

## The Relationship Between Antioxidant Trace Elements (Zn-Cu and Se) and Oxidative Stress in Dogs Affected with Dermatophytosis

Noha M El-Motaily<sup>1</sup>, Heba S Farag<sup>2\*</sup>, Ossama M Abdou<sup>1</sup>, Saber M<sup>1</sup> and Kawkab A Ahmed<sup>3</sup>

<sup>1</sup>Department of Medicine and Infectious Diseases, Internal Medicine, Faculty of Veterinary Medicine, Cairo University, Egypt

<sup>2</sup>Department of Medicine and Infectious Diseases, Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt

<sup>3</sup>Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt

\*Corresponding author: potta\_amr@hotmail.com

Article History: 22-514

Received: 27-Jan-22

Revised: 05-Feb-22

Accepted: 10-Feb-22

### ABSTRACT

Canine dermatologic problems are among the most common disorders seen in canine practice, caused by the fungus. The current study was planned to observe the relationship between antioxidant trace elements (Zn-Cu and Se) and oxidative stress in dogs affected with dermatophytosis. Briefly, this study comprised of 34 dogs. Compared different parameters including hematological, biochemical, histopathological, and the level of antioxidant trace elements in 14 infected dogs with 20 non-infected dogs (control group). Results showed a significantly higher level ( $P < 0.05$ ) of TLC ( $16.33 \times 10^3$  U/L), MCHC (34.65g/dL), RDW (18.25%), and lymphocyte (47.9%) in the dermatophytosis-infected dogs as compared to the control group. Similarly, serum biochemical findings revealed a significant elevation in cortisol level (709.88nmol/L) in infected dogs while a significant decrease in free T4 (8.57pmol/L) in infected dogs compared to the control group. Furthermore, the level of selenium ( $0.02 \pm 0.00$ mg/dL), copper ( $59.725 \pm 3.01$ mg/dL) and zinc ( $60.99 \pm 2.76$ mg/dL) were significantly lower ( $P = 0.00$ ) in dogs infected with dermatophytosis. The study revealed that dermatophytes infection represents a stress factor affecting the level of antioxidant trace elements and the antioxidant defense system of the infected animal.

**Key words:** Canine dermatophytosis, Hematox-biochemistry, Histopathology, Oxidative stress and Antioxidant trace elements.

### INTRODUCTION

Skin is the essential part of the body performing different functions such as immune protection, thermoregulation, and vitamin D production. It acts as a barrier between the body and the environment (Monteiro-Riviere 2010). However, it can be infected with several pathogens, such as bacteria, ecto-parasites and fungi. Dermatologic problems are among the most seen disorders in veterinary hospitals. It has been reported that approximately 25% of the cases presented in small animal clinics are suffering from dermatological disorders (Scott and Paradis 1990). These problems not only threaten the health status of the animal but also pose a serious threat to pet owners because some of the skin infections, such as pinworm infection, are zoonotic in nature (Hill et al. 2006; Kahn 2007).

Dermatophytosis is a contagious superficial fungal skin disease. It is one of the most frequent skin diseases of dogs referred to ringworm (Chermette et al. 2008; Moriello

2019). Dermatophytosis is a zoonotic disease which can be caused by *Microsporum*, *Trichophyton* and *Epidermophyton* spp. (Verde 2005; Outerbridge 2006; Soedarmanto et al. 2017). Clinically, dermatophytosis is characterized by localized, multifocal, or generalized areas of circular, irregular, or diffuse alopecia with variable scaling. The remaining hairs may appear broken off with erythema, papules, crusts, and seborrhea. Pruritus is usually minimal. These infections are frequently developed on the face and limbs of dogs that spend a lot of time outdoors in contact with the ground (Hnilica 2011; Moretti et al. 2013). Diagnosis of this disease mainly depends on microscopic examination of skin scraping from lesions which may reveal fungal hypha or spores (Sekar and Dogan 2011; Sykes and Outerbridge 2014; Sezer et al. 2021) and PCR is also being used for diagnosis (Méndez et al. 2021).

The keratolytic fungi produce keratinases enzymes that invade keratinized tissue and digest the keratin protein complex, allowing the dermatophyte to burrow deeper into the stratum corneum eliciting inflammatory reaction.

