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Antioxidant Status and Lipid Peroxidation in Erythrocyte of Dog Infested with *Demodex canis*

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Abstract

The study was conducted on fifteen dogs (n = 8 demodicosis, n = 7 healthy) brought to Veterinary Clinics, Bihar Veterinary College Clinics to determine Antioxidant Status and Lipid Peroxidation in erythrocyte of Demodex canis infested dog. Activity of catalase (CAT) and superoxide dismutase (SOD) and Thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) concentration were estimated in erythrocytes of dogs. Activity of CAT, SOD and TBARS concentration were higher in infected dogs than healthy dogs while and GSH concentration was higher in healthy dog than infected dogs. The results of the present study suggest that Demodex canis infestation cause oxidative stress in erythrocyte of dogs.

Keywords: *Demodex, SOD, GSH, Antioxidant, CAT*

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INTRODUCTION

Canine demodicosis is a common skin problem of dogs associated with proliferation of *Demodex canis* in canine hair follicles which lead to development of cutaneous lesion. The disease is often localized to one or more discrete foci that regress spontaneously or may progress to widespread generalized cutaneous lesions. The initial lesions include alopecia and scale formation, but secondary bacterial infection often include pustular and crustating dermatitis [1]. Further, canine demodicosis associated with oxidative stress which may contribute to the development of immunosuppression. Oxidative stress is defined as a situation where the production of the free radicals exceeds the antioxidative process necessary to detoxify these toxic molecules, resulting in molecular disruption and tissue damage. The mite triggers the pro-inflammatory cascade leads to excessive generation of the reactive oxidants, free radicals which includes reactive oxygen species (ROS) such as; hydroxyl radical (OH^*), superoxide anion radical (O_2^{*-}) and reactive nitrogen species (RNS) such as nitric oxide radical (NO^*) in the biological system. These free radicals play a key role in host defence against invading parasite, but when generated at high levels they can result in metabolic dysfunction and biomolecular oxidative damage, which contribute to pathological changes in the tissue. In skin diseases, the body possess an array of potent antioxidant protection such as; superoxide dismutase (SOD), catalase (CAT), reduced Glutathione (GSH), GSH-peroxidase and the antioxidant vitamins A, E and C [2]. The measurement of lipid peroxidation in terms of Thiobarbituric acid reactive substances (TBARS), reduced Glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) in biological samples is widely used for determination of oxidative stress in an organism. There is paucity of literature about the antioxidant status in canine erythrocytes infested with canine demodicosis therefore, the present study was undertaken to assess the antioxidant level and lipid peroxidation in erythrocytes of dogs infested with *Demodex canis.*

MATERIALS AND METHODS

This study was performed on eight dogs naturally infested with *Demodex canis* and seven healthy dogs presented to Veterinary Clinics, Bihar Veterinary College, Patna (India). Dogs irrespective of their sex, age and breed were included in the study. The microscopic examination of skin scrapping

showed adults as well as the developmental stages of the mites, but it was free from fungi and yeast. Blood samples were aseptically collected from cephalic vein in heparin coated vial from all dogs and centrifuged at 700 × g for 15 min. Plasma and buffy coat was removed and the erythrocytes were washed thrice with phosphate buffer saline, pH 7.4.

The packed cells were re-suspended in the same solution to give a 33 percent suspension. Thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) concentration were measured in a fresh suspension. Stock hemolysate (1:10) was prepared from cell suspension by mixing 1 volume of cells with 9 volume ice cold water. The erythrocyte hemolysate was used for estimation of CAT and SOD. Hemolysates were stored at -20^0C until the enzyme assays.

Activity of SOD was measured by using MTT reduction microtiter plate method [3]. CAT activity was estimated by the method of Aebi [4] and value was expressed in K/g of hemoglobin (K: rate constant of first order reaction). GSH level was estimated using the method of Prins and Loos [5]. TBARS concentration was measured by the method as described by Stock and Dormandy [6], while Hemoglobin (Hb) concentration was measured by using the Drabkin's solution (Span Diagnostic, India). Data has been presented as mean and standard error (SE). Statistical significance was measured by the independent sample't' test with level of significance of $p < 0.05$ using the statistical package SPSS 17.0 version.

Table 1: *Antioxidant Status and Lipid Peroxidation in Canine Erythrocyte (mean ± SE).*

Enzyme	Control	Infected
SOD activity U/mg Hemoglobin	30.14 ± 0.28^a	33.38 ± 0.18^b
CAT activity K/mg Hemoglobin	94.85 ± 2.4^a	118.37 ± 2.18^b
TBARS concentration nmol/mg Hemoglobin	0.09 ± 0.002^a	0.406 ± 0.019^b
GSH concentration nmol/mg Hemoglobin	0.94 ± 0.01^b	0.55 ± 0.01^a

Means bearing different superscript (a, b) in a row differ significantly (p<0.05).

RESULTS AND DISCUSSION

The results are presented in Table 1. Erythrocyte superoxide dismutase (SOD) and catalse (CAT) activity was significantly higher (P>0.05) in infected group than healthy group. A significant (P>0.05) higher TBARS concentration was found in infected group than healthy group and Healthy group had significantly (P>0.05) higher GSH concentration than infected group. The high erythrocyte SOD and CAT activities in infected group are probably attributed to Oxidative stress caused by *Demodex canis* infestation and an altered antioxidant defence mechanism is under operation. Both CAT and SOD play important role in combating oxidative stress [7]. SOD which exists in three different forms viz. SOD1, SOD2 and SOD3 are known to scavenge both intra and extra cellular superoxide radicals produced by a number of reactions as a part of normal cellular functions [8], the most important being the auto oxidation or oxidation of hemoglobin (Hb-Fe^{2+} in to Hb- Fe^{3+}), which result into continuous formation of superoxide [9]. In the present study, the higher erythrocyte SOD activity in infected group was probably a response to higher superoxide generated in erythrocytes. SOD catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide. Thus a higher SOD activity leads to increase in hydrogen peroxide level in the cells. This hydrogen peroxide is neutralized by a coordinated increase in CAT activity [10]. Present findings are in well concurrence with work published elsewhere [11, 12]. In present investigation a high TBARS concentration in infected group than healthy group indicate increased lipid peroxidation in demodex infested dogs. The present finding is in accordance with earlier research in canine demodicosis [13]. Erythrocyte membrane is rich in polyunsaturated fatty acids. The free radicals, generated during oxidative stress, attack the RBC membrane lipids which results in to initiation of lipid peroxidation [14], which results in formation of lipid centric free radicals. The lipid centric free radicals rearrange and react with molecular oxygen to form lipid hydro peroxide [15]. These hydro peroxides appear in as TBARS and are biochemical markers for lipid peroxidation [16]. GSH play key role in the antioxidative defence system. GSH reacts with

a range of ROS such as H_2O_2 and O_2^- which is the basis of its antioxidant action. Glutathione annihilates oxygen toxicity by interrupting the reaction leading to superoxide formation [17]. The lower GSH in the infected group than in the healthy group observed in the present study may be due higher ROS production in demodex infected dogs. Similar type of finding is also reported in earlier finding in canine demodicosis [13]. The results of the present study suggest that *Demodex canis* infestation causes oxidative stress in the erythrocytes of dog.

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