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Original Research

Vitamin C ameliorates gentamicin-induced acute kidney injury in equines: an experimental study

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Abstract

To date, there is no specific treatment used to protect against gentamicin (Genta)-induced nephrotoxicity. The main objective of the present study was to determine the potential protective effect of vitamin C against Genta-induced acute kidney injury (AKI) in equines using donkey as a model. Nine apparently healthy adult male donkeys (*Equus asinus*) were included in this study. All animals were clinically sound. Three donkeys were randomly selected to receive saline solution and served as controls. The other six donkeys (treated groups) were randomly assigned to receive either vitamin C at a dosage of 30 mg/kg (GVC-group; n = 3) or saline solution (G-group; n = 3) via IV route once daily for 14 days. Animals of treated groups concomitantly received Genta at a dosage of 20 mg/kg IV thrice daily for 14 consecutive days. Blood and urine samples were simultaneously collected at day 14 of Genta administration. Blood samples were used for measuring selected acute phase proteins, cytokines profile, and oxidative stress mediators; while urine samples were used for measuring different urinary analytes. AKI was confirmed by classic laboratory findings. Our results showed that the serum amyloid A, haptoglobin, fibrinogen, C-reactive protein, interleukin (IL)-1β, IL-6, interferon-γ, IL-10, sialic acid, malondialdehyde, blood urea, serum creatinine, alkaline phosphatase, and serum gamma-glutamyl transpeptidase were significantly lower in GVC-group than those in G-group (P < 0.05). In contrast, total antioxidant capacity was much lower in G-group than that of GVC-group; however, they did not reach statistical significance. The results presented herein suggest that vitamin C could have a protective effect against Genta-induced AKI in equines. The ongoing trials orchestrated with improved diagnostic utilities can improve the outcomes of AKI in equines through prophylactic or early use of antioxidant therapy.

**Keywords:** Acute Kidney Injury, Equines, Oxidative Stress, Vitamin C
1. Introduction

Acute kidney injury (AKI) is generally defined as a decline in kidney function resulting in accumulation of waste products in the bloodstream. The main causes of AKI are nephrotoxins, aminoglycosides, oxytetracycline, and non-steroidal anti-inflammatory drugs (NSAIDs) [1].

Gentamicin (Genta) is an aminoglycoside antibiotic routinely used in every day equine clinical practices for the treatment of Gram-negative infections either alone or in a combination with beta-lactam antibiotics [2]. Its low resistance rate and its reasonable cost make it the first antibiotic of choice for life-threatening diseases. Despite its frequent use in equine practices, its potential nephrotoxicity has been a real concern. Genta-induced nephrotoxicity is characterized by morphological and functional alterations of the renal tissue which lead to development of AKI [3]. The early detection of AKI is critical for the effective therapy as well as prognosis. However, the conventional laboratory approaches to detect AKI, such as measuring serum creatinine (S-Cr) and blood urea nitrogen (BUN) concentrations are not efficient for early detection of the cases as there is a time gap between kidney injury and the alterations in these indicators.

To date, there is no specific agent used to protect against Genta-induced nephrotoxicity. In this regard, various medications have been used concomitantly with Genta to prevent AKI in laboratory animal models [3-10]; however in equine medicine, scarce reports are available on the protective therapies against AKI [11]. Several researchers have examined the potential therapeutic role of anti-oxidants in both experimental animal models and human studies of AKI in several clinical settings [3, 5, 6, 8, 9, 12]. Among the different antioxidants tested, vitamin C exhibited a powerful scavenging property against free radicals and activated oxygen species. As an electron donor, vitamin C protects by neutralizing reactive oxygen species (ROS) and decreasing oxidative damages [13]. Vitamin C also exhibits anti-inflammatory effects, prevents
endothelial dysfunction and apoptosis, and reduces the risk of cardiovascular diseases [14]. For example, vitamin C treatments improved kidney function in renal allograft recipients [15], decreased renal inflammation, and improved impaired renal function in salt-sensitive hypertensive rats [16]. In this view, the main objective of the present study was to determine the potential protective effects of vitamin C against Genta-induced AKI in equines using donkey as an experimental model.

