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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(7): 2280-2284

www.thepharmajournal.com
Received: 03-05-2023
Accepted: 08-06-2023

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Effect of intensely higher environmental temperature on changes in endogenous enzymatic antioxidants of liver in Marwari goat

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Abstract
Evaluation of the effect of extremely higher environmental temperature on endogenous enzymatic antioxidants of liver in Marwari goat was executed in the present study. Liver samples were collected from Marwari breed of goat ageing from 3 to 12 months during extremely higher or hot environmental temperature periods. Objectives of the investigation were accomplished by measuring the analytes in the liver during extremely hot period. The endogenous enzymatic antioxidants of liver incorporated different analytes i.e. catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR). A significant ($p \leq 0.05$) upsurge in the mean values of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR was noticed during extremely hot ETP in comparison to respective mean value of comfortable period. Outcome of the study indicated that male Marwari goat in comparison to females had supercilious antioxidant potential of liver. The capability of the goats to bring about the reactions during the extremely hot ETP was exhibited by percent changes in the values. Suggestion can be put forwarded that goats of arid tracts must be kept safe from enlarged exposure to intense hot environmental temperature.

Keywords: Higher environmental temperature, endogenous enzymatic antioxidants, Marwari goat

Introduction
Animal welfare is a burning issue in the area of stress physiology (Kataria and Kataria, 2016) [9]. Workers are trying hard to find out the base mechanism responsible for the manuevering of physiological reactions during abiotic stressors (Kain *et al.*, 2022a) [8]. When fine balance between antioxidant enzymes and oxidants disturbs oxidative stress appears. Free radicals mediate these reactions. They are produced at a massive scale when aerobic metabolism becomes active. Inflammatory progressions are also the key factors in providing contribution to oxidative stress. Due to higher metabolism, free radical production may cause changes in the levels of antioxidant enzymes. Stress from any source is able to decrease the endogenous antioxidants (Kataria *et al*., 2010a) [13]. Oxidative stress can be generated due to environmental temperature governed metabolic parameters alterations through free radicals (Kataria *et al*., 2010 b, c and Kataria *et al*., 2013a) [10]. Assessment of these endogenous antioxidant enzymes of the liver is imperative. Endogenous antioxidant enzymes encompass generally glutathione reductase, catalase, superoxide dismutase etc. Oxidative stress is the basic cause of several disorders (Kataria *et al*., 2012) [11].
Enormous economical value of goat in the form of meat industry and milk production has provoked scientific awareness regarding blood parameters, however, very little attention has been given over the protective features from the stressors. These activities are known to cause emotional stress to the animals. Simultaneously, all the above mentioned stressors are coupled with the most potent stressor, the higher environmental temperature. Present study was executed to set the reference values of antioxidant enzymes of liver of goat which were native to arid regions. Result of this endeavour can benefit researchers and clinicians to put in a nutshell the contemporary implications with relation to oxidative stress and to draw the antioxidative supplementation plans in Veterinary science.

Materials and Methods
Liver tissue was collected from the goat after slaughter with the help of sterile B.P. blade for the measurement of parameters of antioxidant responses.

The liver tissue samples were kept in the box having ice and brought to the Physiology laboratory. After completion of cleaning, each sample was washed with sterile normal saline solution. Then precise weighing of 1 g piece of each tissue sample was done. Into a clean dried test tube, 5 ml of normal saline solution was introduced and 1 g of liver tissue sample was taken into it. A tissue homogenizer was used for proper homogenization of liver tissue with liquid. The final volume was made to 10 ml by adding normal saline solution with mixing. Due care was taken to maintain the temperature from 4 to 8 °C by the use of chilled distilled water. Then this was shifted to small beaker and vibrated at 1000 rpm for 10 minutes in an electronic digital vibrator (Century). Then again it was shifted to a test tube and centrifuged at 4 °C (10,000rpm) for 20 minutes. Then the tube was put in an incubator at 37 °C for 1hour. Proper tissue supernatant was collected in a clean dried test tube. This was a little modification of process described by Cornelius *et al.* (1959) [3] and Bengoumi *et al.* (1997) [2]. This tissue supernatant was used to measure the different liver antioxidant responses as the procedure mentioned for the serum using spectrophotometer (Shimadzu UV-1800). In place of serum, supernatant was taken for the measurement of proteins and antioxidants (Joshi, 2018) [6]. The levels of antioxidants were determined per mg of protein. In the standard procedure employed for serum, value of protein is computed in terms of g proteins per 100 ml. In present exploration, this was first converted to mg per 100 ml and then protein was computed in terms of mg per 1000 ml of supernatant fluid. For each antioxidant, the basic procedure remained same as used for serum determination.