**Cite This Article as:** El-Motaily NM, Farag HS, Abdou OM, Saber M and Ahmed KA, 2022. The relationship between antioxidant trace elements (Zn-Cu and Se) and oxidative stress in dogs affected with dermatophytosis. International Journal of Veterinary Science 11(4): 467-473. <https://doi.org/10.47278/journal.ijvs/2022.137>

It causes excessive generation of the reactive oxidants or free radicals that lead to oxidative stress and consequently the exhaustion of antioxidant system (Chermette et al. 2008; Beigh et al. 2014). These free radicals are involved in the pathogenesis of multiple dermatological disorders (Prado et al. 2008). The overproduction or inadequate removal of reactive oxygen species (ROS) resulted in oxidative stress leading to altered metabolism, tissue damage, lipid peroxidation, protein degradation, and enzyme inactivation (Özben 1998).

During dermatological infections, the body produces various antioxidants that act synergistically to cause degradation of free radicals in combating oxidative damage (Bickers and Athar 2006; Portugal et al. 2007). Usually antioxidants attenuate, delay, or prevent ROS-induced cellular damage. However, the prolonged production of free radical can overwhelm ROS defense mechanisms, contributing to the development of cutaneous diseases (Trouba et al. 2002; Paulsen and Carroll 2013; Nwufoh et al. 2020). Antioxidant trace elements (zinc, copper, selenium) are required for the activity of antioxidant enzymes (glutathione peroxidase, superoxide dismutase). The deficiency of these antioxidant elements results in decreased activity of antioxidant enzymes which ultimately lead to oxidative stress (AL-Qudah et al. 2011). Furthermore, their deficiency result in production of free radicals which cause tissue damage (Donia et al. 2014).

Certain trace elements such as selenium (Se) and zinc (Zn) are common antioxidants (Spears 2011; Abou-Zeina et al. 2014). Selenium acts as beneficial antioxidant that neutralizes free radicals. It acts as integral part of antioxidant enzyme called glutathione peroxidase (GPX), which protects the skin against oxidative damage caused by free radicals. Moreover, Se also inactivates harmful H<sub>2</sub>O<sub>2</sub> via its dissociation to water and oxygen (Case et al. 2011; Butterwick et al. 2015). Similarly, Zn is involved in the synthesis of antioxidant enzymes i.e., (Zn/SOD1), which protects the cell from reactive oxygen species (ROS) and antagonizes the catalytic properties of metals like iron involved in production of oxidative radicals (Saul 2000; Watson et al. 2000; Gaafar et al. 2010).

Copper is another antioxidant element, essential for the activity of antioxidant enzymes like superoxide dismutase (SOD) and ceruloplasmin (Hefnawy and El-Khaiat 2015). It is an essential trace element and is involved in the activation of more than 20 metalloenzymes and metalloproteinases known for the destruction of free radicals, synthesis of connective tissue, formation of myelin and pigmentation (McDowell 1999; Ortolani et al. 2003; Sousa et al. 2012). Both Zn and Cu are important in multiple enzymatic pathways and the activity of enzyme (Cu/Zn SOD1) and GPx is dependent on these two trace elements. Studies have shown that the activity of enzyme (Cu/Zn SOD1) and GPx was lower in Cu-deficient animals and higher in animals supplemented with Cu (Genther and Hansen 2014; Ighotlaro and Akinloye 2018). Dermatophyte infection induces a stress factor in dogs by affecting its antioxidant mechanism system, and consequently antioxidant trace elements level. So, the current study was aimed to observe the correlation between antioxidant trace elements (Zn, Cu, Se) and oxidative stress in dermatophytes infected dog.

## MATERIALS AND METHODS

### Ethical Statement

The authors declare that the present research work was conducted with great consideration to animal welfare and under owner's permission in an ethical manner according to international guiding principles for veterinary medical research.

### Animals Included in the Study

This study was conducted on 34 dogs irrespective of gender and breed. Their age ranged from 2 months to 6 years. Out of 34 dogs, there were 20 healthy dogs used as control group and 14 were dermatophyte-infected dogs which were attended in small animal medicine teaching hospital, Faculty of Veterinary Medicine, Cairo University, Egypt and different private small animal veterinary clinics in Egypt from October 2020 to December 2021.