2. Materials and Methods

2.1. Animals

The present study was conducted in accordance with principles of good clinical practice, and all the procedures were approved by the Ethical Committee for Animal Experiments at Mansoura University, Egypt. Nine apparently healthy adult male donkeys (*Equus asinus*), weighing 150-200 kg BW, were included in this study. All animals were clinically examined. Three donkeys were randomly selected to receive saline solution and served as controls, while the other six donkeys (treated groups) were randomly assigned to receive either vitamin C at a dosage of 30 mg/kg (GVC-group; n = 3) [17] or saline solution (G-group; n = 3) via IV route once daily for 14 days. Animals of treated groups concomitantly received Genta at a dosage of 20 mg/kg IV thrice daily for 14 consecutive days following a previously described protocol [11]. Two weeks prior to the experiment, the selected donkeys were de-wormed by using Ivermectin (Equiveen, ADWIA, Egypt) at an oral dose of 0.2 mg/kg BW. The donkeys were fed on corn silage, bran and tibn. Food and water supply were offered *ad-libitum*. The animals were clinically monitored throughout the experimental period. A special concern was given to the heart and respiratory rates, rectal temperature, mucous membrane color, appetite, water intake, general
demeanor, posture, urination and defecation patterns, hair coat, cecal and colon motility.

Complete blood picture and renal function tests were carried out on all donkeys prior to the study (data not shown). The selected animals exhibited values within the normal reference range.

2. 2. Sampling Protocol

Blood and urine samples were simultaneously collected from each donkey at the 14th day of Genta administration. Briefly, blood was collected from each donkey through jugular vein catheter. Half of the collected blood (10 mL) was added to a plain tube (without anticoagulant) and left at room temperature for 15 min to coagulate and then centrifuged at 3000 x g for 15 min for separation of blood sera. Only non-haemolyzed sera were collected and kept frozen at -20 °C until required for the measurement of circulating cytokines, acute phase proteins (APPs), and classic biochemical profile. The levels of different cytokines including interleukin (IL)-1β, IL-6, interferon gamma (IF-γ) and IL-10 were measured in undiluted sera according to the manufacturer's instructions by using commercially available equine ELISA Kits supplied by Genorise Scientific, Inc., Paoli, PA, USA. The plates were read at 450 nm and a correction wavelength of 550 nm using an automated microplate reader (Bio TEC, USA). Values were expressed in picograms per milliliter (pg/mL). Samples were run in duplicates for all examined cytokines. Serum amyloid A (SAA), haptoglobin (hp), C- reactive protein (CRP) were measured by using equine ELISA kits supplied by (MyBioSource, San Diego, California, USA), and an automated microplate reader (Bio TEC, USA) according to the manufacturer's instructions. Sialic acid (SA) was measured in the serum samples as previously described [18] by using Ehrlich's reagent (made by adding 0.7 g of p-dimethyl aminobenzaldehyde (Sigma Aldrich) to 150 mL of concentrated HCl and 100 mL of distilled water). The absorbance of samples was measured at 525 nm by using Jenway 7305, UV-VIS spectrophotometer (Jenway Scientific Equipments, UK).
Data were expressed as mmol/L. The following biochemical variables were also measured using commercially available kits according to the manufacturer's instructions, including alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (S-GGT), alanine amino transfrease (ALT), total protein, albumin, S-Cr, and BUN. Globulin levels were also calculated. The other half of the blood sample was collected in heparinized tube and was rapidly centrifuged at 3000 x g for 10 min for separation of blood plasma. The collected plasma was used for estimation of fibrinogen (Fb) and selected oxidative stress and anti-oxidant markers. Plasma Fb was measured by using commercially available ELISA Kit supplied by (MyBioSource, San Diego, California, USA) according to the manufacturer's instructions. The kits used for the measurement of malondialdehyde (MDA), total antioxidant capacity (TAC), reduced glutathione (G-SH) and catalase (CAT) were obtained from Biodiagnostic, Egypt.

