

## Impact of Intestinal and Urinary Tracts Obstruction on Oxidative Stress Biomarkers in Dromedary Camels

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### ABSTRACT

The purpose of this study was to evaluate the status of the oxidative stress biomarkers in camels with obstructions of the gastrointestinal and urinary tracts. Eighteen dromedary camels were examined at the Qassim University Veterinary Hospital in Saudi Arabia because of obstructions in the gastrointestinal (n=13) or urinary tracts (n=5). Camels were referred for evaluation because of anorexia, absence of defecation, abdominal distension, anuria and urine dribbling. Ten clinically healthy female dromedary camels were enrolled in this study as controls. A 7mL blood sample was collected in plain tubes from each camel for serum harvesting. Stress biomarkers including malondialdehyde (MDA), catalase (CAT), Glutathione (GSH), and superoxide dismutase (SOD) were determined in the sera. Parallel, lipid profiles including cholesterol, triglycerides (TG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) were also measured. Results showed that the MDA values did not differ significantly among camels with obstructions in the gastrointestinal and urinary tracts. However, the GSH value was significantly lower in camels with intestinal obstruction compared to control. In a similar manner, the SOD value was also significantly lower in animals with intestinal obstruction compared to the healthy ones, with a statistically significant difference. Both GSH and SOD values did not differ significantly between camels with urinary obstruction and control group. The value of CAT in the controls did not differ significantly from the values in both diseased groups. Data of lipid profile analysis showed that the cholesterol and TG values were higher in the diseased groups compared to the healthy group. The HDL value was lower in the group of intestinal obstruction compared to the healthy animals, with a statistically significant difference. However, on the contrary, the LDL value was higher in the group of intestinal obstruction compared to the healthy animals, with a statistically significant. Both HDL and LDL values did not differ significantly between camels with urinary obstruction and control group.

**Key words:** Antioxidant Activity, Biomarkers, Diseases, Oxidative Stress, Stress.

### INTRODUCTION

One of the main causes of camel death due to medical disorders is blockages in the alimentary and urinary tracts. Blockage of the intestines is caused by several etiologies such as hair balls, parasitic infections and foreign bodies (Tharwat and Al-Sobayil 2016a). Compression of the intestine may also occur through external pressure by a big lymph node, abscesses of the abdominal and pelvic cavities and abdominal tumors (Tharwat et al. 2012; Al-Sobayil et al. 2018). The disease is rare in camels (Fowler 2010a). Wool balls are the common etiologies of intestinal blockage in camels having depraved appetite (Tharwat et al. 2012). Colonic

impaction, cecal torsion and dilatation and intestinal strangulation in scrotal hernia are other etiologies of intestinal blockage in camels (Fowler 2010a).

Urinary calculi (urolithiasis, uroliths, nephrolith, bladder stone, cystolith) is common as a subclinical disorder among ruminants raised in management systems where the ration is composed primarily of grain, or where animals graze certain types of grasses (Radostits et al. 2007). In the one humped camel, the requirement for salts is nearly six to eight times than that of other domesticated ruminants (Nigam 1992). The urine of dromedary camels can contain double as salt in sea water because of its high capacity for retention concentration of fluids (Dorman 1986). Thus, small calculi may pass to the ureter or

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urethra causing blockage of the urine flow that may be partial or complete. Urinary stones are formed in either the kidney calices, or commonly in the bladder (Gutierrez et al. 2002; Tharwat and Al-Sobayil 2016b; Tharwat 2021).

Urethral obstruction has been extensively reported in ruminant species; however, there is minimal information about its incidence in camelids. The etiology is unknown but is believed to parallel that for domestic ruminants (Smith 1989). Previous reports of obstructive urolithiasis in llamas have suggested mineral imbalance, castration, and inflammation of the urinary tract as possible contributing factors (McLaughlin and Evans 1989). The calculi may contain a large proportion of calcium (Kock 1985), and in another report, the calculus contained necrotic inflammatory cells with no detectable mineral constituents (McLaughlin and Evans 1989). Urinary bladder rupture and the following occurring uroperitoneum is a common problem in cattle, and in males, urolithiasis is the underlying cause in most cases (Bertone and Smith 1984). Accumulation of urine in the peritoneum may be caused by urine seepage due to over distension and stretching of the urinary bladder wall in prolonged cases of urinary tract obstruction. The condition occurs mostly from single or multiple urinary bladder perforations because of obstruction of the urethra (Fowler 2010b). A series of consequences usually occur following peritoneal accumulation of urine due to failure of excretion of toxic materials together with abnormalities of fluid distribution between fluid in the peritoneum and fluid in the extracellular space. Ascites, stasis of rumen motility, decreased fecal output, depression, and finally uremia usually follows (Roussel and Ward 1985).

