Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.9047

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2024) *12*(1), 35-47

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

Research Article

Oxidative Stress Markers During Summer and Rainy Season in Deccani Sheep

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Received: 7.12.2023 | Revised: 16.02.2024 | Accepted: 24.02.2024

ABSTRACT

The present study was designed to study the effect of two different seasons (summer and rainy) on the activities of some oxidative stress markers (SOD, GSHPx and MDA) in Deccani sheep. The experiment was carried out at the Department of Veterinary Physiology, KNP College of Veterinary Science, and Shirwal. Dist. Satara on eight apparently healthy, non-pregnant, non-lactating Deccani sheep above two years of age maintained under semi-intensive housing conditions at Livestock Farm Complex, KNP College of Veterinary Science, Shirwal, Dist. Satara. The meteorological data recorded during the present study were obtained from the Indian Meteorological Department, Pune. The monthly calculated mean values of THI during the study period revealed significantly (P < 0.01) higher THI during Summer (77.81 ± 0.19) than in the Rainy season (74.92 ± 0.16). In the present study, the mean plasma activity of SOD was significantly (P < 0.01) higher during the summer season (510.93 ± 24.77 U/ ml) than in the rainy season (436.58 ± 26.30 U/ ml). The mean erythrocyte activity of glutathione peroxidase showed significantly (P < 0.05) higher values in the summer season than in the rainy season (102.12 ± 5.03 U/gHb and 85.63 ± 3.89 U/gHb). The mean plasma levels of MDA were significantly (P < 0.01) higher during the summer season (5.29± 0.09 nmol/ml) than in the rainy season (3.27± 0.09 nmol/ml). The finding indicates that oxidative stress markers like SOD, GSHPx and MDA could be used as a sensitive biomarker of oxidative stress caused due to heat stress in Deccani sheep.

Keywords: *oxidative stress, Deccani sheep, SOD, GSHPx, MDA, season.*

INTRODUCTION

Small ruminants constitute a crucial economic and ecological role in agricultural systems and conjointly play a major half in the lives of households in developing countries (Oluwatayo & Oluwatayo, 2012).

Cite this article: Pangaonkar, L.A., Patodkar, V.R., Mehere, P.V., Amle, M.B., Khanvilkar, A.V., Lambate, S.B., & Yeotikar, P.V. (2024). Oxidative Stress Markers During Summer and Rainy Season in Deccani Sheep, *Ind. J. Pure App. Biosci. 12*(1), 35-47. doi: http://dx.doi.org/10.18782/2582-2845.9047