To evaluate impression of intensely higher environmental temperature related changes in endogenous antioxidant enzymes present in liver of Marwari goat, the recordings of various analytes attained during extreme hot environmental temperature were compared with those attained during comfortable ETP, latter to be employed as a control period.

Following parameters were analyzed to accomplish the present study:

Endogenous antioxidants in liver

a) Superoxide dismutase
b) Catalase
c) Glutathione reductase

Superoxide dismutase

A procedure (colorimetric) as per Winterbourn *et al.* (1975) [16] was followed with slight modification (Anonymous, 2016) [1]. Process is instituted upon the fact of capacity of this enzyme to check the reduction of nitroblue tetrazolium.

Procedure

Ten glass dried and clean test tubes in a series were arranged to put supernatant quantities (0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µl correspondingly). Each tube was added with EDTA-cyanide (0.2 ml), NBT (0.1 ml) and phosphate buffer (3 ml). Incubation (5 minutes) was done by using a foil lined light box mounted with 40 W fluorescent bulb (Length 4' long X 8" X 6) producing uniform ambience of light intensity. Then addition of 0.05 ml riboflavin was done. Re-incubation of tubes in the light box for 12 minutes was made. At an interval of one minute in an upward order, % transmission was recorded at 560 mµ wavelength.

$$kU\ L^{-1} = \frac{1000\ (\text{conversion factor})}{\mu l\ (\text{supernatant quantity giving 50 \% transmission})}$$

kU L^{-1}values were changed to k Umg $Protein^{-1}$.

Catalase

Employment of the method as given by Goth (1991) [5] with modest modification (Anonymous, 2016) [1] was done for analysis. Analytical procedure represents a combination of spectrophotometric assay of hydrogen peroxide with an optimized determination of catalase.

Procedure

Incubation (at 37 °C for one minute) of a glass clean and dried test tube (supernatant, 0.1 ml; sodium-potassium phosphate buffer, 0.1 ml; and H_2O_2 solution in buffer, 1.0 ml) was made. Following dilution of supernatant, spectrophotometric procedure of hydrogen peroxide was made by fetching enzymatic reaction to a hault by putting in ammonium molybdate (1.0 ml).

Molybdate and hydrogen peroxide complex was figured out against a blank 3 at 405 nm. Blank 1 consisted of substrate, molybdate, supernatant and sodium-potassium phosphate buffer; blank 2 consisted of substrate, molybdate and buffer and blank 3 included buffer solution, molybdate solution and buffer.

$$\text{Activity of catalase, kU } L^{-1} = \frac{\text{Sample OD- Blank 1OD}}{\text{Blank 2 OD – Blank 3 OD}} \text{ X 2X27.1X10}$$

2: dilution factor; 10: unit conversion factor and 27.1: mg of enzyme per ml

Activity of catalase, kU L^{-1} was converted to kU $mgProtein^{-1}$.

Glutathione reductase (GR)

Procedure

After setting of clean and dried cuvette of spectrophotometer, addition of 2.4 ml buffer, 0.5 ml supernatant and 0.1 ml of coenzyme solution was carried out sequentially. After 2 minutes, substrate (0.1ml) was introduced and optical density was read (340 mµ) by using an interval of one minute with three recordings:

GR Level (kU L^{-1}) = OD $minute^{-1}$ X 1000 X 2X 0.5

(1000: dilution factor; 0.5: quantity of supernatant; 2: minutes for first reaction).

