Assessment of Antioxidant Activity of *WithaniaSomnifera* in Equine

Research article

ABSTRACT

Aims:The present research was done to evaluate the antioxidant activity of aqueous extract of *Withaniasomnifera* (Ashwagandha) in equine. Herbal antioxidants have gained popularity due to their potency and safety, and oxidative stress is a condition that is more prevalent in horses performing physical activities. **Methods:**Twenty clinically healthy horses were selected and equally divided into treatment and control group consisting of 10 animals in each. An aqueous extract was prepared from dried root powder of *Withaniasomnifera* (Ashwagandha) and was continuously administered orally for 15 days to treatment group animals @ 100 mg/kg body weight. Blood samples were collected on 0^{th}, 8^{th}, and 16^{th} day from both the treatment and control group. The plasma was used for the determination of enzymatic oxidants (Catalase (CAT) and superoxide dismutase (SOD) and non-enzymatic antioxidants (reduced glutathione (GSH) and Vitamin C).
Results:A non-significant increase in the values of Catalase, Superoxide Dismutase (SOD), Reduced Glutathione (GSH), and Vitamin C were observed after administration of aqueous extract of *Withaniasomnifera.*
Conclusion: Administration of aqueous extract of *Withaniasomnifera* not significantly modulated the activity of enzymatic and non-enzymatic antioxidants.

Keywords- Horse, Aqueous extract, Withaniasomnifera, Antioxidants, Oxidative stress

1. INTRODUCTION

World Health Organization (WHO) has emphasized the use of medicinal plants as these are considered safe due to their less toxicity, lesser side effects, and being organic andeffective than syntheticdrugs. Oxidative stress is quite common in human and veterinary medicine as it gives rise to the generation of free radicals leading to oxidative damage of body cells and the development of various diseases. in the last few years, several kinds of researches were especially emphasized strengthening the antioxidant defense system of the body. Alternative use of plants as a source of antioxidants was done due to their free radical quenching properties.

"Oxidative stress occurs as the cellular injury and pathologic change that occurs when there is an imbalance favoring oxidants over antioxidants within a living organism. The evaluation of oxidative stress in the horse has been limited primarily to the ischemia-reperfusion injury of the gastrointestinal tract, recurrent airway obstruction, exercise, osteoarthritis, endometritis, equine motor neuron disease, and pituitary pars intermedia dysfunction" (Soffler, 2007). "Physical activities aremore common in horses and 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"(Kinnuenen*et al.*, 2005). "The free radical formation is controlled naturally by various beneficial compounds known as an antioxidant. Antioxidants are substances that can delay or inhibit oxidation. There are several defenses developed in aerobic organisms, which include enzymatic and non-enzymatic antioxidants, that are usually effective in blocking harmful effects of reactive oxygen species. Enzymatic defense is a system of enzymes that includes glutathione peroxidases, superoxide dismutases, and catalase, which decrease the concentration of the most harmful ROS whereas non-enzymatic antioxidants include low molecular

weight compounds such as vitamins C and E, β-carotene, uric acid, and glutathione"(Ozougwu, 2016). "Superoxide dismutase (SOD) is the first step enzyme in the detoxification of oxygen radicals catalyzing the conversion of O_2-, the first product of ROS, to H_2O_2. The increase in SOD enzyme activity corresponds with enhanced resistance to oxidative stress. Catalase is a ubiquitous enzyme found in all organisms having a dual function i.e., decomposition of H_2O_2 to give $H_2O$and O_2. Reduced glutathione (GSH) is a tripeptide and peptide containing glycine (Glu-Cys-Gly). It is the most abundant intracellular thiol, present in virtually every animal cell in millimolar concentrations. GSH plays a key role in antioxidant protection and directly scavenges ROS or enzymatically via glutathione peroxidases and glutathione transhydrogenase. GSH also playsa central role in enhancing antioxidant defenses by regenerating crucial exogenous antioxidants such as Vitamin C and E from their oxidized form"(Lappalainen and Atalay, 2009).

"Herbal plants contain antioxidant compounds which protect cells against degenerative effects of Reactive Oxygen Species (ROS) which is a free radical such as singlet oxygen, superoxide, peroxyl, radicals, and hydroperoxyl radicals" (Aruoma and Cuppett, 1997).*Withaniasomnifera* (Ashwagandha, Family- Solanaceae) popularly known as Indian ginseng/ winter cherry, is an important herb in Ayurveda and indigenous medicinal system for over 3000 years to treat various disorders. In Sanskrit, ashwagandha, the Indian name for *Withaniasomnifera*, means "odor of the horse", probably originating from the odor of its root which resembles that of a sweaty horse. The herb is termed as "Rasayana" in Ayurvedic practice, which means it acts as a tonic for vitality and longevity (Singh *et al*., 2010). "*Withaniasomnifera* has been known for its potent antioxidant and free radical quenching properties in various conditions. The antioxidant effects of *Withaniasomnifera* depend on the presence of steroidal lactones and withanolides, which are the main active components (2.8%)" (Dhuley,1998). As an antioxidant, *Withaniasomnifera*and its active constituents (sitoindoside vii-x and withaferin A) have been proven to increase enzymatic antioxidants i.e. catalase and superoxide dismutase (Bhattacharya *et al*., 2001). The root of*Withaniasomnifera*is a good source of nonenzymatic and enzymatic antioxidant components. *Withaniasomnifera* was evaluated and assayed for enzymatic and non-enzymatic antioxidants. The non-enzymatic potentials (ascorbic acid, reduced glutathione, and tocopherol) and enzymatic potential (superoxide dismutase, ascorbate peroxidase, catalase, and peroxidase) (Jaleel, 2009).

