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Research Article

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ABSTRACT

Antioxidant vitamins may affect an organism's capacity for defense against damage caused by reactive oxygen species (ROS), and biological markers of the dietary exposure to these compounds is of importance. The present study was designed to evaluate the protective effect of vitamin E and vitamin C against the hemolysis induced by hydrogen peroxide (H_2O_2) in camel's red blood cells *in vitro,* and for assessing the vulnerability of camel erythrocytes to oxidative stress (OS). Vitamin E and vitamin C were able to protect the red blood cells against the H_2O_2-induced hemolysis and thiobarbituric acid reactive species (TBARS) formation. The antioxidative property of these vitamins in reducing oxidative injury showed in this work, may suggest that these molecules may be very helpful to fight the ROS during OS induced by transportation, heat, deshydratation, aging, and parasite, infectious and metabolic diseases in dromedary camels.

Keywords: Ascorbic acid; Camel's red blood cells; Hemolysis; Hydrogen peroxide; Oxidative stress; Vitamin E.

List of non-standard abbreviations
AA: ascorbic acid
BCC: blood cells count
BSS: buffered salt solutions
EOF: erythrocytes osmotic fragility
Glu: glucose
HR: heart rate
Ht: hematocrite
LP: lipid peroxidation
MDA: malondialdehyde
OS: oxidative stress
RBCs: red blood cells
ROS: reactive oxygen species
RR: respiratory rate
TBA: thiobarbituric acid
TBARS: thiobarbituric acid reactive species.
TCA: trichloroacetic acid
Tr: rectal temperature

INTRODUCTION

It is well documented that dromedary camel has some biochemical, anatomical and physiological peculiarities due to his adaptation to arid conditions and poor feeding resources (Faye et al., 1992, El Khasmi et al., 21005).

Camel is a good source of meat especially in areas where the climate adversely affects the performance of other animals (Kadim et al., 2006). However, camel meat comes mostly from old males and females and males that are primarily kept for milk, racing, and transportation rather than for meat production (Kurtu, 2004). Furthermore, OS and free radical which may be implicated in the pathogenesis of a variety of diseases and aging (Halliwell and Gutteridge, 1999; Agarwal et al., 2003), are associated with high activities of anti-oxydant enzymatic system and low ascorbic acid (AA) concentrations in tissues and/or blood in camel (Mousa et al., 2006). On the other hand, preslaughter conditions such as road transportation are stressful for camel (El Khasmi et al., 2010; Saeb et al., 2010), and the magnitude of cortisol response under stress transportation is very much higher in old camels than that in young ones (El Khasmi et al., 2009). Transportation and heat stresses have been demonstrated to cause increase of free radical generation (Nazifi et al., 2009) and erythrocyte hemolysis (Adenkola et al., 2010; Minka and Ayo, 2010), and decrease of AA absorption (Naziroglu et al. 2000). When the concentration of antioxidant vitamins decreases, lipid peroxidation (LP) increases in the plasma and tissues leading to oxidative damage of erythrocytic cell membranes (Adenkola and Ayo 2009; Minka and Ayo 2010). The ROS induce oxidative damage of to macromolecules, cells and tissues (Altan et al., 2003) that consequently leads to substantial economic losses (St-Pierre et al., 2003) and hematological changes (Huff et al, 2005) leading to increased morbidity and mortality, poor meat quality and decreased productivity (Franco-Jimenez and Beck, 2007, Rozenboim et al., 2007). Thus, the supplementation of the animal by exogenous antioxidants such as □-tocopherol and AA (Alvarado et al., 2006; Urban-Chmiel et al., 2009; Niki, 2010) improves the antioxidant status, and seems to be very helpful to fight the ROS.

The mammalian erythrocyte is very sensitive to oxidative injury and it is considered as an ideal cell model in which to study stress injury, and composition and behaviour of natural biological membranes since it is enucleated and has a short life span (Bernabucci et al. 2002). Furthermore, hemolysis and the erythrocytic levels of malondialdehyde and hemolysis are considered as markers of LP occurring during OS oxidant stress (Saleh et al., 2011; Mendanha et al., 2012).