GR Level (kU L^{-1}) was converted to k Umg $Protein^{-1}$

Statistical analysis

Present investigation was executed to evaluate the effect of intensely higher environmental temperature on endogenous antioxidant enzymes of liver in Marwari goat. Computer programmes were employed to calculate means and standard error (Kaps and Lamberson, 2004) [7]. Mean changes were also assessed as per Duncan (1955) [4].

Results and Discussion

Data are illustrated in tabular form providing mean changes during comfortable and extremely hot ETPs in Marwari goat.

Superoxide dismutase (SOD)

Values (Mean ± SEM) of liver superoxide dismutase (SOD, kUmg $Protein^{-1}$) in the Marwari goat at the time of

comfortable and extremely hot environmental temperature periods (ETPs) along with percent change during extremely hot ETP

Table 1: Values (Mean ± SEM) of liver superoxide dismutase (SOD, kUmg Protein^{-1}) in the Marwari goat

S. N.	Effects	Mean ± SEM values during different environmental temperature Periods (ETPs)		Percent change during extremely hot ETP
		Comfortable ETP	Extremely hot ETP	
1.	Overall ETP (150)	102.00^{b}±0.39	203.00^{b}±0.40	+ 99.01
2.	Categorization according to gender (I and II categories)			
I.	Males (75), categorization - age groups as a, b & c			
	Males (75)	112.00^{bc}±0.10	213.00^{bd}±0.11	+ 90.17
	3-6 months (25)	102.00^{bd}±0.04	203.00^{bd}±0.06	+ 99.01
	6-9 months (25)	112.00^{bd}±0.04	213.00^{bd}±0.05	+ 90.17
	9-12 months (25)	122.00^{bd}±0.05	223.00^{bd}±0.04	+ 82.78
II.	Females (75), categorization - age groups as a, b & c			
	Females (75)	92.00^{bc}±0.10	193.00^{bc}±0.10	+ 109.78
	3-6 months (25)	82.00^{bd}±0.04	183.00^{bd}±0.05	+ 123.17
	6-9 months (25)	92.00^{bd}±0.05	193.00^{bd}±0.05	+ 109.78
	9-12 months (25)	102.00^{bd}±0.05	203.00^{bd}±0.04	+ 99.01
3.	Categorization according to age groups irrespective of gender			
	3-6 months (50)	92.00^{be}±0.05	193.00^{be}±0.04	+ 109.78
	6-9 months (50)	102.00^{be}±0.04	203.00^{be}±0.05	+ 99.01
	9-12 months (50)	112.00^{be}±0.05	213.00^{be}±0.05	+ 90.17

Fig. in the parenthesis = Number of Marwari goat
ETP = Environmental temperature period.
'b' = Significant ($p \leq 0.05$) differences between mean values for a row.
'c' = Significant ($p \leq 0.05$) differences between overall mean values of males and females for an ETP
'd' = Significant ($p \leq 0.05$) differences among mean values of different age groups of a gender for an ETP
'e' = Significant ($p \leq 0.05$) differences among mean values of different age groups irrespective of a gender for an ETP
+ = Percent increase in the mean value

Catalase (CAT)

Values (Mean ± SEM) of liver catalase (CAT, kUmg Protein^{-1}) in the Marwari goat at the time of comfortable and extremely hot environmental temperature periods (ETPs) along with percent change during extremely hot ETP

Table 2: Values (Mean ± SEM) of liver catalase (CAT, kUmg Protein^{-1}) in the Marwari goat

