

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.706.072>**Effect of Exercise on Oxidative Stress Biomarkers in Horses**

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Corresponding author*ABSTRACT****Keywords**

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Twenty apparently healthy, non-pregnant, indigenous adult female horses having same age group (8-10 years) were selected from a stud farm located in Jaipur district of Rajasthan and divided into two groups of ten animals in each *i.e.* treatment and control group. All the animals were examined for biomarkers of oxidative stress. To study the effect of exercise, all animals of both groups were given trot exercise for 2 hours (for approximately 30 km) for one time. The results of present study revealed a significant ($P \leq 0.05$) effect of exercise on malondialdehyde levels only whereas the level of catalase, reduced glutathione and vitamin C revealed a non-significant effect.

Introduction

Oxidative stress has been defined as a disturbance of the equilibrium between antioxidants and oxidants in favour of oxidants (Sies, 1991). Reactive oxygen species (ROS) or oxidants can be defined as oxygen-containing molecules that are more reactive than the oxygen molecule present in air (Noguchi and Niki, 1999).

Studies in racing horses and horses with airway obstruction have been more systematic including recurrent airway obstruction (RAO), exercise-induced pulmonary haemorrhage (EIPH), racing induced oxidative stress, laminitis, arthritis intestinal strangulation, radiation and physiological challenges such as heat stress also causes oxidative stress (Kiess

and Gallaher, 1998; Punyiczki and Fesus, 1998; Assefa *et al.*, 2005; Gupta and Gollapudi, 2005; Deaton, 2006; Lykkesfeldt and Svendsen, 2007). Deaton *et al.*, (2005) reported that oxidative stress in horses is the phenomenon associated with exercise, environmental oxidants, decrease metabolism, inflammation and deficiency of antioxidant capacity.

According to Robinson (2009) the most important physiological and pathological condition associated with oxidative stress in horses includes intense exercise and training, respiratory tract diseases, perfusion related disorders, gastrointestinal tract reperfusion injury, reperfusion myopathy, laminitis, joint diseases, muscle disorders, neurological disorder, Equine motor neuron diseases,

Equine Cushing's diseases and Equine grass sickness.

Understanding of the role of oxidants and antioxidants in physiological and pathological conditions is continuously increasing and some oxidant-associated or oxidant-mediated processes are now considered as future therapeutic targets (Rahman *et al.*, 2006).

All animals included in the present study were kept under identical feeding, housing and environmental conditions during the complete period of study. The samples for present study were taken from a stud farm located in Jaipur district of Rajasthan and subsequent estimation of oxidative stress was done in the Department of Veterinary Medicine, PGIVER Jaipur and National Research Center on Equine (NRCE), Bikaner.

In order to find out the effect of exercise, all animals of both groups were given trot exercise for 2 hours (for approximately 30 km) for one time and then Pre and Post exercise samples from all animals (n = 20) were collected. Blood in heparinised tubes was also centrifuged at 3000 rpm for 30 minutes to separate the plasma from the whole blood. The plasma was aliquoted into different microcentrifuge tubes, which were stored in a deep freeze at -20°C until the time of analysis. The plasma was used for determination of malondialdehyde (MDA), Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and other biochemical parameters.

Tests for biomarkers of oxidative stress

Catalase

Catalase is a ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase (CAT) is involved in the detoxification of hydrogen per-oxide (H₂O₂). This enzyme

catalyzes the conversion of two molecules of H₂O₂ to molecular oxygen and two molecules of water (catalytic activity). For the determination of catalase activity, a spectrophotometric method was used based on the method of Johansson and Borg (1988). Catalase was determined in plasma samples by "Catalase Assay kit" (Catalog No. 707002) from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbor, MI 48108, USA.

Superoxide dismutase

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism.



Superoxide Dismutase was determined in plasma samples by "Superoxide Dismutase Assay kit" (Catalog No.706002) from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbor, MI 48108, USA.

Malondialdehyde

Estimation of Malondialdehyde was conducted by method developed by Okhawa *et al.*, (1979). Malondialdehyde (MDA) a secondary product of lipid peroxidation, the reaction of lipid peroxides with Thiobarbituric acid (TBA) yields red pigment which can be measured on Colorimeter or Spectrophotometer at 532 nm. MDA was estimated in the plasma separated from freshly collected samples from jugular vein of horses.

