DOI : 10.5958/2277-8934.2020.00037.5 *Vol 27 No 3, p 277-283*

OXIDATIVE STRESS, HISTOPATHOLOGICAL AND HAEMATO-BIOCHEMICAL FEATURES IN DROMEDARY CAMELS INTOXICATED WITH GLIOTOXIN

M.S. Shathele[1], Sabry M. El-Bahr[2,4], A.M. Hereba[1,5], Saad Shousha[2,6], Mahmoud El Sebaei[2,7] and Abdelazim Ibrahim[3,8]

Department of Microbiology[1], Biomedical Sciences[2], Pathology[3], College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

[4]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

[5]Medical Biophysics Department, Medical Research Institute, Alexandria University, Egypt

[6]Department of Physiology, Faculty of Veterinary Medicine, Benha University, Egypt

[7]Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Mansoura University, Mansoura, 35516, Egypt

[8]Department of Pathology, College of Veterinary Medicine, Suez Canal University, Egypt

ABSTRACT

The aim of the current study was to determine the effect of intravenous administration of gliotoxin on oxidative stress, histopathological and haemato-biochemical features in dromedary camels before injection (0h) and at 1, 24, 48 and 72 hours post injection. Five healthy adult female camels (8-10 years old having 300-350 kg b.wt.) were injected intravenously with gliotoxin (0.025µg/kg b.wt.). Blood samples were collected in plain and heparinised vacutainers at all time points (0, 1, 24, 48 and 72h). The whole blood in heparinised vacutainers was used for estimation of haematological parameters. Obtained sera stored frozen at -30°C until used for estimation of biochemical and oxidative stress biomarkers. Liver and heart tissues were collected from one camel died 24 hours post gliotoxin injection and subjected for histopathological examination. The findings revealed significant increase in the values of ALT, AST, urea, creatinine and neutrophils along with significant reduction in values of total leucocyte count (TLC), total erythrocyte count (TEC), hemoglobin (Hb), lymphocytes percentages, glucose, total proteins, albumin and globulin concentrations in gliotoxin treated camels 1, 24, 48 and 72 hour post gliotoxin injection. Gliotoxin induced significant increase in malondialdehyde (MDA) concentration accompanied with significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST) and glutathione reductase (GR) at all time points post injection. Postmortem examination of the dead camel showed congestion and oedema in both lungs. Histopathological findings revealed presence of homogenous eosinophilic fluid within alveoli and congestion of interalveolar capillaries and within the myocardium. In conclusion, the injected dose of gliotoxin was acutely toxic to camels as reflected on disturbed liver and kidney functions, acceleration of oxidative stress and inhibition of antioxidants enzyme activities.

Key words: Camel, gliotoxin, mycotoxin oxidative stress, intoxicated

Fungi and their mycotoxins are widely distributed in the environment. After production, these are adsorbed onto airborne dusts and caused major public health problems (Eshetu *et al,* 2016). Gliotoxin is one of the most serious mycotoxin, as low concentration of this toxin is able to induce adverse effects on animal and human health (Bossou *et al,* 2017) and is produced by a number of fungi including *Aspergillus fumigatus* (Bauer, 1994; Eichner *et al,* 1988). Gliotoxin, a hydrophobic fungal metabolite of the epipolythiodioxopiperazine (ETP) group with a quinoid moiety and a disulfide bridge across the piperazine ring (Waring *et al,* 1988). Intravenous injection of gliotoxin (0.2 µg kg^{-1} b.wt.) once killed all the three camels tested in a pilot study of our previous work (Shathele, 2009). Therefore, half (0.1 µg kg^{-1} b.wt.) of the fatal dose of gliotoxin was injected intravenously once in five camels in the same study (Shathele, 2009). This dose (0.1 µg kg^{-1} b.wt.; Shathele, 2009) did not kill the tested camels but decreased the serum total protein and glucose concentrations accompanied with disturbed liver