Since the hemolysis can be followed by changes in absorbance at 540 nm, the erythrocytes osmotic fragility (EOF) test was frequently used to determine the extent of red blood cells (RBC) hemolysis produced by osmotic stress (Adenkola and Ayo, 2009; Minka and Ayo, 2010). This test has been applied to the diagnosis of haemolytic diseases, studies of membrane permeability, road transport stress and alterations leading to destruction of erythrocytes (Jain et al., 1983; Adenkola and Ayo, 2009) and also as an important indicator of hemolysis in blood vessels (Hanzawa and Watanabe, 2000).

This article reports the protective effects of vitamin E and vitamin C against H_2O_2-induced hemolysis and TBARS (thiobarbituric acid reactive species) formation in Moroccan dromedary camels.

MATERIALS AND METHODS

2.1 Animals and blood sampling

Eleven healthy male camels (6 to 10 years of age old, average weight of 430 ± 40 Kg mean weight) clinically healthy were used in this investigation. They were feed deprived overnight and were slaughtered in the Casablanca Municipality slaughterhouse. Rectal temperature (Tr), heart rate (HR), respiratory rate (RR) and blood samples were taken before slaughter. Rectal temperature was taken in the rectum with a thermometer. The heart rate (beats/min) was determined by auscultation of the heart area or pulse by feeling the tibial, femoral and coccygeal pulse arteries. The respiratory rate (breaths/min) was determined by inspection and auscultation of the respiratory movements of the trachea. Four blood samples were collected by jugular venipuncture from each camel. Two samples were collected in an ethylene diamine tetra acetic acid–dipotassium (EDTA-K2) Vacutainer tubes (analysis of hematocrit and blood cells count) whereas the two other samples were collected in a heparinized tubes (analysis of hemolysis, glucose, calcium, phosphorus and magnesium). Following collection, the tubes were gently inverted to ensure mixing of the sample. After analysis of hematocrit (Ht) and blood cells count (BCC), the plasma was separated by centrifugation at 750 x g for 15 min at 4°C, pipetted into aliquots and then stored at -20°C. until analysis, and the red blood cells suspension was prepared.

2.2 Hematocrit, blood cells count, minerals and glucose analysis

The hematocrit was determined by centrifuging a precise amount of blood in calibrated hematocrit tubes (Hettich Haematokrit D-7200), the report cell mass/plasma was expressed as % by direct reading on the tube: Ht (%) = [(level of pellet)/(overall height)] x 100. A Malassez cell (1 mm^3) was used for counting the blood cells. It comprises five horizontal bands of five lines each, and five vertical bands of 6 lines each. For erythrocyte count, a blood dilution of 1/200 in Marcano's liquid was performed. For leukocyte count, a dilution of 1/20 in Hayen's liquid was used. Erythrocytes were counted in the four rectangles composed of 20 small squares located around the grid (=N) and we averaged the four values found: m = N/4. As the volume of a rectangle is 1/100 mm^3 and the dilution is 1/200, the final result is therefore: m x 100 x 200 erythrocytes/mm^3 or m x 100 x 200 x 10^3 erythrocytes/mL of blood. Leukocytes were counted in five horizontal bands (=N'). As the volume of an horizontal

band is 1/10 mm^3, for 5 counted bands the volume becomes 1/2 mm^3. There are therefore N' x 2 leucocytes/mm^3 of blood diluted to 1/20. The end result is therefore: N' x 2 x 20 leucocytes/mm^3 or N' x 2 x 20 x 10^3 leucocytes/mL of blood. Blood concentrations of glucose (Glu) and plasma levels of Ca, Pi calcium, phosphorus and magnesium (Mg) were measured using commercial kits.

2.3 Erythrocyte separation

Erythrocytes were isolated by centrifugation at 4 °C for 20 min at 1000 g, and at 4 °C. The plasma and buffy coat were carefully removed using a micropipette. The cells pellet was washed three times with 310 mOsm isotonic phosphate buffer (pH 7.4), centrifuged at 4 °C at 1000 g for 10 min at 4°C and finally suspended in an equal volume of isotonic phosphate buffer. This constituted the erythrocyte suspension, which was stored at 4 °C for 24 h until further analysis (Dodge et al., 1963).