Urine (U) samples were collected from each donkey by urethral catheterization without sedation. Each urine sample was divided into two aliquots; one was used for microscopic examination and measuring of specific gravity (U-SG). Whereas, the other aliquot was centrifuged at 1,500 x g for 10 min and the supernatant was stored at -20°C and used within one week for estimation of urea (U-urea), Cr (U-Cr), GGT (U-GGT), Na (U-Na), and U-MDA. The U-SG was measured using (Medi-Test Combi 10, 93067, Germany); while the other urine biochemical variables were spectrophotometrically measured using commercially available kits according to the manufacturer's instructions. U-MDA was estimated by measuring thiobarbituric acid reactive substances (TBARS), a method that has previously been proven to be efficient to measure MDA [19].

2.3. Statistical Analysis
Data were statistically analyzed by using statistical software program (SPSS, version 15, USA). Means and standard deviation (SD) for each of the measured variables were calculated. Significant differences (P<0.05) between groups were calculated by one-way ANOVA and Duncan’s post hoc test.

3. Results

An overview of significant clinical variables as well as biochemical and clinicopathological profiles in donkeys with AKI and controls are presented in Tables 1-4; Figures 1, 2. Clinically, the heart rate, respiratory rate, and rectal temperature showed no significant variations among all examined donkeys when compared with controls. In general, all donkeys did not exhibit detectable clinical abnormalities throughout the study period and showed normal behavior, urination and defecation, hair coat, mucous membrane color, cecal and colon motility. However dental tar, ammonia breath and increased water intake were observed in G-group and GVC-group (Table 1).

Biochemically, the inflammatory mediators including SAA, hp, CRP, Fb, IL-1β, IL-6, INF-γ, IL-10, SA, as well as oxidative stress marker including MDA were significantly higher in G-group than those in GVC-group (P<0.05) (Table 2 & Figure 1, 2). Values of TAC were lower in both groups compared to control (P<0.05). Of note, TAC was much lower in G-group than that of GVC-group; however it did not reach statistical significance. On the contrary, the anti-oxidative parameters including G-SH and CAT were higher in G-group than those in GVC-group (P<0.05) (Figure 2). The microscopic examination of urine samples of G-group and GVC-group revealed the existence of red blood cells, epithelial cells, granular casts, triple phosphate,
and calcium oxalate crystals. While in control group, traces of protein, amorphous phosphate as well as calcium carbonate crystals were also observed.

Although values of serum ALP were higher in G-group than those in GVC-group, they did not reach to statistical significance (Table 3). However, S-GGT values were significantly increased in G-group compared to those in GVC-group (P<0.05). BUN and S-Cr were significantly higher in G-group than those in GVC-group (P<0.05). Total protein and globulin values were significantly higher not only in G-group and GVC-group than controls but also in G-group compared to GVC-group. Albumin levels were however lower in G-group than those of GVC-group and controls (P<0.05) (Table 3). Compared to GVC-group, G-group showed significantly increased values of U-GGT and U-sodium; while, values of U-SG, U-urea, and U-Cr were significantly decreased (Table 4).