Biomarkers are defined as biological indicators of processes, events, or conditions occurring within the body. They can indicate physiological (such as growth and aging), or pathophysiological processes that occur with disease (e.g., cardiac damage and heart failure). In veterinary medicine, an interest in biomarkers is currently emerging, where there is enormous potential for their development and application (Tharwat 2020a; Tharwat and Al-Sobayil 2022a, b). Research is not only relevant to the health and welfare of companion and food-producing animals, but also to broader themes such as global food security. In recent years, the application of these biomarkers has increased in camel medicine, especially their diagnostic value and the extent to which diseased camels respond to treatment or not (Tharwat and Al-Sobayil 2018a, b; Tharwat 2020b-e; Tharwat and Al-Sobayil 2020; Al-Sobayil and Tharwat 2021; Tharwat and El-Deeb 2021).

The oxidative stress (OS) occurs in animals because of stressful activities such as transport, exercise and intensive management (Kirschvink et al. 2002; Tharwat and El-Deeb 2021) or disease conditions such as trypanosomiasis (Hussain et al. 2018; (El-Bahr and El-Deeb 2016), brucellosis (Hussain et al. 2022), babesiosis (Bhutta et al. 2022), etc. Heightened reactive oxygen species (ROS) generation, an impaired antioxidant system, or the two in combination can lead to OS. Under OS, an uncontrolled ROS attack can modify and denature molecular function and structure, resulting in dysfunction and tissue damage (Vaziri 2008; Wang et al. 2022;

Mahmood et al. 2022) and subsequently causing disruptions in the normal cellular detoxification or damage-repair mechanisms of the reactive intermediates (Lands et al. 2000). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase and glutathione (GSH) are mostly the components of the antioxidant system (Abd Ellah et al. 2007; Ghaffar et al. 2021; Li et al. 2022). Under several diverse pathological conditions, free radicals generate tissue damage via the general mechanism of lipid peroxidation (Halliwell and Chirico 1993). Malondialdehyde (MDA) is the most extensively applied common biomarker used in biological and medical science for the assessment of lipoperoxidation (Suttar et al. 2001; Salar-Amoli et al. 2009; Tharwat and El-Deeb 2021). Recently, biomarkers in camel medicine were studied extensively focusing on those markers differing with infection and inflammation, cardiovascular disorders and bone metabolism (Tharwat and Al-Sobayil 2018a, b; Tharwat 2020b-e; Tharwat and Al-Sobayil 2020; Al-Sobayil and Tharwat 2021; Tharwat and El-Deeb 2021). Because obstructions of the gastrointestinal and urinary tracts are considered the most fatal and stressful causes in dromedary camels, the purpose of this study was to evaluate the status of the OS biomarkers in camels with obstructions of the gastrointestinal and urinary tracts.

## MATERIALS AND METHODS

### Ethical Approval

The Animal Ethical Committee, Scientific Research Deanship in the University of Qassim, Saudi Arabia approved the experimental procedures of this study.

### Camels, History, Physical Examination, and Blood Sampling

Eighteen dromedary camels (11 females and 7 males) were examined at the Veterinary Hospital, Qassim University, Saudi Arabia. Animals were admitted because of having obstructions in the gastrointestinal or urinary tracts. Camels with intestinal obstruction (n=13; 10 females and 3 males) were referred with the history of anorexia, absence of defecation and abdominal distension for a period extending from 3 to 5 days. Recurrent tympany was reported in 3 female camels with intestinal obstruction. Camels with urinary obstruction (n=5; 4 males and 1 female) were referred with the history of anorexia, anuria, dribbling and abdominal distension for a period extending from 4 to 7 days. During the clinical examination, the general behavior and physical conditions were observed, auscultation of the heart, lungs, rumen, and intestines was carried out, the heart rate, respiratory rate, and rectal temperature were measured, and finally, swinging and percussion auscultation of both sides of the abdomen and a rectal examination were performed. Ten clinically healthy female dromedary camels were used as controls. A 7mL blood sample was collected in plain tubes from each camel for serum harvesting.

