HVM BIOFLUX
Human & Veterinary Medicine
International Journal of the Bioflux Society

Serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries

Nalini Kataria[1], Anil K. Kataria[2], Nidhi Pandey[3], and Preeti Gupta[3]

[1]Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Bikaner - 334001, Rajasthan, India; [2]Apex Centre for Animal Disease Investigation, Monitoring and Surveillance, College of Veterinary and Animal Sciences, Bikaner - 334001, Rajasthan, India; [3]Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Bikaner - 334001, Rajasthan, India.
Corresponding author: Nalini Kataria, nalinikataria@rediffmail.com

Abstract. Objective: an investigation was carried out in Indian dromedaries (*Camelus dromedarius*) (Linnaeus 1758) in order to find out the defence against reactive oxygen species during environmental stress. Material and Methods: serum levels of reactive oxygen species scavengers like vitamin A, vitamin C, vitamin E, glutathione, catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase were determined. Environmental stress included hot and cold environmental temperature periods. These findings were compared with those obtained during moderate environmental temperature period serving as control. Results: vitamin A, vitamin C, vitamin E and glutathione activity decreased significantly during hot and cold environmental temperature periods. Serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities increased significantly during hot and cold environmental temperature periods. Conclusion: environmental stress induced marked changes in the levels of reactive oxygen species scavengers in the serum of Indian dromedaries.
Key Words: dromedaries, reactive oxygen species scavengers, extremely high and low environmental temperatures.

Rezumat. Obiectiv: determinarea apărării împotriva speciilor de radicali liberi în timpul perioadelor de stres termic extern la dromaderii din India (*Camelus dromedarius*) (Linnaeus 1758). Material şi metodă: S-au determinat nivelurile serice ale vitaminelor A, C, E, glutationului, catalazei, superoxid dismutazei, monoaminoxidazei, glutation reductazei, xantin oxidazei, oxidazei şi peroxidazei. Stresul termic extern a inclus perioadele temperatură scăzută, cât şi ridicată. Datele obţinute în aceste perioade au fost comparate cu cele măsurate în perioadele cu temperatură moderată (control). Rezultate: activitatea vitaminelor A, C, E şi glutationului s-a redus semnificativ în timpul perioadelor cu temperatură ridicată sau scăzută. Activitatea catalazei serice, superoxid dismutazei, monoaminoxidazei, glutation reductazei, xantin oxidazei, oxidazei şi peroxidazei a crescut semnificativ în timpul perioadelor cu temperatură ridicată sau scăzută. Concluzii: stresul termic extern induce modificări marcate ale nivelurilor de antioxidanţi la dromaderii din India.
Cuvinte cheie: dromaderi, antioxidanţi, temperaturi externe scăzute şi ridicate.

Introduction. Reactive oxygen species (ROS) are free radicals that contain the oxygen atom and are highly reactive due to the presence of unpaired valence shell electrons. During environmental stress ROS levels can increase dramatically producing oxidative stress, which is a large rise in the cellular reduction potential (Schafer & Buettner 2001). It can result in a decrease in antioxidant defense contributing to health disorders. Heat and cold are common stressors to the animals in arid and semi-arid tracts. To combat the stress, physiological changes occur according to the priorities of the body (Kataria & Kataria 2005).

Dromedary camels contribute immensely in short distance transport in western states of India and face extreme variations in environmental temperatures. Oxidative stress is extremely dangerous (Kataria et al 2010a) and can be a prelude to many

disease conditions. To save the animals from such situations, their stress free management in arid tracts is very essential. For this assessment of oxidative stress is mandatory. As it can be identified by means of laboratory tests only, several serum biomarkers are available to monitor the oxidative stress in the form of antioxidants and pro-oxidants which can be assessed in blood (Kataria et al 2010c). Superoxide dismutase (SOD), catalase, xanthine oxidase, peroxidase, monoamine oxidase, oxidase, glutathione reductase, vitamin E, vitamin C, and glutathione are considered as measures of oxidative stress allowing the assessment of real status of physiological defenses against reactive oxygen species or oxidative stress. Further the changes in serum biomarkers of oxidative stress can alter the immune status of animals by increasing susceptibility to infectious agents and by reducing the rate of wound healing (Kataria et al 2010b). Looking towards the paucity of literature on this aspect in Indian dromedaries, the present investigation was planned to find out the physiological defense against reactive oxygen species during environmental stress.

This investigation attempted at providing biomarkers of physiological defense against ROS at one platform. This will provide normal range for future studies and will be helpful in making the strategies to protect the animals from oxidative stress.

