A variety of factors and widely varied causes can lead to camel lameness, including direct trauma, nutritional problems, punctured foot, abnormal limb conformation, and infectious disease (Gahlot and Chouhan, 1992; Ramadan, 1994; Gahlot, 2007; Al- Juboori, 2011a; Al-Juboori, 2011b; Al-Juboori, 2010; Mostafa and Khalil, 2018; Mostafa, 2020). Lameness in camels is characterised by partial or non-weight bearing by one or more limbs, swelling of the joints, pain upon palpation, protruding toes, shivering while sitting, and an asymmetrical pelvis (Gahlot, 2007). In addition to decreased milk production, decreased reproductive performance, growth retardation, and culling from farms or competition, lame camels also suffer from diminished physiological vitality and need additional treatment and care costs (Manefield and Tinson, 1997; Al-Ani, 2004 and Al-Juboori, 2010). Camel lameness differs from that of cattle and horses owing to its unique anatomy, biomechanics, geoclimatic adaptations, and use (Gahlot, 2000). There is increasing evidence that oxidative damage contributes to many diseases, such as atherosclerosis, cancer, liver damage, rheumatoid arthritis, immunological incompetence, neurodegenerative disorders, and aging (Dalle-Donne et al, 2003; Habif et al, 2001). Oxidative stress (OS) is defined as RONS (Reactive Oxygen/Nitrogen species) that exceed the body’s antioxidant capacity (Finaud et al, 2006).

The body produces RONS as a result of its normal metabolic processes and through exposure to an array of environmental and physiological stressors. The tissue’s specific antioxidant defenses and the specific macromolecules targeted by RONS will determine the degree of damage and disease generation that may accompany OS. The total antioxidant capacity of the body provides protection against the excessive production of reactive oxygen species.

**INVESTIGATION OF LAMENESS IN RACING DROMEDARY CAMELS (Camelus dromedarius) AND ASSOCIATED OXIDATIVE STRESS BIOMARKERS**

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**ABSTRACT**

In racing camels, lameness is measured to be a major health issue and an economically important problem for many camel owners. This study aimed to investigate different oxidative stress and antioxidant biomarkers in the blood of racing lamed dromedary camels. Moreover, to highlight their role in lameness diagnosis, pathogenesis and to emphasise its role to monitor treatment response. Thirty five out of 315 racing camels exhibited clinical lameness. The serum levels of malondialdehyde (MDA) and nitric oxide (NO) in lame dromedary camels with different perceived causes of lameness (punctured foot, traumatic injury) were remarkably over than those detected in the control healthy dromedary camels. However, lame dromedary camels had significantly lower levels of serum superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and total antioxidant capacity (TAC) when compared with the control healthy camels. The serum levels of MDA, NO, SOD, GSH, CAT, and TAC markers in lame dromedary camels pre-and post-treatment were measured in this study. An obvious decline was detected in serum levels of MDA and NO of lame camels after 10 days of treatment, whereas, the levels of antioxidant markers (SOD, GSH, CAT, and TAC) were significantly increased toward normal values. The ROC curves were created. The AUCs were assessed to evaluate the accuracy of each variable to distinguish diseased and healthy camels. Based on the ROC curves and AUCs; MDA, SOD, GSH, CAT, TAC, and NO were considered highly diagnostic and predictive biomarkers of lame dromedary camels. Moreover, the addition of antioxidants to the treatment protocol of lameness may enhance the treatment response in camels.

**Key words:** Camel, catalase, glutathione, lameness, malondialdehyde, super oxide dismutase
and nitrogen species (RONS) by cells (Bloomer and Fisher-Wellman, 2008). Endogenous and exogenous compounds contribute to antioxidant capacity (Halliwell and Gutteridge, 1999; Possamai et al., 2007). The former recognised antioxidant compounds include uric acid, superoxide dismutase, catalase, and glutathione peroxidase. Over the last decade, various tests for the measurement of OS have been developed to assess the oxidative status of an individual under normal or pathological conditions or after any kind of intervention. By measuring the levels of markers for ongoing oxidative damage in serum and plasma samples, we can get a complete picture of the OS state in the body (Argqelles et al., 2004).

