



BLOOD PICTURE AND SELECTED OXIDATIVE STRESS BIOMARKERS IN DROMEDARY CAMELS NATURALLY INFECTED WITH TRYPANOSOMA EVANSI

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ABSTRACT

Additional Biomarkers are required for estimation of the oxidative stress status in camel trypanosomiasis. Therefore, the present study aimed to determine lipid peroxidation, enzymatic antioxidants level and hematological indices in camels naturally infected with Trypanosoma evansi and trypanosome free camels (control). The clinical examinations revealed that all the infected camels showed signs of loss of appetite, diarrhea and loss of weight with poor body condition. Hematological analysis revealed a significant decrease ($P=0.022-0.031$) in the values of total erythrocytic count (TEC), hemoglobin (Hb) and Packed cell volume (PCV) in trypanosoma infected camels ($5.0 \pm 0.5 \times 10^{12}/L$; 5.7 ± 0.5 g/dl; $21.8 \pm 1.0\%$) compared to control group ($9.6 \pm 0.6 \times 10^{12}/L$; 10.3 ± 0.5 g/dl; $29.1 \pm 1.5\%$). However, the values of total leucocytic counts (TLC) and differential counts were comparable to the control values except for Eosinophils value which were significantly ($P=0.023$) increased in trypanosome infected camel ($6.0 \pm 1.9\%$) compare to the control ($1.8 \pm 0.8\%$). Biochemical analysis indicated that, lipid peroxidation level was significantly ($P=0.021$) increased in trypanosoma infected camels as reflected on higher values of malonaldehyde (MDA; $6.3 \pm 0.3\mu M$) as compare to the control ($0.07 \pm 0.01\mu M$). The activity of glutathione reductase was significantly ($P=0.025$) increased in trypanosoma infected camels (1.3 ± 0.01 nmol/ml) compare to the control (0.8 ± 0.2 nmol/ml) whereas, the activity of super oxide dismutase (SOD) remained unchanged ($P=0.072$) in trypanosoma infected camels compare to the control healthy

animal. The present findings concluded that *Trypanosoma evansi* infection in camels was associated with lipids peroxidation and oxidative stress. In addition, the present study suggests that glutathione reductase may use as oxidative stress biomarker in *Trypanosoma evansi* infection in camels.

Keywords: Trypanosomiasis, Camel, Oxidative stress, Lipid peroxidation, Anemia.

Contribution/ Originality

This study is one of very few studies which have investigated lipid peroxidation, enzymatic antioxidants level and hematological indices in camels naturally infected with *Trypanosoma evansi*.

1. INTRODUCTION

Dromedary camels are mainly live in arid area of Africa and Asia and considered the important component of desert ecosystem. They are used not only for transportation but also for racing and meat and milk production [1, 2]. Camel trypanosomiasis, also known as Surra, is a disease caused by *Trypanosoma evansi*. Camels are most often affected in the Middle East and Africa [3]. Surra is the most economically important disease of camel herds with morbidity of up to 30% and mortality of around 3% [4]. The prevalence of the disease in Saudi Arabia was demonstrated by Al-Khalifa, et al. [5]. The same authors reported that Asir region camels were disinfected while those of the Eastern Jazan, Northern Frontiers, Riyadh and Tabook regions were infected (5-40%). The disease occurred in acute and chronic forms, however, chronic form is the most common one and characterized by anemia, emaciation, lacrymation, lymphadenitis, and sometimes abortions [6]. The Anemia is mainly macrocytic and hypochromic [7]. The primary causes of anemia are reported to be due to either Dyshemopoiesis or erythrophagocytosis in ruminants trypanosomiasis [8]. Taylor and Authie [9] explain that, the possible causes of Erythrophagocytosis are attributed to damage and increased rate of removal of red cells from the circulation by hemolytic factors released by dying trypanosomes, immune complexes bound to RBCs, together with fever and mechanical damage to RBCs by trypanosomes. Many investigators have shown alterations in hematology of infected camels [10-13]. Recently, chronic camel trypanosomiasis was associated with a state of oxidative stress process. Oxidative stress is caused by free radicals, reactive oxygen species which damage DNA, biomembrane lipids, proteins and other macromolecules [14]. The primary source of reactive oxygen species is leakage of electron from the respiratory chain during the reduction of molecular oxygen to water generating superoxide anion [14, 15]. However, free radicals can be scavenged by the use of antioxidant system including non-enzymatic components and a series of antioxidant enzymes [14]. Non enzymatic components include glutathione, selenium, Vitamin C and E. The antioxidant enzymes include glutathione peroxidase, catalase and superoxide dismutase which are the most major antioxidant enzymes that are capable to minimize oxidative stress in the organelles [16]. The degree of lipid peroxidation is often used as an indicator of ROS mediated damage [17] and the concentration of MDA in blood and tissues are generally used as biomarkers of lipid peroxidation

[18]. If the pro-oxidant burden overwhelms the endogenous antioxidant defenses of the organism, the arising imbalance between pro- and antioxidants is resulted which defined as oxidative stress [19]. Trypanosomiasis in camels resulted in significant increase of serum MDA, significant reduction SOD and non-significant increase in CAT compared to control values [13]. The publications regarding the estimation of the oxidative stress status in *T. evansi* infection in camels are scarce [13] therefore, additional Biomarkers are required. Therefore, the present study aimed to determine lipid peroxidation, enzymatic antioxidants level and hematological indices in camels naturally infected with *Trypanosoma evansi*.

