Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Corpuscular oxidation in camels naturally infected with *Dipetalonema evansi*

Mostafa A. Saleh[*], M. H. Rateb[*], Elham A. Abd-Allah[**], Osman M. Mahran[***], Ghada A.E. Mohamed[*]

[*]Biochemistry Unit, Regional Animal Health Research Laboratory, Animal Health Research Institute, Agriculture Research Center, Assiut, 71514, Egypt.
[**]Department of Zoology, Faculty of Science, New-Valley University, 72511 Egypt.
[***]Department of Parasitology, Animal Health Research Institute, Agriculture Research Center, Giza, 12618 Egypt.

Received in 18/5/2022
Received in revised from 14/6/2022
Accepted in 27/6/2022

Keywords:
Camel
Dipetalonema evansi
Oxidative Stress

ABSTARACT

Dipetalonema evansi (*D. evansi*) is an important filarial nematode especially in camels and represents a significant health problem. This study aimed to determine the effect of natural infection with *D. evansi* on the oxidative status in plasma and erythrocytes of camels (*Camelus dromedarius*). In this study, 22 female dromedaries were classified according to blood parasitological examinations into infected group (n = 10) and healthy control one (n = 12). Compared to values of the control group, a significant reduction in red blood cell count ($P < 0.05$), hemoglobin concentration ($P < 0.01$) and packed cell volume ($P < 0.01$) was noticed in the infected camels. Plasma total oxidant status (TOS) was significantly increased ($P < 0.05$) in the infected camel group compared to the control one. Plasma total antioxidant capacity (TAC) was significantly decreased ($P < 0.05$) whereas the erythrocytic activity of the antioxidant superoxide dismutase and erythrocytic lipid peroxidation as denoted by malondialdehyde concentration were increased ($P < 0.05$) in the infected group when compared to the corresponding control values. In conclusion, erythrocyte of camels infected by *D. evansi* is a subject of peroxidation, which may contribute to pathogenesis of the disease.

INTRODUCTION

Camels (*Camelus dromedarius*) are multipurpose animals used for milk and meat production in addition to working, racing, transportation and tourism. They are well adapted to harsh environments in arid regions. According to the FAO official statistical data for live animals (FAOSTAT), the worldwide camel population is ~37.5 million heads (FAO, 2021).

Dipetalonema evansi (*D. evansi*) was first discovered in the blood of Indian dromedaries by Evans under the name of Filaria evansi. Later also detected in Egyptian camels **(Mason, 1906, 1911 and Nagaty, 1947)**. *D. evansi* is a nematode parasite transmitted by mosquitoes. The mature worm inhibits the blood vessels of the epididymis, testicles, lungs and heart caus-

Mostafa A. Saleh, Biochemistry Unit, Regional Animal Health Research Laboratory, Animal Health Research Institute, Agriculture Research Center, Assiut, 71526, Egypt
E-mail address:
DOI:

ing pathological damages whereas the sheathed microfilariae circulate in the peripheral blood stream **(Mason, 1906 and Nagaty, 1947).** *D. evansi* is an endemic disease affecting camels in several countries all over the world and represents a significant health problem, impaired working capacity and lowered productivity of camels **(Sazmand and Joachim, 2017).**

Inflammatory response and immunological disorders are evident as a host defense mechanism against the parasitic infections **(Chakraborti et al. 2019).** Unfortunately, these mechanisms in the host can enhance free radical production and impair the antioxidative potential **(Halliwell and Gutteridge, 2015**). The discrepancy between radical-generating and scavenging activities can result in an oxidative stress (OS) response, formation of oxidative by -products and modification of the disease outcome **(Abd Ellah, 2018).** The group of lipid peroxides are of these by-products. The most prevalent of this group is the malondialdehyde (MDA) macromolecules. Lipid peroxides are formed by peroxidation of lipids specially polyunsaturated fatty acids **(Halliwell and Gutteridge, 2015).**

Previous reports showed that OS plays an important role in host defense against filarial infection **(Mukherjee et al. 2019).** Increased pro-oxidants, decreased antioxidant defense and enhanced an OS state are reported during filarial infection in ruminants **(Nasr Eldeen, 2015)** and humans **(Mukherjee et al. 2019).**

