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Serum Monoamine Oxidase Activities of Marwari Sheep from Arid Tracts During Extreme Ambient Temperatures

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Abstract: Serum Monoamine Oxidase (MAO) activities were determined in the Marwari breed of sheep from arid tracts in India during extreme ambient temperature periods. Animals were categorised according to sex and age. Blood samples were collected during moderate, extreme hot and cold ambient temperatures to harvest sera. The overall mean value of MAO was 288.88±5.10 U L^{-1} which was obtained from 630 animals irrespective of sex and age. The range was 102-600 U L^{-1}. Further mean values were determined according to ambient temperatures irrespective of sex and age. Moderate overall mean value was 162.14±4.61 U L^{-1} which was obtained from 210 animals during moderate ambience. The mean value of serum MAO was significantly ($p \leq 0.05$) higher during hot and cold ambient temperatures as compared to moderate mean value. The sex and age effects were significant ($p \leq 0.05$) in all ambient temperatures. The mean values were significantly ($p \leq 0.05$) higher in male animals than female animals. 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 sex and age effects were significant ($p \leq 0.05$) in all ambient temperatures. Pattern of variations in serum MAO activities indicated that oxidative stress developed in sheep due to extreme ambient temperatures. Impact of hot ambient temperature was greater than cold ambient temperature.

Key words: Ambient temperature, cold, hot, Marwari sheep, mono amine oxidase, serum

INTRODUCTION

Ageing and life span of animals are related to production of oxidants together with the ability of organisms to respond to oxidative stress (Finkel and Holbrook, 2000). Each cell of the animal maintains a reducing environment with the help of enzymes through a constant input of metabolic energy. An imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage can cause oxidative stress. Oxidative stress can influence the metabolism of cells in vital organs of the animals (Kataria *et al*., 2010). Oxidative stress resulting from increased production of reactive oxygen species leads to damage of biological macromolecules and disruption of physiological processes (Trevisan *et al*., 2001). Although, reactive oxygen species are used by the immune system as a way to attack and kill pathogens by cell signaling (Nathan and Shiloh, 2000) but when they are produced at a faster rate, antioxidant mechanism is highly activated to neutralize them. Increased levels of oxidative species may be deleterious to cells.

Monoamine oxidases catalyse oxidative deamination of several monoamines resulting in significant reactive oxygen species production contributing to oxidative stress (Weyler *et al*., 1990). Increased oxidation of dopamine by monoamine oxidase is associated with an oxidant stress expressed as a rise in the level of oxidized glutathione. Oxidation of glutathione is suppressed by MAO inhibitors (Cohen, 1990). Many scientists have conducted studies on monoamine oxidase activity which is considered as degradation enzyme of dopamine, norepinephrine and epinephrine (Pastorova and Varady, 1996; Molnarova *et al*., 2000).

The evaluation of the extent of oxidative stress in the blood can be useful to define its role in different diseases and can be used for clinical diagnosis. These serum

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markers are considered to be minimally invasive peripheral markers (Gutierrez *et al*., 2006). The inexorableness of sheep to experience extreme ambient temperatures of arid and semiarid tracts makes oxidative stress associated with extreme ambiences an apposite ground to investigate adaptive physiological measures. Latter may have implications in health management during extreme temperatures. Marwari breed of sheep represents a major fraction of the sheep population in western part of Rajasthan and plays critical role in the economy of arid and semi arid tract. Physiological mechanisms need proper investigation to formulate health strategies. Tyrannical hot and cold ambient temperatures may lead to changes in enzymes regulationg important reactions of the body and monoamine oxidase is one of them. The present investigation was planned to determine serum monoamine oxidase levels to assess oxidative stress in Marwari sheep and to set its normal values for the use in veterinary clinical physiology.

MATERIALS AND METHODS

Animals: The study was carried out in 630 apparently healthy Marwari sheep of both sex between 6 months to 4.5 years of age to determine monoamine oxidase 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) ambient temperatures. 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 categorized gender wise as male (105) and non-pregnant female (105) and age wise 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). Blood samples were collected in sterilized and dried test tubes and sera were harvested by centrifugation (10 min at 2700 rpm).

Analysis: Serum monoamine oxidase was determined by the colorimetric method of Green and Haughton (1961). The method is based on the measurement of the aldehyde formed during the enzymic oxidation of tyramine. The unstable aldehyde is prevented from decomposing by the presence of semicaebaz-ide which is then converted into the corresponding 2,4-dinitrophenylhydrazone. The colour of this material is intense in alkaline solution which provides a sensitive measure of the enzymic activity.