These are considered as integral part of the pastoral production systems and square measure significantly relevant for subsistent agricultural systems, involving the importance of their distinctive biological attributes, as well as short biological time, high fruitfulness, ascension rate, high feed conversion potency, high diseases resistance capability also as straightforward marketability (Sejian et al., 2013; & Adams & Ohene-Yankyera, 2014). Deccani sheep belongs to native geographic region of Maharashtra, Andhra Pradesh, Telangana and Karnataka province states of India are medium sized, coarse wool sheep (Acharya, 1982), which inhabit mainly in five districts of Maharashtra viz., Ahmednagar, Kolhapur, Satara, Sangli and Solapur are known as Sangamneri, Kolhapuri, Lonand, Madgyal and Solapuri strains, respectively (Gokhale, 2003). They are predominantly off-white or dark brown in colour, a dwarf in size with stumpy legs and have been used for coarse wool production and mutton production. The warmest month in Satara district is May, during which typical temperature reaches upto 43^{o}C (109.4^{o}F) or more, and the monsoon lasts from June to September with moderate to high precipitation, during which temperature ranges from 28^{o}C to to 32^{o}C (82.4^{o}F to 89.6^{o}F) along with 80% to 90 % relative humidity (Ambade, 2019; & Pangaonkar et al., 2023). Sheep are less heat tolerant than goats as they have shorter bodies and legs, short and thick ears, tight skin, and dense fleeces/wool, which significantly reduces evaporation by sweating so reducing heat loss through connective tissue phase transition cooling mechanism that leaves them with the choice of relying heavily on metastasis phase transition cooling mechanism for thermoregulation in these animals (Hooda & Upadhyay, 2014; Gesualdi Junior et al., 2014). According to Taylor (1992), the thermoneutral zone of sheep is set between 12^{O}C and 32^{O}C.The temperature humidity index (THI) becomes relevant under conditions of high temperature and high humidity as the evaporation rate is directly proportional to the humidity, which is also considered as an important tool for measuring the heat stress in the farm animals and, therefore, temperature-humidity index (THI) is a good indicator of stressful thermal climatic conditions(Ambade, 2019; & Pangaonkar et al., 2023). Oxidative stress results from increased production of free radicals and ROS and a decrease in antioxidant defense. Oxidation is essential to nearly all cells in the body to provide energy for vital functions. Approximately 95 to 98% of the oxygen consumed is reduced to water during aerobic metabolism, but the remaining fraction may be converted to oxidative by-products, i.e., reactive oxygen species that may damage DNA and contribute to degenerative changes. Further, biomarkers to assess oxidative stress include lipid peroxides (MDA) and antioxidant enzymes like superoxide dismutase and glutathione peroxidase (Celi, 2011; & Ganai et al., 2013). Superoxide dismutases (SODs) are a category of closely connected enzymes that change the breakdown of the anion into O_2 and H_2O_2 in the majority of aerobic cells (Rahman, 2007). Glutathione Peroxidase (GSHPx) is a selenium-dependent antioxidant enzyme which converts H_2O_2 to water. The increased production of H_2O_2 due to increased activity of SOD during heat stress resulted in a coordinated increase in $GSHP_X$. Plasma GSHPx activity contributes to the oxidative defence of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides and was also considered an indicator of oxidative stress (Halliwell & Chirico, 1993). Glutathione peroxidase (GSHPx) functions in cellular oxidation-reduction reactions to protect the cell membrane from oxidative damage caused by free radicals, and SOD catalyzes the dismutation of superoxide to hydrogen peroxide (H_2O_2), and it is considered the first defence against pro-oxidants. Lipid peroxidation (MDA) is commonly measured in terms of Thiobarbituric Acid Reactive Substance (TBARS). Erythrocytes, being rich

in Polyunsaturated Fatty Acids (PUFA), are exposed to high oxygen concentrations and are highly susceptible to peroxidation damage (Clemens & Waller, 1987). MDA is a sensitive biomarker of oxidative stress; therefore, the elevated MDA levels documented in heat-stressed animals strongly indicate that the heat period was stressful to these animals and negatively impacted immune responses. Heat stress can stimulate extracellular and intracellular superoxide generation (Rathwa et al., 2017; & Yeotikar et al., 2019) and Reactive Oxygen Species (ROS) synthesized by superoxide can attack membrane lipid composition and therefore initiate lipid peroxidation (Halliwell & Chirico, 1993). In sheep, the available literature also reveals no reports yet with respect to seasonal changes in oxidative stress markers in Deccani Sheep and hence, it is proposed to undertake the present study the seasonal effect on SOD, MDA and GSHPx during summer and rainy seasons.

MATERIALS AND METHODS

The experiment was conducted on eight (08) apparently healthy, nonpregnant, non-lactating adult Deccani sheep with an age above two years and similar body weight maintained under a semi-intensive farming system at Livestock Farm Complex (LFC), KNP College of Veterinary Science, Shirwal, Dist. Satara.

Recording of Meteorological variables: Meteorological variables like temperature and relative humidity were recorded. The observations were used to calculate the temperature humidity index (THI). On the basis of mean temperature and relative humidity, THI was calculated using the formula of Mader et al. (2006).

$$THI = (0.8 \times Tdb) + [(RH/100) \times (Tdb-14.4)] + 46.4$$

where, Tdb = dry bulb temperature and RH = relative humidity.

Blood Sampling Schedule: The protocol of this experiment was approved by the Institutional Animal Ethics Committee of KNP College of Veterinary Science, Shirwal. Dist. Satara vide No. **IAEC/15/KNPCVS/01/2019.** Eight (08) whole blood samples (06 ml each) were collected aseptically for analysis of MDA and enzymatic antioxidants (SOD and GSHPx) fortnightly during Summer (March, April and May) and Rainy seasons (July, August and September) in the year 2019. All the samples were immediately carried to the laboratory on ice for further processing after blood collection and were processed within one hour of collection.