The erythrocytes had an efficient antioxidant system that scavenges free radicals and modifies prooxidants into substantially less reactive intermediates **(Podsiedlik et al. 2020).** The corpuscular antioxidant system primarily consists of the enzymatic and non-enzymatic potential that act together in harmony against the enhanced prooxidants **(Fujii et al. 2021).** Redox imbalance and oxidative stress causes oxidative injury to erythrocytes and results in membrane lipids and proteins damage, cell destruction and cytotoxisity and hemolysis **(Fujii et al. 2021).**

The present work aimed to study proxidants, antioxidant status and erythrocytic lipid peroxidation levels in camels (*Camelus dromedarius*) naturally infected by microfilariae of *D.evansi*.

MATERIALS and MWETHODS

Animals

This study was carried out in El-Wdi El-Gadid governorate in Upper Egypt. Twenty-two non-pregnant non-lactating female camels (*Camelus dromedarius*, 8–10 years) were selected in this study. According to clinical and laboratory diagnosis, these animals were classified into two groups. The first group (n = 10) was infected with *D. evansi* (microfilariae infected group). The second group (n = 12) was apparently healthy and parasite free (negative control group).

Blood sampling:

Blood was sampled from each camel by jugular vein puncture into 10ml heparinized sterile vacuum tubes in the early morning. Samples were sent immediately in ice box to the laboratory. Two-ml of blood was separated for immediate hematological investigations and preparation of erythrocyte hemolysate for determination of erythrocytic lipid peroxides concentrations and superoxide dismutase (SOD) activity. The remaining blood samples were centrifuged at 1500 rpm for 15 min at 4°C. Plasma was separated and stored at -20 ºC until used for determination of total oxidant status (TOS) and total antioxidant capacity (TAC).

Parasitological examination:

Microhematocrit centrifugation technique, wet blood film and Giemsa-stained thin and thick blood smears were prepared according to standard procedures for microflaria detection and reject cases with other hemoparasites.

Microfilarae are sometimes not easily detected, so Knott's procedure **(Knott 1939)** was applied to detect rare infections. It was performed by adding 9ml of 2% formalin on 1ml blood in a 15ml conical tube. The mixture was properly mixed and moderately centrifuged for 10 min at 650 ×g. Methylene blue (2 drops) were mixed with the sediment and examined microscopically.

Hematological investigations

Standard methods of hematology were used for estimation of red blood cell count (RBC), hemoglobin concentration (Hb) and packed cell volume (PCV) according to Jain (1986).

Erythrocytic hemolysate was prepared by centrifugation of one-ml of blood sample at 650 ×g for 15 min at 4°C. The packed erythrocytes were washed several times in isotonic phosphate-buffered saline. Then they were lysed by nine-ml of cold bi-distilled water as a hypotonic shock to prepare 10% hemolysate.

Biochemical analysis

Total oxidant status (TOS)

Plasma TOS was measured as total peroxides concentration after the method of Erel (2005). In this method, the oxidative molecules oxidize Fe^{+2}-*o*-dianisidine complex to Fe^{+3} forming a colored complex with xylenol orange, which can be read at 560 nm. TOS was expressed as µmol H_2O_2 Equiv/L.

Total antioxidant capacity (TAC)

Plasma TAC was measured after the method of **Miller et al. (1993).** Colorimetric ABTS assay kit (Beyotime Institute of Biotechnology, Haimen, China) was used. TAC was expressed as mmol Trolox equiv/L.

Erythrocytic superoxide dismutase (SOD)

Corpuscular superoxide dismutase (SOD) activity was estimated in the hemolysate according to the method described by **Misra and Fridovich (1972).** This method depends on the ability of SOD to inhibit the autoxidation of epinephrine to adrenochrome in an alkaline medium (pH 10.2). OD was measured at 480 nm. SOD activity was expressed as U/mg Hb.

Erythrocytic lipid peroxides

Erythrocytic lipid peroxides was determined in the hemolysate after Placer et al. (1966). The method is based on forming a colour complex between the products of lipid peroxidation and thiobarbituric acid, which can be read at 548 nm and expressed as nmol MDA/g Hb.