### Clinical Examination

At time of admission, case history and vitals of all the animals were recorded. All the dogs were observed physically for skin lesions and skin changes. In addition, fecal samples were also examined microscopically.

### Skin Scraping Samples

Crusts and plugged hairs were collected from the periphery of the lesions on a clean glass slide with few drops of 10% KOH and covered with clean cover slip. Each specimen was examined under the microscope (40X) for the presence of spores (Hnilica and Patterson 2016).

### Blood Samples and Hematological Analysis

#### Whole Blood Samples

Blood samples were collected from the cephalic vein of dogs and preserved in EDTA tubes for the evaluation of hematological parameters including total erythrocyte count (TEC), packed cell volume (PCV), hemoglobin (Hb), total Leucocyte count (TLC), differential leucocyte count (DLC) and platelets by using Diatron hematology analyzer, USA.

#### Serum Samples

Blood samples were also collected in plain tubes and serum was separated for the biochemical analysis of alanine amino transferase (ALT), blood urea nitrogen (BUN), creatinine, thyroid stimulating hormone (TSH), free thyroxine (FT4) and cortisol level. Furthermore, the level of trace elements like copper (Cu) and zinc (Zn) was checked by using specific kits (spectrum diagnostic, Egypt) according to manufacturer's instructions in Diatron chemistry analyzer, USA. Similarly, Selenium (Se) level was measured in serum samples according to method described by Lavu et al. (2012) and Van Zelst et al. (2015) in Thermo scientific ICE 3300 Atomic Absorption spectrometer, Germany.

### Skin Biopsy and Histopathological Examination

#### Histopathological Examination

After skin biopsy, specimens were collected (using 3mm circular punch at a depth of 2mm) and fixed in 10%

neutral buffer formalin. After that, the specimens were washed, dehydrated, cleared, and embedded in paraffin. The paraffin embedded blocks were sectioned at 4-5µm thickness and stained with Hematoxylin and Eosin (H&E) for light microscopic examination (Olympus BX50, Tokyo, Japan (Bancroft et al. 2012)).

### Statistical Analysis

Statistical analyses were performed by SPSS version 20 (IPM Inc., Chicago). Descriptive statistics were measured as mean±SE. Variables of diseased animals, blood parameters and trace elements were compared to that of control animals using student T-test. P<0.05 value was considered as significant.

## RESULTS

### Clinical Examination

During careful scrutinization of dermatophytosis infected dogs, the most obvious clinical signs observed were alopecia, scaly, encrusted, circumscribed focal or multifocal lesions. Erythema, papules and seborrhea were also observed as shown in Fig. 1. Pruritus was usually minimal or absent.

### Microscopic Examination

Microscopic examination of specimens obtained from skin scraping of lesions showed presence of fungal hyphae and spores as shown in Fig. 2.

### Histopathological Findings of Affected Skin Lesions

Sections of skin biopsy from lesions showed pronounced histopathological alterations described as laminar orthokeratotic hyperkeratosis and acanthosis. Vacuolar degeneration of keratinocytes and fungal hyphae were seen in some examined cases. Moreover, slight dermal edema associated with few inflammatory infiltrate cells were also recorded as shown in Fig. 3-5.

### Hematological Examination Findings

Hematological profile showed significantly higher (P<0.05) level of TLC (16.325±1.20), MCHC (34.645±0.35), RDW (18.25±0.57) and lymphocytes (47.9±10.47) in dermatophytosis infected dogs as compared to control group (non-infected) as shown in Table 1. However, other parameters have not shown any significant difference in control and infected group of dogs.

### Biochemical Analysis Findings

Biochemical findings showed a significant decrease in freeT4 (8.57±3.62pmol/L) along with significant increase in cortisol level (709.88±67.01nmol/L) in infected dogs as

shown in Table 2. However, no significant difference (P>0.05) was observed in case of ALT, BUN, Creatinine and TSH in both infected and control group.

### Antioxidant Trace Elements Analysis

The level of antioxidant trace elements was significantly lower (P>0.05) in dermatophytosis as compared to control group. The level of the antioxidant trace elements recorded in infected dogs were Zn= 60.99±2.76mg/dL, Cu 59.725±3.01mg/dL and Se= 0.02±0.00mg/dL as shown in Table 3.