Estimation of Oxidative Stress Markers: Blood samples for estimation of oxidative stress parameters were obtained between 08.00 am and 09.00 am to avoid diurnal influences by jugular venipuncture into vacutainers containing K3EDTA (in 10:1 ratio) as an anticoagulant. After centrifugation at 750 x g for 15 minutes, the supernatant plasma was separated and stored at -20^{o}C to estimate plasma superoxide dismutase and lipid peroxidation.

Preparation of Hemolysate: The erythrocyte lysate was prepared by the method described by Ivanov (1999). After triple washing of erythrocyte mass with physiological saline (0.9 % NaCl), 0.5 ml of cell suspension was dissolved in 2 ml cold distilled water for lysis of erythrocytes. Haemoglobin was then precipitated by adding 1.8 ml water and 0.2 ml ethanol/chloroform (3:5/v:v) to 0.2 ml lysate. The tubes were shaken for 5 minutes and centrifuged at 750 x g for 20 minutes. The supernatant was used for the determination of glutathione peroxidase activity and stored at -20^{o}C.Estimation of Haemoglobin in hemolysate was carried out by using the cyanomethamoglobin method described by Dacie and Lewis, 1975.

Estimation of Plasma Superoxide dismutase activity: Superoxide dismutase activity was determined by using simple and rapid method (Marklund & Marklund, 1974; & Li, 2012). Absorption was read at the wavelength of 420 nm against Tris-EDTA buffer at zero time and after 1 minute of the addition of Pyrogallol immediately.

Calculation of SOD Activity:

$$\% \text{ Inhibition of pyrogallol autoxidation} = \frac{\Delta A \text{ test}}{\Delta A \text{ control}} \times 100\%$$

$$\textbf{SOD Activity (U/ml)} = \frac{\% \text{ inhibition of pyrogallol autoxidation}}{50\%}$$

Estimation of Erythrocyte Glutathione Peroxidase activity: The GSHPx activity in hemolysate was measured using the method described by Paglia and Valentine (1967). The UV-visible Spectropotometer (SYSYTRONICS -117) was adjusted to read zero using a blank prepared as per protocol. The depletion of NADPH at 340nm was recorded for 3 minutes (at an interval of every 30 seconds). The enzyme activity was calculated as µM NADPH oxidized/minute/gmHb with the molar extinction coefficient of $6.22 \times 10^3 M^{-1} CM^{-1}$.

Calculation of GSHPxActivity:

$$\text{GSHPx activity} = \frac{\Delta \text{ OD} \times \text{Volume of assay} \times 1000}{6.22 \times \text{volume of enzyme source} \times \text{gmHb}}$$

GSHPx activity is expressed **as µM NADPH oxidized /minute/gmHb**

Estimation of Plasma Lipid Peroxidation: In this study, the assay was conducted using EZ Assay™ TBARS Estimation Kit supplied by Hi Media Cell Culture Limited, Mumbai. This assay was based on the reaction of MDA with a chromogenic agent thiobarbituric acid (TBA) at high temperature and acidic conditions to form MDA-TBA adduct (1:2). This complex has maximum absorption at 530-540 nm and was directly proportional to the TBARS concentration in the sample. The average values were determined from triplicate readings at 532 nm and corrected absorbance was calculated as follows.

Corrected absorbance = Absorbance (Test/standard) – Absorbance (Blank).

The standard curve was obtained by plotting the corrected absorbance of standards on Y-axis against MDA concentrations on X-axis (**Figure 1**).

Calculations: The slope of standard curve was determined (y = mx + c).

The MDA concentration of each sample was calculated by using the following equation of the standard curve.

$$\textbf{MDA (µM)} = \frac{\text{(corrected absorbance)} - \text{(y-intercept)}}{\text{Slope.}}$$

Plasma Lipid peroxidation is expressed as **nanomoles of MDA/ml**.

