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Superoxide Dismutase Profiling During Extreme Ambiences in Marwari Sheep from Arid Tracts

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Abstract: Serum Superoxide Dismutase (SOD) profiling was carried out in the Marwari breed of sheep from arid tracts in India. Animals were categorized according to sex and age. Blood samples were collected during moderate, extreme hot and cold ambiences to harvest sera. Mean value of serum SOD during moderate ambience was 171.76±2.72 kU L^{-1}. Moderate mean value was considered as control. The mean value of serum SOD was significantly ($p \leq 0.05$) higher during hot and cold ambiences as compared to control. Mean value was 2.57 times higher in hot ambience and 1.54 times higher in cold ambience as compared to control. The sex and age effects were significant ($p \leq 0.05$) in all ambiences. The mean values were significantly ($p \leq 0.05$) higher in male animals than female animals. In each ambience the age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the animals of 2.5-4.5 years of age. Increased SOD activities during extreme ambiences indicated the development of oxidative stress. Present study generated data of SOD which can be used as reference values for future studies and for diagnostic purposes. The pattern of variation of SOD activity indicated modulation of adaptive mechanisms to protect the body from changes in ambient temperatures and on this basis use of antioxidants in the animals during changing environments is recommended.

Key words: Serum superoxide dismutase, Marwari breed, moderate, hot ambience, antioxidants

INTRODUCTION

Antioxidant enzyme activities are sensitive markers of oxidative stress as their levels may increase or decrease in response to reactive oxygen species. Superoxide dismutase is one of the antioxidant enzymes which catalyses the dismutation of superoxide and therefore becomes important in the defense mechanisms against oxidative stress (Halliwell and Chirico, 1993). Oxidative stress commonly occurs following heat stress in tropical regions and affects the animals (Dehghan *et al*., 2010). It causes serious physiolo gical dysfunction that may result in a decline in animal performance. Hyperthermia has been proposed to be responsible for stimulating reactive oxygen species production because of similarities in the expression patterns of genes including heat shock, oxidative stress proteins or both (Schiaffonati *et al*., 1990). Reactive oxygen species can modulate metabolic reaction due to heat stress producing oxidative stress. Peroxidation of lipid is one such mechanism by which free radicals induce tissue damages resulting into varieties of pathologies (Halliwell and Chirico, 1993).

The evaluation of the extent of oxidative stress in the blood can be useful to define its role in different biotic and abiotic stresses and can be used for clinical diagnosis. Serum superoxide dismutase is considered as one of the non invasive peripheral markers to assess oxidative stress (Kataria *et al*., 2010a). Researchers are also using superoxide dismutase profiling to determine the extent of oxidative stress in heat stressed animals (Kataria *et al*., 2010b, c). The inevitability of exposure of sheep to extreme hot and cold ambiences of arid

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tracts makes profiling of SOD associated with extreme ambiences an appropriate field of investigation to explore adaptive physiological measures of the body and their use in health management and clinical diagnosis. Marwari breed of sheep constitutes a major portion of the sheep population in Western part of Rajasthan and plays an important role in the economy of arid and semi arid tract.

Despite of its immense quality characteristics very little scientific savoir faire is there about normal and clinical variations in the values of serum superoxide dismutase. To understand the real worth of these animals and to explore the productive potential, establishment of their own norms becomes very important in the field of veterinary clinical physiology. Therefore, the present investigation was planned to carry out profiling of serum superoxide dismutase during extreme ambiences in Marwari sheep and to set normal values for the use in veterinary clinical physiology and for future research.

MATERIALS AND METHODS

Animals: The study was carried out in 630 apparently healthy Marwari sheep of either sex between 6 months to 4.5 years of age to determine glutathione reductase enzyme in the serum during moderate (mean maximum ambient temperature 30.34±0.20°C), hot (mean maximum ambient temperature 45.10±0.09°C) and cold (mean minimum ambient temperature 4.83±0.03°C) ambiences. Blood samples were collected through jugular vein during slaughtering from private slaughter houses (Bikaner, Rajasthan, India) where all the animals were kept in similar conditions of management. In each ambience, 210 blood samples were obtained to harvest the serum samples and the animals were categorised gender wise as male (105) and non-pregnant female (105) and agewise as below 1 year (35 male and 35 female); 1-2 years (35 male and 35 female) and 2.5-4.5 years (35 male and 35 female).

Analytical method: Superoxide dismutase was determined by colorimetric method of Winterbourn *et al.* (1975) with the little modification. The method is based upon the ability of superoxide dismutase to inhibit the reduction of nitroblue tetrazolium by superoxide. One unit is defined as that amount of enzyme causing half the maximum inhibition of nitroblue tetrazolium reduction. In the modification, instead of the Michaelis-Menten type of plot, a linear plot was obtained when the reciprocal values for the percentage inhibition of Nitroblue Tetrazolium (NBT) reduction were plotted against the serum samples. This plot was found to give precise values for the volumes of samples required for 50% inhibition of NBT reduction.

