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STATUS OF OXIDATIVE STRESS BIOMARKERS OF KACHCHHI CAMEL DURING EXERCISE

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ABSTRACT

The study was conducted on 5 adult clinically healthy Kachchhi camels (BW 450-550 kg) to assess the effect of different payloads (L_1, L_2 and L_3), seasons (S_1, S_2 and S_3) and work rest cycles (WR_1 and WR_2) on oxidative stress biomarkers (GPX, SOD and TBARS). The lowest level of GPX (Unit/g protein) was 4.74 ± 0.513 and 5.55 ± 0.510 during S_3, while during S_1 and S_2 the values were 7.44 ± 0.473 and 8.02 ± 0.622 and 10.12 ± 0.677 and 11.10 ± 0.692 in WR_1 and WR_2, respectively. The SOD (Unit/g Hb) level increased at the rate of 9.87 and 8.46% (S_1), 19.65 and 19.10 % (S_2), and 31.91and 14.03% (S_3) in WR_1 and WR_2, respectively. TBARS level (nmol/ml) increased at the rate of 31.37 and 27.56% (S_1), 70.04 and 77.69 % (S_2) and 52.42 and 59.45% (S_3) in WR_1 and WR_2, respectively. The Kachchhi camel worked comfortably under WR_1 as compared to WR_2.

Key words: Exercise stress biomarkers, Kachchhi camel, payloads, seasons, work rest cycles

Exercise induced stress also promotes lipid peroxidation where by Reactive Oxygen Species (ROS) induces tissue damage by interesting with plasma membrane. Antioxidative enzymes are the sensitive markers for detecting oxidative stress which is regulated upon production of free radicals and down regulated upon its utilisation. The plasma thiobarbituric acid-reactive substances (TBARS) / malondealdihyde (MDA) measurement is widely used technique for the assessment of lipoperoxidation level (Lykkesfeldt and Svendsen, 2007). At the cellular level antioxidants work in a coordinated fashion to protect muscle fibres from oxidative injury during periods of increased oxidant production. Superoxide dismutase forms the first line of defense against superoxide radicals as SOD dismutates superoxide radicals to form hydrogen peroxide (H_2O_2) and oxygen (O_2). Endurance exercise training/ higher intensities and/or longer daily durations of exercise promotes 20–112% and 20–177% increases in the activities of both SOD and Glutathione Peroxidase (GP_X) activity in the exercised muscles.

The objective of present study was to assess the effect of different payloads (L_1, L_2 and L_3), seasons (S_1, S_2 and S_3) and work rest cycles (WR_1 and WR_2) on oxidative stress biomarkers (GPX, SOD and TBARS) clinically healthy Kachchhi camels.

Materials and Methods

Experimental location and Climate

The present experiment was conducted at Instructional farm, Department of Livestock Production, Veterinary College, Anand during hot dry, hot humid and winter season. Anand, a mid-Gujarat Zone has a subtropical climate. The climatic condition of Anand was based on observation during last 50 years (1956 to 2005) is cold and dry (Max. temp 27.81°C to 32.40°C and THI-73.45 to 78.16) in winter (October to mid February). Summer commences from middle of February and ends in middle of June (Max temp 34.64°C to 40.08°C and THI-77.98 to 87.08), feeling quite hot and dry. Monsoon is hot and humid, prevails from about mid-June to mid-October (Max temp 31.13°C to 35.08°C and THI-82.05 to 84.73).

Experimental Animals and Management

This study was conducted on 5 adult clinically healthy Kachchhi camels of similar body weight and age (450-550 kg and 7-8 years, respectively). The camels, camel cart and driver were hired from the local market. The camel was acclimatised for route of transport. The camels were given the feed as per the ICAR (1985) guidelines.

Duration

The present experiment was conducted in 3 seasons namely S_1 - hot dry (15th May -30th June), S_2

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- hot humid (15th Sept - 31st Oct) and S_3 - winter (1st Dec – 15th Jan).