Statistical analysis: All the data of the present study were analyzed using the computerized Web-based Agricultural Statistics Software Package, WASP. 2.0, by applying the completely randomized design cited by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Temperature Humidity Index (THI): Table No. 1 displays the average values of the THI recorded during the research period (summer and rainy seasons). During the study period, the monthly estimated mean values of THI

showed THI was substantially ($P < 0.01$) greater than during the rainy seasons during the summer. THI, commonly used as a heat stress index for livestock production, incorporates ambient temperature (wet/dry bulb) and relative humidity. It has been extensively utilized as an indicator of thermal stress in cattle (Vaidya et al., 2010). THI levels between 70 and 78 are regarded as pleasant, 75 and 78 as stressful, and values above 78 as extremely distressing, causing the animals to lose their ability to maintain their normal body temperature or their thermoregulatory system (Silanikove, 2000). The thermoneutral zone for sheep is 12^0C to 32^0C (Taylor, 1992), depending on a number of variables, including the animal's breed, age, body weight, diet, level of health, and physiological state (Kolacz & Dobrzanski, 2006). The present study revealed that Deccani sheep experienced more stress in the summer than in the rainy season due to elevated THI in Summer at the experimental place.

Oxidative Stress Markers:

Plasma Superoxide dismutase activity: The plasma activity of superoxide dismutase (SOD) enzyme (U/ml) in Deccani sheep reported during two different seasons is presented in **Table 2.** The statistical analysis of the fortnightly data revealed significantly ($P < 0.01$) higher plasma SOD activity during the summer season (THI = 77.81 ± 0.19) than in the rainy season (THI = 74.92 ± 0.16) which indicates that in the summer season because of the higher THI, Deccani sheep were under heat stress that occurs when core body temperature of these animals exceeds its range required for normal activity that resulted from a total heat load. Heat stress stimulated excessive production of ROS, such as superoxide anion (O_2^-), hydroxyl ion (OH^-) and hydrogen peroxide (H_2O_2), which were continuously produced in the course of normal aerobic metabolism and these free radicals could damage healthy cells (Chetia et al., 2017). The enzymatic antioxidants, mostly metalloenzymes are the first line of defence system that counteracts the oxidative damage of the intracellular constituents induced by ROS (Das et al., 2016). Hence, the higher value of SOD reported in the present study in the summer season might be due to increased free radicals and ROS as a result of oxidative stress developed due to heat stress. This stress is induced by the synthesis of free radicals, especially superoxide anion, and could also be due to the auto-oxidation of haemoglobin, resulting in the generation of the superoxide anion in the blood. Being considered as the first line of defence against oxidative stress, the higher activity of serum SOD during summer might be aimed to prevent oxidative injury to the cells (Ambade, 2019). A significant increase in the plasma SOD activity observed during the summer season than in the rainy season reported in the present study could also be attributed to the sparing of the endogenous SOD during oxidative stress, indicating the beneficial effects of SOD during the thermal stress in Deccani sheep. The significantly ($P<0.01$) higher activity of SOD during summer season than other seasons reported in the present study are in accordance with the findings of Maan et al. (2013), Rathwa et al. (2017), Vasava et al. (2017), Di-Trana et al. (2006), Manish et al. (2011), Ocheja et al. (2017) in the small ruminants. Similarly, the significantly ($P<0.01$) higher activity of SOD during summer season than other seasons reported in the present study also corroborated with the findings of Bernabucci et al. (2002), Odedara et al. (2016), Thankachan (2007), Chigerwe et al. (2013), Lallawmkimi (2009), Chetia et al. (2017), Chaudhary et al. (2015), Yatoo et al. (2014), Ambade (2019), Lakhani et al. (2016), Yeotikar et al. (2019) in the large ruminants. On the contrary, Yaqub et al. (2019), Cai et al. (2019), Ghosh et al. (2013), Megahed et al. (2008), Sunil Kumar et al. (2010), Sakatani et al. (2012), Kumar et al. (2016) and Shakirullah et al. (2017) reported the lower values of SOD during summer season than other seasons which were not corroborated with the results of the present study. The variations in the contradictory findings of SOD reported by various researchers might be due to alterations in the factors like physiological status,

nutrition, altitude, location, sex, age, species, type of tissue and chosen method of estimation of the enzyme.