**Table 1:** Comparison of hematological profiles in dermatophytosis infected dogs and healthy dogs (Control Group)

Parameters	Units	Control (n=20)	Dermatophytosis (n=14)	P-value
HB	g/dL	14.685±0.34	15.01±1.21	0.80
RBCs counts	×10 <sup>6</sup> /L	6.98±0.15	6.93±0.61	0.93
PCV	%	45.845±1.07	43.39±3.75	0.55
MCV	fL	65.22±0.94	62.99±2.19	0.37
MCH	pg	21.23±0.25	21.86±0.69	0.42
MCHC	g/dL	33.55±0.30	34.645±0.35*	0.03
RDWcv	%	15.26±1.21	18.25±0.57*	0.03
Plts counts	×10 <sup>3</sup> U/L	297.8±21.53	209.25±41.22	0.08
WBCs counts	×10 <sup>3</sup> U/L	11.24±0.57	16.325±1.20*	0.00
Neutrophils	%	66.89±1.30	43.075±10.27	0.05
Staff	%	2.7±0.21	3.0±0.21	0.16
Segmented	%	64.19±1.23	40.08±10.27	0.05
Lymphocytes	%	22.035±1.11	47.9±10.47*	0.04
Eosinophils	%	7.2±0.53	5.475±0.77	0.09
Monocytes	%	3.875±0.28	3.55±0.70	0.68

Values (mean±SE) bearing asterisk differ significantly (P<0.05) than control values.

**Table 2:** Comparison of serum biochemical findings in dermatophytosis infected dogs and healthy dogs (Control group)

Parameters	Units	Control (n=20)	Dermatophytosis (n=14)	P-value
ALT	U/L	50.9±5.50	44.01±12.63	0.63
BUN	mg/dL	19.9±1.18	18.62±2.47	0.65
Creatinine	mg/dL	1.015±0.06	1.05±0.14	0.83
TSH	ng/mL	0.33±0.04	0.31±0.13	0.89
Free T4	pmol/L	21.15±2.18	8.57±3.62*	0.01
Cortisol	nmol/L	197.6±9.86	709.88±67.01*	0.00

Values (mean±SE) bearing asterisk differ significantly (P<0.05) than control values.

**Table 3:** Comparison of antioxidant trace elements of dermatophytosis infected dog and healthy dogs (Control group)

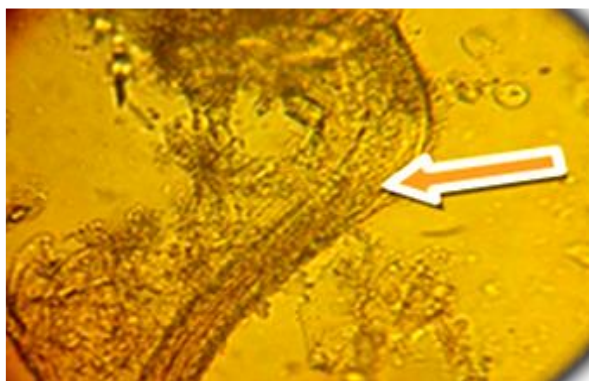
Parameters	Units	Control (n=20)	Dermatophytosis (n=14)	P-value
Selenium	mg/dL	0.256±0.010	0.02±0.00*	0.000
Copper	mg/dL	111.15±3.34	59.725±3.01*	0.000
Zinc	mg/dL	102.7±2.39	60.99±2.76*	0.000

Values (mean±SE) bearing asterisk differ significantly (P<0.05) than control values.



**Fig. 1:** Localized and generalized circumscribed multifocal, alopecia with scaly, crusted lesions of dermatophytosis.





**Fig. 2:** From the under microscopic picture showing the presence of fungal hypha and spores is confirmative of the fungal disease.