SEND REPRINT REQUEST TO SABRY M. EL-BAHR email: selbahar@kfu.edu.sa; sabryelbahr@hotmail.com

and renal function in dromedary camels 2 days post injection. Furthermore, half dose (0.05 µg kg^{-1} b.wt.) of gliotoxin was used in our previous work (Shathele, 2009) where it was injected intravenously for 3 days in five camels (Shathele, 2011). This dose (0.05 µg kg^{-1} b.wt.; Shathele, 2011) led to significant reduction in serum total protein, albumin, globulin, leukocytes, lymphocytes, neutrophils and lysosomal activity on day 7 post injection. The previous studies did not focus on the influence of gliotoxin injection on oxidative stress biomarkers and histopathology. Therefore, the present study was aimed to investigate the effect of intravenous administration of half dose (0.025µg/kg b.wt.) of the last examined dose of gliotoxin (Shathele, 2011) on oxidative stress, histopathological and haemato-biochemical examination before injection (0h) and at 1, 24, 48 and 72 hours post injection in dromedary camels.

Materials and Methods

Experimental Animals and gliotoxin injection

Five healthy adult female camels (8-10 years old, 300-350 kg body weight) were used in the present study. The animals were provided by camel research centre, King Faisal University, Saudi Arabia, kept in an open yard with free access to food and water. Animals were injected intravenously with Gliotoxin (0.025µg/kg b.wt; Sigma, UK). Blood samples were collected in plain and heparinised vacutainers before injection (0h) and at 1, 24, 48 and 72 hours post injection. The whole blood in heparinised vacutainers were used for estimation of haematological parameters. After centrifugation (3000rpm/10 minutes), the obtained sera samples were stored frozen at -30°C until used for estimation of biochemical and oxidative stress biomarkers. Liver and heart tissues were collected from one camel died 24 hours post gliotoxin injection and subjected for histopathological examination.

Haematological and biochemical analysis

Complete blood picture was determined by using electronic cell counter (VetScan HM5 Haematology system). Commercial diagnostic kits (United Diagnostic Industry, Dammam, Saudi Arabia) were used for determination of serum glucose (EP37L-660), total proteins (EP56-660) and albumin (EP03-570), ALT (EP07-500), AST (EP15-500), BUN (EP20-420), Uric acid (EP61-620) and Creatinine (EP33K-660) on ELIPSE full automated chemistry analyser (Rome, Italy). Concentration of the biochemical constituents were calculated according to the manufacturer's instruction.

Analysis of oxidative stress biomarkers

The ELISA kits of Cayman Chemical Company, USA were used for determination of all serum oxidative stress biomarkers. The concentration of MDA (catalogue #10010263) and the activities of total SOD (U/ gram tissue; catalogue #706002), CAT (nmol/min/gram tissue; catalogue #707002), GPX (nmol/min/ gram tissue; catalogue #703102), GST (nmol/min/ gram tissue; catalogue #703302) and GR (µM; catalogue #703202) were measured in the serum by using an ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, USA). The results were calculated according to the manufacturer's instruction.

Histopathological analysis

Lung and heart tissues were cut into small pieces and immersed in neutral buffered formalin for 24 hours. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinised and rehydrated using standard techniques (Bancroft and Gamble, 2002). The extent of gliotoxin toxicity was evaluated by assessing the morphological changes in the lung and sections stained with haematoxylin and eosin (H&E) using standard techniques (Bancroft and Gamble, 2002).

Statistical analysis

All data were presented as the mean ± standard error of the mean (SEM) using analysis of variance (ANOVA). All tests were performed using a statistical analysis system program (SAS, 2002).