There have only been a few studies comparing antioxidant capacity and OS in domestic animals, particularly in racing dromedary camels (Mousa et al., 2006). Our study measured (malondialdehyde) MDA as well as nitric oxide (NO) (as markers of OS), super oxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and total antioxidant capacity (TAC) levels (as markers of antioxidant status) in lame racing dromedary camels and healthy ones. Moreover, the study monitored the oxidant-antioxidant status in lame dromedary camels after 10 days of treatment.

Materials and Methods

Camels’ selection and sampling protocol

A total of 315 racing dromedary camels, aged 5.5–8.1 years, were investigated between January and September 2019 in one camel herd in the eastern region of Saudi Arabia. A total of 35 out of 315 racing camels exhibited clinical lameness. Punctured feet and traumatic injuries were diagnosed as lesions responsible for clinical lameness in these racing camels. Bovine hoof testers and walking on sandy, pebbled or hard tracks were used to diagnose lame camels (Gahlot, 2000 and 2007). Moreover, 20 clinically healthy racing dromedary camels were carefully chosen as a control group.

Whole blood samples were collected from clinically lame dromedary camels (N = 35) and the control healthy group (N = 20) for further biochemical analysis. Lame dromedary camels were subjected to a treatment protocol which included the use of non-steroidal anti-inflammatory drugs (NSAIDS) (2.2 mg flunixin per kg of camel body weight, IV injection), cleaning, and disinfection of lesions for five consecutive days, with complete rest of the affected camels.

Evaluation of oxidative stress and antioxidant biomarkers

With the help of commercial ELISA kits (Cayman, USA), malondialdehyde, reduced glutathione, catalase, and superoxide dismutase activities were determined in serum. After nitrate has been reduced to nitrite by the Griess reagent, nitric oxide (NO) is determined as the nitrite concentration in serum. Using sodium nitrite as a standard, the reaction was measured at nm (Ding et al., 1988). A commercial test kit (Bayer diagnostics) was used to measure total serum protein concentration. The NO concentration in serum was measured using a Nmol/mg protein unit. Beers and Sizer (1952) method, CAT activity was measured in the RBC hemolysate. CAT activity was measured by measuring the difference in absorbance per min/mg Hb following the decomposition of H2O2. Spectrophotometric analysis of TAC was conducted using kits supplied by Biodiagnostic®, Egypt.

Data Analysis

All parameters were expressed as mean ± SD. Comparisons in mean were performed by Kruskal–Wallis ANOVA on Ranks followed by Dunn’s multiple comparisons. The different means were significant at P<0.05. Statistical analysis was performed using JMP software version 11.0.0 (SAS Institute, Cary, NC, USA). Spearman’s rank correlation test was used to investigate the correlation between parameters. Each assay’s diagnostic accuracy was evaluated by creating the ROC (receiver operator characteristics) curve and determining the area under the curve (AUC). An AUC of 0.7 to 0.9 was considered moderately accurate, an AUC of >0.9 highly accurate, and an AUC of 1 perfect.

Results

The serum levels of MDA and NO in lame dromedary camels with different causes were remarkably (P 0.0001) higher than those detected in the control healthy dromedary camels (Fig 1). However, we found that lame dromedary camels had significantly lower levels of serum SOD, GSH, CAT, and TAC when compared with the control healthy camels. (Fig 1). The serum levels of MDA, NO, SOD, GSH, CAT, and TAC markers in lame dromedary camels pre-and post-treatment were measured in this study. A significant decline was detected in serum levels of MDA and NO of lame camels after ten days of treatment, whereas the levels of antioxidant markers (SOD, GSH, CAT, and TAC) were significantly increased towards normal values (Fig 2). Spearman’s correlation was estimated for
the study biomarkers in clinically lame camels and healthy ones and in lame camels before and after 10-days of treatment (Table 1). There was a significant appositive correlation between TAC, SOD, GSH, and CAT. However, significant negative correlations were observed between OS markers (MDA and NO) and all tested antioxidants (TAC, SOD, GSH, and CAT) in camels under investigation.1

The diagnostic test characteristics of OS and parameters in racing dromedary camels with clinical lameness were explored in Table 2. The ROC curves were created. The AUCs were assessed (Fig. 3) to evaluate the accuracy of each variable in distinguishing diseased and healthy camels. Based on the ROC curves and AUCs, MDA (AUC = 0.910), SOD (AUC = 0.922), GSH (AUC = 0.901), TAC (AUC = 1), and NO (AUC = 0.994) were similarly highly diagnostic and predictive for treatment response in lame dromedary racing camels.