## DISCUSSION

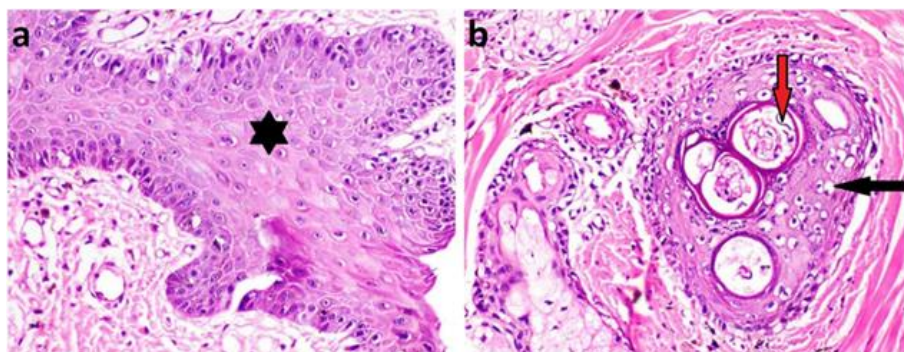
The present study was conducted to observe the association between antioxidant trace elements and the oxidative stress in dogs infected with dermatophytosis. For this purpose, 34 dogs were subjected to hematological and serum biochemical analysis. Most importantly, the level of antioxidant trace elements was checked in dermatophytosis infected dogs and health dogs. For the confirmation of dermatophytosis in dogs, clinical examination of all the participants was carried out and suspected dogs were subjected to microscopic examination of skin scrapings.

In our study, 14 dogs showed alopecia, redness and swelling of the infected skin indicating dermatophytosis infection. Dermatophytosis in these dogs was confirmed by microscopic examination of skin scraping which indicated the presence of fungal hypha and spores (ectothrix and endothrix). Such clinical signs and presence of fungal spores in dermatophytosis have also been reported by many scientists (Hnilica 2011; Moretti et al. 2013; Sykes and Outerbridge 2014). Furthermore, histopathological examination of affected skin lesions indicated the presence

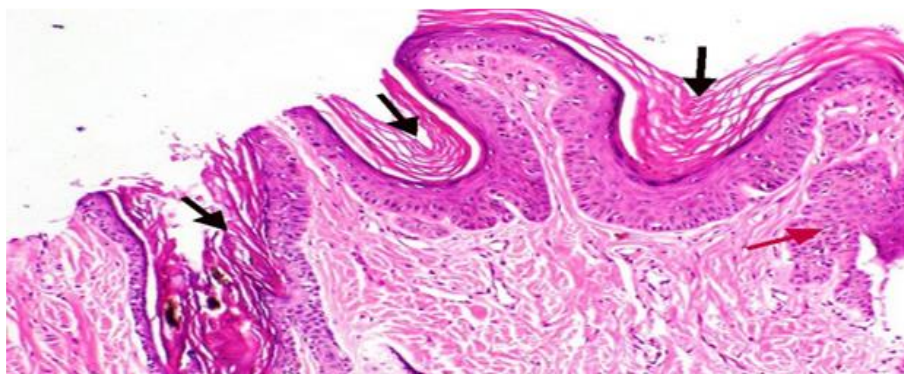
of laminar orthokeratotic hyperkeratosis and acanthosis. Similar results were found in the study of Alkaragoly (2016) and Moriello et al. (2017) in which vacuolar degeneration of keratinocytes and fungal hyphae were seen in infected animals.

During hematological examination of animals in our study, erythrogram (PCV, Hb conc and RBCs count) showed no significant difference ( $P>0.05$ ) in both infected and control group dogs. Similar results have also been reported in the past (Nair and Nauriyal 2007; Remi et al. 2012; Devi and Vijayakumar 2013; Sykes and Outerbridge 2014). However, some studies have reported a significant decrease in the erythrogram (Wilkinson 1979; Ibrahim et al. 1984; Sindha et al. 2015; Salem et al. 2020; Dina 2021). Previously, Král and Schwartzman (1964), Stockham et al. (2003) and Nair and Nauriyal (2007) have reported insignificant difference in the TLC and DLC in dermatophytosis infected dogs. In contrast to these reports, our study showed significant increase in the TLC count ( $16.325 \times 10^3/L$ ) in infected dogs.