Results and Discussion

The effect of intravenous administration of Gliotoxin (0.025µg/kg b.wt.) on haematological parameters before (0 h) and at 1, 24, 48 and 72 hours post injection are presented in table 1. Total erythrocyte counts, total leucocyte counts, hemoglobin and haematocrit percentage were significantly decreased at 1, 24, 48 and 72 hours post injection as compared to its value before injection (0 hour). The values of these parameters at 24 hours post injection and onwards were significantly lower than that at 1-hour post injection. The current findings were similar to that observed earlier in dromedary camels (Shathele, 2011), rams (Dönmez *et al,* 2012) and broilers (Mohamed and Mohamed, 2009). The reductions of RBC and WBC were observed previously (Chattopadhyay *et al,* 2013) in all *Fusarium* mycotoxins treated rats. Data presented in table 2 showed a significant decrease in lymphocyte percentage accompanied

Table 1. Effect of intravenous administration of Gliotoxin (0.025µg/kg b.wt.) on haematological parameters before (0 h) and at 1, 24, 48 and 72 hours post injection.

Variables	0 h	1 h	24 h	48	72
TEC (10^{12}/L)	8.9 ± 0.3[a]	7.4 ± 0.2[b]	5.6 ± 0.2[c]	5.9 ± 0.2[c]	6.0 ± 0.2[c]
Hb (g/dl)	11.1 ± 0.3[a]	9.1 ± 0.3[b]	7.1 ± 0.4[c]	7.4 ± 0.4[c]	7.2 ± 0.4[c]
Hematocrit (%)	28.4 ± 0.9[a]	25.1 ± 0.5[b]	21.8 ± 0.3[c]	21.4 ± 0.3[c]	21.6 ± 0.3[c]
TLC (10^9/L)	15.6 ± 0.2[a]	10.6 ± 0.3[b]	8.1 ± 0.3[c]	8.3 ± 0.3[c]	8.0 ± 0.3[c]
Neutrophils (%)	66.0 ± 1.1[a]	53.0 ± 0.9[b]	49.0 ± 1.1[c]	49.0 ± 1.2[c]	49.0 ± 1.1[c]
Lymphocytes (%)	29.1 ± 1.6[a]	42.1 ± 2.0[b]	46.1 ± 0.9[b]	46.1 ± 1.1[c]	46.1 ± 1.1[c]
Monocytes (%)	2.1 ± 1.2	2.3 ± 1.2	2.3 ± 1.2	2.3 ± 1.2	2.3 ± 1.2
Basophils (%)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Eosinophils (%)	1.5 ± 0.5	2.0 ± 0.5	2.1 ± 0.5	2.1 ± 0.5	1.9 ± 0.5

Values are mean ± SEM of 5 camels

[a-c]Within the same raw with different superscripts differ significantly (P <0.05).

TEC: total erythrocyte count; Hb: hemoglobin; TLC: total leucocyte count.

Table 2. Effect of intravenous administration of gliotoxin (0.025µg/kg b.wt.) on selected serum biochemical parameters before (0 h) and at 1, 24, 48 and 72 hours post injection.

Parameters	0 h	1 h	24 h	48 h	72 h
Glucose (mg/dl)	83.33 ± 1.0[a]	72 ± 1.9[b]	65 ± 2.1[c]	66 ± 2.5[c]	68 ± 2.4[c]
Total Protein (g/l)	8.0 ± 0.3[a]	6.0 ± 0.3[b]	4.9 ± 0.3[c]	5.0 ± 0.4[c]	5.1 ± 0.4[c]
Albumin (g/l)	6.0 ± 0.1[a]	5.5 ± 0.1[b]	4.7 ± 0.1[c]	4.8 ± 0.1[c]	4.8 ± 0.1[c]
Globulin (g/l)	2.0 ± 0.1[a]	1.5 ± 0.0[b]	0.2 ± 0.0[c]	0. 2 ± 0.0[c]	0.2 ± 0.0[c]
ALT (U/l)	22.44 ± 1.1[a]	34.7 ± 1.1[b]	40.7 ± 1.1[c]	40.7 ± 1.0[c]	38.7 ± 1.0[c]
AST (U/l)	45.21 ± 2.4[a]	70.70 ± 3.1[b]	86.70 ± 2.5[c]	87.70 ± 1.8[c]	85.70 ± 2.8[c]
BUN (mg/dl)	18.70 ± 1.6[a]	25.5 ± 1.4[b]	35.5 ± 1.6[c]	34.5 ± 1.3[c]	37.5 ± 1.9[c]
Creatinine (mg/dl)	1.0 ± 0.1[a]	1.4 ± 0.1[b]	1.8 ± 0.1[c]	1.8 ± 0.1[c]	1.8 ± 0.1[c]

Values are mean ± SEM of 5 camels

[a-c]Within the same raw with different superscripts differ significantly (P < 0.05).

