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ORIGINAL ARTICLE

Serum xanthine oxidase profile in stressed *Marwari* sheep from arid tracts in India

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The present investigation was aimed to determine serum xanthine oxidase profile in stressed *Marwari* breed of sheep belonging to arid tracts in Rajasthan, India. Extreme hot and cold ambiences were considered as stress conditions to the animals. Blood samples were collected to obtain sera during moderate, extreme hot and cold ambiences. The mean value of serum xanthine oxidase during moderate ambience was 93.33±1.11 mU L^{-1}.The mean value of serum xanthine oxidase was significantly ($p \leq 0.05$)higher during hot and significantly ($p \leq 0.05$) lower during cold ambiences as compared to moderate mean value serving as 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 males than females. 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. The effects of extreme ambiences were observed on the male and female animals of all age groups as revealed by various interactions studied *viz*. ambience X age; ambience X sex and age X sex ($p \leq 0.01$). Further sex effect was present in the animals of each age group. It can be concluded that serum xanthine oxidase can be used as an effective marker to assess oxidative stress in these animals. Mean values obtained from large number of animals during moderate ambience will help in providing physiological reference values for future research and clinical interpretations.

Key words: ambience, cold, hot, Marwari, sheep, serum, xanthine oxidase

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Exposure of the animals to dire changes in ambient temperatures is a major concern in the health management of the breeds which are native to arid tracts. Oxidative stress through greater free radical generation complicates the health issues of the animals. The occurrence of oxidative stress due to extreme hot and cold ambiences is documented in the animals (Kataria *et al*., 2010a). However, an understanding of the physio-biochemical mechanism in the formation of oxidative stress yet requires exploration for native breeds. *Marwari* breed of sheep plays an important role in the economy of arid tracts in Rajasthan state of India. However, physiological mechanisms are modulated

severely as these animals encounter drastic variations in ambient temperatures. Research in the field of veterinary medicine, though few reports, have reached to an assumption that free radicals are proven to be detrimental in exacerbating the stress in the animals (Kataria *et al*., 2010b). Determination of stress markers is gaining importance so that degree of stress can be assessed by a clinician to take timely measures.

Xanthine oxidase (XO) plays an important role in the catabolism of purines. It catalyses the conversion of hypoxanthine to xanthine and that of xanthine to uric acid, which are the last steps in purine metabolism. The by product of these reactions is a toxic superoxide radical. This reaction is considered as potential source of oxygen free radical (Zhang *et al.,* 2010). The uric acid product from xanthine oxidase catalysis contributes to the antioxidant capacity of the blood. The reduction of O_2 and H_2O_2 in the xanthine oxidase catalysis has been proposed as a central mechanism of oxidase injury (Mccord, 1985).Free radicals not only cause damage but they also have a role in cell signalling (Murrantand Reid, 2001). The redox-sensitive transcription factor NF-κB is activated during stress in animals. Various experiments conducted from time to time in humans and animals have shown the increased concentration of XO during exercise or stress, which is found to be a source of free radicals (Heunks *et al.,* 1999). Ardan *et al.,* (2004) discussed its role in oxidative stress by generating reactive oxygen species, therefore in stressed animals higher serum xanthine oxidase may indicate oxidative stress (Kataria *et al.*, 2010c). Further inhibition of XO has been found to prevent damage due to free radicals (Gomez-Cabrera *et al.,* 2005).

Xanthine Oxidase has also been implicated in several physiological and pathological cases (Izotov *et al*., 1991). Serum XO is more sensitive than serum amino transferases in detecting acute liver damage (Ramboer *et al.,* 1972). Xanthine oxidase has also been considered as a trypanocidal serum protein in some animals (Muranjan *et al.,* 1997 and Black *et al*., 1999), whereas the low content of xanthine oxidase has been related to lack of trypanocidal activity in cattle (Wang *et al.,* 1999). Hydrogen peroxide, that is generated during substrate catabolism by xanthine oxidase, could kill trypanosomes. It is generated by reduction of oxygen during oxidation of hypoxanthine and xanthine to uric acid.

Xanthine oxidase activity of purified sheep's milk is found to be low relative to that of the bovine milk (Benboubetra *et al.,* 2004). To date very few studies have been carried out to relate the serum XO activity with development of oxidative stress in sheep. It is on this background of the several applications of xanthine oxidase and paucity of research in *Marwari* breed of sheep, that the present study is designed to determine the profile of serum xanthine oxidase activities in male and female *Marwari* sheep of various age groups during extreme hot and cold ambiences.

MATERIALS AND METHODS

The present investigation was carried out in six hundred and thirty apparently healthy *Marwari* sheep of either sex, between 6 months to 4.5 years of age during extreme ambiences. Blood samples were collected during slaughtering (jugular vein) from private slaughter houses (Bikaner, Rajasthan) to harvest sera in morning hours during moderate, hot and cold ambiences. In each ambience 210 blood samples were collected (male, 105 and non

pregnant female, 105). Further each group was divided according to age as as below 1year (35 male and 35 female); 1-2 years (35 male and 35 female) and 2.5-4.5 years (35 male and 35 female). Mean maximum temperature during moderate ambience was 30.34±0.20°C and hot ambience was 45.1±0.09°C. Mean minimum temperature during cold ambience was 4.83 ± 0.30°C.