A series of ten test tubes were set and various quantities of each serum sample (0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 μL) were added. In each tube 0.2 mL of EDTA-cyanide reagent (0.1M EDTA and 0.3 mM sodium cyanide), 0.1 mL Nitroblue Tetrazolium (NBT) reagent (1.5 mM) and 3 mL of phosphate buffer (0.067 M, pH 7.8) were added. The tubes were incubated by placing them in light box providing uniform light intensity. For this a foil-lined box (4' long×8"×6") with an internally mounted 40 W fluorescent bulb was used. The tubes were incubated for 5 min to achieve a standard temperature. Then, 0.05 mL riboflavin (0.12 mM) was added. All tubes were again incubated in the light box for 12 min. Then at 560 μm wavelength, the % transmission of each tube was determined at 1 min interval in an increasing order. The amount of sample resulting in 50% of transmission was determined by a curve and put in the formula to calculate SOD units as follows:

$$\text{Units/mL} = \frac{1000}{\text{Microlitter of serum resulting in 50\% transmission}}$$

Here, 1000 = Conversion factor. After calculation the values were converted into kU/L.

Statistics: The changes in the means were measured by using multiple mean comparison procedures (Duncan, 1955; Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Mean±SEM values are presented in Table 1. Moderate mean value of serum SOD was 171.76±2.72 kU L^{-1}. The range of serum SOD was from 150-460 kU L^{-1} including moderate hot and cold ambiences.

Effect of hot and cold ambiences on serum SOD: The mean value of serum SOD was significantly ($p \leq 0.05$) higher during hot and cold ambiences as compared to moderate mean value. Mean value was 2.57 times higher in hot ambience and 1.54 times higher in cold ambience as compared to moderate ambience.

In present study, the higher values of serum SOD during hot and cold ambiences were most likely to scavenge the free radicals produced. High environmental temperature is known to stimulate excessive production of free radicals especially super oxide anion radicals and hydrogen peroxide (Siva Kumar *et al.*, 2007). Hot environment induced oxidative stress on the basis of higher activity of SOD has been reported by earlier researchers (Bernabucci *et al.*, 2002; Kataria *et al.*,

Table 1: Mean±SEM values of serum Super Oxide Dismutase (SOD, kU/L) in Marwari sheep

	Ambiences		
Effects	Moderate	Extreme hot	Extreme cold
Ambience	171.76±2.72[b]	441.58±4.87[b]	265.74±1.39[b]
Sex			
Male (105)	177.31±2.37[d]	453.71±4.95[d]	270.71±2.01[d]
Female (105)	166.21±2.51[d]	429.45±4.85[d]	260.76±1.82[d]
Age			
Below 1 year (70)	160.40±2.53[f]	393.33±2.40[f]	246.12±1.62[f]
1-2 years (70)	170.77±2.99[f]	435.71±5.77[f]	257.65±1.76[f]
2.5-4.5 years (70)	184.12±2.58[f]	495.71±5.02[f]	293.44±2.27[f]

Figures in the parenthesis indicate number of animals. [B]Marks significant ($p \leq 0.05$) differences among ambience overall mean values of a parameter; [d]Marks significant ($p \leq 0.05$) differences between male and female mean values of a parameter within an ambience; [f]Marks significant ($p \leq 0.05$) differences among mean values of different age groups of a parameter within an ambience

2010b, c). High ambient temperature could increase oxidative stress by increasing lipid peroxidation and decreasing antioxidant defense (Bhat *et al*., 2008). Influence of varying environmental temperatures on antioxidant enzyme activities was observed by many researchers (Harmon *et al*., 1997; Burke *et al*., 2009). Extreme ambient temperatures in extreme hot and cold ambiences probably produced enormous stress to the animals resulting in an increase in serum SOD values. Earlier workers have used serum SOD as important parameter to find out biotic stress (Kataria *et al*., 2010a; Kataria and Kataria, 2012a, b; Kataria *et al*., 2012). Higher activities show physiological upregulation of this enzyme in an attempt to mitigate superoxide radical challenge (Kahlon and Singh, 2003). A study by Sakatani *et al.* (2011) in black cow revealed that high ambient temperature during summer increases both body temperature and oxidative stress. Scientists evaluated the effect of hyperthermia in altering the oxidative stress on the basis of superoxide dismutase. The high ambient temperature beyond the range of comfort zone causes environmental heat stress and produces serious physiological dysfunction that may result in heat-related diseases and even death. Persistent higher ambient temperature may result in hepatic dysfunction. Thermal load via oxidative stress may deteriorate of liver functions (Das, 2011). On the basis of earlier discussion it can be reiterated that extreme ambiences generated free radicals to produce oxidative stress in sheep in the present study.

Effect of sex and age on serum SOD: The sex and age effects were significant ($p \leq 0.05$) in all ambiences. The mean values were significantly ($p \leq 0.05$) higher in male animals than female animals. In each ambience the age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the animals of 2.5-4.5 years of age.

Higher activity in male animals suggested higher rate of formation of free radicals. Influence of age on serum SOD activity was also observed by earlier researchers (Nazifi *et al*., 2009) who suggested that age influenced the level of enzyme antioxidant defense due to generation of free radicals. De and Darad (1991) conducted a study in rats and observed that SOD activity decreased with the advancement in age.

CONCLUSION

Pattern of serum SOD activities clearly showed the development of oxidative stress. Its magnitude was greater during hot ambience than cold ambience. Effect of extreme ambiences was potent that it affected animals of both sexes and all age groups. Modulation of adaptive mechanisms was greater in male animals and in the animals of 2.5-4.5 years of age. Present study generated data of SOD which can be used as reference values for future studies and for diagnostic purposes. The pattern of variation of SOD activity indicated modulation of adaptive mechanisms to protect the body from changes in ambient temperatures and on this basis use of antioxidants in the animals during changing environments is recommended.

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