan parasite *Theileria annulata* [18,19,21]. The infection is widespread, particularly in the Mediterranean Europe, Middle East, India, middle Asia and even China which impose economic losses to dairy industries [12,20]. Like other parasitic diseases involve erythrocytes, biochemical and hematological changes are conceivable in theileriosis too [5,11,16]. Besides of immune mediated reactions contributing in pathogenesis of theileriosis [10], there are also so many studies in which evaluated several actions and/or interrelationship of oxidant process by antioxidant enzymes [4,15,17]. The antioxidant system consists of several antioxidant substances such as SOD, catalase, GSH-Px and etc. Antioxidants may act by scavenging the radicals directly and sustaining the activity of antioxidant enzymes or inhibiting the activity of oxidizing enzymes [1]. Intra-erythrocytic parasite, *Theileria annulata* [3,8,12,13,14] and *Babesia bovis* [7] infection in metabolize hemoglobin and produce free radicals, which, in turn, cause increased oxidative stress as indicated by a significant increase in lipid peroxidation in erythrocytes [2,9].

The purposes of this study was to evaluate the activities of the key antioxidant enzymes (SOD, GSH-Px and catalase), TAC in the serum naturally infected cattle with *T. annulata*.

## MATERIALS AND METHODS

### Animals

This study was performed in the veterinary hospital of Tabriz Azad University. The study group (Group 1) and healthy control group (Group 2) consisted of 37 theileriosis infected (17 males, 20 females) and 35 healthy Holstein cattle (11 males, 24 females), respectively. Disease confirmation was based on clinical sign, staining of peripheral and lymph node punctures.

### Biochemical analysis

Non coagulated blood samples of two groups were collected through the jugular vein using disposable 14 needles and plastic syringes. The specimens were centrifuged at 1800 ×g and the non hemolyzed sera were stored at -20 °C until biochemical analysis.

Serum activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase and total antioxidant capacity (TAC) of both groups were analyzed on Randox diagnostic's kits by automated chemistry analyzer.

### Statistical analysis

Statistical analysis concluded analysis of variance and comparison of mean ± SD by t-test and correlation coefficient between variables were performed using the SPSS version 18 statistical package, 2010, (SPSS Inc. Chicago, IL, USA).

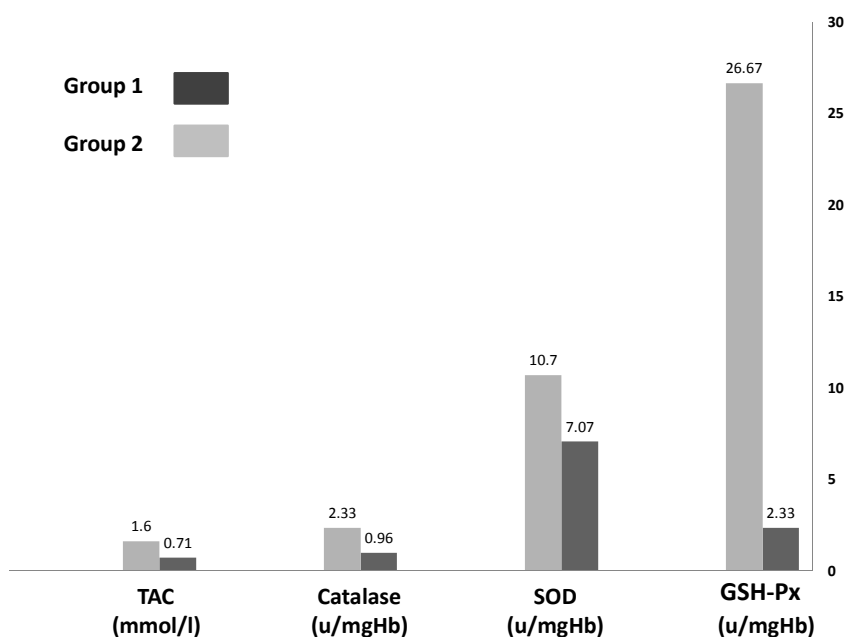
## RESULTS

Statistical analyses of variables are presented in Table 1. The results revealed significant decrease ( $p < 0.001$ ) in the serum levels of SOD and TAC of affected cattle. Serum levels of GSH-Px and catalase were also significantly lower in this group ( $P < 0.0001$ ). There was a positive correlation between serum catalase antioxidant activity and total antioxidant capacity of theileria infected animals ( $r = 0.389$ ,  $P = 0.049$ ).

**Table 1: The comparison of mean ± SD results for serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and total antioxidant capacity (TAC) in patients and healthy cattle**

Variable	Group 1	Group 2	P value
SOD (u/mgHb)	7.07 ± 1.26	10.70 ± 1.98	0.001
GSH-Px (u/mgHb)	2.33 ± 0.89	26.67 ± 0.497	0.0001
Catalase (u/mgHb)	0.96 ± 0.497	2.33 ± 0.89	0.0001
TAC (mmol/l)	0.71 ± 0.21	1.6 ± 0.35	0.001

\* Group 1, patients; Group 2, healthy cattle.