Statistical Analysis

The packaged SPSS program for windows version 20.0.1 (SPSS, Chicago, IL, USA) was used for the statistical procedures. Data were analyzed by using Student t-test and expressed as mean ± standard error (SE). The level of significance was set at $P < 0.05$.

RESULTS

Clinical and parasitological findings:

Infected camels showed pale mucous membranes of the eye and oral cavity indicating signs of anemia. These signs were accompanied by debilitation, dullness and loss of appetite. The rectal temperature of the infected animals was higher (38.4-39.9 °C) than the control group within (38 °C). Sometimes animals showed loss of coordination and sternal recumbency. Sheathed microfilaria was shown in Giemsa-stained thin blood smears (Fig. 1).

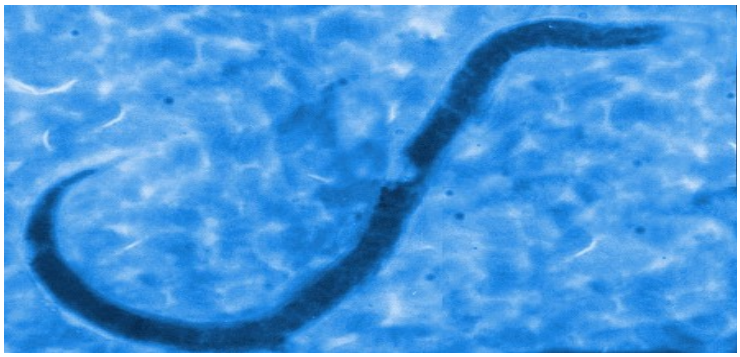

Fig 1. Sheathed microfilaria, Giemsa-stained thin blood smear (x 400)

Hematological findings:

Results of the hematological indices are presented in table (1). A significant reduction in the mean values of red blood cell count ($P < 0.05$), hemoglobin concentration ($P < 0.01$) and packed cell volume ($P < 0.01$) was noticed in the infected camels group compared to values of the control one.

Table 1. Mean values (± SE) of hematological indices in *D. evansi* infected camels group and control one.

Parameter	Control (n = 12)	Infected (n = 10)
RBC (10^{12}/L)	9.12 ± 0.34	7.89 ± 0.361 *
Hb (g/L)	101.7 ± 3.811	89.09 ± 3.401 *
PCV (L/L)	0.314 ± 0.006	0.291± 0.007 **

*, ** difference between means of control and infected groups is significant at P<0.05 and P<0.01, respectively.
RBC: red blood cell count, Hb: hemoglobin concentration, PCV: packed cell volume.

Redox biomarkers findings

The results of the redox indices including plasma concentrations of total oxidant status (TOS), total antioxidant capacity (TAC), erythrocytic superoxide dismutase (SOD) activity and erythrocytic lipid peroxides concentration as indicated by malondialdehyde (MDA) in the infected and control camels are presented in table 2. The mean value of TOS significantly increased ($P < 0.05$) in the infected camels compared to the control value. On the contrary, the mean value of the TAC was significantly decreased ($P < 0.05$) in the infected camels when compared to the control results. The erythrocytic activity of SOD significantly increased ($P < 0.05$) in the infected camels compared to the control value. Similarly, there was a significant increase in erythrocytic MDA ($P < 0.01$) in the infected camels compared to the control result.

Table 2. Mean values (± SE) of pro-oxidants and antioxidants in plasma (p) and erythrocytes (e) control and infected camels

Parameter	Control (n = 12)	Infected (n = 10)
p TOS (µmol H_2O_2 Equiv/L)	16.23 ± 0.693	18.5 ± 0.712 *
p TAC (mmol Trolox equiv/L)	1.223 ± 0.061	1.049 ± 0.054 *
e SOD (U/mg Hb)	4.26 ± 0.26	5.04 ± 0.39 *
e MDA (nmol MDA/g Hb)	143.9 ± 9.871	198.1 ± 14.11**

TOS: total oxidant status, TAC: total antioxidant capacity, SOD: superoxide dismutase, MDA: malondialdehyde.
*, **, difference between means of healthy and infected camels is significant at P<0.05 and P<0.01 respectively.
p: plasma e: erythrocytes

DISCUSSION

The present study showed that erythrocytes of camels infected with microfilarae of *D. evansi* are subjected to oxidation as indicated by increased erythrocytic lipid peroxide concentration. Blood of infected camels had increased prooxidant substances coupled with inhibited antioxidant potential.