2.4. Oxidative stress indices

2.4.1. Hemolysis

To induce an oxidative stress, a 5% (v/v) suspension of washed, packed erythrocytes in buffer (310 mOsm) was mixed with the same volume of 0.5, 2 and 8 mM H_2O_2. H_2O_2 that is normally generated in vivo mainly by the autoxidation of hemoglobin and dismutation of superoxide gives rise to radicals like hydroxyl ions (Djordjevic, 2004). The mixtures were incubated at 37 °C for 2 h. One sample of each concentration was pretreated with 1 mM sodium azide, an inhibitor of catalase, at 37 °C for 10 min before inducing oxidative stress. The reagent solutions were gently shaken every 15 min during incubation.

To evaluate the protective effect of antioxidant molecules, erythrocytes were previously incubated for 30 min at 37°C with vitamin E (2 mM) or vitamin C (60 mM) before the oxidation process.

The hemolytic level was determined spectrophotometrically. After the sample was oxidized, the reagent solutions were centrifuged for 10 min at 300 g and at 25°C and the absorption of the supernatant (A) was measured at 540 nm. Percent RBC lysis was determined according to the following equation: % hemolysis = Aa - Ac_1 / Ac_2 - Ac_1, where Ac_1 is the control sample (0 % H_2O_2), Ac_2 is the sample completely hemolyzed with distilled water and Aa is the sample with the desired H_2O_2 concentration (Senturk et al., 2001).

2.4.2. Erythrocytes osmotic fragility

The procedure of EOF was a slightly modified method of O'Dell et al. (1987). A 100 □l aliquot of washed erythrocyte suspension was added to test tubes containing 5 mL of 0.2 %, 0.3 %, or 0.9 % buffered salt solutions (BSS, pH 7.4). The contents of these tubes were gently mixed by inverting them five times and were allowed to stand at 37 °C for 30 min. There after,Then these tubes were centrifuged at 1270 x g for 10 min to pellet the cells. The supernatant was then transferred into a glass cuvette and the absorbance was measured at 540 nm, measure data wave length of 540 nm using a spectrophotometer by reading the absorbance. The profile of the EOF of our camels was previously analysed by using a BSS (pH 7.4) concentrations, ranging from 0.1 % to 0.9 %.

Hemolysis in each tube was expressed as a percentage, taking as 100 % the maximum value of absorbance of distilled water. BSS (0.9 %) was considered as a control sample. The percent hemolysis was calculated according to Fraukner and King (1970) as follows: (Optical density of test/ Optical density of distilled water) x 100 EOF curve was obtained by plotting percent hemolysis against the saline concentrations.

2.4.3. Lipid peroxidation: determination of thiobarbituric acid reactive species

Malondialdehyde (MDA) production was measured according to the following protocol (Gilbert et al., 1984). After incubating the RBCs in solutions containing H_2O_2, 10 % trichloroacetic acid (TCA (w/v) final concentration) was added to 1.5 mL of the supernatant solution of erythrocytes. The solutions were then centrifuged for 10 min at 300 *g* and 1 mL thiobarbituric acid (TBA, 1 % in 0.05 M NaOH) was added to the supernatant. The reagent solution was heated to 95°C for 30 min. After cooling to 4°C, 1 mL n-butanol was added to each tube to extract the MDA-TBA complex. TBARS concentration was obtained by subtracting 20% of the absorbance at 453 nm from the absorbance at 532 nm, using a molar extinction coefficient of 1.56 x 105 $M^{-1}cm^{-1}$.

2.5. Statistical analysis

All values were expressed as mean and standard error of mean (SEM) (SE), and statistical significance was determined using one-way analysis of variance followed by student's *t*-test. Differences were considered significant when $p < 0.05$.

RESULTS

3.1. Physiological parameters

The values of physiological parameters (rectal temperature, heart rate, respiratory rate, BCC, hematocrit, glucose, calcium, phosphorus and magnesium RT, HR, RR, BCC, Ht, Glu, Ca, P an Mg) measured in the camels used in this investigation are were presented in the in normal range (table 1). The curve of osmotic fragility of camel's erythrocytes in absence of H_2O_2 showed strong fall between 0.2 and 0.4% NaCl concentration (figure 1). is shown in figure 1. Hemolysis (6 % ± 2) started at 0.4 % NaCl and becamed higher (98 % ± 6) at 0.2 % NaCl.

Table 1: Physiological parameters in the population of camels used in this investigation (Mean ± SEM, n = 11).