Workrest cycle and Total loads

The camels worked for 6 hrs daily from morning 08.00 hrs to 16.00 hrs in 2 work rest cycles *viz.* WR_1 : 2h (W) - 1h (R) + 2h (W) - 1h (R) + 2h (W) - 1h (R) (Singh, 1999) and WR_2: 1h (W) - 15 min (R) - 1h (W) - 15 min (R) - 1h (W) - 1h (R) - 1h (W) - 15 min (R) - 1h (W) - 15 min (R) - 1h (W) (Traditional) on strait coal tar road of about 5.2 km/round. The 3 total loads (L_1 - 1500, L_2 - 2000 and L_3 - 2500 kg) were placed on the camel cart. The total load was the sum of payload + weight of cart + weight of driver. The bags filled with gravels and concentrate mixture were used to fix the pay loads on the cart. The camel worked with three pay loads in two work rest cycles for 6 days (two days for each pay load).

Blood sampling and processing

Blood (10ml) was collected before work at 08.00 hrs (morning session) and immediately after work at 16.00 hrs (evening session). Blood samples were collected by jugular vein puncture into vacutainers containing EDTA. The blood tubes were placed on ice till estimation. The blood samples were centrifuged at 2000 rpm for 20 minute, and then the plasma was pipetted into different aliquots and stored at -70°C until analysis for plasma content of SOD and GP_X. The estimation of serum TBARS was performed from blood samples collected from jugular vein and kept in centrifuge tube in tilted position till separation of serum (2 hrs) and finally centrifuged at 2000 rpm for 20 minute and estimated immediately.

Plasma SOD estimation

The auto oxidation reaction was started by addition of freshly prepared pyrogallol solution to tris–Hcl buffer at pH 8.5. The 50% inhibition of pyrogallol by SOD present in haemolysate was measured by spectrophotometer at 420 nm. The activity was expressed as U/g HB (Chaudhari *et al,* 2003).

Plasma GP_X estimation

Reaction cocktail containing 1.0 mM sodium azide, glutathione reductase and 200 mM reduced glutathione was prepared in a vial containing beta-NADPH. After adjusting pH at 7.0, the 10.0 mM sodium phosphate buffer with 1.0 mM dithiothreitol and GPx was added to the cocktail, mixed by inversion and equilibrated to 25°C. The reaction was monitored at A340nm until constant, using a suitably thermostated spectrophotometer. The 30% hydrogen peroxide was added to the reaction and mixed by inversion. The decrease in A340 nm was recorded for approximately minutes and the activity was expressed as U/g of protein (Wendel, 1980).

Serum TBARS estimation

The Thiobarbituric acid (TBA) reacting substances (TBARS) assay was used as an indicator of lipid peroxidation. The assay was based upon the reaction of TBA with malondialdehyde (MDA), one of the aldehyde products of lipid peroxidation. The serum sample was heated with TBA under acidic conditions and the amount of MDA-TBA adduct produced was measured. The complex was extracted with butanol to increase the sensitivity and measured spectrophotometrically at 530 nm. The values were expressed in terms of malondialdehyde (n Mol/ ml) (Satoh, 1978).

Statistical analysis

The data are presented as mean ± standard error (SE). All means and SE were estimated as per the

Table 1. Effect of Work Rest Cycle -1 and 2 on GP_X (Unit/g protein) of Kachchhi camel.