4. Discussion

The findings of the present study suggest that vitamin C could have a potential protective effect against Genta-induced AKI in equines. In the past decade, most of the advances in the area of Genta-induced nephrotoxicity were focused on the molecular biology, genetic basis, and pathophysiology of nephrotoxicity [20-22]. However, several researchers have explored the potential therapeutic role of anti-oxidants in AKI in both experimental animal models and human clinical studies. In this regards, several trials have been performed to evaluate the protective effects of vitamin C on kidneys in humans and laboratory animal models [3, 15, 23]. However, such effect has not previously been evaluated in equines with AKI. We hypothesized that vitamin C might have a potential protective effect against Genta-induced nephrotoxicity in equines. In the current study, the examined donkeys did not exhibit any clinical illness throughout the 14 day-
study period. Similar finding was reported by Hinchcliff et al., where 5 ponies out of 7 did not
develop physical or behavioral abnormalities after 14 days of Genta administration, although the
ultrastructural abnormalities of the proximal tubular epithelium were apparent in all ponies [24].
A decrease in specific gravity of the urine in both the G-group and GVC-group would suggest a
loss of concentrating ability of the kidneys and would explain the increase in water intake. The
Genta dose used in the present study is considered higher than the previously applied therapeutic
dose where the conservative dose regimen of 3 mg/kg BW three times followed by 4.5 mg/kg
BW twice daily for five consecutive days did not cause significant renal toxicity in healthy
ponies [2]. Here we aimed at developing a model of AKI as previously described [11].

The classical findings of serum, urinary biochemical profile confirmed the occurrence of
AKI after 7 days of Genta exposure (data not shown) and became apparent after 14 days of
Genta administration. The significant elevation of BUN and S-Cr concurrently with severe
reduction in U-urea and U-Cr in Genta-treated animals is an indicator of severe renal tubular
necrosis. The increased GGT levels, a brush border marker, suggest tubular damage and direct
toxic injury. The observed changes in the proteinogram could be attributed to alterations in the
acute phase response and the associated systemic inflammatory response (as indicated by the
results of acute phase proteins and circulating cytokines). These findings appear in part
controversy to those previously obtained by other researchers [9]. Treatment with vitamin C
resulted in less deterioration in the biochemical variables most likely due to its cytoprotective
effect by inhibition of free radical production. A similar biochemical profile has previously been
reported in horses with NSAID-induced nephropathy and those with exertional rhabdomyolysis
[25, 26]. The development of AKI in the examined donkeys could be attributed to the toxic
accumulation of Genta in the epithelial cells of the renal proximal tubules. It was previously
suggested that the accumulation of Genta in the proximal tubules of the kidney might interfere with lysosomal, mitochondrial, and Na/K/ATPase functions [1].

In this study, G-group and GVC-group exhibited marked inflammatory and oxidative stress responses concomitantly with lower TAC and higher values of anti-oxidative parameters. However, the derangements in both inflammatory response as well as the oxidative status were more prominent in G-group compared to those in GVC-group. The increase in G-SH and CAT values in G-group could be a response towards increased ROS generation. The up-regulation of antioxidant enzymes seems to render the cell more resistance to the subsequent oxidative damage. Our findings were in part similar to those reported in rats with CCl4-induced oxidative damage [27]. However, our findings were opposite to those reported by other researchers who showed that G-SH and CAT were significantly decreased in rats with Genta-induced nephrotoxicity [12]. It is believed that the body has an effective defense mechanism to prevent and neutralize free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes that constitute defensive team against ROS. It is suggested that administration of vitamin C could ameliorate the Genta-induced kidney damage. It has also been shown that vitamin C alone could protect against Genta-induced renal damage [15]. Other investigators advocated that co-administration of moderate doses of vitamin C and vitamin E could protect against Genta-induce nephrotoxicity in rats [28]. Whereas, supplementation of vitamin E, either alone or in combination with vitamin C, was found to be effective in preserving the activity of superoxide dismutase in Genta-treated rats [29]. It was also recently suggested that ascorbic acid could provide a protection against contrast-induced AKI in humans [30].