Physiological parameters	Mean ± Ecart type SEM
Heart rate	48 ± 2 beats/min
Respiratory rate	12 ± 2 breaths/min
Rectal temperature	37.8 ± 0.4 °C
Hematocrit	32.6 ± 3.5 %
Red blood cells count	7.8 ± 0.95 (x 10^9) milliard/mL
White blood cells count	7.76 ± 3.14 (x 10^6) billion/mL
Glycemia	5.8 ± 1.7 mmol/L
Calcemia	2.6 ± 0.3 mmol/L
Phosphoremia	1.2 ± 0.1 mmol/L
Magnesemia	0.8 ± 0.2 mmol/L

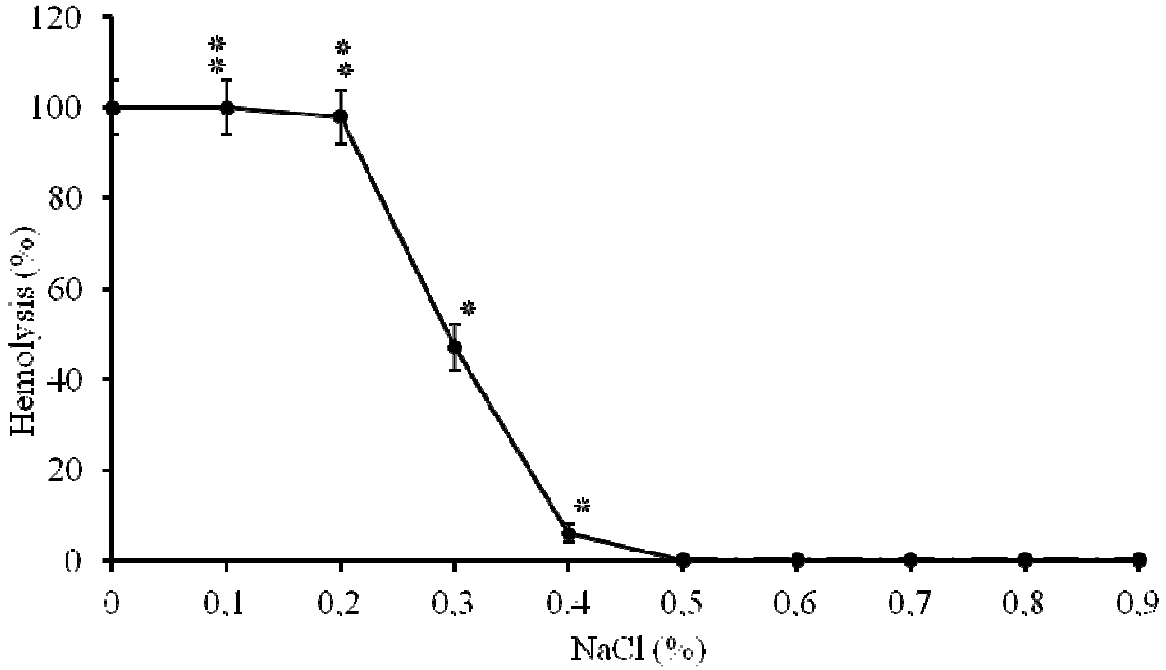

Figure 1: The erythrocyte osmotic fragility in dromedary camels at a pH value of 7.4 and temperature of 37°C. Values are expressed as mean ± SEM of 11 animals. Changes between the isotonic and other NaCl concentrations are analyzed by one-way ANOVA, and are significantly different at $p < 0.05$ (*) or $p < 0.005$ (**).

3.2. Oxidative stress and protection by antioxidant vitamins

The levels of H_2O_2-induced oxidative hemolysis measured at 5 % hematocrit (table 2) indicate that erythrocyte damage occurs in the concentration range of 0.5 to 8 mM H_2O_2. Oxidative damage induced by this free radical initiator can be presented with reference to hemolysis, osmotic fragility and lipid peroxidation LP. The erythrocyte hemolysis (%) induced by 0.5, 2 and 8 mM H_2O_2 was significantly very higher (48.7 ± 5.2, 65.6 ± 6.7 and 93.4 ± 8.1 respectively) than that of control (18.7 ± 1.4) ($p < 0.05$) and was associated to with a significant increase ($p < 0.05$) of TBARS formation (□M/mL) (1.16 ± 0.10; 3.82 ± 1.13 and 4.54 ± 1.21 respectively) by comparison to control (0.12 ± 0.08) (table 2).

