

ISSN 1819-1878

Asian Journal of
Animal
Sciences

Fungal Mycotoxin (Gliotoxin) Immunomodulating Effects on One-humped Camel (*Camelus dromedarius*) in Al-Ahsa, Saudi Arabia

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ABSTRACT

The main aim of this study was to determine fungal mycotoxin (Gliotoxin) immunomodulating effects on one-humped camel (*Camelus dromedarius*) in Al-Hassa, Saudi Arabia. Intravenous administration of 0.05 $\mu\text{g kg}^{-1}$ gliotoxin for 3 days in camels has significantly resulted in decreased serum total protein, albumin and globulin on day 7 after gliotoxin injection. The toxin also caused reduction of leukocyte, lymphocyte and neutrophil count and lysosomal activity compared to saline treated controls. It is suggested that gliotoxin at a dose of 0.05 $\mu\text{g kg}^{-1}$ could produce an immunosuppressive effect in the camel.

Key words: *Aspergillus fumigatus*, fungi, camels, gliotoxin, immunosuppressive

INTRODUCTION

With the increasing number of immune compromised patients and increased use of highly immune suppressive regimens to treat a variety of illnesses, fungi have emerged as major causes of human and animals disease (Jouany and Diaz, 2005). These pathogens are largely opportunists, causing infection when host defenses are breached. Filamentous fungi, including species of *Aspergillus* and *Fusarium*, the Zygomycetes and the dark walled fungi, generally cause invasive disease in hosts. *Aspergillus* species are ubiquitous molds with worldwide distribution. The most common infecting species is *Aspergillus fumigatus* (AF), followed by *A. flavus* and *A. niger* (Marr *et al.*, 2002; Husain *et al.*, 2003). AF produces several mycotoxins, including gliotoxin, tremorgens Ochratoxin A and Patulin that are toxic to cattle (Cole *et al.*, 1977). The secondary metabolite gliotoxin is hypothesized as a unique virulence factor (Rementeria *et al.*, 2005; Tsitsigiannis *et al.*, 2005; Ward *et al.*, 1999).

Gliotoxin (GT), a hydrophobic metabolite which belongs to the class of epipolythiodioxopiperazines compounds, is characterized by the presence of a quinoid moiety and disulfide bridge across a piperazine ring (Latge, 1999; Waring and Beaver, 1996). Gliotoxin at a dose of 0.1 mg kg^{-1} body weight is acutely toxic to camels, affecting liver and kidney functions (Shathele, 2009). This study was designed to investigate the effect of evasion of an immunomodulator mycotoxin (Gliotoxin) on the immune system in camels.

MATERIALS AND METHODS

The study was carried at the Department of Microbiology, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia during 2009-2010.

Selection of animals: Twenty adults camels (age between 4-5 years) were divided into four groups for the study. The camels were kept in an open pen in the vicinity of camel research unit. Animals were fed on grass hay (Rhoades) and water available *ad libitum*.

Mycotoxin administration: Gliotoxin (Sigma Chemicals, UK) was administered intravenously. The dose were 0.0 (saline) and 0.05 $\mu\text{g kg}^{-1}$ for group 1 (control) and 2, respectively for 3 consecutive days.

Collection of blood samples: Blood samples were collected frequently in EDTA tubes for hematology and in plain tubes for serum. Serum was then separated and stored at -30°C for analysis.

White blood cell count: The total blood leukocyte count was determined in a haemocytometer after dilution. Blood was smeared on glass slides, air dried and stained with Gimsa. Differential count was made on 200 white blood cells.

Serum lysozyme activity: Serum lysozyme concentration was measured using micrococcus lysodieticus as a substrate (Lysozyme reagent kit, Washington Biochemical, Co. Freehold, NJ) according to the manufacturer's recommendations. The percentage changes in transmission (at 510 nm) per min were immediately recorded using spectrophotometer (Hitachi, Japan). The values were compared to a standard curve simultaneously prepared using known concentration of egg white lysozyme.