In a 25 mL capacity conical flask, 1 mL of 0.05 M semicarbazide hydrochloride,1.6 mL of 0.2 M phosphate buffer and 0.1 mL serum were taken. It was placed in a water bath for 10 minutes at 25°C and shaken continuously. Then, 0.4 mL of 0.1 M tyramine hydrochloride was added into the flask and the mixture was shaken in the bath for a further of 30 min. Then, 1 mL of 0.5 N acetic acid was added to stop the reaction. The flask contents were transferred to a centrifuge tube and heated in boiling water for 3 min to precipitate the protein which was then removed by centrifuging. In a test tube, 2 mL of supernatant was taken and to this 2 mL of 2,4-dinitrophenylhydrazine (500 mg in 2 N HCl to make 1 L) was added.

The mixture was kept at room temperature for 10 min. Then 5 mL of benzene was added to extract dinitrophenylhydrazone which was formed. Then, 4 mL of the benzene layer was removed and shaken with 4 mL of 0.1 N NaOH. In both extraction steps the mixtures were centrifuged to ensure complete separation of the 2 layers. The benzene layer was then discarded and the alkaline layer was heated at 80°C for 10 min to convert the initial red form of the dinitrophenylhydrazone into the orange-yellow form. After cooling the solution was transferred to 1 cm cuvette of spectrophotometer and its extinction was measured at 450 μm. The blank was processed in the same manner however, distilled water was taken instead of substrate. The optical densities of sample and blank were taken against distilled water and then blank OD was subtracted from sample to get corrected OD. Activity of the enzyme was then determined directly from the standard curve which was plotted between variable enzyme concentrations (U L^{-1}: 100, 200, 300, 400 and 500) and their respective optical densities.

Statistics: The main effects were classified as ambient temperatures, sex and age groups. The subsets of ambiences were moderate, hot and cold periods of sex were male and female and of age groups were below 1, 1-2 and 2.5-4.5 years. For each subset data were expressed as mean±SE of mean. The changes in the means were measured by using multiple mean comparison procedures. For this Duncan's new multiple range test (Duncan, 1955; Steel and Torrie, 1980) was used.

RESULTS AND DISCUSSION

Mean±SEM values of serum monoamine oxidase during different ambient temperatures, sex and age groups are presented in Table 1. The overall mean value of MAO was 288.88±5.10 U L^{-1} which was obtained from

Table 1: Mean±SEM values of serum Monoamine Oxidase (MAO) in Marwari sheep

	MAO (U L^{-1})		
Effects	Moderate (210)	Hot (210)	Cold (210)
Ambient tempertaure (630)	162.14±4.61[b]	415.01±8.87[b]	289.52±5.70[b]
Sex			
Male (105)	206.19±5.20[d]	439.57±8.09[d]	324.76±6.61[d]
Female (105)	118.09±4.60[d]	390.52±8.33[d]	254.28±5.55[d]
Age			
Below 1 year (70)	108.57±4.70[f]	237.85±7.15[f]	160.71±5.21[f]
1-2 years (70)	133.57±5.41[f]	420.71±9.30[f]	241.42±5.50[f]
2.5-4.5 years (70)	244.28±3.60[f]	586.42±8.63[f]	466.42±6.20[f]

Figures in the parenthesis indicate number of animals; [b]Marks significant ($p \leq 0.05$) differences among ambient temperatures mean values; [d]Marks significant ($p \leq 0.05$) differences between male and female mean values within an ambient temperature period; [f]Marks significant ($p \leq 0.05$) differences among mean values of different age groups within an ambient temperature period

630 animals irrespective of sex and age. The range was 102-600 U L^{-1}. Further mean values were determined according to ambient temperatures irrespective of sex and age. Moderate overall mean value was 162.14±4.61 U L^{-1} which was obtained from 210 animals during moderate ambience.

There is paucity of research on serum MAO in sheep. The activity in the serum can be related with oxidative stress as it catalyses the breakdown of dopamine in brain. During stress activity of MAO increases showing pathological alterations in various diseases and physiological fluctuations in normals (Veral *et al*., 1997). The mean value of serum MAO was significantly ($p \leq 0.05$) higher during hot and cold ambient temperatures as compared to moderate mean value. Serum MAO activity was used as a marker to assess oxidative stress in the animals (Kataria *et al*., 2010). Higher serum value of MAO can indicate oxidative stress. MAOs catalyse oxidative deamination of several monoamines resulting in significant reactive oxygen species production contributing to oxidative stress (Weyler *et al*., 1990). Increased oxidation of dopamine by monoamine oxidase is associated with an oxidant stress, expressed as a rise in the level of oxidised glutathione. Scientists have confirmed the presence of oxidative stress on the basis of increased concentration of serum MAO (Chen *et al*., 2013).