S. N.	Effects	Mean ± SEM values during different environmental temperature periods (ETPs)		Percent change during extremely hot ETP
		Comfortable ETP	Extremely hot ETP	
1.	Overall ETP(150)	59.00^{b}±0.62	163.00^{b}±0.58	+ 176.27
2.	Categorization according to gender (I & II categories)			
I.	Males (75), categorization - age groups as a, b & c			
	Males (75)	69.00^{bc}±0.24	173.00^{bd}±0.24	+ 150.72
	3-6 months (25)	60.00^{bd}±0.04	162.00^{bd}±0.06	+ 170.00
	6-9 months (25)	68.00^{bd}±0.04	173.00^{bd}±0.05	+ 154.41
	9-12 months (25)	79.00^{bd}±0.05	184.00^{bd}±0.04	+ 132.91
II.	Females (75), categorization - age groups as a, b & c			
	Females (75)	49.00^{bc}±0.25	153.00^{bc}±0.23	+ 212.24
	3-6 months (25)	38.00^{bd}±0.05	144.00^{bd}±0.06	+ 278.94
	6-9 months (25)	50.00^{bd}±0.06	152.00^{bd}±0.05	+ 204.00
	9-12 months (25)	59.00^{bd}±0.05	163.00^{bd}±0.05	+ 176.27
3.	Categorization according to age groups irrespective of gender			
	3-6 months (50)	49.00^{be}±0.06	153.00^{be}±0.05	+ 212.24
	6-9 months (50)	59.00^{be}±0.06	162.50^{be}±0.06	+ 174.57
	9-12 months (50)	69.00^{be}±0.05	173.50^{be}±0.05	+ 150.72

Fig. in the parenthesis = Number of Marwari goat.
ETP = Environmental temperature period
'b' = Significant ($p \leq 0.05$) differences between mean values for a row.
'c' = Significant ($p \leq 0.05$) differences between overall mean values of males and females for an ETP
'd' = Significant ($p \leq 0.05$) differences among mean values of different age groups of a gender for an ETP
'e' = Significant ($p \leq 0.05$) differences among mean values of different age groups irrespective of a gender for an ETP
+ = Percent increase in the mean value

Glutathione reductase (GR)

Mean ± SEM values of liver glutathione reductase (GR, Umg Protein^{-1}) in the Marwari goat during comfortable and extremely hot environmental temperature periods (ETPs) along with percent change during extremely hot ETP

Table 3: Mean ± SEM values of liver glutathione reductase (GR, Umg Protein^{-1}) in the Marwari goat

S. No.	Effects	Mean ± SEM values during different environmental temperature Periods (ETPs)		Percent change during extremely hot ETP
		Comfortable ETP	Extremely hot ETP	
1.	Overall ETP(150)	7.00^{b}±0.08	15.00^{b}±0.08	+ 114.28
2.	Categorization according to gender (I and II categories)			
I.	Males (75), categorization - age groups as a, b & c			
	Males (75)	8.00^{bc}±0.02	16.00^{bd}±0.02	+100.00
g	3-6 months (25)	7.00^{bd}±0.006	15.00^{bd}±0.006	+114.28
h	6-9 months (25)	8.00^{bd}±0.004	16.00^{bd}±0.005	+100.00
i	9-12 months (25)	9.00^{bd}±0.005	17.00^{bd}±0.004	+88.88
II.	Females (75), categorization - age groups as a, b & c			
	Females (75)	6.00^{bc}±0.02	14.00^{bc}±0.02	+133.33
g	3-6 months (25)	5.00^{bd}±0.006	13.00^{bd}±0.005	+160.00
h	6-9 months (25)	6.00^{bd}±0.005	14.00^{bd}±0.006	+133.33
i	9-12 months (25)	7.00^{bd}±0.005	15.00^{bd}±0.005	+ 114.28
3.	Categorization according to age groups irrespective of gender			
g	3-6 months (50)	6.00^{be}±0.006	14.00^{be}±0.005	+133.33
h	6-9 months (50)	7.00^{be}±0.005	15.00^{be}±0.005	+114.28
i	9-12 months (50)	8.00^{be}±0.005	16.00^{be}±0.006	+100.00

Figures in the parenthesis = Number of Marwari goat.
ETP = Environmental temperature period
'b' = Significant ($p \leq 0.05$) differences between mean values for a row.
'c' = Significant ($p \leq 0.05$) differences between overall mean values of males and females for an ETP
'd' = Significant ($p \leq 0.05$) differences among mean values of different age groups of a gender for an ETP
'e' = Significant ($p \leq 0.05$) differences among mean values of different age groups irrespective of a gender for an ETP
+ = Percent increase in the mean value

Evaluation of the effect of intensely higher environmental temperature on endogenous antioxidant enzymes of liver in Marwari goat

In the present study, several parameters of endogenous antioxidant responses of liver i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), exhibited significant ($p \leq 0.05$) changes in the mean values during extremely hot ETP as compared to respective comfortable ETP mean values.