Over the past years, we began to understand the underlying mechanisms behind the development of AKI. The generation of ROS and oxidative stress have been shown to play a key
role in the pathogenesis of AKI in different clinical settings [25, 26, 31]. It was also hypothesized that Genta-induced AKI is associated with generation of free radicals [4, 8, 32, 33], increase in intracellular calcium concentration, production of numerous inflammatory markers, and induction of apoptosis and necrosis [34]. In addition, Genta has been shown to induce iron release from renal cortical mitochondria [32], which is known to be a potent catalyst to generate free radicals. Moreover, Genta enhances the generation of ROS that is believed to cause nephrotoxicity [31] and cell death in many different pathological states including glomerular disease and renal ischemia reperfusion injury [35, 36, 37]. Taken together, it could be suggested that Genta induces nephrotoxicity is associated with disturbances of the renal tubular function, alterations in the antioxidant enzyme activities, and formation of lipid peroxides. Co-administration of vitamin C could have an ameliorative effect against Genta-induced AKI.

5. Conclusion

The data presented herein suggest that vitamin C has a potential protective effect against Genta-induced AKI in equines. However further studies are required to explore this potential. The ongoing trials in concert with improved diagnostic utilities can improve the outcomes of AKI in equines through prophylactic or early therapeutic use of antioxidants. Further studies are needed to evaluate the potential ameliorative effect of other agents in horses with AKI.

6. Disclosure

The authors of this paper have no financial or personal relationship with other people or organizations that could appropriately influence or bias the content of the paper.
7. Acknowledgements

The authors would like to thank Mr. Mohamed Abdu, Mrs. Al-Shaeimaa Farag and Mr. Mohamed Al-Hoot for their kind assistance during collection of the samples. Sincere gratitude also extends to Mrs. Gehad Abd El-Galil for her kind clinicopathological assistance.
8. References


Table 1

Significant clinical variables in donkeys with experimentally induced acute kidney injury (GVC-group and G-group) compared to controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 3)</th>
<th>G-group (n = 3)</th>
<th>GVC-group (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (b/min)</td>
<td>32.75 ± 0.6$^a$</td>
<td>34.45 ± 2.0$^a$</td>
<td>33.33 ± 1.0$^a$</td>
</tr>
<tr>
<td>Respiratory rate (c/min)</td>
<td>12.02 ± 1.0$^a$</td>
<td>12.66 ± 0.4$^a$</td>
<td>12.40 ± 0.5$^a$</td>
</tr>
<tr>
<td>Rectal temperature C$^0$</td>
<td>37.46 ± 0.03$^a$</td>
<td>37.52 ± 0.04$^a$</td>
<td>37.53 ± 0.03$^a$</td>
</tr>
<tr>
<td>Visible mucous membranes</td>
<td>Moist, pink in color</td>
<td>Moist, pink in color</td>
<td>Moist, pink in color</td>
</tr>
<tr>
<td>Dental tar</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Ammonia odor at the buccal cavity</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Water intake</td>
<td>Normal</td>
<td>Increased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

$^a$ Variables with different superscripts in the same row are significantly different at $P < 0.05$.

G-group represents animals received gentamicin plus saline solution; GVC-group represents animals received gentamicin plus vitamin C.
Table 2
Mean values ± SD of selected inflammatory markers in donkeys with experimentally induced acute kidney injury compared to controls

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Control (n = 3)</th>
<th>G-group (n = 3)</th>
<th>GVC-group (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum amyloid A (µg/mL)</td>
<td>9.98 ± 2.77a</td>
<td>146 ± 27b</td>
<td>43.26 ± 25a</td>
</tr>
<tr>
<td>Haptoglobin (mg/dL)</td>
<td>47.56 ± 10.77a</td>
<td>267 ± 47b</td>
<td>118 ± 36c</td>
</tr>
<tr>
<td>C reactive protein (mg/L)</td>
<td>4.96 ± 0.72a</td>
<td>8.46 ± 0.41b</td>
<td>5.30 ± 0.26a</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>164 ± 4.93a</td>
<td>342 ± 27b</td>
<td>220 ± 3.42c</td>
</tr>
<tr>
<td>Sialic acid (mmol/L)</td>
<td>0.117 ± 0.006a</td>
<td>0.170 ± 0.005b</td>
<td>0.130 ± 0.002c</td>
</tr>
</tbody>
</table>

* Variables with different superscripts in the same raw are significantly different at $P < 0.05$.