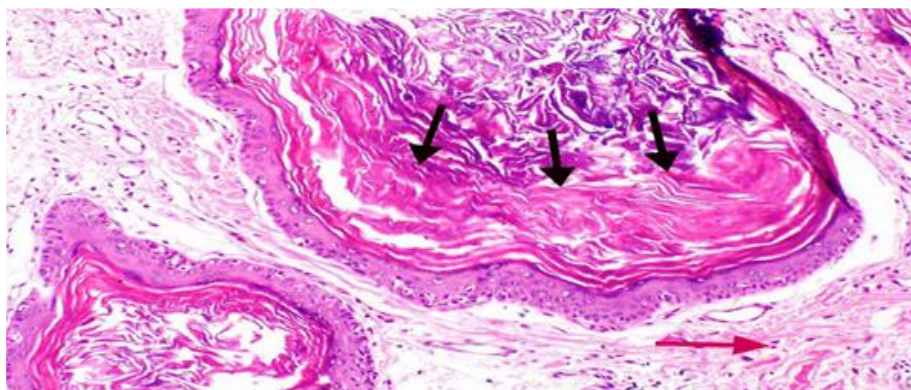
It has been reported that 80% of hypothyroid dogs were associated with dermatological problems characterized by dry skin, changes in coat color and quality, alopecia or seborrhea (Miller et al. 1992; Guptill-Yoran 2000; Srikala and Kumar 2014). The measurement of fT4 concentration as a test for hypothyroidism has higher sensitivity and accuracy than measurement of the tT4 (Peterson et al. 1997; Ramsey et al. 1997). In current study, the level of fT4 in dermatophytosis infected dogs was measured as  $8.57 \pm 3.62 \text{ pmol/L}$  which was significantly lower ( $P<0.05$ ) than control group i.e.,  $21.15 \pm 2.18 \text{ pmol/L}$ . This low level of fT4 in the dermatophytosis infected dogs may be due to the weaker immune system. Bansal et al. (2007) stated that the thyroid gland plays an important role in the regulation of the body immune system and in case of any problem in the thyroid functioning, the body is exposed to dermatophytosis due to immune suppression (Reis et al. 2019). Furthermore, the level of cortisol was significantly



**Fig. 3:** Photomicrographs of a skin biopsy of dermatophytosis infected mixed breed dog showing a) acanthosis of prickle cells of the epidermis (asterisk) and b) vacuolar degeneration of keratinocytes (black arrow) with presence of fungal hyphae (red arrow). (H & E stain, X 200).



**Fig. 4:** Photomicrographs of a skin biopsy of dermatophytosis infected dog showing laminar orthokeratotic hyperkeratosis (black arrows) and acanthosis (red arrow). H & E stain X200.



**Fig. 5:** Photomicrographs of a skin biopsy of dermatophytosis infected dog showing marked hyperkeratosis (black arrows) and slight dermal edema associated with few inflammatory cells infiltration (red arrow). H & E; X200.

higher ( $P < 0.05$ ) in dermatophytosis infected animals in our study and the results were in accordance to the previous studies (Khaled et al. 2010).

Antioxidant trace element analysis of our study showed significant decrease ( $P < 0.05$ ) in dermatophyte infected dogs as compared to non-infected dogs. Similar results were found by Beigh et al. (2014) and Sharma et al. (2017). However, in contrast of our study, some studies have shown no significant difference in the Zn level of infected dogs (Ural et al. 2009; Dina 2021).

### Conclusion

There are two double mechanisms; first, the oxidative stress has a degenerative effect on skin layers, facilitating infections with dermatophytes. Second, the dermatophytes are keratolytic fungi which elaborates keratinases enzymes allowing the dermatophyte to burrow deeper into the stratum corneum eliciting inflammatory reaction that produce free radicals resulting in oxidative stress. These double mechanisms resulting in a state of significant oxidative stress, consequently the exhaustion of antioxidant system with significant changes in antioxidant trace elements level especially zinc and copper.

### Author's Contribution

Ossama M Abdou conceptualized the idea of the research. Noha M El-Motaily collected the samples and the data. Kawkab A Ahmed performed the histopathology tests. Saber M analyzed the data. Heba S Farag wrote and edited the manuscript.

### Acknowledgment

The authors are thankful to Internal Medicine and Infectious Diseases, Department and Pathology Department, Faculty of Veterinary Medicine, Cairo University for their help and guidance during the research period. Grateful to Dr. Tamer Fawzy, Department of Behavior, Faculty of Veterinary Medicine, Cairo University for his help in statistical analysis of data.

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