Table 1. Correlation matrix using spearman test among different oxidative stress biomarkers in racing dromedary camels with clinical lameness.

<table>
<thead>
<tr>
<th></th>
<th>sMDA</th>
<th>CAT</th>
<th>GSH</th>
<th>SOD</th>
<th>NO</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>sMDA</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>-0.557</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>-0.527</td>
<td>0.524</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>-0.542</td>
<td>0.497</td>
<td>0.556</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>0.589</td>
<td>-0.613</td>
<td>-0.515</td>
<td>-0.588</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>-0.557</td>
<td>0.633</td>
<td>0.518</td>
<td>0.64</td>
<td>-0.597</td>
<td>1</td>
</tr>
</tbody>
</table>

Serum malondialdehyde (sMDA), catalase (CAT), reduced glutathione (GSH), super oxide dismutase (SOD)
Discussion

The clinical signs of lame camels in this study are in concurrence with the clinical picture of lame camels reported in other studies (Gahlot, 2007; Al-Juboori, 2011a and Al-Juboori, 2011b; Al-Juboori, 2013; Mostafa and Khalil, 2018; Mostafa, 2020).

There are several types of stress that athletes of all species encounter, including OS. According to the results presented in this study, circulating OS is generated by increased free radical generation (increased NO levels), lipid peroxidation enhancements (increased MDA levels), combined...
with abnormalities of antioxidant status (reduced GSH, SOD, CAT, and TAC) in racing dromedary camels clinically affected by lameness. As far as we know, this study is the first to describe OS status in lame dromedary racing camels. It is known that OS is involved in the pathogenesis of lameness in dairy cows (Al-Qudah and Ismail, 2012; Zhao et al., 2015).

In particular, polyunsaturated lipids are prone to oxidation. Biomarkers of lipid peroxidation are considered the best indicators of OS since lipids are one of the most vulnerable substrates to free radical damage (Georgieva, 2005).

During the radical-induced breakdown of polyunsaturated fatty acids, MDA forms. The reaction between MDA and thiobarbituric acid produces thiobarbituric acid reactive substances (TBARS), which facilitates the measurement of these substances by spectrophotometry (Janero, 1990). In this study, increased levels of serum MDA and NO in lame racing dromedary camels under the same environment, diet, training management, and feeding regimen proved that OS influences lameness in dromedary racing camels. In addition, significantly reduced levels of tested antioxidants (SOD, GSH, CAT, and TAC) markers were observed in lame racing dromedary camels, providing yet another support for the notion that oxidative damage may be involved in lameness pathogenesis.

OS is any disturbance in the normal redox state of cells that will cause veritably bad effects due to the yield of peroxides, and free radicals, leading to damage of all factors in the cell, including proteins, lipids, and DNA (Kowaltowski and Vercesi, 1999). Accordingly, OS can cause disturbances in normal mechanisms of the cellular capability to detoxify the reactive interceders or to repair the cell.

Fig 3. Receiver operating characteristic (ROC) curve analysis of malondialdehyde (MDA); catalase (CAT), reduced glutathione (GSH); super oxide dismutase (SOD), nitric oxide (NO) and total antioxidant capacity (TAC) in healthy and lame dromedary racing camels.
damage (Kowaltowski and Vercesi, 1999). As proved previously and verified in this study, a complex association exists between OS and inflammation. Moreover, OS is a consequence of the imbalance between reactive oxygen species (ROS) and product and antioxidant capacity. This can be because of either heightened ROS generation detected, a disturbed antioxidant system, or a combination of both (as perceived in this study). In the presence of OS, uncontained ROS attacks modify and denature functional and structural molecules, leading to cell injury (Vaziri, 2008).

Comparable to our findings, it was reported that MDA levels were increased in varieties of inflammatory conditions in dromedary camels like acute, and chronic cystitis (Abd Ellah, et al, 2012; El-Deeb and Buczinski, 2015), liver abscess (El-Deeb et al., 2014), Coxiella burnetii infection (El-Deeb et al, 2019) and Trypanosoma evansi infection (Saleh et al, 2009; El-Deeb and Elmoslemany, 2015; El-Bahr and El-Deeb, 2016).