Pay Load	Season												Mean	
	Summer				Hot – Humid				Winter					
	WR_1		WR_2		WR_1		WR_2		WR_1		WR_2		WR_1	WR_2
	BW	AW	BW	AW	BW	AW	BW	AW	BW	AW	BW	AW		
L_1	6.46± 1.57	7.22 ±1.51	4.63 ±1.70	8.20 ±1.55	8.48 ±1.24	9.62 ±1.54	12.50 ±2.10	14.06 ±2.39	4.04 ±0.83	5.40 ±1.03	3.78 ±0.53	5.66 ±0.79	6.87 ±0.60	8.14 ±0.95
L_2	7.14 ±0.73	9.77 ±0.90	5.92 ±1.11	9.42 ±1.04	12.0 ±1.70	14.34 ±1.84	8.32 ±0.93	9.66 ±1.04	2.64 ±0.56	5.02 ±1.19	3.99 ±0.78	6.73 ±1.55	8.48 ±0.88	7.34 ±0.55
L_3	6.44 ±0.83	7.64 ±1.01	9.43 ±1.05	10.47 ±1.32	7.38 ±0.87	8.94 ±0.86	9.70 ±0.72	12.36 ±1.50	4.81 ±1.69	6.54 ±1.73	5.94 ±1.22	7.25 ±1.86	6.96 ±0.51	9.19 ±0.63
Mean(Se)	6.68 ±0.60^A	8.24 ±0.69^B	6.68 ±0.89^A	9.36 ±0.75^B	9.28 ±0.88^A	10.96 ±1.02^A	10.17 ±0.88^A	12.02 ±1.05^B	3.83 ±0.65^A	5.65 ±0.74 B	4.57 ±0.54^A	6.54 ±0.80^B		
Mean(S)	7.44±0.47^b		8.02±0.62^b		8.02±0.62^b		11.10±0.69^c		4.74±0.51^a		5.55±0.51^a			

GP_X mean with different superscripts (A, B) in row differ at $p < 0.05$
GP_X mean with different superscripts (a, b) in row differ at $p < 0.05$

procedure outlined in SPSS® 11.00 statistical packages (SPSS, 2001). The level of significance and significance between means and their combined interaction effect of different treatment effect were assessed using the 4 factorial completely randomised design (CRD) procedures (Snedecor and Cochran, 1980).

Results

The GP_X (Unit/g protein) was significantly ($p < 0.05$) influenced by season, session of work and interaction effects of S x L, W x L and S x W x L but work rest cycles and load independently did not produce any significant effects (Table 1 and 2). The lowest level of GP_X (Unit/g protein) was 4.74 ±0.513 and 5.55 ± 0.510 was observed during S_3 in WR_1 and WR_2, respectively. The corresponding values for S_1 and S_2 were 7.44 ± 0.473 and 8.02 ± 0.622 and 10.12 ± 0.677 and 11.10 ± 0.692. The GP_X level remained high during S_2 followed by S_1 and S_3. When simultaneous effect of season and load on GP_X studied together, the GP_X level significantly ($p < 0.05$) lower under L_1 during S_1 only. The GP_X increased by 56.92 and 113.50% in S_1 and S_2, respectively as compared to S_3 in WR_1. The corresponding values in WR_2 were 44.50 and 100%. The GP_X level increased significantly ($p < 0.05$) after exercise. The percentage increment in GP_X after exercise was observed to be 23.35, 40.11, and 18.10 and 18.19, 47.52 and 41.13, respectively during S_1, S_2 and S_3 in WR_1 and WR_2.

The level of SOD (U/g HB) was significantly ($p < 0.05$) influenced by Se, while it was not influenced by S, W and L (Table 3). The SOD level increased at the rate of 9.87 (S_1), 19.65 (S_2) and 31.91% (S_3) in WR_1 after whole day of exercise. The corresponding values in WR_2 were 8.46 (S_1), 19.10 (S_2) and 14.03 % (S_3).

The concentration of TBARS was significantly ($p < 0.05$) affected by S, L, Se, interaction effects of S x L, S x Se and W x L (Table-4). The TBARS level increased at the rate of 31.37 (S_1), 70.04 (S_2) and 52.42 % (S_3) in WR_1 after whole day of exercise. The corresponding values in WR_2 were 27.56 (S_1), 77.69 (S_2) and 59.45 % (S_3) at the end of work. The concentration of TBARS increased to maximum in S_2 followed by S_3 and S_1. The TBARS level was at par between L_1 and L_2 in WR_1 but increased significantly ($p < 0.05$) when camel was put to work under L_3, where as in WR_2, the

Table 2. Interaction effects of S x L, W x L and S x W x L on GP_X (Unit/g protein) of Kachchhi camel.