Material and Method. The blood samples were collected to harvest the serum during morning hours from 150 adult healthy dromedaries (*C. dromedarius*) of either sex of arid region (Rajasthan, India) managed in similar conditions of feeding and watering by the private farmers kept for the purpose of farming and load carrying. The samples were collected during moderate environmental temperature period (Mean maximum temperature ranged from 27 to 29^{o} C), hot environmental temperature period (Mean maximum temperature ranged from 45 to 47^{o} C), and cold environmental temperature period (Mean minimum temperature ranged from 0.5 to 3^{o} C). Each environmental temperature period consisted of 25 male and 25 female dromedaries.

Serum biomarkers included vitamin A, vitamin C, vitamin E, catalase, monoamine oxidase, glutathione reductase, superoxide dismutase, glutathione, peroxidase, xanthine oxidase and oxidase. They were determined by the methods of Varley (1988) for vitamin A and vitamin C, Nair & Magar (1955), Goldblith & Proctor (1950), Green & Haughton (1961), King (1965), Winterbourn et al (1975), Owens & Belcher (1965), Snell & Snell (1954), Litwack et al (1953) and Snell and Snell (1954), respectively, with the modifications as described by Kataria et al (2010 c).

The mean values obtained during moderate environmental period were considered as control. The mean value of each parameter during hot and cold environmental period was compared from the respective control mean value. The statistical significance was determined as per Snedecor & Cochran (1967).

Results. The mean values of serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries are presented in Table 1.

Results indicated that vitamin A, vitamin C, vitamin E and glutathione activity decreased significantly ($p \leq 0.05$) during hot and cold environmental temperature periods in both male and female dromedaries as compared to moderate (control). Serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities increased significantly ($p \leq 0.05$) during hot and cold environmental temperature periods in dromedaries as compared to moderate environmental temperature period.

Table 1

Mean ± SEM values of serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries

Serum Biomarkers	Moderate Environmental Temperature Period (50)		Hot Environmental Temperature Period (50)		Cold Environmental Temperature Period (50)	
	Male (25)	*Female (25)*	*Male (25)*	*Female (25)*	*Male (25)*	*Female (25)*
Vitamin A, μmol L^{-1}	1.91 ± 0.03	1.8 ± 0.02	1.22 ^{b} ± 0.01	1.15 ^{b} ± 0.05	1.18 ^{b} ± 0.02	1.11 ^{b} ± 0.04
Vitamin C, μmol L^{-1}	25.70 ± 1.1	25.10 ± 1.0	18.2^{b} ± 1.0	18.3^{b} ± 1.0	20.1^{b} ± 1.0	21.0^{b} ± 1.1
Vitamin E, μmol L^{-1}	6.20 ± 0.1	6.1 ± 0.1	5.0^{b} ± 0.09	5.0^{b} ± 0.03	5.3^{b} ± 0.03	5.2^{b} ± 0.04
Glutathione, μmol L^{-1}	4.5 ± 0.1	4.4 ± 0.1	3.1 ^{b} ± 0.2	3.0 ^{b} ± 0.1	3.5 ^{b} ± 0.1	3.3 ^{b} ± 0.2
Superoxide dismutase, kU L^{-1}	132.10 ± 6.1	130.2 ± 5.1	170.5^{b} ± 6.5	165.1^{b} ± 6.0	164.0^{b} ± 6.5	163.9^{b} ± 5.3
Monoamine oxidase, U L^{-1}	310.0 ± 9.34	309.0 ± 7.0	357^{b} ± 9.0	351^{b} ± 8.0	353^{b} ± 7.3	355^{b} ± 9.3
Catalase, kU L^{-1}	75.00 ± 4.2	72.00 ± 2.2	97.0^{b} ± 4.0	98.3^{b} ± 3.3	93.2^{b} ± 4.6	94.2^{b} ± 3.3
Glutathione reductase, kU L^{-1}	2.5 ± 0.1	2.3 ± 0.2	3.9 ^{b} ± 0.05	3.8 ^{b} ± 0.06	3.4 ^{b} ± 0.04	3.5 ^{b} ± 0.03
Peroxidase, mU L^{-1}	75.0 ± 5.8	72.0 ± 5.0	99.0^{b} ± 4.0	98.5^{b} ± 4.1	95^{b} ± 4.2	94^{b} ± 4.0
Xanthine oxidase, mU L^{-1}	51.0 ± 2.5	50.0 ± 2.0	79 ^{b} ± 3.9	80 ^{b} ± 3.9	73 ^{b} ± 3.6	74 ^{b} ± 3.9
Oxidase, U L^{-1}	63.0 ± 4.1	62.0 ± 4.5	87.4 ^{b} ± 5.9	86.1 ^{b} ± 4.3	82.4 ^{b} ± 4.3	84.3 ^{b} ± 4.6

Superscript 'b' on the means showed the significant ($p \leq 0.05$) difference from the respective mean value of moderate ambience (control).
Figures in the parentheses indicate number of animals.