Table 2: Percent hemolysis and thiobarbituric acid reactive species (TBARS) induced by different hydrogen peroxide (H_2O_2) concentrations in a camel erythrocyte suspension at 5 % hematocrit. Values are expressed as mean ± SEM of 11 animals. Changes between the control (0 mM H_2O_2) and H_2O_2 are were analyzed by one-way ANOVA, and are significantly different at $p < 0.05$ (*).

	H_2O_2			
	Control	0.5 mM	2 mM	8 mM
% Hemolysis	18.7 ± 1.4	48.7 ± 5.2*	65.6 ± 6.7*	93.4 ± 8.1*
TBARS (□M/mL)	0.12 ± 0.08	1.61 ± 0.10*	3.82 ± 1.13*	4.54 ± 1.21*

The stress oxidant hemolysis is showed in the table 3. This hemolysis was related to changes in the EOF of our camel erythrocytes exposed to H_2O_2 (table 3). Membrane fragility increased significantly ($p < 0.05$) with the increase of H_2O_2 concentration. For example, the EOF at 0.3 % NaCl in the presence of 2 and 8 mM H_2O_2 was 21.2 % ± 2.3 and 28.4 % ± 3.4 respectivly, however, in the same conditions, the EOF of control was 14.2 % ± 2.2 (table 3).

Table 3: Hydrogen peroxide – induced osmotic fragility of camel erythrocytes. Values are expressed as mean ± SEM of 11 animals. Changes between the control and H_2O_2 for the same concentration of NaCl are were analyzed by one-way ANOVA, and are significantly different at $p < 0.05$ (*).

	NaCl concentration		
	0.2 %	0.3 %	0.9 %
H_2O_2 concentration			
Control	15.1 ± 2.0	14.2 ± 2.2	10.5 ± 1.2
0.5 mM	24.4 ± 2.5*	18.0 ± 2.0*	12.8 ± 1.3
2 mM	30.2 ± 4.5*	21.2 ± 2.3*	16.6 ± 2.1*
8 mM	36.5 ± 4.3*	28.4 ± 3.4*	22.3 ± 2.6*

A second set of experiments were conducted to test the potential of vitamins E and C for reducing oxidative stress when the cells were exposed to H_2O_2. We pre-incubated the erythrocytes with these antioxidants before incubating them with H_2O_2. Vitamins E (2 mM) and C (60 mM) reduced significantly ($p < 0.05$) the hemolysis levels (26.4 % and 42,9 % respectively) and the TBARS formation (80.4 % and 73.3 % respectively) by comparison with values observed in cells exposed to 2 mM H_2O_2 (table 4). The protective effects of the antioxidants vitamins C and E at the used concentrations (60 and 2 mM respectively) against oxidative hemolysis induced by 2 mM H_2O_2, showed that ascorbic acid provided the higher effect.

Table 4. Protective effects of vitamin C (60 mM) and vitamin E (2 mM) on percent hemolysis induced by the hydrogen peroxide H_2O_2 (2 mM) and thiobarbituric acid reactive species (TBARS) (□M/mL) formation in camel erythrocyte suspension. Values are expressed as mean ± SEM of 11 animals. Changes between H_2O_2 alone and H_2O_2 in combination with vitamins E or C are were analyzed by one-way ANOVA, and are significantly different at $p < 0.05$ (*).

	Control	H_2O_2	H_2O_2 + vitamin E	H_2O_2 + vitamin C
% Hemolysis	8.7 ± 1.4	65.6 ± 6.7	48.3 ± 5.7*	37.4 ± 4.2*
TBARS	0.12 ± 0.08	3.82 ± 1.13	0.75 ± 0.09*	1.02 ± 0.10*