Erythrocyte Glutathione Peroxidase Activity: The erythrocyte activity of glutathione peroxidase (GSHPx) reported in Deccani sheep during two different seasons is presented in **Table 3.** The statistical analysis of the fortnightly data revealed significantly ($P < 0.05$) higher erythrocyte glutathione peroxidase activity during the summer season (THI = 77.81 ± 0.19) than in the rainy season (THI = 74.92 ± 0.16). These findings of seasonal variation recorded in the present study indicated that both hot dry and hot humid seasons are stressful for Deccani sheep, as seen by the associated increase in levels of SOD and GPx hence, it can be concluded that heat stress due to high ambient temperature in summer season at Satara district of Maharashtra is more as compared to hot humid season even if the THIs are comparable (Pangaonkar et al., 2023). The results obtained in the present study might be due to summer stress stimulated excessive production of ROS such as superoxide anion (O_2^-), hydroxyl ion (OH^-) and hydrogen peroxide (H_2O_2), which were continuously produced in the course of normal aerobic metabolism and these free radicals could damage healthy cells and can cause some altered biochemical metabolites if not being eliminated from the animal's body. Therefore, the increase in the GSHPx enzyme during the summer season might be due to the thermal stress and GSHPx enzyme activities are thus sensitive markers of oxidative stress as their level may increase or decrease in response to ROS (Bernabucci et al., 2002). Being a selenium-dependent antioxidant enzyme, GSHPx converts H_2O_2 to water. The increased production of H_2O_2 due to increased activity of SOD during heat stress resulted in a coordinated increase in GSHPx (Brummer et al., 2013) indicating positive and significant correlation exists between GSH-Px and SOD activities during two seasons. The increased levels of serum GSHPx could be used as a sensitive marker of oxidative stress. The significantly ($P < 0.01$) higher activity of GSHPx enzyme during summer season than rainy season reported in the present study are in accordance with the findings of Chauhan et al. (2014), Rathwa et al. (2017), Vasava et al. (2017) and Di-Trana et al. (2006) in the small ruminants. Similarly, the significantly ($P < 0.01$) increased activity of GSHPx during summer season than rainy season reported in the present study are in accordance with the findings of Bernabucci et al. (2002), Odedara et al. (2016), Chigerwe et al. (2013), Lallawmkimi (2009), Chetia et al. (2017), Chaudhary et al. (2015), Yatoo et al. (2014), Ambade (2019), Tej et al. (2017) in the large ruminants. However, Alonso et al. (1997), Andres et al. (1999), Masoudi et al. (2010), Manish et al. (2011), Cai et al. (2019), Yaqub et al. (2019), Ocheja et al. (2017), Shakirullah et al. (2017), Colakoglu et al. (2017), Bhat et al. (2008), Burke (2007) and Sakatani et al. (2012) reported the lower values of GSHPx during summer season than other seasons which were contradictory with the findings of the present study.

PlasmaLipid Peroxidation (in terms of MDA): The plasma levels of MDA (nmol/ml) in Deccani sheep during summer and rainy seasons is shown in **Table 4.** The statistical analysis of the fortnightly data revealed significantly ($P<0.01$) higher plasma MDA levels during the summer season (THI = 77.81 ± 0.19) than in the rainy season (THI = 74.92 ± 0.16) which indicated that in the summer season because of the higher THI, Deccani sheep were under heat stress that occurs when core body temperature of these animals exceeds its range required for normal activity that resulted from total heat load. In the present study, LPO in the form of MDA production was found to be higher in the summer season in Deccani sheep raised under hot-dry conditions. Thiobarbituric acid reactive substances (TBARS) are also an indicator of LPO, which was also found to increase in the summer season (Bernabucci et al., 2002). The MDA level indicates the lipid peroxidation process in tissues, where the fatty acids in the cell membrane lose hydrogen molecules that increase the amount of MDA in