ALT: alanine transaminase; AST: aspartate transaminase; BUN: blood urea nitrogen

by significant increase in neutrophil percentage at 1, 24, 48 and 72 hours post injection as compared to its value before injection (0 hour). The observed lymphocytopenia and neutrophilia at 24 hours post injection and onwards were augmented significantly as compared to their values at 1 hour post injection. Observed lymphocytopaenia may be attributed to the toxic effects of gliotoxin on cells in the peripheral circulation or suppression of bone marrow and lymphoid organs function as indicated earlier for aflatoxicosis (Valchev *et al,* 2018). The mycotoxins lead to haematopoietic suppression and anaemia by reduction of red blood cells and hemoglobin (Reddy and Waliyar, 2012). Reduced haematocrit, haemoglobin, erythrocytes and lymphocyte percentage was observed in broiler chickens with experimental aflatoxicosis, probably due to the inhibitory effect of aflatoxins on haematopoietic organs (Mohamed and Mohamed, 2009). This decrease in red blood parameters may result from inhibition of protein biosynthesis (Kubena *et al,* 1993; Abdel-Wahhab, *et al,* 2002) or the faster degradation of erythrocytes in the spleen (Mokif and Muiz, 2015). Increase in the neutrophils percentages might indicate a tendency of the organism to compensate for the decrease of its resistance (Sova *et al,* 1991). The effect of intravenous administration of gliotoxin (0.025µg/kg b.wt.) on selected biochemical parameters before (0 h) and at 1, 24, 48 and 72 hours post injection were presented (Table 2). Total proteins, albumin, and globulin were decreased significantly at 1, 24, 48 and 72 hours post injection compared to its value before injection (0 hour). The values of these parameters at 24 hours and onwards post injection were significantly lower than that at 1-hour post injection. The current findings were similar to that observed earlier in dromedary camels (Shathele, 2009; 2011) and fish (Kaur and Saxena, 2018). This inhibition in protein biosynthesis (total proteins, albumin and globulin) supports the reduction of haematological