**Figure 1: Serum total antioxidant capacity (TAC), catalase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) changes in Group 1 (patients) and Group 2 (control).**

**Table 2: Pearson Correlation between activities of antioxidant enzymes and total antioxidant capacity of theileria infected animals.**

Variable	SOD	GSH-Px	Catalase	TAC
SOD	1			
GSH-Px	0.003 (0.988)	1		
Catalase	0.062 (0.716)	-0.01 (0.995)	1	
TAC	-0.182 (0.282)	-0.066 (0.696)	0.389 (0.049)	1

\*\* SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; TAC, total antioxidant capacity.

\*\*\* P-values are presented in parenthesis.

## DISCUSSION AND CONCLUSION

Theileriosis is one of the prevalent protozoans infectious diseases in country which imposes significant economic losses to dairy industries and already its anemia pathogenesis have been explained [10]. Moreover, free radicals and antioxidants enzymes interactions [3] may play integral roles in different aspects of pathogenesis and finally in a patient destiny.

In the present study, serum GSH-Px activity of group 1 (patients) was significantly lower than group 2 (Table 1). This finding is agreement with other studies [3,13,19]. GSH-Px activity is a major mechanism for intracellular decomposition of lipid peroxides and has a crucial role in membranes protection from them [3,19]. Reduced glutathione is required for the disposal of H<sub>2</sub>O<sub>2</sub> from erythrocytes by a reaction catalyzed by GSH-Px. This reaction is important because accumulation of H<sub>2</sub>O<sub>2</sub> might decrease the lifespan of erythrocytes by increasing the rate of oxidation of hemoglobin to methemoglobin [3,19]. G6PD enzyme is the principal source of NADPH, which helps in maintaining glutathione in the reduced state, thus protecting erythrocytes from oxidative stress. G6PD serves as an antioxidant enzyme and decreased activity of G6PD has been associated with increased hemolysis in buffaloes affected with theileriosis and increased oxidative stress in endothelial cells [3]. Incompatible with our results, Grewal *et al.*, (2005) reported that GPX activity exhibits a significant rise in cattle naturally infected with *T. annulata*, whereas SOD and catalase showed no substantial changes. They concluded that the increased level of GPX during parasitemia could be due to the fact that this enzyme activity is the major mechanism for intracellular destruction of lipid peroxides rather than SOD or catalase activity. Also, they believed that the activity of catalase might have a role in concert with GPX to scavenge peroxides. In our finding the maximum lessen is related to this enzyme [8]. According to this finding, we speculate that this highly reduction is related to its mentioned crucial role in membranes protection. By this standpoint, our finding is also agreement [20].

In our study, serum SOD of group 1 was significantly disturbed compared with group 2 by infection [7.07±1.26 vs. 10.70±1.98 u/mgHb (P=0.001), respectively]. The decrease in the activity of SOD may suggest a contributory role of oxidative stress in development of haemolytic anaemia in infected cattle. These results are in accordance with the findings of other researches [3,12].

In agreement with other studies [6,14], catalase activity of affected animals (group 1) were apparently lower than sound cows (group 2), Table 1. The catalytic activity of SOD and catalase enzymes allows the transformation of superoxide anion into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and water, thereby inactivating important amounts of oxidants [17]. A significant decrease in SOD and catalase levels was observed in the present study. The measurement of antioxidant enzyme activities like SOD and catalase are appropriate indirect ways to assess the status of antioxidant defense.

In contrast with our study, there were not significant difference between SOD and catalase activity of naturally affected theileria and healthy cows [8]. They speculated that, SOD might not be the major antioxidant enzyme for protection of erythrocytes in oxidative stress. However, according to Asri, Nazifi and Esmaeilnejad studies, the results of our study also indicated that catalase might be acting in relevance with GSH-Px and SOD [3,7,12,13,14]. Moreover, corresponded to El-Deeb study, there was significant (p<0.001) reduction in the levels of TAC in infected cows. This reduction may be attributed to the reduction in antioxidant enzymes as they are consumed by excessive free radicals in the infected animals [6].

There was only a positive significant correlation coefficient relationship between serum catalase activity and TAC levels of affected cows (P=0.049, r=0.389) but there were no significant correlation between examined traits (Table 2). In contrast with our finding, there were significant correlations between these variables in other studies [3,7]. It is likely; these inconsistent results may be due to different procedure of measurement. We have evaluated antioxidant enzymes in serum but they performed their studies on whole blood. Using Tert-Butyl Hydroperoxid as an oxidative

substrate evaluates total glutathione peroxidase (dependent and independent selenium), but by using Cumene Hydroperoxide as an oxidative substrate selenium dependent glutathione peroxidase could be measured. In some studies H<sub>2</sub>O<sub>2</sub> have been used for this purposes. The second reason which may be relevant to these finding is sample size and grouping of the affected animals.

In conclusion, our findings could demonstrate that the dynamic balance between oxidation and antioxidation process in theileriosis were severely damaged. Therefore, antioxidant agent recommendation, such as vitamin C and vitamin E may alleviate oxidative damages [22]. These vitamins are free-radical scavengers and could have a protective effect against further oxidative damage and might be better in clinical improvement.

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