Interaction effects of S x L on GP_X (Unit/g protein) of Kachchhi Camel			
	L_1	**L_2**	**L_3**
S_1	$6.63±1.12^{1}$	$8.07±0.78^{2}$	$8.49±0.92^{2}$
S_2	$11.16±0.87^{3}$	$11.08±0.65^{3}$	$9.59±1.01^{3}$
S_3	$4.72±1.24^{1}$	$4.59±0.56^{1}$	$6.13±0.89^{1}$
Interaction effects of W x L on GP_X (Unit/g protein) of Kachchhi Camel			
W_1	$6.87±0.89^{Y}$	$8.48±0.78^{Y}$	$6.96±0.51^{Y}$
W_2	$8.14±0.67^{Y}$	$7.34±0.41^{Y}$	$9.19±0.34^{Z}$
Interaction effect of S x W x L on GP_X (Unit/g protein) of Kachchhi Camel.			
S_1W_1	$6.84±0.91^{M}$	$8.45±0.78^{M}$	$7.04 ±0.51^{M}$
S_1W_2	$6.41±0.64^{M}$	$7.70±0.47^{NM}$	$9.95±1.20^{N}$
S_2W_1	$9.05±0.35^{M}$	$13.17±1.39^{N}$	$8.16±0.98^{M}$
S_2W_2	$13.28±0.76^{NM}$	$8.99±0.79^{M}$	$11.03±0.49^{M}$
S_3W_1	$4.72±0.57^{M}$	$3.83±0.32^{M}$	$5.67±0.99^{M}$
S_3W_2	$4.72±0.29^{M}$	$5.35±0.48^{M}$	$6.59±1.01^{M}$

Interaction mean of S x L with different superscripts (1, 2 and 3) in column differ at $p<0.05$

Interaction mean of W x L with different superscripts (Y and Z) in column differ at $p<0.05$

Interaction mean of S x W x L with different superscripts (M and N) in column differ at $p<0.05$

Table 3. Effect of Work Rest Cycle -1 and 2 on SOD (Unit /g HB) of Kachchhi camel.

Pay Load	**Season**												**Mean**	
	Summer				**Hot – Humid**				**Winter**					
	WR_1		**WR_2**		**WR_1**		**WR_2**		**WR_1**		**WR_2**		**WR_1**	**WR_2**
	BW	**AW**	**BW**	**AW**	**BW**	**AW**	**BW**	**AW**	**BW**	**AW**	**BW**	**AW**		
L_1	7.16 ±0.24	7.37 ±0.24	8.02 ±0.16	7.37 ±0.24	5.73 ±0.58	7.19 ±0.40	6.46 ±0.31	7.52 ±0.48	6.39 ±1.00	7.34 ±0.98	7.18 ±1.07	8.49 ±1.19	6.91 ±0.27	7.50 ±0.29
L_2	7.13 ±0.37	7.39 ±0.17	7.78 ±0.24	7.39 ±0.17	6.51 ±0.46	8.09 ±0.46	6.32 ±0.40	7.27 ±0.20	5.51 ±0.52	8.41 ±0.75	6.16 ±0.71	6.19 ±0.61	7.31 ±0.27	6.85 ±0.20
L_3	6.99 ±0.43	6.87 ±0.81	7.67 ±0.73	6.87 ±0.81	6.98 ±1.02	7.73 ±0.95	6.08 ±0.64	7.67 ±0.64	5.76 ±0.21	7.58 ±0.60	6.55 ±0.51	8.00 ±0.29	7.08 ±0.30	7.14 ±0.26
Mean(Se)	$7.09 ±0.19^{a}$	$7.21 ±0.27^{a}$	$7.82 ±0.24^{b}$	$7.21 ±0.27^{a}$	$6.41 ±0.41^{a}$	$7.67 ±0.36^{b}$	$6.28 ±0.25^{a}$	$7.48 ±0.26^{b}$	$5.89 ±0.37^{a}$	$7.77 ±0.44^{b}$	$6.63 ±0.44^{a}$	$7.56 ±0.50^{b}$		
Mean(S)	7.44±0.20		7.52±0.19		7.04±0.29		6.88±0.21		6.83±0.33					