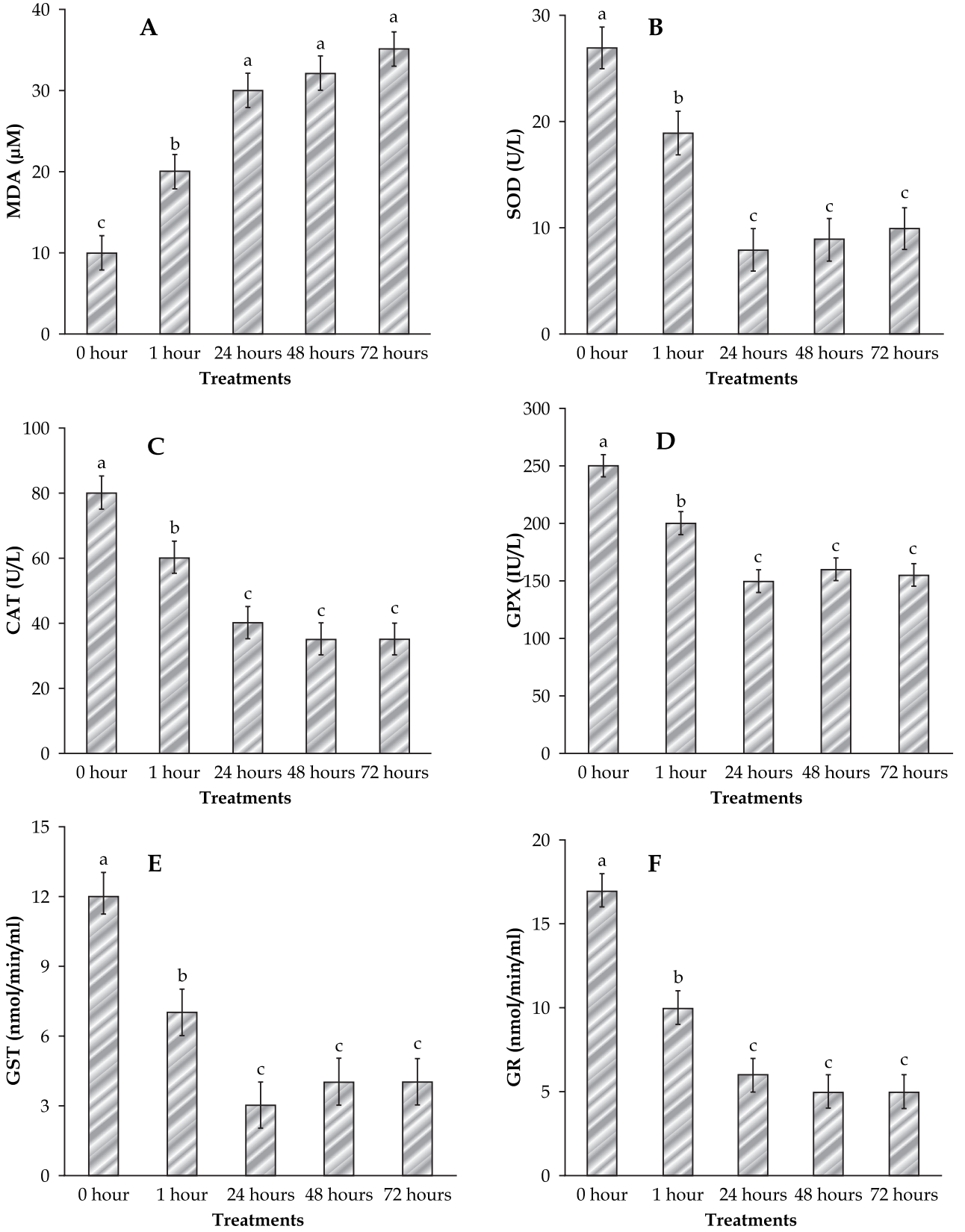

Fig 1. Values of serum oxidative stress biomarkers **(A)** MDA, **(B)** SOD, **(C)** CAT, **(D)** GPX, **(E)** GST and **(F)** RG before injection (0h) and at 1h, 24h, 48h, and 72 hours post intravenous administration of Gliotoxin (0.025µg/kg b.wt.) in dromedary camels. MDA; malonaldhyde; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; GST: glutathione-s-transferase; GR: glutathione reductase.

