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Oxidative Stress Biomarkers in Dermatophytosis Infected Dogs

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ABSTRACT

Skin affections especially of fungal etiology are common problem faced on daily basis in canine practice. This work aimed to investigate selected oxidative stress biomarkers in dermatophytosis infected dogs. Clinical studies were applied on 20 dog and for investigation of hematological, biochemical and oxidative stress biomarkers. Leukocytosis ($p \leq 0.01$)along with monocytopenia($p \leq 0.05$) and significant elevation in ALP($p \leq 0.05$) were the most recorded hemat-biochemical alterations. The oxidative stress biomarkers showed significant decrease in glutathione peroxidase (GPX)($p \leq 0.05$) and plasma zinc levels($p \leq 0.05$). Dermatophytes infection represents a stress factor to the animal affecting its antioxidant mechanism system.

Key words: Dog, skin affections, hematology, biochemistry, oxidative stress biomarkers.

INTRODUCTION

Skin status considered a mirror of animal health (Merck and Merial, 2007). In canine practice, skin affections may be originated either from infectious or non-infectious causes (Verde, 2005). Dermatophytosis is a superficial mycosis affecting the skin, many fungal agents are incriminate; however, Microsporum, Trichophytonor Epidermophyton fungi are the mostcommon in canine population.These fungi required keratin for their growth, they can be isolated from skin, hair and nails (Hainer, 2003).
Dermatophytes infection is known to cause circumscribed pruritic zone with elevated erythematous margins and scabbed center (Wright and Allingham, 1976).

Oxidative stress is a mechanism resulted from overproduction of Reactive oxygen species (ROS), which responsible for biomolecular damage involved in pathological changes in cells and tissues. It can be manifested by lipid peroxidation, DNA mutations/breakage, enzyme inactivation/activation, and protein oxidation/degradation (Özben , 1998).
So, this work aim to investigate hematological, selected biochemical and oxidative stress biomarkers (Superoxide dismutase (SOD), Glutathion eperoxidase (GPX), Catalase (CAT) and plasma zinc levels) in dermatophytosis infected dogs.

MATERIALS AND METHODS

a) Experimental animals:
Twenty dog of different breeds (15 German shepherd, 2 Boxer, 1 pit-bull, 1 Mastiff, and 1 Golden retriever), ages ranged from 2 months- 4.5 years and of both sex (13 male and 7 female) were referred to a small animal medicine teaching hospital, faculty of veterinary medicine, Cairo University, Egypt. At time of admission, clinical signs were recorded, animals exposed to a comprehensive clinical examination, stool and skin scraping samples were examined microscopically (Houston 2000). Animals were classified into:
1) Normal control group:
Includes 10 apparently healthy dogs.
2) Dermatophytosis infected group:
Includes 10 diseased dogs.

b) Hematological analysis:
Venous blood was collected from the cephalic vein; complete blood picture was done within 2 hours according to methods described by Feldman et al., (2000).

c) Biochemical analysis:
Serum samples were collected from affected animals to estimate ALT, ALP, BUN and creatinine using respective test kits (Spectrum diagnostics, Egypt).

d) Oxidative stress biomarkers estimations:
Blood samples were collected using EDTA and heparinzed anticoagulant tubes. Erythrocytes lysates preparationand heparinized plasma samples were made according to manufacturer instructions (BioDiagnostic Company-Egypt).

Erythrocytes lysates were used for estimation of levels of superoxide dismutase (SOD) and glutathione peroxidase (GPX) enzymes according to methods described by Nishikimi et al., (1972) and Paglia and Valentine, (1967) respectively. Heparinized plasma samples were taken to determine catalase and zinc levels. Plasma catalase level was determined according to Fossati et al., (1980) and Aebi (1984) using Catalase kits and plasma zinc level was determined according to Hayakawa (1961) with respective test kit.

e) Statistical analysis:

The obtained data were analyzed using ANOVA test using SPSS , statistical program version 16.1 and tabulated as mean value ± SE at levels of significance $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$; the values have the same symbol are not significantly different.