SOD Mean with different superscripts (a and b) in row differ at $p < 0.05$

Table 4. Effect of Work Rest Cycle -1 and 2 on TBARS (n Mol /ml) of Kachchhi camel.

Pay Load	Season												Mean	
	Summer				Hot – Humid				Winter					
	WR_1		WR_2		WR_1		WR_2		WR_1		WR_2		WR_1	WR_2
	BW	AW	BW	AW	BW	AW	BW	AW	BW	AW	BW	AW		
L_1	1.85 ±0.13	2.74 ±0.33	1.73 ±0.40	2.15 ±0.46	2.13 ±0.91	3.17 ±1.39	1.54 ±0.15	2.85 ±0.45	1.91 ±0.15	2.65 ±0.37	1.69 ±0.09	2.66 ±0.28	2.41 ±0.28^{a}	2.10 ±0.16^{a}
L_2	1.96 ±0.18	2.43 ±0.29	2.70 ±0.59	3.59 ±0.54	1.80 ±0.51	2.99 ±0.67	2.59 ±0.35	4.76 ±0.74	1.82 ±0.19	2.82 ±0.19	1.93 ±0.28	3.39 ±0.39	2.31 ±0.17^{a}	3.16 ±0.25^{b}
L_3	2.31 ±0.13	2.84 ±0.12	2.33 ±0.20	2.88 ±0.29	2.88 ±0.41	5.43 ±0.78	3.67 ±0.64	6.24 ±0.71	2.47 ±0.29	3.95 ±0.63	3.03 ±0.53	4.58 ±0.75	3.31 ±0.26^{b}	3.79 ±0.32^{c}
Mean(Se)	2.04 ±0.10^{A}	2.68 ±0.15^{B}	2.25 ±0.25^{A}	2.87 ±0.28^{B}	2.27 ±0.37^{A}	3.86 ±0.61^{B}	2.60 ±0.33^{A}	4.62 ±0.51^{B}	2.06 ±0.14^{A}	3.14 ±0.28^{B}	2.22 ±0.24^{A}	3.54 ±0.35^{B}		
Mean(S)	2.35±0.104^{a}		2.56±0.20^{a}		3.07±0.38^{b}		3.61±0.35^{b}		2.60±0.183^{a}		2.88±0.24^{a}			

TBARS mean with different superscripts (A, B) in row differ at $p < 0.05$
TBARS mean with different superscripts (a, b and c) in row and column differ at $p < 0.05$

concentration of TBARS was increased progressively and significantly ($p < 0.05$) as the payload increased from L_1 to L_3. This indicated that Kachchhi camel worked comfortably under L_1 and L_2 in WR_1 and under L_1 in WR_2, respectively. Irrespective of work rest cycle and session of work, the S x L (Table 5) interaction revealed that the concentration of TBARS was at par up to L_2 in all 3 three seasons but became significantly ($p < 0.05$) higher under L_3 during S_2 while the concentration of TBARS increased significantly ($p < 0.05$) in all 3 seasons after the whole day exercise suggesting increase in lipid peroxidation (Table 5). The TBARS level under L_1 and L_3 in both the work rest cycles was at par but the level of TBARS under L_2 was significantly ($p < 0.05$) higher in WR_2 (Table 6).