Infected camels in the current study showed clinical signs similar to those observed earlier in the Egyptian camels **(Mason 1906, 1911).** The increased body temperature during the parasitemia in camels come in agreement with the findings of several studies **(Elamin et al. 1993 and Abou El-Ela, 2003).**

Pale mucus membranes of the eye and oral cavity in addition to declined values of RBCs, Hb and PCV suggest that infected camels were suffering from anemia. These results agreed with the findings of **Mason (1906).** This anemia may be attributed to filarial antigen-induced immuno-pathological disorders **(Muhammad et al. 2004).**

In the course of pathogenesis originating from host–parasite interaction, the circulating microfilaria interact with immune cells **(Pionnier et al. 2016).** Accordingly, proinflammatory cytokines are triggered and enhance the production of ROS to fight the parasite **(Mukherjee, et al. 2019).** ROS including superoxide anion radical, hydrogen peroxide and hydroxyl radical play an important role in the normal function of biomolecules including nucleic acids, cell membrane phospholipids and proteins **(Mavangira and Sordillo, 2018).** Excessive production of free radicals results in cytotoxicity and irreversible damage to the cell **(Mavangira and Sordillo, 2018).** ROS have been implicated in the pathogenesis of a variety of parasitic diseases **(Abd Ellah, 2013 and Chakraborti, et al. 2019).** In the present work, the increased circulating peroxides in infected camels suggest augmentation of free radical production during the course of microfilaria circulation. Previous reports showed that ROS increased in human filariasis **(Mukherjee, et al. 2019).**

As a protection, the body has adequate enzymatic and non-enzymatic antioxidant range that counteract the continuous production of prooxidants **(Halliwell and Gutteridege, 2015).** Measurement of the total antioxidant capacity (TAC) is considered as a collective action of all antioxidants and gives relevant descriptive information on the dynamic equilibrium between pro-oxidants and antioxidant **(Erel, 2005).** Although highly effective, the antioxidant defenses have restricted capacity and can be crushed by free radical attacks. The decreased TAC in the present study is similar to the findings in buffalo filariasis **(Nasr Eldeen, 2015)** and suggests impaired antioxidant defenses in plasma of camels infected with *D.* evansi. However, the present results showed increased erythrocytic SOD activity in infected camels. These results contradict the findings observed by **Nasr Eldeen (2015)** who found decreased serum SOD in filarial infection in buffalo. This might be related to the virulence of the invading parasite, the nature of the enzymatic antioxidants and species susceptibility **(Abd Ellah, 2013).**

When free radical generation exceeds antioxidant defenses, oxidative stress (OS) condition is enhanced **(Halliwell and Gutteridege, 2015).** In the present work, TOS was higher and TAC was lower in the infected camels group than the healthy one, suggesting a creation of OS condition in filarial infection with subsequent peroxidation of macromolecules like proteins, lipids and nucleic acids resulting in cytotoxicity and pathology.

Lipids are main targets of oxidation and toxicity by the chain reactions of free radical to give a diverse scale of the highly reactive toxic by-products, lipid peroxides of which MDA is the most obvious **(Mavangira and Sordillo, 2018).** Enhanced lipid peroxide was reported in buffalo filariasis **(Nasr Eldeen, 2015).** The enhanced erythrocytic MDA observed in this study is an indicator of corpuscular membrane lipid peroxidation. Oxidation of the erythrocytes induces membrane lipid injury, cytotoxicity and destruction of the cell **(Fujii et al. 2021).** The enhanced OS and accumulation of lipid peroxides in the erythrocytes can result erythrocytic damage and anemia **(Fujii et al. 2021).** So that further researches are required to study if the infection with *D. evansi* is considered as a hemolytic agent in the camel. In

conclusion, erythrocytes of camels infected with *D. evansi* is a subject of peroxidation.