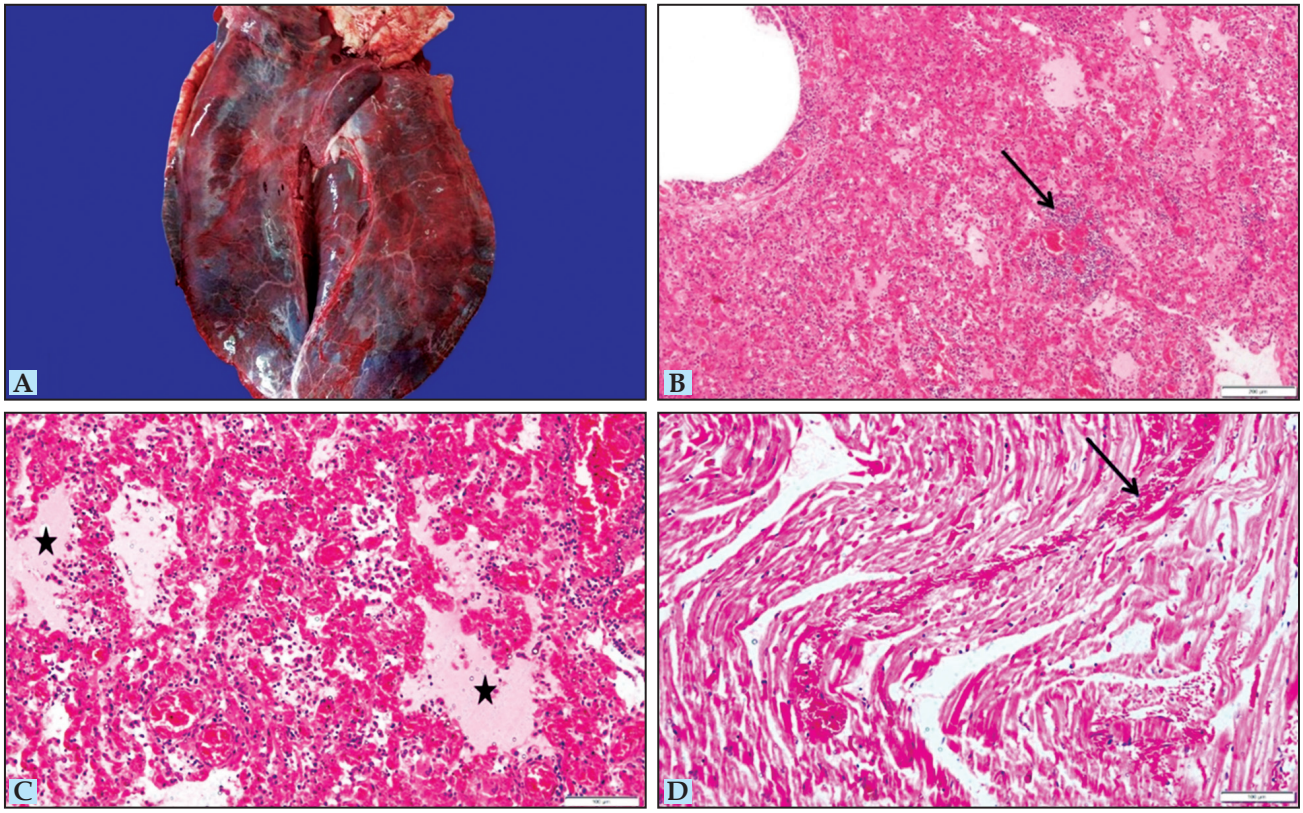

Fig 2. Images from camels injected with gliotoxin (0.025µg/kg b.wt.). **(A)** The whole lungs were congested and oedematous. **(B)** Loss of normal architecture of the pulmonary tissue as well as perivascular cuffing with lymphocytes (arrow). **(C)** Severe congestion of the interalveolar capillaries and homogenous eosinophilic material filing alveoli (asterisk). **(D)** Congestion of the capillaries of cardiac myofibres.

parameters of present study. The significant decrease in globulin supports the immune-depressant effect of gliotoxin in mice (Hussain *et al,* 2020). The AST and ALT are well-known biomarkers of hepatic damage (Recknagel *et al,* 1989). In this study, intravenous administration of gliotoxin (0.025µg/kg b.wt.) caused an increase in ALT and AST activities in camel liver at 1, 24, 48 and 72 hours post injection compared to its value before injection (0 hour). The values of these parameters at 24 hours post injection and onwards were significantly higher than that at 1-hour post injection. This finding was consistent with the findings of previous studies (Shathele, 2009, 2011) in camels intoxicated with gliotoxin (0.05µg/kg b.wt. and 0.1µg/kg b.wt., respectively). As indicated in Fig 1A, the intravenous administration of gliotoxin induced significant elevation in serum MDA levels at 1, 24, 48 and 72 hours post injection compared to its value before injection (0 hour). The MDA values from 24 hours post injection and onwards were significantly higher than its value at 1-hour post injection. These findings indicated that injected camels were subjected to a state of oxidative stress or lipid peroxidation due to gliotoxin injection. This state of oxidative stress reached its maximum 24 hours post injection. This finding was consistent with previous studies in the livers of rats (El-Bahr, 2015) intoxicated with AFB1. The superoxide radicals are converted to H_2O_2 by SOD. Furthermore, either CAT or GPX converts H_2O_2 to molecular oxygen and H_2O. Moreover, GPX can reduce lipid peroxides and other organic hydroperoxides that are highly cytotoxic products (Mujahid *et al,* 2005; Lu *et al,* 2010). Thus, SOD, CAT, GPX and GST constitute the principal components of the antioxidant system (Ahmad *et al,* 2012). In the present study, the activities of antioxidant enzymes such as total SOD (Fig 1B), CAT (Fig 1C), GPX (Fig 1D), and GST (Fig 1E) and GR (Fig 1F) in camels intoxicated with gliotoxin decreased significantly at 1, 24, 48 and 72 hours post injection compared to its value before injection (0 hour). The maximum decline of activities of these enzymes observed at 24, 48 and 72 hours post injection. The observed increase in lipid peroxidation was followed by decrease in the activities of enzymatic antioxidants (SOD, CAT, GPX, GST and GR). Thus, the significant increase in lipid peroxidation (MDA) could be due to a significant reduction in the activities of enzymatic