RESULTS AND DISCUSSION

a) Clinical examination:

The most consistent clinical signs observed during the examination of dermatophytosis infected dogs are circumscribed lesion localized on the back as shown in photo (1), accompanied with itching, the lesion of dermatophytes infection was described as a circular up to 5 cm. in diameter, pruritic area may be observed with elevated erythromatous margins, hyper pigmented centers, alopecia, scales and crusts might be found (Wright and Allingham, 1976).

b) Microscopic examination:

Microscopic examination findings are shown in photo (2),the presence of fungal spores (exothrix and endothrix) under microscope is confirmative of the disease (Lappin and Turnwald, 2004).

c) Hematological examination:

Hematologic findings of infected dogs were compared to that of control dogs table (1),significant increase in leucocytes ($p \leq 0.01$)along with statisticaly significant decrease in monocytes ($p \leq 0.05$)were noted among infected dogs but within reference range. The acute steroid response in the stress conditions might be implicated in leucogram changes (Morag, 2008). However, the erythrogram showed insignificant changes. Dermatophytosis is not known to cause anemia (Cheesborough, 2005).

d) Biochemical analysis:

Serum biochemical findings are shown in table (2),significant elevation in ALP value($p \leq 0.05$) was recorded, and this change has been reported also by Abo El Foutah et al., (2012) who found significant increase in liver enzymes (AST, ALT, and ALP) in dermatophytes infected animals($p \leq 0.05$).Also, agreed with Gera et al., (2009) who concluded that stress of dermatosis reflected on serum enzyme (higher in ALT) in affected dogs, while renal parameters (BUN , Creatinine) remain unaffected.

e) Oxidative stress biomarkers analysis:

Oxidative stress analysis was shown in table (3), significant decrease in glutathione peroxidase (GPX) ($p \leq 0.05$) and plasma zinc levels ($p \leq 0.05$) were documented. However similar changes in enzymatic and non-enzymatic oxidative stress biomarkers were recorded by (Khaled et al., 2010).While (Kerem et al., (2009) claimed no change occurred in plasma zinc level.

In conclusion, dermatophytes infection contributes in stress-related skin diseases in dogs was found to affect the antioxidant mechanism system resulting in significant changes in oxidative stress biomarkers activities.

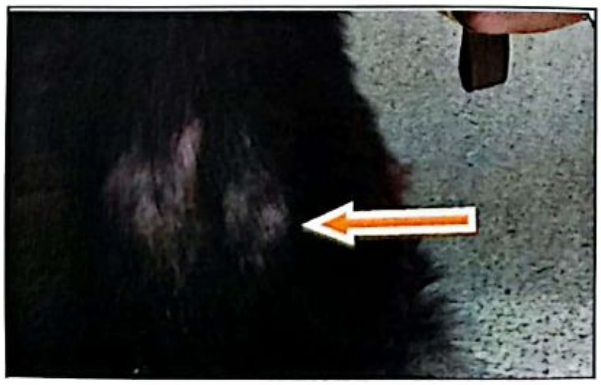

Photo (1). Localized alopecia, scaly, crusted and erythematous lesion to the back of Dermatophytosis infected German shepherd dog.

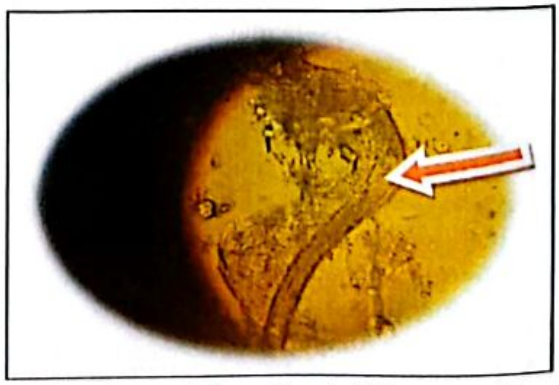

Photo (2). Exothrix and endothrix spores of dermatophytosis under light microscope at 40x.

