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Evaluation of exercise-induced oxidative stress in horses

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Abstract
The present study aims to study the effect of exercise on oxidative stress in terms of variation in enzymatic and non-enzymatic antioxidants. Based on prior clinical examination and deworming, twenty healthy horses were selected and distributed equally in group I and group II. In the only group I a single event of trot exercise was given for two hours and the second group was kept as control without giving any exercise. Blood samples were collected from both groups and various enzymatic (Catalase (CAT), Superoxide Dismutase (SOD)) and non-enzymatic antioxidants (Reduced glutathione (GSH), and Vitamin C) were estimated in plasma. Statistical analysis of before and after exercise values of enzymatic antioxidants *viz.* catalase and superoxide dismutase and non-enzymatic antioxidants *viz.* reduced glutathione and vitamin c in group I revealed no significant change. A single episode of trot exercise failed to produce any effect of exercise in inducing oxidative stress in horses based on enzymatic and non-enzymatic antioxidants activity.

Keywords: Exercise, oxidative stress, enzymatic, antioxidants

Introduction
Horses possess the amazing ability and endurance to perform running, jumping, and other physical activities better in comparison to other animals of similar body size. Pack, riding, chariot, war, race, and even plowing horses were frequently mentioned in the Vedic age (1500–1000 BC).

Oxidative stress is an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions (Sies, 1997) [24]. Normally, there is a balance between free radicals and antioxidant defense systems. But, if several free radicals exceed the capacity of the antioxidant defense systems for various reasons it may lead to oxidative stress. Lipids, proteins, and DNA components of the cell may undergo oxidative damage due to free radicals (Halliwell and Chirico, 1993) [12]. Free radicals can be defined as reactive chemical species having a single unpaired electron in an outer orbit (Riley 1994) [23]. Free radicals include hydroxyl (OH•), superoxide(O_2•$^-$), nitric oxide (NO•), nitrogen dioxide (NO_2•), peroxyl (ROO•), and lipid peroxyl (LOO•). Hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH), are non-free radicals, but can easily lead to free radical reactions in living organisms (Genestra, 2007) [10].

Antioxidants act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, an enzyme inhibitors, synergists, and metal-chelating agents. Living organisms have developed non-enzymatic (Glutathione, Vit C, and Vit E) and enzymatic (Superoxide Dismutase and Catalase) complex antioxidant defense systems against reactive oxygen species (Onmaz *et al.*, 2011) [20]. Superoxide dismutase (SOD) dismutates superoxide (O_2•$^-$) and catalase detoxifies H_2O_2. Reduced glutathione can neutralize oxidants and provides first-degree protection against oxidants by acting as the cofactor for several protective enzymes. Vitamin C is located in the aqueous phase of cells, it scavenges radicals and recycles reduced forms of Vit E (Pham-Huy, 2008) [21]. Antioxidant enzymes including glutathione peroxidases, glutathione reductase (GR), superoxide dismutase (SOD), and catalase are used as measures of antioxidant status and ROS production (Maral *et al.*, 1977) [18]. In horses oxidative stress is the phenomenon associated with exercise, environmental oxidants, decrease metabolism, inflammation, or deficiency of antioxidant capacity.

In equine respiratory research, ozone exposure has been shown to induce oxidative stress (Deaton *et al*., 2005) [4]. There is a linear relationship between speed and oxygen uptake in horses during exercise. However, in horses, the maximal oxygen uptake can increase up to 30 to 40 folds within 60 seconds of the onset of exercise (Dermans and Noakes, 1994) [7]. Due to the dense capillary network of regularly trained muscles and high oxygen uptake, the oxygen flux in the active peripheral skeletal muscle fibers may increase up to100-fold during exercise. It has been estimated that 1 to 5% of inhaled oxygen in aerobes will form ROS (Halliwell and Gutteridge, 2007) [13]. Different studies revealed the importance of oxidative stress in disease development and incidence of malignancies and autoimmune disorders, and increase susceptibility to bacterial, viral, and parasitic diseases (Rahal *et al*., 2014) [22]. An increase in oxygen utilization during exercise results in a proportional increase in reactive oxygen species (ROS) production, thus potentially causing oxidative stress. A state where the increased generation of ROS overwhelms body antioxidant protection results in lipid, protein, and DNA damage.