The sex and age effects were significant ($p \leq 0.05$) in all ambient temperatures. The mean values were significantly ($p \leq 0.05$) higher in male animals than female animals. 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.

In present study, the higher serum MAO concentration could be due to androgenic influence (Ellis *et al*., 1972). Tryding *et al*. (1969) observed the effect of sex and age on serum MAO activity being higher in males than females and adults than children. Alterations in the activities of MAO due to sex can be correlated with the influence of sex hormones. Scientists have reported that oestrogen also modify the enzymatic activity of monoamine oxidase in the hypothalamus and striate region. Changes in the activities of monoamine oxidase after application of the hormonal preparations like LH have been observed earlier (Pastorova, 2009). Ellis *et al*. (1972) correlated the MAO activity with age in rats.

CONCLUSION

The findings on the basis of levels of serum MAO triggered the assumption that extreme ambient temperatures produced oxidative stress in sheep. Impact of hot ambient temperature was greater than cold ambient temperature. The extreme ambient temperature impact was observed on the animals of both sexes and all age groups.

REFERENCES

Chen, S., L. Zou, L. Li and T. Wu, 2013. The protective effect of glycyrrhetinic acid on carbon tetrachloride-induced chronic liver fibrosis in mice via up regulation of Nrf2. PLoS One, Vol. 8. 10.1371/journal.pone.0053662.

Cohen, G., 1990. Monoamine oxidase and oxidative stress at dopaminergic synapses. J. Neural. Transm. Suppl., 32: 229-238.

Duncan, D.B., 1955. Multiple range and multiple F test. Biometrics, 11: 1-42.

Ellis, L.C., A.W. Jaussi, M.H. Baptista and R.L. Urry, 1972. Correlation of age changes in monoamine oxidase activity and androgen synthesis by rat testicular minced and teased-tubular preparation *in vitro*. Endocrinology, 90: 1610-1618.

Finkel, T. and N.J. Holbrook, 2000. Oxidants oxidative stress and the biology of ageing. Nature, 408: 239-247.

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

Gutierrez, L.L.P, N.G. Mazzotti, A.S.R. Araujo, R.B. Klipel, T.R.G. Fernandes, S.F. Llesuy and A. Bello-Klein, 2006. Peripheral markers of oxidative stress in chronic mercuric chloride intoxication. Braz. J. Med. Biol. Res., 39: 767-772.

Kataria, N., A.K. Kataria, R. Maan and A.K. Gahlot, 2010. Evaluation of oxidative stress in brucella infected cows. J. Stress Physiol. Biochem., 6: 19-25.

Molnarova, M., B. Pastorova, A. Stanikova, J. Halagan and J. Buleca, 2000. Monoamine oxidase activity in some brain areas of sheep after hormonal stimulation. Acta Vet. Hung., 48: 107-111.

Nathan, C. and M.U. Shiloh, 2000. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc. Natl. Acad. Sci. USA., 97: 8841-8848.

Pastorova, B. and J. Varady, 1996. Catecholamine levels and activity of monoamine oxidase in some hypothalamic structures and in the pineal gland of sheep after administration of FSH. Physiolo. Res., 45: 131-136.

Pastorova, B., 2009. The influence of pmsg and antisergon on monoamine oxidase activity in some hpothalamic-hypophysiary structures and in the epiphysis of sheep in the estrus period. Slovak J. Anim. Sci., 42: 72-75.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw-Hill Kogakusha, Ltd., Tokyo, pp: 187-190.

Trevisan, M., R. Browne, M. Ram, P. Muti, J. Freudenheim, A.M. Carosella and D. Armstrong, 2001. Correlates of markers of oxidative status in the general population. Am. J. Epidemiol., 154: 348-356.

Tryding, N., S.E. Nilsson, G. Tufvesson, R. Berg, S. Carlstrom, B. Elmfors and J.E. Nilsson, 1969. Physiological and pathological influences on serum monoamine oxidase level: Effect of age, sex, contraceptive steroids and diabetes mellitus. Scand. J. Clin. Lab. Invest., 23: 79-84.

Veral, A., G. Alper, G. Mentes and B. Ersoz, 1997. Age and sex related alterations in serum and platelet monoamine oxidase. Eur. J. Clin. Chem. Clin. Biochem., 35: 265-268.

Weyler, W., Y.P. Hsu and X.O. Breakefield, 1990. Biochemistry and genetics of monoamine oxidase. Pharmacol. Ther., 47: 391-417.