Protein estimation: Fresh sera were used for protein electrophoresis (Henry *et al.*, 1974). Using titan III cellulose acetate plate at pH 8.8, stained by Panceau S stain and scanned by autodensometer at 525 nm. Absolute values of the fractions were correlated from their percentage of total proteins. Total proteins was estimated by biuret reaction (Henry *et al.*, 1974).

The data (Mean \pm SD) was examined for statistical differences by analysis of variance (ANOVA) tests (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Results of the effect of gliotoxin administration in camels on the concentration of proteins, leukocyte counts and lysosomal activity were summarized (Table 1). The toxin significantly ($p < 0.05$) resulted in decreased total protein, albumin and globulin on day 7 after gliotoxin injection. The toxin also caused reduction of leukocytes, lymphocytes and neutrophil count and lysosomal activity. The saline administration did not cause any effect on different serum variables.

Table 1: Concentration of proteins, leukocytes count and lysosomal activity one week after gliotoxin administration

Parameter	Saline treated	Gliotoxin treated
Total protein (g dL ⁻¹)	7.55 \pm 0.31	5.20 \pm 0.25*
Albumin (g dL ⁻¹)	3.55 \pm 0.21	2.40 \pm 0.15*
Total globulin (g dL ⁻¹)	4.00 \pm 0.20	2.80 \pm 0.15*
α -globulin (g dL ⁻¹)	0.92 \pm 0.07	0.70 \pm 0.05*
β -globulin (g dL ⁻¹)	0.96 \pm 0.08	1.47 \pm 0.11*
γ -globulin (g dL ⁻¹)	2.12 \pm 0.12	4.60 \pm 0.22*
Lysosomal activity (U)	8.34 \pm 0.45	5.40 \pm 0.21*
Leukocytes ($\times 10^9$)	9.25 \pm 0.31	5.40 \pm 0.21*
Lymphocytes ($\times 10^9$)	5.20 \pm 0.12	2.90 \pm 0.06*
Neutrophils ($\times 10^9$)	4.00 \pm 0.13	2.20 \pm 0.02*

*Significantly different from control ($p < 0.05$)

Administration of gliotoxin to camels has resulted in reduced total protein, albumin and globulins one week after administration. Many investigators reported similar results. For example: Aflatoxins have produced decreased; levels of proteins and albumins in dairy calves (Brucato *et al.*, 1986), swine's (Harvey *et al.*, 1994), rabbits (Kweon *et al.*, 2003) and camels (Shathele, 2009). These effects may be due to liver impairment as hepatic toxicity has been demonstrated recently in camels receiving gliotoxin (Shathele, 2009). Gliotoxin has caused leucopenia, lymphopenia and neutropenia in camels. Similar findings have been observed in animals treated with aflatoxin B1 (Reddy *et al.*, 1987) or gliotoxin (Eichner *et al.*, 1988). Serum lysosomal activity decreased significantly in camels treated with gliotoxin. Serum lysosomal activity is considered to be an index of macrophage function (Currie and Eccles, 1976). Suppression of macrophage activity by methyl palmitate was associated with reduction of lysosome enzyme release in serum (Kokoshis and Di Luzio, 1979). These findings suggest that gliotoxin may have an immunosuppressive effect in camels. Gliotoxin has been shown to possess a number of immunosuppressive activities (Watanabe *et al.*, 2003) in rabbits can induce apoptosis on various cell lineages (Kweon *et al.*, 2003) including thymocytes (Sutton *et al.*, 1995), macrophages (Eichner *et al.*, 1988) and spleenocytes (Kamei and Watanabe, 2005) and produce immunosuppressed immune function in mice and rabbits (Reddy *et al.*, 1987).

CONCLUSIONS

Administration of gliotoxin to camels significantly ($p < 0.05$) reduced total protein, albumin and globulins one week after administration. Furthermore, the toxin also caused reduction of leukocytes, lymphocytes and neutrophil count and lysosomal activity. The saline administration did not cause any effect on different serum variables.

ACKNOWLEDGMENTS

The author thanks the Deanship of Scientific Research for financial support of the Project No. 110028 and to Professor Abdulgadir Homeida for revising the paper.

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