### Determination of Stress Biomarkers and Lipid Profile

Levels of serum malondialdehyde (MDA, Elabscience), cholesterol (Quimica Clinica Aplicada, S.A.), triglycerides (TG, Linear Chemicals, S.L.U.), high-

density lipoproteins (HDL, Cintronic GmbH), low-density lipoproteins (LDL, TRI-(TRI/5+HDL) and  $\beta$ -hydroxybutyric acid ( $\beta$ HBA, Biochemical Enterprise) as well as glutathione (GSH, Elabscience), and superoxide dismutase (SOD, Elabscience) activities were measured calorimetrically using kits.

### Statistical Analysis

Data were analyzed statistically by using the *t*-test (SPSS for Windows 2009, statistical software program). A *P* value of less than 0.05 was considered significant.

## RESULTS

In camels with intestinal obstruction, clinical examination revealed anorexia, recurrent tympany, clean rectum and bilateral abdominal distension (Fig. 1). In the group of obstruction in the urinary tract, clinical findings included anorexia, abdominal distension, anuria, dribbling and arched stance (Fig. 2). Fig. 3 shows mean $\pm$ SD of the stress biomarker MDA in camels with obstructions of the gastrointestinal and urinary tracts compared to controls. Compared to a value of 177 $\pm$ 55 mmol/g Hb in the controls, the MDA values did not differ significantly among camels with obstructions of the gastrointestinal or urinary tracts (*P*=0.32).

Fig. 4 shows mean $\pm$ SD of the stress biomarker GSH in camels with obstructions of the gastrointestinal and

urinary tracts compared to controls. The GSH value was significantly lower in camels with intestinal obstruction compared to control (2.7 $\pm$ 1.6 mmol/g Hb vs. 6.02  $\pm$ 1.6 mmol/g Hb; *P* = 0.008). In a similar manner, the SOD value was also significantly lower in animals with intestinal obstruction compared to the healthy ones, with a statistically significant difference (4.9 $\pm$ 2.1 mmol/g Hb vs. 10.92 $\pm$ 3.9 mmol/g Hb; *P*=0.0001) (Fig. 5). Both GSH and SOD values did not differ significantly between camels with urinary obstruction and control group (*P*>0.05). The value of CAT in the controls (0.97 $\pm$ 0.7U/mg Hb) did not differ significantly from the values in both diseased groups (*P*>0.05) (Fig. 6).

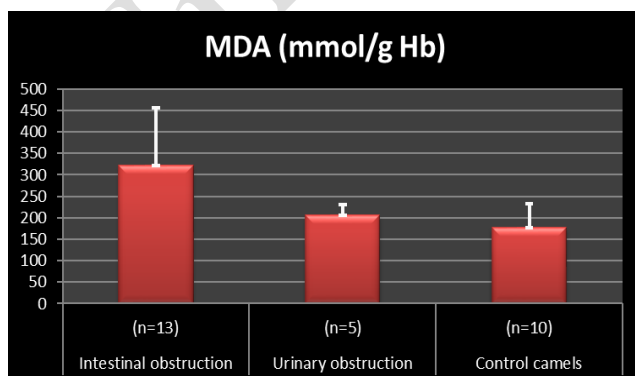
Table 1 summarizes the values of the lipid profiles. Cholesterol and TG values were higher in the diseased groups compared to the healthy group (160 $\pm$ 13mg/dL, 157 $\pm$ 14mg/dL vs. 108 $\pm$ 7mg/dL, 139 $\pm$ 24mg/dL; *P*<0.0001). The HDL value was lower in the group of intestinal obstruction compared to the healthy animals, with a statistically significant difference of 11 $\pm$ 511mg/dL vs. 36.46 $\pm$ 8.8mg/dL; *P*<0.0001). However, on the contrary, the LDL value was higher in the group of intestinal obstruction compared to the healthy animals, with a statistically significant difference of 104 $\pm$ 22mg/dL vs. 59 $\pm$ 16mg/dL; *P*=0.0002). Both HDL and LDL values did not differ significantly between camels with urinary obstruction and control group (*P*>0.05).