G-group represents animals received gentamicin plus saline solution; GVC-group represents animals received gentamicin plus vitamin C.
Table 3

Mean values ± SD of selected clinicopathological variables in donkeys with experimentally induced acute kidney injury compared to controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 3)</th>
<th>G-group (n = 3)</th>
<th>GVC-group (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>411 ± 64.20a</td>
<td>689 ± 2.51b</td>
<td>500 ± 92b</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>20.66 ± 3.05a</td>
<td>33.0 ± 0.01b</td>
<td>22.3 ± 0.57a</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>11.99 ± 1.54a</td>
<td>13.65 ± 1.45a</td>
<td>14.22 ± 1.12a</td>
</tr>
<tr>
<td>S-Urea (mmol/L)</td>
<td>4.93 ± 0.09a</td>
<td>9.03 ± 0.33b</td>
<td>6.60 ± 0.166c</td>
</tr>
<tr>
<td>S-Cr (µmol/L)</td>
<td>84.27 ± 16.42a</td>
<td>157.35 ± 7.07b</td>
<td>122.27 ± 3.53c</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>8.43 ± 1.01a</td>
<td>16.0 ± 2.64b</td>
<td>12.48 ± 0.56c</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.41 ± 0.19a</td>
<td>3.76 ± 0.15b</td>
<td>4.30 ± 0.10a</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>4.01 ± 0.9a</td>
<td>12.23 ± 2.51b</td>
<td>8.18 ± 0.63c</td>
</tr>
</tbody>
</table>

a, b, c Variables with different superscripts in the same row are significantly different at $P < 0.05$. ALP: alkaline phosphatase; GGT: gamma-glutamyl transpeptidase; ALT: alanine amino transferase; S-Cr: serum creatinine; G-group represents animals received gentamicin plus saline solution; GVC-group represents animals received gentamicin plus vitamin C.
Table 4

Mean values ± SD of urine biochemical variables in treated (GVC-group) versus not treated (G-group) donkeys with experimentally induced acute kidney injury compared to controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>G-group</th>
<th>GVC-group</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG</td>
<td>1.032 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.008 ± 0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.014 ± 0.005&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>U-GGT (U/L)</td>
<td>21.6 ± 7.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.10 ± 5.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.00 ± 7.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>U-Cr (mmol/L)</td>
<td>17.36 ± 1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.30 ± 1.52&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>U-GGT/Cr ratio</td>
<td>1.24</td>
<td>13.77</td>
<td>4.93</td>
</tr>
<tr>
<td>U-Urea (mmol/L)</td>
<td>21.64 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.30 ± 0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.30 ± 0.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>U-Na (mmol/L)</td>
<td>19.33 ± 8.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.6 ± 3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.3 ± 7.63&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Variables with different superscripts in the same row are significantly different at P < 0.05; USG: urine specific gravity, U-GGT: urine gamma-glutamyl transpeptidase; U-Cr: urine creatinine; U-Na: urine sodium.
Figure 1

Mean values ± SD of selected acute phase cytokines in donkeys with experimentally induced acute kidney injury compared with controls. Bars labeled with different letters are statistically significant (P<0.05).
Figure 2

Mean values ± SD of oxidative stress mediators in donkeys with experimentally induced acute kidney injury compared with controls. Bars labeled with different letters are statistically significant (P<0.05).
• Acute kidney injury was experimentally induced in six donkeys by using gentamicin 10%.
• These animals were concomitantly received either vitamin C (n = 3) or saline (n = 3).
• Blood samples were used for measuring inflammatory markers and oxidative stress mediators.
• Animals received vitamin C exhibited less inflammatory and oxidative stress responses.
• Administration of vitamin C in equines could have a protective effect against gentamicin-induced kidney injury.