Discussion

Oxidative stress may exacerbate psychological and physiological demands brought about by stressful conditions. Oxidative stress, involving an imbalance between the production of free radicals and the capability of an organism to absorb their excess, has been proposed to play a role in the pathogenesis of several infectious diseases of domestic animals (Miller, 1993). Measure of oxidative stress allows estimation of the real status of physiological defence and prevention of the appearance of correlated pathologies (Piccione *et al,* 2007). Oxidative stress promotes the insurgence of serious pathologies as a result of the degenerative damage of cellular structures (Freidovich, 1999; Matsuo and Kaneko, 2000; Mc Cord, 2000). Lipid peroxidation is a general mechanism whereby free radicals induce tissue damages, and implicated under several diverse pathological conditions (Halliwell and Chirico, 1993).

Table 5. Interaction effects of S x L on TBARS (n Mol /ml) of Kachchhi camel.

	S_1	S_2	S_3
L_1	2.120±0.25^{Y}	2.424±0.34^{Y}	2.229±0.24^{Y}
L_2	2.676±0.63^{Y}	3.041±0.21^{Y}	2.495±0.29^{Y}
L_3	2.594±0.28^{Y}	4.561±0.50^{Z}	3.513±0.35^{Y}
Interaction effects of S x Se on TBARS (n Mol /ml) of Kachchhi camel.			
Se_1	2.151±0.41^{M}	2.440±0.23^{M}	2.145±0.45^{M}
Se_2	2.776±0.27^{N}	4.244±0.41^{N}	3.346±0.65^{N}

Interaction mean of S x L with different superscripts (Y and Z) in column differ at $p<0.05$.

Interaction mean of Se x S with different superscripts (M and N) in column differ at $p<0.05$.

Table 6. Interaction effects of W x L on TBARS (n Mol /ml) of Kachchhi camel.

	L_1	L_2	L_3
WR1	2.410±0.711	2.310±0.211	3.317±0.251
WR2	2.106±0.231	3.165±0.252	3.794±0.391

Interaction Mean of W x L with different superscripts (1 and 2) in column differ at $p<0.05$.

The low percentage increment in GP_X level during S_2 as compared to S_1 and S_3, was because of higher level of GP_X in morning session itself. This again reflected that animals were under stress condition before the start of work during S_2 irrespective of work rest cycles and session of work. The GP_X level was at par between L_1 and L_2 in WR_1 and WR_2 but became significantly ($p < 0.05$) higher in WR_2 as compared to WR_1 under L_3. When interaction effect of S x W x L discussed together, it revealed that the GP_X level was at par in WR_2 during S_1 and S_2 up to L_2. Sentürk *et al* (2005) observed changes in GP_X status in the blood after exercise

in humans. They found that the trained men who were exercised to exhaustion had increased blood amounts of GP_X. The highest level of GP_X 11.62 (U/G of protein) was observed in S_2. Increased GP_X activity at elevated temperatures during S_1 has been reported in cattle (Bernabucci *et al,* 2002) and horses (Susanna Kinnunen *et al,* 2005). The GP_X was significantly ($p<0.05$) negatively correlated with Hb and TBARS, suggesting association of higher GP_X activity with low serum concentration of TBARS. Thus increase in the GP_X level may help to reduce the peroxide level during stress condition.

Mn-SOD levels are found to be increased after acute exercise in men and rats (Akkus, 2001), and also have been validated by checking expression profile of SOD gene (Morikawa *et al,* 2004). Moreover, the higher erythrocyte SOD activity in hot condition has been reported in cattle (Bernabucci *et al,* 2002).

The high level of glucocorticoids and adrenaline induces pathways of aerobic energy production in stress and exacerbate MDA concentration in camel during hot temperature (Nazifi *et al,* 2009). The maximum changes in stress related markers after work load treatment was observed during S_1 (GP_X), S_2 (TBARS) and S_3 (SOD). This investigation highlight that elevated level of stress related biomarkers and haematological parameter are oxidative challenge for camel. Thus, it has becomes necessary to reduce such oxidative stress in concern to maintain homeostatic environment of animals by supplementing with different antioxidant like vitamin E and C (Ryan *et al,* 2010).

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