The NO levels in lame dromedary racing camels’ cases compared to those in the control group, a statistically highly significant increase (P<0.0001) was observed. NO and peroxynitrite are free nitrogen derivatives among the reactive nitrogen species (RNS) (Tabakoglu and Durgut, 2013). Yarim et al (2006) described NO as a free radical that mediates both physiological and pathological events in the body. Despite its primary role in defending against bacteria, viruses, and parasites, NO has been reported to suppress the immune system. Accordingly, NO may protect or damage tissues depending on its concentration (Bozukluhan et al., 2013). In the presence of bacterial infections, macrophages produce large amounts of nitric oxide and exhibit antibacterial properties (Bozukluhan et al, 2016). A different viewpoint suggests that nitric oxide has anti-inflammatory property (Miranda et al, 2001). A major component of inflammatory diseases is peroxynitrite, a strong oxidising agent in tissues and organs. Sezer and Keskin (2014) described peroxynitrite as a highly toxic form of nitric oxide. According to a other research study, neutrophils released from diseased animals produce high levels of NO and myeloperoxidase, which ultimately leads to nitrotyrosine formation (i.e., protein damage) (Wessely-Szponder et al, 2004).

As detected in this study, increased NO production was also reported in humans with other inflammatory skin diseases and cutaneous infections (Serarslan et al, 2005; Bickers and Athar, 2006). In harmony with our findings, synovial fluid NO levels were significantly higher in 20 arthritic calves than in the control group (Yurdkul and Sartas, 2013). According to Yarim et al (2006) and Bozukluhan et al (2013) serum NO levels were higher in cattle with foot-and-mouth disease than in the control group, which was attributed to NO release and immune system stimulation. Conclusively, the increase in NO levels in this study suggests that it contributes actively to the development of racing dromedary camel lameness.

Since SOD catalyses the dismutation of superoxide to hydrogen peroxide (Halliwell et al, 1993), it is an important first line of defense against pro-oxidants. In this study, the levels of SOD, GSH, CAT, and TAC were significantly decreased in lame camels when compared with controls. Observed reductions in SOD, and CAT activities in the serum of lame racing camels in our study correlate with lower glutathione levels in the serum. The levels of tested antioxidants significantly increased toward normal values following the lameness treatment protocol. The present study provides evidence that levels of examined antioxidants can be used to evaluate the state of OS in lame camels, and to guide the treatment response.

Several methods have been developed to determine total antioxidant capacity (TAC) because it is difficult to measure each antioxidant component separately and their interaction within the plasma. The antioxidant capacity is based on the cumulative effect of all antioxidants present in plasma, and body fluids (Ghiselli et al, 2000). In this study, we detected that TAC is significantly decreased in lame dromedary camels and that it positively correlates significantly with all tested antioxidants, which gives a new and easy tool to evaluate the antioxidant status in lame camels, and to monitor treatment response.

The antioxidant state is the product of numerous distinct chemicals and systemic metabolic interactions interacting together (Ghiselli et al, 2000). TAC, as a single metric, provides useful information about the dynamic equilibrium between pro-oxidants and antioxidants in the plasma compartment (Ghiselli et al, 2000; Cao and Prior, 1998). Comparable to our results, TAC is an effective method for assessing stress in transported calves (Pregel et al, 2005). To conclude, evaluating TAC in camels may be a more precise and efficient indicator of camel lameness than measuring a single metric, which may reveal individual variances.

Present investigation has established a link between OS and lameness. Compared with our
results, OS biomarkers were similarly higher in sheep with hoof disorders (Talukder et al., 2015). Several inflammatory pathways are known to be activated by changes in the redox state of cattle (Sordillo and Aitken, 2009; Shi et al., 2015).

In present investigation it was revealed that the levels of MDA (AUC = 0.910) and antioxidant biomarkers (AUCs ranged from 0.901–1) were considered sensitive and specific biomarkers for differentiating lame camels from healthy ones and this might help monitoring the progress of treatment. This study found that lame camels have higher levels of MDA and NO biomarkers and lower serum levels of SOD, GSH, CAT, and TAC than healthy camels. The results of this study suggest that the measurement of MDA, NO, and antioxidant biomarkers in addition to physical examinations of camels with lameness may be a putative diagnostic and predictive tool for lameness in racing dromedary camels. Moreover, the addition of antioxidants to the treatment protocol may enhance the treatment progress in lame camels.

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