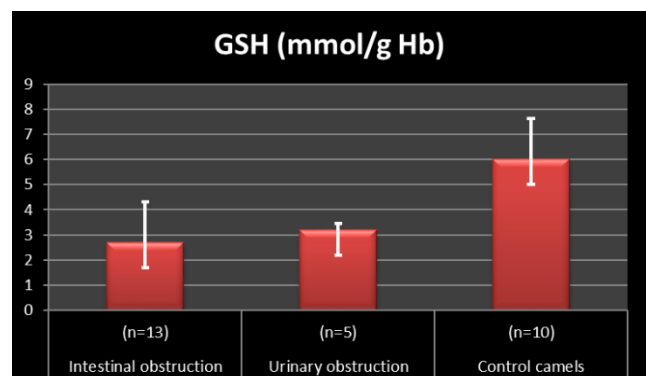
**Fig. 1:** Bilateral abdominal distension in female camel with 7-day intestinal obstruction.



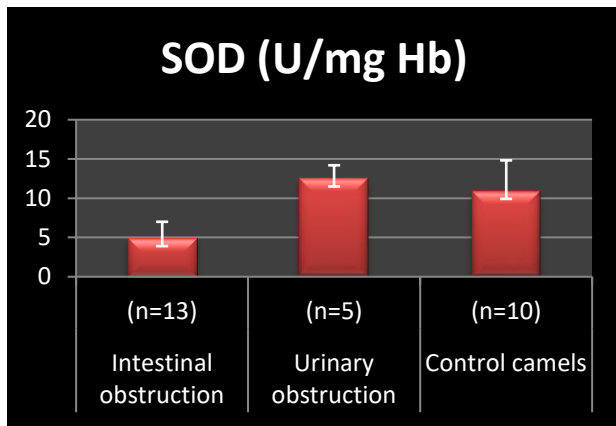
**Fig. 2:** Arched stance and urine dribbling in a female camel with obstruction in the urinary tract due to compression of the bladder neck by a pelvic mass.



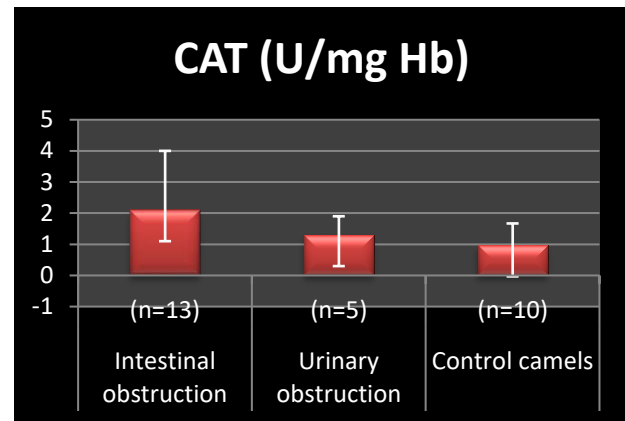
**Fig. 3:** Mean $\pm$ SD of the stress biomarker malondialdehyde (MDA) in camels with obstructions of the gastrointestinal and urinary tracts compared to controls (*P*=0.32).



**Fig. 4:** Mean $\pm$ SD of the stress biomarker glutathione (GSH) in camels with obstructions of the gastrointestinal and urinary tracts compared to controls (*P*=0.008).



**Fig. 5:** Mean±SD of the stress biomarker superoxide dismutase (SOD) in camels with obstructions of the gastrointestinal and urinary tracts compared to controls (P=0.0001).



**Fig. 6:** Mean±SD of the stress biomarker catalase (CAT) in camels with obstructions of the gastrointestinal and urinary tracts compared to controls (P=0.45).

**Table 1:** Lipid profiles (mg/dL) in camels with obstructions of the gastrointestinal and urinary tracts compared to controls

Parameters	Intestinal obstruction (n=13)	Urinary obstruction (n=5)	Control camels (n=10)	P value
Cholesterol	160.00±13.0***	157.00±14.0***	108.00±7.0	<0.0001
Triglycerides	219.00±18.0***	220.00±15.0***	139.00±24.0	<0.0001
High density lipoproteins	14.00±11.0*	25.00±13.0	36.46±8.8	<0.0001
Low density lipoproteins	104.00±22.0**	88.00±6.0	59.00±16.0	0.0002

Values (mean±SD) marked with \*, \*\* and \*\*\* differ significantly at P<0.05, P<0.01 and P<0.001, respectively in the same row compared with the control group.