DISCUSSION

The present study was designed to induce OS in RBCs membrane lipid by H_2O_2 either alone or in combination with vitamin E or AA and to assess the vulnerability of camel RBC to OS. The results of the physiological and hematological parameters observed here obtained in this study (table 1), were similar and within the normal ranges of values recorded in camels (Amin et al., 2007; Hussein et al., 2010), which suggested that our camels were healthy. The pattern of the osmotic fragility of camel's RBC in various hypo-osmotic solutions of NaCl was basically decreased with decreasing concentration (figure 1) and showed that RBC of our camels were characterized by a significant increase of osmotic resistance by comparison with that measured in other mammalian species. Thus, for example, hemolysis started at 0.4 % NaCl in camel and at 0.85 %, 0.70 % and 0.55 % NaCl in sheep, cattle and humans respectively (Arikan, 2003: Livne and Kuiper, 1973; Mirgani, 1992; Al-Qarawi, 1999) which demonstrates that camels have more resistant erythrocytes. On the other hand, it has been established that RBC of males are more susceptible to hemolysis than those of females in domestic fowl (March et al., 1966; Oyewale and Durotoye, 1988), cattle (Olayemi, 2007) and camel (Amin et al., 2012). According to Amin et al. (2007), in camel, the osmotic resistance increased significantly during the green season. Camel erythrocytes are able to expand to 240 % of their original volume without rupturing in hypotonic solutions (Perk, 1966) and have an oval shape (Jain and Keeton, 1974). So, this animal has an exceptional ability to rapidly replace water lost during prolonged periods of dehydration within a few minutes of access to drinking water without hemolysis (Schmidt-Nielson et al., 1956). The exceptional osmotic resistance of camel RBC may be due to the high concentration of total lipids, cholesterol, proteins, sphingomyelin and phosphatidylcholine in the RBC membranes of camels when compared with the concentrations of these parameters in the RBC membranes of sheep and goats (Al-Qarawi and Moussa, 2004). In addition, the augmented water-binding associated with the high hydrophilicity of camel haemoglobin, may contribute to this high osmotic resistance. In fact, according to Bogner et al. (2005), the proportion of osmotically non-removable water in camel erythrocytes is nearly 3-fold greater than that in human RBC (approximately 65 vs approximately 20%). On the other hand, erythrocytes of camels show a very low water contents (1.1 – 1.3 g water/g dry mass) (Yagil et al., 1974; Weiser et al., 1992; Perk, 2000) and a difference in the major intrinsic membrane water-soluble protein ‘‘spectrin’’ which appears to be very tightly bound to the membrane by comparison to those of humans and bovine species (Ralston, 1975).

The camel RBC subjected to H_2O_2 showed an increase of hemolysis, membrane fragility and TBARS production (table 2). The EOF test is a commonly used technique to detect changes in the RBC content and membrane flexibility, and is a sensitive marker of change in osmotic stress of RBC (Adenkola and Ayo, 2009; Minka and Ayo, 2010). Earlier studies have also suggested that osmotic fragility is related to pathological situations, and that these changes in cell integrity may be applied to the study of OS injury and diagnosis of hemolytic diseases (Sato et al., 1998; Kolanjiappana et al., 2002). In dromedary camels, the high concentration of total lipids, cholesterol, proteins, sphingomyelin and phosphatidylcholine, and the higher ratio of proteins to total lipids and cholesterol to phospholipids in the RBC membranes of camels may have a role in the lower osmotic fragility and the stability of the camel RBC (Al-Qarawi and Mousa, 2004). But, several environmental factors (transportation, stress, heat and moisture) are able to provoke excessive generation of ROS or free radicals as a result of increased metabolism (Ramnath et al., 2008). In dromedary camels, transportation and heat stress, might cause an oxidative challenge by inducting a significant increase of plasma concentrations of malondialdehyde and whole blood glutathione peroxidase activities (Nazifi et al., 2009) and increase susceptibility for infections such as pneumonia (Werner and Kaaden, 2002).

In the present investigation, camel erythrocytes showed increased fragility in response to H_2O_2 (0.5 – 8 mM) (table 3). To interpret our results on the mechanisms of oxidative stress through camel RBC *in vitro*, we can consider three factors, could be considered namely, (i) the impact of H_2O_2 as a free radical initiator on hemolysis and EOF, (ii) the influence of this compound on LP in cell membrane and (iii) the effect of antioxidants, vitamin E and AA in suppressing the mechanisms induced by H_2O_2 in the lipid regions of RBC. H_2O_2 is known by its ability to penetrate biological membranes and then increase LP (Nordberg and Arner, 2001) and decrease RBC deformability. In fact, Snyder et al. (1988) showed that H_2O_2 induces the formation of a spectrin-hemoglobin complex in human RBC membranes associated with a progressive alteration in the cell’s shape to echinocytic morphology and decreased cell deformability. The camel erythrocytes which were subjected to OS with H_2O_2 were characterized by increased hemolysis and TBARS formation (tables 2 and 3). These membrane dynamic changes may suggest a peroxidative breakdown of phospholipid fatty acids in the RBC membrane (Ramazan et al., 2000). In the face of a profound external OS to the on RBC, the plasma membrane is often the initial site of damage, and the resulting peroxidation of membrane lipids causes hemolysis and cross-links between protein and lipid molecules to different extents. Further, H_2O_2 is largely known by its toxicity due to its ready conversion to the reactive hydroxyl radical (OH^-), either by exposure to ultraviolet light or by interaction with a range of transition metal ions, of which the most important in vivo is probably iron (Djordjevic, 2004). In addition, oxidized hemoglobin may bind to the RBC membrane and increase its rigidity (Mendanha et al., 2012). On the other hand, a significant increase of LP, TBARS production and hemolysis was recently observed in camel with OS inducted by Sarcoptic mange (Saleh et al., 2011) or liver cystic echinococcosis (Heidarpour et al., 2012).