Extremely hot ETP in the present investigation affected the contents of parameters of endogenous liver antioxidant responses with a higher propensity. Antioxidant responses were found to be influenced during extremely hot ETP. It was surmised that depletion of antioxidants (glutathione, vitamin A, & vitamin E) presented a biological crusade between free radicals and antioxidants. Body attempted to increase the responses of antioxidant potential by raising the manufacture of enzymatic antioxidants like superoxide dismutase; catalase and glutathione reductase (Kataria *et al*., 2010b) [12]. Suppositions can be derived from the interpretation of the observations collected in the present study regarding the values of enzymatic antioxidants in the liver. Percent changes were observed to be utmost for each parameter during extremely hot ETP in the female goats and in goats of 3-6 months.

In the present investigation, amongst all the parameters explored, the most sensitive parameter was found to be liver catalase which showed the maximum percent change. Scientists have revealed the involvement of catalase as an important component of the first line of antioxidant defense of the body and related its higher levels with the extremely hot ambience associated oxidative stress (Promila, 2018 and Joshi, 2018) [15, 6]. Excessive free radical production can change internal defending antioxidant system of the body can culminate in oxidative stress. All the time, antioxidant arrangement can not be adequate to safeguard the body from gratuitous free radical formation and consequently to oxidative insult. For that reason, amplification of antioxidant hoard may be required to remodel antioxidant profile.

Changes in the antioxidant responses of liver indicated alterations in the antioxidant potential in an overall mode pointing towards a combined force of the liver cells to impede oxidative stress and typified the toughened antioxidant position of the Marwari goat during extremely hot environmental temperature period. Conclusion of the investigation suggested about the magnitude of presence of oxidative stress, which was noted to be higher during extremely hot environmental temperature period in females and in 3-6 months aged.

The findings of this research have attempted to offer a new insight to endogenous antioxidant responses of liver during extremely hot environmental temperature will help in making the plans for the health improvement of native breeds of arid tracts. Exploration of the previous research clarified the scarcity of research on this aspect, hence the upshots of the present investigation will assist in developing antioxidant supplementation plans during harsh climatic conditions. The research has provided an imminent approach to the liver functions in goats by finding the possible modulations in the reactions influencing the antioxidant responses, particularly during the presence of abiotic stressors.

Conclusion

The conclusion of the exploration will confer aid in supervising the stratagem for placate of the native breeds of goat. Eloquent stipulations of the present study have attempted to divulge that liver cells are fraught with a blend of antioxidants. Resolution of research have tried to focus on the relative worth of the changes in the levels of antioxidants associated with the extremely hot environmental temperature. This may be the first investigation where parameters of antioxidant responses of liver have been appraised at one stage in Marwari goat. Research contribution of the present study can be employed in strengthening the clinical aspect of Physiology in Veterinary sciences in the field of antioxidant status and in systematizing the scientific supervision of the animals during adverse ambiences. The vibrancy of alterations regarding antioxidant responses of liver revealed

the existence of oxidative stress. Findings acquired in the investigation will assist in increasing the endorsement of contrivances to have gist about the damaging effects of harsh ambiences in the goat. Results will be temptingly valuable in crafting scientific tactics for Marwari goat to help the poor farmers and goat owners of arid tracts. Similarly researchers associated in the execution of skills in goat sector will also be benefitted. It can be concluded that present study evaluated efficiently the effect of intensely higher environmental temperature on antioxidant enzymes present in liver of Marwari goat.

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