antioxidants (SOD, CAT, GPX, GST and GR). The same finding were demonstrated in liver tissue of rats intoxicated with AFB1-intoxicated (El-Bahr, 2015). Gross examination of died camel revealed that both lungs were diffusely congested, oedematous and oozing foamy exudate in cut section with presence of large focally extensive area of consolidation in the cranioventral part. The mucosa of the nasal passages and trachea were moderately congested. Small amount of clear straw yellow fluid was seen within the abdominal and thoracic cavities. Microscopically, the main histopathologic lesions were seen in the lungs. The inter-alveolar capillaries as well as peribronchiolar arterioles were greatly dilated and filled with blood. Approximately, 75% of the alveoli were filled with homogenous eosinophilic fluid and some foamy macrophages. Lymphocytes were noticed infiltrating the lung tissue and surrounding hyperemic capillaries and arterioles. Many desquamated and necrotic epithelial cells were seen within the bronchiolar and alveolar lumen. Some alveoli were lined with pneumocyte type II. The cardiac capillaries between myofibers were congested in association with multifocal area of hemorrhages. The observed lung histopathological changes were similar to that observed in lung of mice injected with gliotoxin (Hussain *et al,* 2020). In conclusion, the injected dose of gliotoxin was acutely toxic to camels as reflected on disturbed liver and kidney function, acceleration of oxidative stress and inhibition of antioxidants enzyme activities.

Acknowledgements

The authors thank the Deanship of Scientific Research, King Faisal University, Saudi Arabia for financial support (Project # DSR 140108). Thanks are extended to staff member of Camel Research Centre, King Faisal University, Saudi Arabia for technical assistance during the study. Authors thank Dr. Ayman Alnahas, Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University for gliotoxin injection to camels.

References

Abdel-Wahhab MA, Nada SA and Khalil FA (2002). Physiological and toxicological responses in rats fed aflatoxin contaminated diet with or without sorbent materials. Animal Feed Science and Technology 97: 209-219.

Ahmad H, Jinke T, Wang J, Wang Y, Zhang L, Khan MA and Wang T (2012). Effect of dietary sodium selenite and selenium yeast on antioxidant enzymes and oxidative stability of chicken breast meat. Journal of Agricultural and Food Chemistry 60:7111-7220.

Bancroft JD and Gamble M (2002). Theory and Practice of Histological Techniques, (5th edn). Churchill Livingstone, London, New York and Philadelphia.

Bauer J (1994). Moglichkeiten zur entigifting mykotoxinhaltiger futtermittel. Montash Veterinar Med. 49:175-181.

Bossou YM, Serssar Y, Allou A, Vitry S, Momas I, Seta N, Menotti J and Achard S (2017). Impact of mycotoxins secreted by *aspergillus* molds on the inflammatory response of human corneal epithelial cells. Toxins 9: 197.