Reduced glutathione

Estimation of reduced glutathione was conducted by method developed by Beutler *et al.*, (1971).

Vitamin C

Estimation of Vit C was conducted by method developed by Denson and Bowers (1961).

Results and Discussion

Effect of exercise on biomarkers of oxidative stress

The Pre and Post exercise Mean \pm SE Values of different Biomarkers of Oxidative Stress in horses were presented in Table 1.

Catalase (nanomol/min/ml)

Pre and post exercise Mean \pm SE values of catalase were observed as 30.00 \pm 0.34 and 30.69 \pm 1.81 (nanomol/min/ml), respectively.

A non-significant change after exercise was observed in the values of catalase.

The findings of the present study are in agreement with those reported in horses by Ono *et al.*, (1990), Ji (1997), Schneider *et al.*, (2005), Andriichuk *et al.*, (2013) and Andriichuk *et al.*, (2014).

Superoxide dismutase (U/ml)

Mean \pm SE values of superoxide dismutase at pre and post exercise were as 2.38 \pm 0.26 and 2.75 \pm 0.21 (U/ml), respectively. There was no significant difference in values of SOD before and after exercise. These findings were in concurrence with that reported by Ono *et al.*, (1990); McMeniman and Hintz (1992); Balogh *et al.*, (2001); De Moffarts *et al.*, (2004); Schneider *et al.*, (2005) and Ordonez *et al.*, (2006).

Malondialdehyde (nanomol/ml)

The Mean \pm SE values of malondialdehyde were observed as 2.58 \pm 0.15 and 3.47 \pm 0.29 (nanomol/ml), respectively. There malondialdehyde level at post exercise was significant ($P\leq 0.05$) higher than that at pre exercise stage. Finding of present study are in agreement with the findings reported by McMeniman and Hintz (1992); Mills *et al.*, (1996); Chiaradia *et al.*, (1998); Hargreaves *et al.*, (2002); Marlin *et al.*, (2002) and Williams *et al.*, (2005) in horse. According to Tozzi-Ciancarelli *et al.*, (2002), strenuous exercise enhances accumulation of secondary products of lipid peroxidation in horses.

Table.1 Effect of exercise on biomarkers of oxidative stress

Biomarkers of Oxidative Stress	Pre Exercise n=20	Post Exercise n=20
	Mean \pm SE (Range)	Mean \pm SE (Range)
Catalase (nanomol/min/ml)	30.00 \pm 0.34 (26.66-32.51)	30.69 \pm 1.81 (0.840-42.12)
Superoxide Dismutase (U/ml)	2.38 \pm 0.26 (0.160-3.80)	2.75 \pm 0.21 (0.970-3.86)
Malondialdehyde (nanomol/ml)	2.58 \pm 0.15 ^b (1.07-3.50)	3.47 \pm 0.29 ^a (2.28-6.95)
Reduced glutathione (mg/dl)	1.85 \pm 0.089 (1.00-2.24)	2.05 \pm 0.020 (1.89-2.19)
Vitamin C (mg/liter)	3.16 \pm 0.20 (1.24-5.76)	2.68 \pm 0.17 (0.960-3.66)

Observations with different superscripts (a, b) differ significantly ($P\leq 0.05$) between pre and post exercise. (n=no of animal)

Reduced glutathione (mg/dl)

Pre and post exercise Mean \pm SE values of reduced glutathione were observed as 1.85 ± 0.089 and 2.05 ± 0.020 (mg/dl), respectively. Statistical analysis revealed a non-significant difference in the levels of reduced glutathione after exercise. It was in agreement with the findings of Leeuwenbergh and JI, (1995); Balogh *et al.*, (2001); Deaton *et al.*, (2002) and Siqueria *et al.*, (2014).

Vitamin C (mg/liter)

The Mean \pm SE values of vitamin C pre and post exercise were observed as 3.16 ± 0.20 and 2.68 ± 0.17 (mg/liter), respectively. There was no significant ($p < 0.05$) difference in the levels of vitamin C before and after exercise. Our findings were in concord with the findings of McMeniman and Hintz (1992); White *et al.*, (2001) and Dedar (2012) in horses.

It can be concluded that trot exercise of 30 kilometer have a significant ($P \leq 0.05$) effect of exercise on malondialdehyde levels only whereas the level of catalase, reduced glutathione and vitamin C revealed a non-significant effect.

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