2. MATERIALS AND METHODS

2.1. Animals

Forty camels (7–10 years) were used in this study. All camels were reared in Riyadh Province which are of the one humped type (*Camelus dromedaries*). All camels subjected to careful clinical and laboratory investigations. Accordingly, the selected camels were divided into 2 groups (20 each); the first was naturally infected with *T. evansi*, and the second was healthy (control group). No abnormal clinical signs were observed in the control group and they had a good body condition on the physical examinations.

2.2. Sampling

Blood samples were collected from the jugular vein, one in heparinized vacutainers for hematology and the second in plain vacutainers for subsequent biochemical analysis of oxidative stress biomarkers and lipid peroxidation. The blood samples collected in heparinized vacutainers were used for parasitological examination and hematological investigations. Blood collected in plain vacutainers was centrifuged at 3000 rpm for 10 min for serum harvesting. The harvested serum was stored at -20°C until the time of analysis.

2.3. Samples Analysis

Card agglutination test (CATT/*T. evansi*) were used for the detection of trypanosomes according to the manufacture instructions (Institute of Tropical Medicine, Antwerp, Belgium). TLC and differential leucocytic counts were determined using electronic cell counter (VetScan HM5 Hematology system). The ELISA commercial kits were used for the determination of SOD (U/ml; Cayman Chemical Company, USA, Catalog No. 706002; [20]), Glutathion Reductase (nmol/ml; Cayman Chemical Company, USA, Catalog No. 703202; [21]) and MDA (μM ; NWK-MDA01, Vancouver, WA USA; [22]) by BioTek® ELISA reader (ELx 800TM, USA).

2.4. Statistical Analysis

All data were presented as mean \pm standard error of mean (SEM) using one way analysis of variance (ANOVA). The level of significance was set at $P \leq 0.05$. All tests were performed using computer package of the statistical analysis system SAS [23].

3. RESULTS

3.1. Clinical Findings

The clinical examinations revealed that all the infected camels showed signs of loss of appetite, diarrhea and loss of weight with poor body condition. Infected animals were anemic and the anemia was identified by paleness of mucus membranes. In addition, lymphadenitis and lacrimation were also observed. No abnormal clinical signs were observed in the control group and they had a good body condition on the physical examinations.

3.2. Hematological Findings

The hematological parameters are shown in Table 1. These data indicated that, there was a significant decrease ($P < 0.05$) in the values of total erythrocytic count (TEC), hemoglobin (Hb) and Packed cell volume (PCV) in trypanosoma infected camels ($5.0 \pm 0.5 \times 10^{12}/L$; $5.7 \pm 0.5g/dl$; $21.8 \pm 1.0\%$) in comparison with the control group ($9.6 \pm 0.6 \times 10^{12}/L$; $10.3 \pm 0.5g/dl$; $29.1 \pm 1.5\%$).

However, the values of total leucocytic counts (TLC) and differential counts were comparable to the control values. These values in control and infected animals, respectively were 13.2 ± 0.7 and $13.4 \pm 0.9 \times 10^3/mm^3$ for TLC; 66.0 ± 4.0 and $64.1 \pm 3.5\%$ for Neutrophils; 29.1 ± 1.5 and $27.1 \pm 1.9\%$ for Lymphocytes; 2.3 ± 1.2 and $2.2 \pm 1.0\%$ for Monocytes, 0.5 ± 0.5 and $0.6 \pm 0.5\%$ for Basophils. In other hand, Eosinophils values were significantly increased ($P < 0.05$) in trypanosome infected camel ($6.0 \pm 1.9\%$) compare to the control ($1.8 \pm 0.8\%$).

3.3. Biochemical Findings

The value of lipid peroxidation and antioxidant enzymes are shown in Table 2. ELISA colorimetric estimation indicated that, lipid peroxidation level was significantly ($P < 0.05$) increased in trypanosoma infected camels as reflected on higher values of malonaldehyde (MDA; $6.3 \pm 0.3\mu M$) when compared with the control ($0.07 \pm 0.01\mu M$). The activity of glutathione reductase was significantly ($P < 0.05$) increased in trypanosoma infected camels ($1.3 \pm 0.01nmol/ml$) compare to the control ($0.8 \pm 0.2nmol/ml$) whereas, the activity of super oxide dismutase (SOD) remained unchanged ($P > 0.05$) in trypanosoma infected camels compare to the control healthy animal.

