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Ambience-associated variation in serum biomarkers of oxidative stress in donkeys of arid tracts in India

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Abstract

An investigation was carried out in donkeys to discover serum biomarkers of oxidative stress during moderate and extremely hot conditions. Serum biomarkers included vitamin A, vitamin C, vitamin E, glutathione, catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase. These findings were compared with those obtained during the moderate conditions that served as a control. Serum vitamin A, vitamin C, vitamin E and glutathione activity decreased significantly during hot conditions, while serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities all increased significantly. It was concluded that hot ambient stress induced marked changes in the levels of biomarkers in the serum of donkeys, indicating oxidative stress.

Keywords:

Introduction

Heat stress during hot environmental conditions in arid habitats is a problem of great concern among animal owners as it affects reproduction and efficiency of the animals. Donkeys are more prone to physical stress during such conditions due to the type of work they carry out. Heat stress is one of the factors resulting in oxidative stress, disturbance in the prooxidant–antioxidant balance which leads to potential cellular damage. Measuring oxidative stress can be difficult due to the presence of complex endogenous systems for correction and repair. A brief elevation in oxidative stress rapidly induces various antioxidant defenses, particularly antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, that quickly reduce the stress. Oxidative stress can result from diminished antioxidant protection as well as increased production of free radicals. Therefore investigating antioxidant depletion as a biomarker of oxidative stress may involve the assessment of decreases in antioxidant concentrations or increases in their metabolites.

Serum biomarkers are used as tools to detect various disease or stress processes. Selection of an antioxidant intervention depends upon the measurement of parameters relevant to the status of antioxidant defenses and oxidative stress. Exposure to extreme ambient conditions causes intracellular damage indicative of oxidative stress, with consequences for mutagenic activity as well as aberrant changes. Oxidation of cellular lipids and proteins can adversely affect several metabolic steps leading to variety of diseases with changes in various cell regulatory and signaling functions (Kataria *et al*. 2010a). Biomarkers of oxidative stress are sufficient to establish pathological changes related to disease and therefore should be employed to inform the design and outcome of clinical trials in veterinary medicine. Identification and application of suitable biomarkers should shorten the time it takes to demonstrate that an agent has a beneficial, untoward, or null effect on health promotion and disease prevention or a therapeutic value in disease treatment. The aim of the present study was to establish reference values for the selected biomarkers of oxidative stress in donkeys living in arid and semi-arid habitats, and to find out the effect of variations in ambient temperatures on these animals.

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Materials & Methods

Blood samples were collected from the jugular vein to harvest serum during the morning hours from donkeys found in arid habitats of Rajasthan state, India. The animals were maintained in similar management and feeding conditions. Blood samples were collected during moderate (maximum temperature of 26 – 27 ºC) and hot ambient conditions (max 44 – 47 ºC) from 30 adult male animals free from endo-parasites (as assessed by faecal examination).

Serum biomarkers included vitamin A, vitamin C, vitamin E, catalase, monoamine oxidase, glutathione reductase, superoxide dismutase, glutathione, peroxidase, xanthine oxidase and oxidase. Vitamin A and vitamin C were determined by the methods of Varley (1988): the others were determined using the methods of 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 modifications as described by Kataria *et al.* (2010b). The mean values obtained during moderate ambient conditions were considered as controls. The mean value of each parameter during hot ambient conditions was compared from the respective control mean value, with statistical significance determined as per Snedecor & Cochran (1967).

Results

The mean values of serum biomarkers of oxidative stress in donkeys are presented in Table 1. Results indicated that vitamin A, vitamin C, vitamin E and glutathione activity decreased significantly ($p \leq 0.05$), whereas serum superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities increased significantly ($p \leq 0.05$) during hot ambient conditions as compared to moderate conditions. Catalase also increased, but the difference was not quite significant (see Table 1).

Biomarkers of oxidative stress	Environmental conditions		Statistical analysis (ANOVA)		
	moderate	hot	F	df	p
Vitamin A, µmol L^{-1}	2.10 ± 0.01	1.49 ± 0.01	1845.1	1	<0.001
Vitamin C, µmol L^{-1}	22.0 ± 2.4	13.8 ± 1.9	7.1	1	<0.01
Vitamin E , µmol L^{-1}	5.21 ± 0.11	2.22 ± 0.04	656.7	1	<0.001
Glutathione, µmol L^{-1}	4.01 ± 0.12	2.11 ± 0.01	247.2	1	<0.001
Catalase, kU L^{-1}	71.3 ± 10.0	100.2 ± 11.1	3.7	1	0.058
Superoxide dismutase, kU L^{-1}	121.1 ± 10.1	350.3 ± 12.3	207.1	1	<0.001
Monoamine oxidase, U L^{-1}	285.0 ± 10.0	453.5 ± 13.0	105.4	1	<0.001
Glutathione reductase, kU L^{-1}	3.35 ± 0.11	5.95 ± 0.12	254.9	1	<0.001
Xanthine oxidase, mU L^{-1}	51.2 ± 2.0	80.3 ± 5.9	22.0	1	<0.001
Oxidase , U L^{-1}	63.2 ± 6.0	93.1 ± 3.2	19.2	1	<0.001
Peroxidase, mU L^{-1}	70.2 ± 5.1	105.1 ± 5.1	23.3	1	<0.001

Table 1: Mean ± SEM values of serum biomarkers of oxidative stress in donkeys (n=30).