Discussion. In present study serum vitamin A, vitamin C, vitamin E and glutathione levels were lower during hot and cold environmental periods which indicated their depletion in the process to prevent oxidative stress. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells along with alteration in the immune status of animals by increasing susceptibility to infectious agents (Kataria et al 2010b). Stress can use up large quantities of vitamin C (Kataria et al 2010a). Vitamin A has an effect on lipid peroxidation whereas Vitamin C protects the body against oxidative stress (Padayatty et al 2003). As ascorbate free radical reacts poorly with oxygen, a superoxide is not created. Instead two semi dehydro ascorbate radicals will react and form one ascorbate and one dehydroxy ascorbate. With the help of glutathione, dehydroxyascorbate is converted back to ascorbate. Therefore the presence of glutathione is crucial since it spares ascorbate and improves antioxidant capacity of blood (Gropper et al 2004). Glutathione (GSH) is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining other antioxidants such as vitamins C and E in their reduced or active forms. Vitamin E stops the production of reactive oxygen species during oxidation of fat. It protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. The oxidised α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. These reactions made the vitamin E to be important in cell homeostasis and antioxidant pathways (Galli & Azzi 2010).

Superoxide dismutase, monoamine oxidase, catalase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities showed similar trends. The activities in the serum increased during hot and cold environmental temperature periods. Superoxide dismutase is responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways and its higher concentration in the serum is an indicator of oxidative stress (Bauer & Bauer 1999). It is considered as the key antioxidant enzyme and known to reverse fibrosis, perhaps through reversion of myofibroblasts back to fibroblasts (Vozenin-Brotons et al 2001).

Monoamine oxidases catalyse the oxidative deamination of monoamines and play an important role in the inactivation of neurotransmitters. During stress activity of MAO increases (Kataria et al 2010c). This could be the possible reason of maximum activity in the camels during hot and cold environmental temperature periods.

Catalase functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al 2004). Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other, less dangerous substances. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani et al 1996). Glutathione reductase (GSR or GR) is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant (Meister 1988). The activity of glutathione reductase is used as an indicator for oxidative stress.

Xanthine oxidase (XO) is a form of xanthine oxidoreductase that generates reactive oxygen species (Ardan et al 2004). It is not a scavenger of free radical but along with free radical scavengers it can be used as a marker of oxidative stress. Serum XO is more sensitive than serum aminotransferases in detecting acute liver damage (Ramboer et al 1972). An oxidase is any enzyme that catalyzes an oxidation/reduction reaction involving molecular oxygen (O_2) as the electron acceptor. In these reactions, oxygen is reduced to water (H_2O) or hydrogen peroxide (H_2O_2). Therefore it is considered as good marker of oxidative stress. Peroxidase is a hemoprotein catalyzing the oxidation by hydrogen peroxide of a number of substrates such as ascorbate, ferrocyanide, cytochrome C etc. Serum peroxidase activity is considered as the main indicator of the antioxidant activity (Podil'chalk et al 1996).

Conclusions. It was concluded that environmental stress was able to induce marked changes in the levels of serum biomarkers in an order to evoke physiological defense.

Decrease in the levels of antioxidants strongly pointed towards the existence of oxidative stress in dromedaries. The present investigation also attempted to provide baseline values of serum biomarkers of physiological defense against oxidative stress in healthy dromedary camel for future studies to help in the clinical diagnosis, as there is paucity of these values in dromedaries. It is suggested that decrease in the levels of antioxidants can be prevented by supplementing them during extreme temperature periods.

References

Ardan T., Jejková J., 2004 Comparative histochemical and immunohistochemical study on xanthine oxidoreductase/xanthine oxidase in mammalian corneal epithelium. Acta histochem **106**(1):69-75.

Bauer V., Bauer F.,1999 Reactive oxygen species as mediators of tissue protection and injury. Gen Physiol Biophys **18**:7-14.

Chelikani P., Fita I., Loewen P. C., 2004 Diversity of structures and properties among catalase. Cell Mol Life Sci **61**(2):192–208.

Gaetani G., Ferraris A., Rolfo M., Mangerini R., Arena S., Kirkman H., 1996 Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood **87**(4):1595–1599.

Galli F., Azzi A., 2010 Present trends in vitamin e research. Biofact **27**: (in press).

Goldblith S. A., Proctor B. E., 1950 Photometric determination of catalase activity. J Biol Chem **187**:705-709.