Table (1). Hematological profiles of dermatophytosis infected dogs.

Animal group / Hemogram	Normal control dogs (n = 10) Mean± SE	Dermatophytosis infected dogs (n=10) Mean± SE
RBCs(× 10^6/mm^3)	7.09 ± 0.381	7.42 ± 0.764
PCV %	46.10 ±1.187	45.70 ± 2.418
Hemoglobin(gm %)	17.35 ±0.476	15.27 ± 1.567
MCV (fl)	68.47 ±3.468	67.34 ± 6.967
MCH (pg)	22.35 ±0.908	21.31 ± 1.550
MCHC (g %)	34.86 ±1.471	33.52 ± 3.007
WBCS (10^3/mm^3)	10.09 ±0.995	17.54 ± 1.663 [b]
Neutrophiles %	67.00 ±2.472	68.29 ± 3.828
Lymphocytes %	25.22 ±1.770	23.43 ± 3.728
Monocytes %	3.22 ± 0.147	2.29 ± 0.360 [c]
Esinophiles %	6.11 ± 0.676	5.14 ± 1.079
Basophiles %	0.000	0.000

Table (2). Biochemical Constituent of dermatophytosis infected dogs.

Animal group / Serum constituents	Normal control dogs (n = 10) Mean± SE	Dermatophytosis infected dogs (n=10) Mean± SE
ALT (u/l)	13.96 ± 1.830	51.85 ± 21.870
ALP (u/l)	123.26 ±10.622	225.63 ±31.536 [c]
BUN(mg/dl)	13.16 ± 0.913	17.25 ± 3.233
Creatinine (mg/dl)	1.10 ± 0.104	2.62 ±0.686

Table(3). Selected Oxidative stress biomarkers of dermatophytosis infected dogs.

Animal group / Oxidative stress biomarkers	Normal control dogs (n = 10) Mean± SE	Dermatophytosis infected dogs(n=10) Mean± SE
SOD (u/ml)	129.49 ±22.841	227.15 ±44.433
GPX(Mu/ml)	513.57±101.837	253.30 ±35.672 [c]
Catalase(u/l)	351.80 ± 67.453	341.86 ± 60.478
Plasmazinc (µg/dl)	204.78 ±8.176	128.17 ± 29.504 [c]

[c]: Weakly significant ($p \leq 0.05$).
[b]: Significant ($p \leq 0.01$).
[a]: Highly significant ($p \leq 0.001$).

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الملخص العربي

دلائل الإجهاد التأكسدى فى الكلاب المصابة بالفطريات الجلدية

تعتبر العدوى الفطرية من اشهر مسببات الأمراض الجلدية التى تواجهنا فى مجال الكلاب .يهدف هذا العمل الى تقييم دلائل الاجهاد التأكسدى فى الكلاب المصابة بالفطريات الجلدية . حيث اجريت هذه الدراسة على عدد 20كلب لاستبيان صورة الدم , المكونات البيوكيميائية و دلائل الاجهاد التاكسدى . أظهرت النتائج زيادة معنوية فى عدد كرات الدم البيضاء ($p\leq0.01$) و مستوى انزيم الالانين امينو ترانفيريس ($p\leq0.05$) مع وجود نقص معنوى فى الوحيدات ($p\leq0.05$) . و أوضحت أيضا وجود نقص معنوى فى مستوى انزيم الجلوتاثايون بيروأوكسديز ($p\leq0.05$) و مستوى زنك البلازما ($p\leq0.05$). نستخلص من هذه الدراسة أن العدوى بالفطريات الجلدية تمثل اجهاد على الحيوان و تؤثر بالسلب على آلية عمل مضادات الأكسدة.