In the present study, the role of exercise in inducing oxidative stress in horses was estimated on the basis of variation recorded in the values of enzymatic and non-enzymatic antioxidants.

Materials and Methods

Equine farm established in Jaipur was selected to carry out the planned study. With the consent of the owner, twenty clinically healthy adult nonpregnant mares kept in similar environment, housing, and feeding were selected and distributed equally into group I and group II. Only group I was given 30 kilometers of single episode of trot exercise for two hours to study the exercise-induced oxidative stress and group II was used as control.

All laboratory work was done in the department of veterinary medicine, P.G.I.V.E.R, Jaipur and N.R.C.E, Bikaner (Rajasthan). Blood samples were collected in heparinized tubes was centrifuged at 3000 rpm for 30 minutes, to separate the plasma for the proposed experiment**.** The sample collected from each horse was marked and aliquoted into different microcentrifuge tubes, which were later stored in a deep freezer at -20°C. Various enzymatic and nonenzymatic antioxidants parameters *viz*. Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and Vitamin C. CAT and SOD were estimated in plasma samples by "Catalase Assay kit" (Catalog No.707002) ,"Superoxide Dismutase Assay kit" (Catalog No.706002) from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbour, MI 48108, USA. GSH and Vitamin C were determined by using the method developed by Beutler *et al.* (1971) [3] and Denson and Bowers (1961) [6]. The statistical analysis of all observations was done as per methods described by Snedecor and Cochran (1994) [25]**.**

Results and Discussion

Mean values and ranges of plasma catalase, superoxide dismutase, reduced glutathione and vitamin c obtained in the study were found more or less similar to Ganaie (2012) [9], and White *et al.* (2001) [26]. Statistical analysis of values of enzymatic antioxidants *viz*. catalase, and superoxide dismutase and non-enzymatic antioxidants *viz*. reduced glutathione and vitamin c in group I increased after exercise, but not significantly. No variation in these values was observed in group II also, which was taken as control. The results obtained in the present study were supported by various authors McMeniman and Hintz (1992) [19]; Ji (1995); Krumrych (2005) [15]; Andriichuk *et al.* (2013) [2] and Andriichuk *et al.* (2014) [1] reported no effect of exercise on catalase and SOD activity. Catalase is a cytoplasmic protein that catalyses the reduction of hydrogen peroxide in the body, converting it to oxygen and water (Guemouri *et al*., 1991) [11]. GSH is present intracellularly at concentrations typically from 2 to 8 mM, while negligible amounts are found in plasma. Leeuwenbergh and JI (1995) observed that the values of plasma GSH remain relatively stable during prolonged exercise at moderate intensity. The present results were in accordance with other studies (McMeniman and Hintz, 1992; White *et al.*, 2001; Deaton *et al.*, 2002) [5, 26, 19] that reported no effect of race on plasma ascorbate values.

Mean ± SE of values of catalase, superoxide dismutase, reduced glutathione and vitamin c in the group I and group II are presented in Table 1.

Table 1: Mean ± SE of values of enzymatic and non-enzymatic antioxidants in horses.

	Group I (n=10)		Group II (n=10)	
	Before exercise	**After two hours of exercise**	**Before exercise**	**After two hours of without exercise**
Enzymatic antioxidants				
CAT (nmol/min/ml)	32.77±0.88	34.56±1.18	31.41±1.11	31.55±0.95
SOD /(U/ml)	3.10±0.36	3.24±0.36	3.37±0.27	3.38±0.27
Non-enzymatic antioxidants				
GSH (mg/dl)	2.40±0.10	2.41±0.11	2.53±0.09	2.53±0.09
Vit C (mg/l)	2.39±0.26	2.23±0.28	2.45±0.23	2.46±0.22

Conclusion

The event of exercise given to horses only once, did not produce exercise-induced oxidative stress on the basis of enzymatic and non-enzymatic parameters estimated in the present study. However, future research could be done by experimenting with different types of exercise and other oxidative stress parameters.

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