the environment (Deveci & Guven, 2008; & Celi, 2011). The oxidative damage results in the structural alteration in the membrane release of cell and organelle contents and essential fatty acids loss, which is seen with cytosolic aldehyde and peroxide product formation and the end product of free radical reactions on membrane fatty acids is called MDA (Bahrami et al., 2015). As a result of the loss of some endogenous antioxidants in the process of fighting free radicals, the process of lipid peroxidation was enhanced and the production of MDA increased in the summer season in the present study. The present findings of the significant ($P < 0.01$) increased levels of MDA during summer is in accordance with findings reported by Rathwa et al. (2017), Ocheja et al. (2017), Vasava et al. (2017), Shakirullah et al. (2017), Teama (2018), Cai et al. (2019), Yaqub et al. (2019) and Shi et al. (2020) in the small ruminant animals. Similarly, Altan et al. (2003) reported that when broilers were exposed to an ambient temperature of 38 ± 1^{o}C for 3 hours at 35 and 36 days of age with ad-libitum water and removal of feed, a significant increase ($P < 0.05$) in MDA levels was observed after exposure to heat stress and it was revealed that increased antioxidant enzyme activities may be considered as a protective mechanism against heat-induced free radical production and lipid peroxidation. The significantly ($P < 0.01$) increased levels of MDA reported in the present study during summer also corroborated with the findings of Megahed et al. (2008), Sunil Kumar et al. (2010), Sakatani et al. (2012), Chaudhary et al. (2015), Kumar et al. (2016), Lakhani et al. (2016), Odedara et al. (2016), Colakoglu et al. (2017), Lakhani et al. (2018) and Yeotikar et al. (2019) indicating that hot dry season is more stressful as compared to other seasons in the large ruminant animals. However, Lektib et al. (2016) in camels, Zhang and Wang (2017) in Tibetan sheep and Okeke et al. (2020) in Nigerian sheep reported significantly decreased levels of MDA during summer which were not in accordance with the findings of the present study.

Table1. Seasonwise Mean ± S. E. values of Temperature Humidity Index

Summer			**Rainy**		
March	April	May	July	August	September
76.16	77.87	79.47	74.47	75.47	74.47
Mean ± SE= 77.81^{a}± 0.19			**Mean ± SE= 74.92^{b}± 0.16**		

The meanTHI values with different superscripts differ significantly at 1% and 5 % significance level.

Table 2.Season wise Mean ± S. E. values of Plasma SOD (U/ml) In Deccani Sheep (n = 08)

Fortnight	**Summer** (March, April and May)	**Rainy** (July, August and September)
I	310.75	235
II	336.87	251.25
III	430.25	627
IV	592.75	676
V	685	502.5
VI	710	327.75
Mean ± SE	**510.93^{a} ± 24.77**	**436.58 ^{b} ± 26.30**

The mean values of Plasma SOD differ significantly at1% and 5% level of significance.

Table3.Season wise Mean ± S. E. values of Erythocyte GSHPx (U/ gHb) in Deccani Sheep (n =08)

Fortnight	Summer (March, April and May)	Rainy (July, August and September)
I	73.84	68.46
II	79.73	72.91
III	87.24	113.42
IV	91.10	118.24
V	134.03	77.59
VI	146.78	63.19
Mean ± SE	**102.12 ^{a} ± 5.03**	**85.63^{b} ± 3.89**

The mean values of Erythrocyte GSHPx differ significantly at 5% significance level.

Table4.Seasonwise Mean ± S. E. values of Plasma LPO (nmol of MDA/ml) in Deccani Sheep (n =08)

Fortnight	Summer (March, April and May)	Rainy (July, August and September)
I	4.91	2.91
II	5.22	3.08
III	5.25	4.10
IV	5.03	3.86
V	5.71	2.98
VI	5.60	2.68
Mean ± SE	**5.29 ^{a}± 0.09**	**3.27 ^{b}± 0.09**

The mean values of Plasma LPO differ significantly at1% and 5% level of significance.