The ROS are compounds with high potential to damage almost all types of cellular constituents by increasing LP, resulting induction and/or amplification of a number of tissular lesions (Bernabucci et al., 2002;

Lata et al., 2004). The mammalian RBC are able to defend themselves against these compounds, by an effective and complex antioxidant system, including protective enzymes and biological antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, AA, and vitamin E. However, when the capacity of the RBC to repair oxidative damage is limited, these cells become very sensitive to oxidative damage and constitue an ideal cell model in which to study OS (Sato et al., 1998). The protective effects of AA and α-tocopherol against the oxidative damage of camel RBC when exposed to H_2O_2 were marked by a significant decrease of hemolysis and TBARS formation (table 4). In fact, the supplementation of the animal by exogenous antioxidants such as these vitamins, improves the antioxidant status, cellular immune activity and cytokine production as well as inflammatory responses, and seems to be very helpful to fight the ROS generated in the membrane cells (Alvarado et al. 2006; Urban-Chmiel et al., 2009).

Several researchers have suggested that the use of AA and/or vitamin E as antioxidants in combating OS by reducing LP and increasing the stabilization of cell-membrane,. This has been documented in birds (Ajakaiye et al., 2010), cows (Urban-Chmiel et al., 2009), goats (Minka and Ayo, 2010) and pigs (Adenkola and Ayo, 2009). In addidtion, AA administration has been shown to decrease hemolysis and reduce oxidative damage to RBC (Adenkola and Ayo, 2009; Minka and Ayo, 2010) by stabilizing the membrane integrity of the cells and decreasing the membrane susceptibility to LP (Tauler et al., 2003). AA is involved in the defence against free radicals, modulates the immune response as it influences phagocytic cell mobility and chemotaxis, and its status may be important in relation to animal health and disease (Dragsted, 2008). Thus, in several studies, it had been showed that camels with naturally acquired parasite infections such as trypanosomiasis, Sarcoptic mange, and helminthiasis (Mohamed and Beynen 2002; Saleh et al., 2009; 2011), *brucellosis* (Hasab et al., 2011), echinococcosis (Heidarpour et al., 2012) and Sarcoptes scabiei (Saleh et al., 2011) had a lowered AA status. In addition, sheep with *Fasciola hepatica* (Gameel, 1982), cows with endometritis (Kolb et al., 1994) and calves with pneumonia (Jagos et al., 1977) have low AA concentrations in plasma. AA can also indirectly protect cell membranes from LP, protect the cell membrane and decreases hemolysis by reducing the tocopheroxyl free radicals of the membrane bilayer (Massaeli et al., 1999). Vitamin E, a constituent of the plasma membrane, then an effective antioxidant, scavenges peroxide free radicals and converts them to less toxic lipid hydroperoxides, thereby protecting the cell membrane and decreasing hemolysis (Niki and Noguchi, 2004). The benefits of this vitamin have been reported in human RBC when challenged with phenylhydrazine hydrochloride (Claro et al., 2006). However, according to Mendanha et al., (2012), AA and α-tocopherol are able to prevent oxidative hemolysis and TBARS production, but can not decrease the membrane rigidity when RBCs are exposed to H_2O_2.

Taken together, our study suggests that vitamin E and AA could be used in reducing OS in camel, and that it's camel erythrocytes could be considered *in vitro* models to evaluate the mechanisms of oxidative injury.

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