Chattopadhyay P, Aadesh U, Amit A, Sanjeev K, Danswerang G and Vijay V (2013). Comparative hematoxicity of *fusirium* mycotoxin in experimental sprague-Dawley Rats. Toxicology International 20(1):25-29.

Dönmez N, Dönmez HH, Keskin E and Kısadere İ (2012). Effects of Aflatoxin on some haematological parameters and protective effectiveness of esterified glucomannan in merino rams. The Scientific World Journal. Article ID 342468, 4 pages.

Eichner RD, Tiwari-Palni U, Waring P and Mullbacher A (1988). Detection of the immunomodulating agent gliotoxin in experimental aspergillosis. In: J. M Torres-Rodriguez (ED) pp 133-137. Proceedings of the Tenth Congress of International Society Human and Animal Mycology, Barcelona. Prous Scientific, Barcelona.

El-Bahr SM (2015). Effect of *curcumin* on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin B1. Phytotherapy Research 29:134-140.

Hussain AF, Sulaiman GM, Dheeb BI, Hashim AJ, Abd Alrahman ES, Seddiq SH and Khashman BM (2020). Histopathological changes and expression of transforming growth factor beta (TGF-b3) in mice exposed to gliotoxin. Journal of King Saud University – Science 32:716-725.

Kaur R and Saxena A (2018). Estimation of variations in the haematological and serum-biochemical parameters of *Labeo rohita* fingerlings due to mold infested feed. Journal of Entomology and Zoology Studies 6(1):1709-1713.

Kubena L, Harvey RB, Phillips TD and Clement BA (1993). Effect of hydrated sodium calcium aluminosilicates on aflatoxicosis in broiler chicks. Poultry Science 72: 651-657.

Lu T, Piao XL, Zhang Q, Wang D, Piao XS and Kim SW (2010). A protective effects of *Forsythia suspensa extract* against oxidative stress induced by diquat in rats. Food and Chemical Toxicology 48:764-770

Mohamed AH and Mohamed MH (2009). Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. Veterinaria Italiana 45:323-37.

Mokif SA and Muiz AMI (2015). Effect of some medicinal plants in reducing aflatoxicosis on erythrocytes and organ weights in Albino Rat. European Journal of Academic Essays 2:98-101.

Mujahid A, Yoshiki Y, Akiba Y and Toyomizu M (2005). Superoxide radical production in chicken skeletal muscle induced by acute heat stress. Poultry Science 84:307-314.

Recknagel RO, Glende EA, Dolak JA and Waller RL (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacology and Therapeutics 43:139-154.

Reddy SV and Waliyar F (2012). Properties of aflatoxin and its producing fungi. Aflatoxins. http://www.icrisat.org/aflatoxin/aflatoxin.asp (Accessed on 8th June 2012).

SAS (2002). Statistical Analysis System. (1st edn). SAS Institute Inc., Cary, NC, USA.

Shathele MS (2011). Fungal mycotoxin s(Gliotoxin) immunomodulating effects on one-humped camel (*Camelus dromedarius*) in Al-Ahsa, Saudi Arabia. Asian Journal of Animal Sciences 5:285-288.

Shathele MS (2009). Effect of administration of fungal mycotoxin (gliotoxin) on clinical and serobiochemical parameters in camels. Interational Journal of Pharmacology 5:381-383.

Sova Z, Pohunkova'H, Reisnerova H, Slamova A and Haisl K (1991). Haematological and histological response to the diet containing aflatoxin B1 and zeolite in broilers of domestic fowl. Acta Veterinaria Brno 60:31-40.

Valchev I, Groseva N, Kanakov D, Hristov Ts, Lazarov L and Binev R (2018). Effect of experimentally induced aflatoxicosis on haematological parameters and bone marrow morphology in mulard ducks. Agricultural Science and Technology 10(3):208-214.

Waring P, Eichner RD and Mullbacher A (1988). The chemistry and biology of the immunomodulating agent gliotoxin and related epipolythiodioxopiperazines. Medical Research Review 8:499-524.