REFERENCES

Abd Ellah MR. 2013. Involvement of free radicals in parasitic infestations. J. Appl. Anim. Res. (41): 69-76.

Abd Ellah MR. 2018. Involvement of oxidative stress in cardiovascular diseases. J. Adv. Vet. Res. (8): 1-5.

Abou El-Ela A. 2003. Trace elements status in healthy and diseased camels in New-Valley governorate. Ph.D. thesis, Assiut Univ, Egypt

Chakraborti S, Chakraborti T Chattopadhyay D, Shaha C. 2019. Oxidative Stress in Microbial Diseases. Springer Nature Singapore Pte Ltd.

Elamin EA, Mohamed GE, Fadl M, Elias S, Saleem MS, Elbashir MOA., 1993. An outbreak of cameline filariasis in the Sudan. Br. Vet. J. (149):195-200.

Erel O. 2005. A new automated colorimetric method for measuring total oxidant status. Clin. Biochem. (38):103-111.

FAO 2021. FAOSTAT, Food and Agriculture Organization of the United Nation Statistical Database: Live Animals. https:// www.fao.org/faostat/en/#data/GU (accessed June 24, 2021)

Fujii J, Homma T, Kobayashi S, Warang P, Madkaikar M, Mukherjee MB. 2021. Erythrocytes as a preferential target of oxidative stress in blood. Free Radic. Res. (55):781-799.

Halliwell B, Gutteridge JM. 2015. Free Radicals in Biology and Medicine. 5th ed. Oxford University Press, USA.

Jain NC. 1986. Hematologic techniques. In: Schalm OW, Jain NC. (Ed.), Schalm's Veterinary Hematology, 4th ed. Lea and Febiger, Philadelphia pp. 20-86.

Knott J. 1939. A method for making microfilarial surveys on day blood. Trans. R. Soc. Trop. Med. Hyg. (33): 191-196.

Mason FE, 1906. Filariæ in the Blood of Camels in Egypt. J. Comp. Pathol. Ther. (19): 118 -120

Mason FE. 1911. A further note on filariae in the blood of camels in Egypt J. Comp. Pathol. Ther. (26): 329-39

Mavangira V, Sordillo LM. 2018. Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. Res. Vet. Sci. (116): 4-14.

Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Miller A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. (84): 407-412.

Misra HP, Fridovich I. 1972. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. (247): 3170–3174.

Muhammad SA, Farooq AA, Akhtar MS, Hayat CS. 2004. Dipetalonemiasis in a dromedary camel and its treatment. Pak. Vet. J. (24):205-206

Mukherjee S, Joardar N, Babu SPS. 2019. Redox Regulatory Circuits as Targets for Therapeutic Intervention of Bancroftian Filariasis: Biochemical, Molecular, and Pharmacological Perspectives. In Chakraborti, S; Chakraborti, T; Chattopadhyay, D. and Shaha, C. Oxidative Stress in Microbial Diseases (pp. 185-208). Springer Nature Singapore Pte Ltd.

Nagaty HF, 1947. *Dipetalonema evansi* (Lewis, 1882) and its microfilaria from Camelus dromedarius. Parasitology, (38): 86-92.

Nasr Eldeen NAM., 2015. Clinicopathological Studies on the Effect of Filariasis in Egyptian Buffaloes. Zag. Vet. J. (43):126-34.

Pionnier N, Brotin E, Karadjian G, Hemon P, Gaudin-Nome F, Vallarino-Lhermitte N, Chollet-Martin S. 2016. Neutropenic mice provide insight into the role of skin-infiltrating neutrophils in the host protective immunity against filarial infective larvae. PLoS neglected tropical diseases, 10(4), e0004605.

Placer ZA, Cushman LL, Johnson BC. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. (16): 359–364.

Podsiedlik M, Markowicz-Piasecka M, Sikora J. 2020. Erythrocytes as model cells for biocompatibility assessment, cytotoxicity screening of xenobiotics and drug delivery. Chem. Biol. Interact. 1, (332):e109305.

Sazmand A, Joachim A. 2017. Parasitic diseases of camels in Iran (1931–2017)–a literature review. Parasite, 24. (21): e2017024.