4. DISCUSSION

The anemia observed in the present study come in accordance with those demonstrated previously [7, 13]. In the present study, oxidative stress was happened as evidenced by higher

level of MDA as lipid peroxidation biomarker and stimulation of antioxidants enzymes to counteract the active free radicals in dromedary camels naturally infected with *T. evansi*. Plasma lipids are also sensitive to peroxidation [24]. Parallel to the current study, earlier researches demonstrated higher level of MDA in *T. brucei* infected mice [25], humans infected with *Trypanosoma cruzi* [26-28] and in naturally infected camel with *trypanosoma evansi* [13]. In healthy aerobic organisms production of free radicals is approximately balanced with antioxidant defense system [29]. Thereby, we can say that antioxidants control the level of reactive species rather than eliminate them. Animals have a multi-leveled ROS defense network of enzymes and non-enzymatic antioxidants. Enzymatic antioxidants acts as primary defense whereas non enzymatic antioxidants act as secondary against ROS [30]. Superoxide dismutase (SOD), catalase, and peroxidases are the main enzymes incorporated in defense mechanism against ROS. SOD dismutase $\cdot O_2$ while catalase and peroxidases detoxify H_2O_2 [24, 31-33]. The present study reported no significant changes in the activity of SOD in trypanosoma infected camel compare to the control. Parallel to the current study, blood SOD activity was not affected in acute *T. evansi* infection in rats [34]. In the contrary, a 60% decrease of SOD activity in blood of humans chronically infected with *T. cruzi* was reported [28]. In addition, Saleh, et al. [13] demonstrated that, chronic *T. evansi* infection in camels resulted in inhibition of the antioxidant status (albumin, ascorbate, GSH and SOD). Glutathione (GSH) is a tripeptide called glutamylcystenylglycine, widely distributed in both plants and animals. GSH serves as a nucleophilic co substrate to GSH transferases in the detoxification of xenobiotics and is essential electron donor to GSH peroxidases in the reduction of hydroperoxide. GSH is also involved in amino acid transport across membranes. Glutathione reductase (GR. EC.1.6.4.2) is a flavoprotein that catalyzes the NADH dependent reduction of which maintains adequate levels of reduced cellular GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress [21]. The present study indicated that GR activity was increased in trypanosoma infected camels compare to the control. ROS caused lipid peroxidation as reflected on MDA level, might stimulate the increase in GR activity to counteract the oxidative stress situation. When Saleh, et al. [13] has observed a decrease in SOD activity, he postulated that, the antioxidants are likely consumed as free radical scavengers during the oxidative process in the natural chronic *T. evansi* infection in camels. We suggested that, both ways of discussion might be correct depending on the course of the disease and time of blood collection. By another way, one researcher might be collect the blood during the course of the disease whenever the antioxidants enzymes are stimulated to counteract the oxidation process. In another work, blood might be collected during the course of the disease whenever the antioxidants capacity was depleted. The anemia observed in trypanosma infected camel in the present study are reflected on decreased values of TEC, Hb and PCV. This decrease might be attributed to oxidation of the erythrocyte membrane and the formation of methemoglobin [35]. Changes of hemoglobin to methemoglobin caused hemolytic anemia and clearance of these cells in the spleen [36]. Parallel to the current findings, *T. brucei* caused

osmotic fragility, reduced life span and destruction of the red cells in mice [37]. In addition, hemolytic anemia was previously described in *T. evansi*-infected camels [6, 13].

5. CONCLUSION

The present findings concluded that *Trypanosoma evansi* infection in camels was associated with lipids peroxidation and oxidative stress. In addition, the present study suggested that, glutathione reductase can be used as oxidative stress biomarker in *Trypanosoma evansi* infection in camels.

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Table-1. Hematological parameters in control (n=20), and trypanosoma infected camels (n=20)

Variables	Control	Diseased	P values
TEC ($10^{12}/L$)	9.6 ± 0.6	5.0 ± 0.5*	0.022
Hb (g/dl)	10.3 ± 0.5	5.7 ± 0.5*	0.027
PCV (%)	29.1 ± 1.5	21.8 ± 1.0*	0.031
TLC ($10^9/L$)	13.2 ± 0.7	13.4 ± 0.9	0.190
Neutrophils (%)	66.0 ± 4.0	64.1 ± 3.5	0.076
Lymphocytes (%)	29.1 ± 1.5	27.1 ± 1.9	0.087
Monocytes (%)	2.3 ± 1.2	2.2 ± 1.0	0.199
Basophils (%)	0.5 ± 0.5	0.6 ± 0.5	0.102
Eosinophils (%)	1.8 ± 0.8	6.0 ± 1.9*	0.023

*Means are significantly different at the level ($p < 0.05$) when compare with the control.
TEC: Total erythrocyte count; Hb: Hemoglobin; TLC: Total leucocytes count

Table-2. Lipid peroxidation and antioxidants enzymes in control (n=20), and trypanosoma infected camels (n=20)

Parameter	Control	Diseased	P values
MDA (μM)	0.07 ± 0.01	6.3 ± 0.30*	0.021
GR (nmol/ml)	0.80 ± 0.20	1.3 ± 0.01*	0.025
SOD (U/ml)	1.80 ± 0.20	2.1 ± 0.10	0.072

*Means are significantly different at the level ($p < 0.05$) when compare with the control.
MDA: Malonaldehyde; GR: Glutathione reductase; SOD: Superoxide dismutase.

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