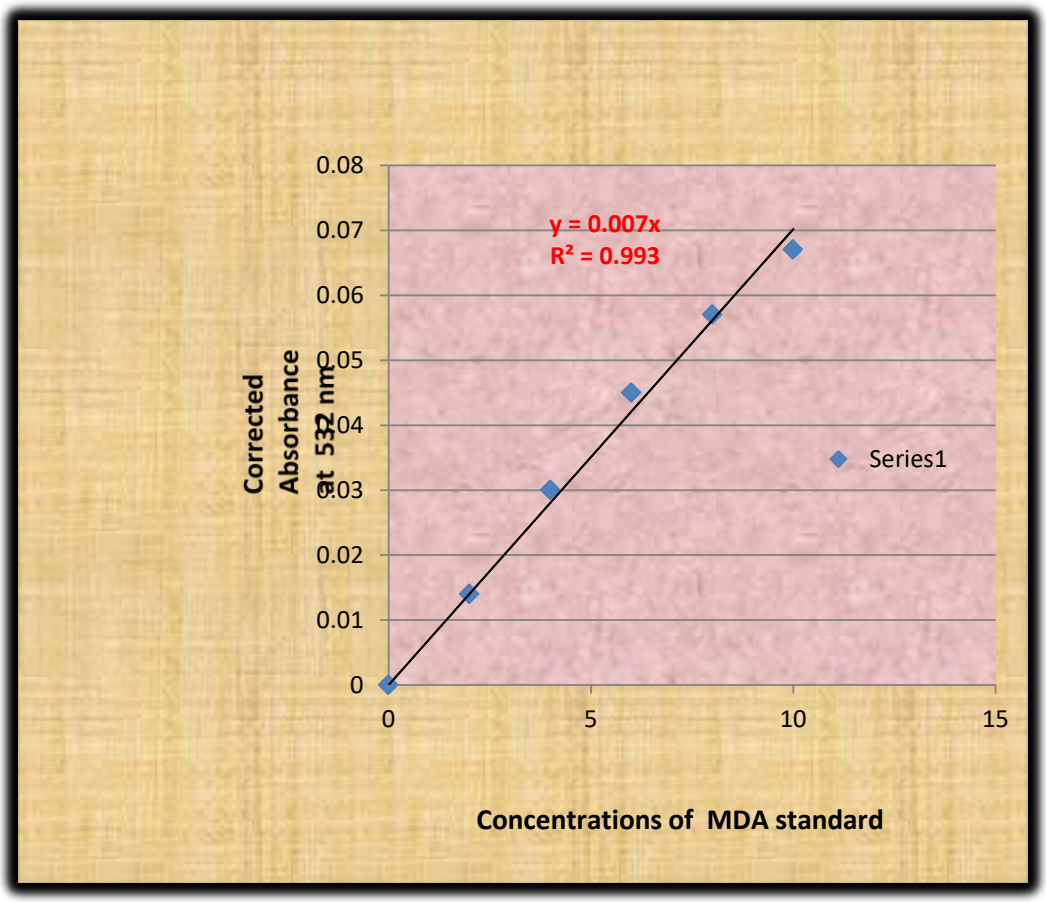

Figure1. Standard Curve Of MDA

CONCLUSION

The markers of oxidative profile such as SOD, MDA and GSHPx in this study were found to increase during the summer season as compared to the rainy season, indicating that Deccani sheep have experienced elevated oxidative stress in the summer season than in the rainy season. Hence, the activities of these oxidative stress markers, which are influenced by THI of the seasons and its up-regulation was maintained during elevated THI in summer than in the rainy season, could also be used as a sensitive biomarker of oxidative stress caused due to heat stress in Deccani sheep.

Acknowledgement:

The authors are thankful to the Department of Veterinary Physiology and CIAF and the Associate Dean, KNP College of Veterinary Sciences, Shirwal Dist. Satara (Maharashtra), for providing the necessary facilities for research work.

Funding: NIL.

Contribution of Authors:

Dr. L.A. Pangaonkar has worked on the project during his PhD studies at Dept of Veterinary Physiology, KNPCVS, Shirwal and has prepared the manuscript thus, he is a corresponding author. Dr. V.R. Patodkar, Professor, Dept of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Dist. Satara (MS) was a major advisor to PhD scholar. Dr. P.V. Mehere, Dr. A.V. Khanvilkar, and Dr. M.B. Amle worked as a Minor advisor of PhD scholar. Dr. P.V. Yeotikar, Associate Professor, Dept of Veterinary Biochemistry, COVAS, MAFSU, Parbhani and Dr. P.V. Mehere have edited the manuscript.

Conflict of Interest:

The authors hereby declare that there is no conflict of interest amongst them or with others regarding this work.

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