## DISCUSSION

Blockage of the GIT occurs in camels due to many etiologies. Volvulus is one of the causes that may affect the small intestine in camels, with or without involving the large intestine. Sometimes, volvulus only involves the distal part of the jejunum and the upper part of the ileum. Complications of volvulus include distension of the intestines and abdomen, compression of the blood vessels, necrosis of the intestines and finally gangrene unless surgery is performed (Fowler 2010). Another cause of intestinal blockage in camels is the intussusception that may occur through the entrance of one part of the intestine into the neighboring part. The jejuno-jejunal intussusception is commonly found in camels with intestinal obstruction as in cows (Tharwat et al. 2012). On the other hand, blockage of the urinary tract commonly occurs due to urolithiasis that is usually associated with feeding high concentrates. Clinical examination of camels with obstruction of the urinary tract may include severe abdominal pain, dysuria, urine dribbling, colored urine, uroperitoneum and bladder and/or urethral rupture (Tharwat 2021).

Biomarkers of stress are one of the very important markers in camel medicine. Some of these markers differ significantly in diseased camels compared to healthy ones (Tharwat and El-Deeb 2021). For example, in camels with Johne's disease, the serum levels of SOD, CAT and reduced glutathione (RGS) are significantly low compared to healthy camels. Opposite, the marker of lipid peroxidation MDA was tested significantly higher in camels with paratuberculosis compared to control camels (El-Deeb et al. 2014). Similar results were also found in camels affected with trypanosomiasis where SOD, CAT, and RGS were reported to be significantly reduced in diseased camels versus healthy ones, whereas lipid

peroxidation marker MDA was reported to be increased significantly in diseased camels versus healthy animals (El-Bahr and El-Deeb 2016). Biomarkers of stress also differ in camels with skin affections versus those with healthy skin. For example, the serum level of MDA is significantly higher in camel either having moderate or severe forms of sarcoptic mange but normal in mild affections compared to healthy camels. Other stress markers as SOD and CAT are significantly higher in mild cases of sarcoptic mange and significantly lower in either moderate or severe affections versus non-infected camels. Similarly, GSH serum levels tested higher in mild and lower in moderate and severe cases versus healthy camels (Saleh et al. 2011; Tharwat and El-Deeb 2021).

This study investigated the status of OS biomarkers in camels with intestinal and urinary obstructions. Because MDA is one of the products of lipid peroxidation, the degree of lipid peroxidation is most frequently determined by evaluating MDA levels (Lata et al. 2004). Although the MDA serum levels were higher in both diseased groups versus controls confirming enhanced lipid peroxidation, the change was statistically nonsignificant. In dromedary camels with a normal periparturient period, it was also reported that the levels of MDA did not differ significantly between the prepartum and postpartum periods. However, it was reported that MDA levels were increased in a variety of inflammatory conditions like acute and chronic cystitis in camels (Abd Ellah et al. 2012), and urinary tract infection in human patients (Kurutas et al. 2005). The other OS biomarker GSH however was lower in diseased groups compared to controls. The third SOD value was also significantly lower in camels with intestinal obstruction compared to the healthy controls. Similar findings were also reported in camels with infection of the urinary tract (El-Deeb and Buczinski 2015). The last OS marker CAT did not differ

significantly between diseased camels in both groups and controls. Importance of OS biomarkers in diagnosis and prognosis was investigated in camels with infection of the urinary tract, where CAT, SOD, and GSG levels were significantly lower in diseased camels versus healthy control camels (El-Deeb and Buczinski 2015). In the later study, it was concluded that OS biomarkers can be used as diagnostic and prognostic indicators in the management of camel suffering from infection of the urinary tract.

Concerning the lipid profile, the serum levels of cholesterol, TG and LDL were significantly higher, and HDL was significantly lower in camels with obstruction of the gastrointestinal tract compared to controls. In the group of urinary tract obstruction, cholesterol and TG were significantly higher in diseased camels compared to controls. However, the HDL and LDL values did not differ significantly. Both groups of diseased camels in this study were admitted with a history of anorexia pointing to a status of negative energy balance (NEB). The NEB in the camels with intestinal obstruction was confirmed in this study by the significant increases in cholesterol, TG, and LDL and decreases in HDL compared to the control values. However, the NEB in the camels with urinary tract obstruction was confirmed by the significant increases in cholesterol and TG compared to the control values.

#### Conflict of Interest

The authors declare that there is no conflict of interest.

#### Authors Contribution

Almundarij TI designed the study, carried out the practical and laboratory work, and read and revised the manuscript draft. Tharwat M. designed the study, carried out the practical and laboratory work, and wrote the manuscript draft. Both authors revised and approved the manuscript.

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