Green A. L., Haughton T. M., 1961 A colorimetric method for the estimation of monoamine oxidase. Biochem J **78**:172-175.

Gropper S. S., Smith J. L., Grodd J. L., 2004 Advanced nutrition and human metabolism. 9th edition. Thomson wadsworth, belmont, (pp. 260-275) CA, USA.

Kataria N., Kataria A. K., Maan R., Gahlot A. K., 2010a Evaluation of oxidative stress in brucella infected cows. J Stress Physiol Biochem **6**:19-31.

Kataria A. K., Kataria N., Maan R., 2010b Correlation of serum IgE with stress in Indian dromedaries affected with skin wounds. J Stress Physiol Biochem **6**(3):17-24.

Kataria N., Kataria A. K., Maan R., 2010c Evaluation of oxidative stress due to hot environmental condition in healthy Marwari goats from arid tracts in India. Philipp J Vet Anim Sci **36**(2):175-184.

Kataria N., Kataria A. K., 2005 A psychophysiological approach to alleviate stress in cattle. Indian Cow **2**(6):2-5.

King J., 1965 Practical clinical enzymology. D.van nostrand company ltd., (pp 1-301) London.

Linnaeus C., 1758 Tomus I. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Holmiae (Laurentii Salvii) **1-4**:1-824

Litwack G., Bothwell J. W., Williams J. N. Jr., Elvehjem C. A., 1953 A colorimetric assay for xanthine oxidase in rat liver homogenates. J Biol Chem **200**(1):303-310

Meister A.,1988 Glutathione metabolism and its selective modification. J Biol Chem **263** (33):17205–17208.

Nair P. P., Magar N. G., 1955 Detemination of vitamin E in blood. J Biol Chem **220**(1):157-159.

Owens C. W. I., Belcher R. V., 1965 A colorimetric micro method for the determination of glutathione. Biochem J **94**(3):705-711.

Padayatty S., Katz A., Wang Y., Eck P., Kwon O., Lee J., Chen S., Corpe C., Dutta A., Dutta S., Levine M., 2003 Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Col Nutr **22**(1):18–35.

Podil'chalk M. D., Vdovychenko V. I., Terlets'ka L. M., 1996 Lipid peroxidation and blood serum peroxidase activity in diseases of the hepatobiliary system. Lik Sprava **1-2**:110-112.

Ramboer C., Piessens F., De Groote J., 1972 Serum xanthine oxidase and liver disease. Digestion **7**(3-4):183-195.

Schafer F. Q., Buettner G. R., 2001 Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med **30** (11):1191–1212.
Snedecor G. W., Cochran W. G., 1967 In: statistical methods. 6th edition. Oxford & ibh publishing co. (pp45-83). New Delhi
Snell F. D., Snell C. T., 1954 Colorimetric methods of analysis 3rd edn D. Van Nostrand company (pp 512-518), New York.
Varley H., 1988 Practical Clinical Biochemistry. 4th Edition. Cbs publishers (pp349-393), New Delhi.
Vozenin-Brotons M. C., Sivan V., Gault N., Renard C., Geffrotin C., Delanian S., lefaix J. L., Martin M., 2001 Antifibrotic action of Cu/Zn sod is mediated by tgf-beta1 repression and phenotypic reversion of myofibroblasts. Free Radic Biol Med **30** (1):30–42.
Winterbourn C., Hawkins R., Brian M., Carrell R., 1975 The estimation of red cell superoxide dismutase activity. J Lab Clin Med **85**:337-340.

Received: 23 September 2010. Accepted: 05 November 2010. Published online: 12 November 2010.
Authors:
Nalini Kataria, In-Charge Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Bikaner - 334001, Rajasthan, India. Phone: 0091 – 151- 2546399. E-mail: nalinikataria@rediffmail.com
Anil Kumar Kataria, In-Charge, Apex Centre for Animal Disease Investigation, Monitoring and Surveillance, College of Veterinary and Animal Sciences, Bikaner - 334001, Rajasthan, India. Phone : +919460073909. E-mail: akkataria1@rediffmail.com
Nidhi Pandey, Department of Veterinary Physiology, College of Veterinary and Animal Science, Bikaner 334001, Rajasthan , India. Phone: +919461880599. E-mail: nidhip83@rediffmail.com
Preeti Gupta , Department of Veterinary Physiology, College of Veterinary and Animal. Science, Bikaner 334001, Rajasthan , India. Phone: +919414790040. E-mail: preetphy@rediffmail.com
How to cite this article:
Kataria N., Kataria A. K., Pandey N., Gupta P., 2010 Serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries. HVM Bioflux **2**(2):55-60.