Discussion

Serum vitamin A, vitamin C, vitamin E and glutathione levels were lower during hot ambient conditions, which indicated their depletion in the process to prevent oxidative stress. The decrease in the value of serum vitamin A reflects its antioxidant role in neutralizing oxygen-derived free radicals. Vitamin C is a strong reducing agent and an endogenous antioxidant

which also helps the animals protect against oxidative stress. Vitamin E or α-tocopherol is another important endogenous antioxidant which inhibits the production of reactive oxygen species formed when fat undergoes oxidation, protecting cell membranes from oxidation. The oxidised α-tocopheroxyl produced in this process can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Kataria *et al*. 2010b). The antioxidant glutathione protects cells from free radicals by participating directly in their neutralization.

Superoxide dismutase, monoamine oxidase, catalase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities in serum increased during hot ambient conditions. Superoxide dismutase is the key antioxidant enzyme responsible for the quenching of superoxide radicals produced during various metabolic pathways. The activity of monoamine oxidase helps maintain neuron firing rates throughout the body within homeostatic limits. A relevant source of free radicals in mitochondria is represented by monoamine oxidases (Youdim *et al.* 2006), and its higher concentration may reflect oxidative stress.

Catalase is frequently used by cells to catalyze rapidly the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. Alteration in the levels is suggestive of oxidative stress (Kataria *et al*. 2010b), and the increase recorded here is suggestive but not conclusive. Glutathione reductase is an enzyme that reduces glutathione disulfide to the sulfhydryl form, an important cellular antioxidant. The increased activity of glutathione reductase indicates oxidative stress (Maan *et al*. 2013). During oxidative stress xanthine oxidase is shed from liver and released into plasma. In this way it can play an important role as an indicator of oxidative stress, as in fact can any oxidase. Peroxidase catalyses the oxidation by hydrogen peroxide of a number of substrates and its activity is considered an indicator of antioxidant activity (Kataria *et al*. 2010b). Heat stress and oxidative stress can be damaging agents reported to induce an adaptive response in animals (Maan *et al*. 2013). Coupling of oxidative stress with abiotic stress can hinder the growth and productive potential of animals living in arid habitats. Therefore it is essential to diagnose oxidative stress at an early stage, but this is possible only by laboratory means.

It is concluded that hot ambient conditions most likely modulate the mechanisms evolved to neutralize free radicals. This was evident in the form of reduced levels of major endogenous antioxidants. Hot conditions possibly result in the development of oxidative stress. The present investigation also attempted to provide baseline values of serum biomarkers of oxidative stress in healthy donkeys for future studies to help in clinical diagnosis.

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الملخص العربي

الاختلافات المصاحبة لضغوط الأكسدة للمؤشرات الحيوية فى سيريم دم الحمير فى المناطق الإستوائية بالهند

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تمت هذه الدراسة على الحمير لاكتشاف المؤشرات الحيوية لسيريم الدم تحت ضغوط الأكسدة أثناء ظروف الحرارة المتوسطة والشديدة. شملت المؤشرات الحيوية للسيريم كل من : فيتامين أ - فيتامين ج - فيتامين ى - جلوتاثايون - كاتاليس - سوبر ديميتيز - مونوأمين أوكسيديز - جلوتاثايون ريديكتيز - كسانثن أوكسيديز - أوكسيديز - بيرأوكسيديز. تم مقارنة النتائج لهذه المؤشرات مع التى تم الحصول عليها خلال ظروف الحرارة المتوسطة والتى تم استخدا مها كمجموعة ضابطة. نقصت مؤشرات فيتامين أ - فيتامين ج - فيتامين ى - جلوتاثايون بصورة معنوية أثناء ظروف الحرارة الشديدة، بينما زادت كل من كاتاليس - سوبر ديميتيز - مونوأمين أوكسيديز - جلوتاثايون ريديكتيز - كسانثن أوكسيديز - أوكسيديز - بيرأوكسيديز بصورة معنوية. ولذا يمكننا القول بأن ظروف الحرارة تمثل ضغطاً على الحمير مما يتسبب فى حدوث تغييرات ملحوظة فى كل مستويات المؤشرات الحيوية لسيريم الدم مما يوضح ضغوط الأكسدة.