Serum xanthine oxidase was determined by the colorimetric method (Litwack *et al.*,1953) with little modification. In a test tube, 1 ml serum, 0.3 ml potassium phosphate buffer (0.067 M, pH 7.8) and 0.6 ml xanthine solution (0.038 M) were mixed and incubated at 37°C for 40 minutes. Then 1 ml of 40% sodium tungstate, 5 ml distilled water and 1 ml 2N H_2SO_4 were added. Tube was centrifuged (5 minutes, 2000 rpm) and 0.5 ml of supernatant was separated. To this 2.5 ml of distilled water and 1 ml of the diluted Folin-Ciocalteu reagent (1:1 diluted with distilled water) were added. The colour was developed by the addition of 5 ml of saturated sodium carbonate solution. A control was also prepared for each sample by replacing xanthine solution with distilled water. A reagent blank was prepared by taking 1 ml of 40 per cent sodium tungstate, 5 ml of distilled water and 1 ml of 2 N H_2SO_4. From this mixture, 0.5 ml was taken and then processed like samples. The optical densities of sample and control were determined at 660 mμ wave length in a spectrophotometer against the blank.

$$\text{Xanthine oxidase } (\mu L^{-1}) = \frac{\text{Test OD-Control OD X 1000 X 1 X5 X 1000}}{1.22X10^4 \text{ X } 0.6}$$

Mean changes in serum XO levels of stressed animals were compared from those of healthy animals by using statistical significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The mean ± SEM values are presented in table 1.The range of serum xanthine oxidase was 11-120 mUL^{-1} considering the observations of all the ambiences. The mean value of serum XO was significantly ($p \leq 0.05$) higher during hot and significantly ($p \leq 0.05$) lower during cold ambiences as compared to moderate mean value.

Its increased activity during hot ambience indicated oxidative stress probably due to higher formation of free radicals (Kataria *et al*., 2010a). Kataria *et al.* (2010c) also related higher serum XO activity to oxidative stress in cows. Scientists have correlated enhanced serum XO activities with the lowered antioxidant enzymes and development of oxidative stress and on that basis recommended the antioxidant therapies in stressed human cases (Zhang *et al.,* 2010).

The ubiquitous enzyme xanthine oxidase can generate superoxide radical in the presence of hypoxanthine and oxygen (McCord and Fridovich, 1968). It is now clear that intracellular redox mechanisms involve xanthine oxidase along with other intracellular antioxidant defense pathways collectively orchestrate a redox balance system whereby reactive oxygen and nitrogen species integrate cues controlling vascular growth and remodeling (Bir *et al.*, 2012). Studies on rats have revealed that liver releases xanthine oxidase at higher rates, which is not the result of a nonspecific protein leakage, but is due to some pathological process. Scientists have demonstrated experimentally the involvement of XO with oxidative stress in the hyperthermia. A causal relationship was observed between the generation of superoxide by XO, produced by hyperthermic perfusion of rat liver (Powers *et al.,* 1992). Xanthine

oxidase has been implicated as an important source of oxidant production and plays an essential role in several inflammatory and oxidative stress-related diseases (Vida *et al.,* 2011). Scientists have showed that this enzyme is involved in free radical production (Heunks *et al.,* 1999).

In present study hot ambience related higher concentration of xanthine oxidase reflected towards free radical generation. It can be assumed that in stressed animals higher serum xanthine oxidase may indicate oxidative stress, therefore, it can be used as one of the potential markers of oxidative stress.

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. Fano *et al.* (2001) reported higher oxidative stress in males and related this gender difference to hormones. Earlier studies have suggested that androgens are required during puberty for full expression of hepatic xanthine oxidase activity, and furthermore, an ovarian suppressive effect is evident. In a study by Levinson and Chalker (1980), the effect of maturation, castration, and sex hormonal treatment on hepatic xanthine oxidase activity was evaluated in rats, which was greater in mature males than in mature females.

Table 1: Serum levels of xanthine oxidase in *Marwari* sheep

Ambiences	XO, mU L^{-1}
Moderate (210)	93.33±1.11[b]
Sex	
Male (105)	99.95±1.13 [d]
Female (105)	86.72±1.14[d]
Age	
Below 1 Year (70)	86.14±1.21 [f]
1-2 Years (70)	90.45±1.94[f]
2.5-4.5 Years (70)	103.41±1.93[f]
Hot (210)	99.28±1.57 [b]
Sex	
Male (105)	104.38±1.79 [d]
Female (105)	94.18±1.8[d]
Age	
Below 1 Year (70)	90.48±1.57 [f]
1-2 Years (70)	92.485±1.86[f]
2.5-4.5 Years (70)	114.87±1.84 [f]
Cold (210)	38.88±1.81[b]
Sex	
Male (105)	50.23±1.67 [d]
Female (105)	27.53±1.9[d]
Age	
Below 1 Year (70)	16.59±1.22 [f]
1-2 Years (70)	38.25±2.89[f]
2.5-4.5 Years (70)	61.81±2.28[f]

Vida *et al.* (2011) observed age related changes in xanthine oxidase activity in mice. They suggested that the age-related increase in the xanthine oxidase activity in liver and cerebral cortex of mice was due to its role in the acceleration of the oxidative damage in these organs with age and its possible contribution to the patho-physiological changes associated to the process of ageing. It is known that the increasing levels of oxidants cause the chronic oxidative stress characteristic of the ageing process. The interactions between ambience X age; ambience X sex and age X sex were highly significant ($p \leq 0.01$) which showed the effect of ambience on the animals of both sexes and all age groups. Further sex effect was present in the animals of each age group.

Through this investigation, authors have tried to explain the enzymatic mechanism involved in the formation of free radicals in the stressed animals. These results may have clinical significance in the prevention of oxidative stress during extreme ambiences. This will help in protecting the animals from other infectious diseases which affect the immune compromised animals during extreme ambiences. As the study involved large number of animals, it can be concluded that serum xanthine oxidase activity can be used as a potential marker to assess the oxidative stress.

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