The proposed study aims at investigating the antioxidant activity of the aqueous extract of *Withaniasomnifera*on the biomarkers of oxidative stress (enzymatic and non-enzymatic antioxidants).

2. MATERIAL AND METHODS

The location where the study was performed was a private equine farm and all parameters were estimated in the Department of Veterinary Medicine, P.G.I.V.E.R, Jaipur, and National Research Centre on Equine (NRCE),Bikaner, Rajasthanfrom 01-01-2017 to 31-5-2017.Twenty clinically healthy horses (8-10 years of age) were selected and divided equally into treatment and control group. All the clinically healthy horses taken for this study were allowed to access the same type of environment, housing, and feed throughout the research. To study the antioxidant activity of aqueous extract of *Withaniasomnifera*(Ashwagandha) in horses, the roots of ashwagandha were dried in the laboratory in the incubator at 40°C to remove excess moisture and then grounded into powder form. An aqueous extract was prepared using the Soxhlet apparatus (hot extraction method)as proposed by Handa*et al.* (2008).For administration, an aqueous extract prepared from roots of *Withaniasomnifera*(Ashwagandha)was diluted with distilled water and given orally @ 100 mg/kg body weight daily, once a day in the morning, and was given continuously for 15 days to all animals belonging to treatment group. Blood samples were collected on 0^{th}, 8^{th}, and 16^{th} day after daily administration of aqueous extract of *Withaniasomnifera*(Ashwagandha).

The plasma was used for the determination of enzymatic oxidants such as Catalase (CAT), andsuperoxide dismutase (SOD). Non-enzymatic antioxidants estimated in the study were reduced glutathione (GSH), and Vitamin C. Catalase was determined in plasma samples by "Catalase Assay kit" (Catalog No.707002) and Superoxide Dismutase by "Superoxide Dismutase Assay kit" (Catalog No.706002) from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbour, MI 48108, USA. Estimation of reduced glutathione and Vitamin C was conducted by the method developed by Beutler*et al.* (1971)) and Denson and Bowers (1961) respectively.

The statistical analysis of collected observations will be done as per methods described by Snedecor and Cochran (1994).

3. RESULTS AND DISCUSSION

Mean ± SE values of biomarkers of oxidative stress on 0^{th}, 8^{th} and 16^{th} days in the treatment and control group are presented in Table 1.

`Table 1: Mean ± SE values of biomarkers of oxidative stress on 0^{th}, 8^{th} and 16^{th} days in the treatment and control group

Biomarkers of Oxidative Stress	Group	Days of sampling		
		0^{th} day	**8^{th} Day**	**16^{th} Day**
		Mean±SE (Range)	Mean±SE (Range)	Mean±SE (Range)
Enzymatic antioxidant				
CAT (nmol/min/ml)	Treatment	32.77±0.88 (26.98-35.6)	33.18±1.01 (27.30-36.48)	33.39±0.10 (27.84-38.01)
	Control	31.41±1.11 (25.11-35.98)	31.98±0.80 (28.34-35.91)	32.05±1.25 (25.51-38.73)
SOD (U/ml)	Treatment	3.10±0.36 (1.25-4.98)	3.21±0.35 (1.58-5.74)	3.64±0.42 (1.25-6.15)
	Control	3.37±0.27 (2.34-5.17)	3.20±0.25 (1.66-4.43)	3.07±0.32 (1.09-5.14)
Non-Enzymatic antioxidant				
GSH (mg/dl)	Treatment	2.40±0.10 (2.03-3.17)	2.44±0.13 (1.98-3.45)	2.54±0.16 (2.14-3.8)
	Control	2.53±0.091 (2.21-3.04)	2.48±0.09 (2.16-2.98)	2.43±0.08 (2.12-2.89)
Vit C (mg/l)	Treatment	2.39±0.26 (1.08-3.52)	2.51±0.28 (1.09-3.81)	2.64±0.26 (1.57-4.04)
	Control	2.45±0.23	2.34±0.22	2.32±0.25

		(1.48-3.36)	(1.06-3.26)	(1.25-3.33)

The values of plasma Catalase (CAT), Superoxide Dismutase (SOD), Reduced Glutathione (GSH), and Vitamin C were recorded on 0^{th}, 8^{th} and 16^{th} days in the treatment and control group. A non-significant increase in the values of Catalase, Superoxide Dismutase (SOD), Reduced Glutathione (GSH), and Vitamin C was observed on 8^{th} and 16^{th} days in the treatment group after administration of an aqueous extract of *Withaniasomnifera*, with respect to control group.Similarly, a non-significant increase was observed in these parameters within the treatment group on 8^{th} and 16^{th} days.

Non-significant effect on CAT and SOD activity was observed on treatment with *Withaniasomnifera* root extract as compared to untreated animals (Sharma *et al.,* 2011) supported the present study. However, a significant increase in the plasma CAT and SOD activity was observed by Das *et al.* (2010) and El-Sabbagh *et al*. (2022)reporting that the herb possesses antioxidant properties, and administration of an aqueous root extract of *Withaniasomnifera* for 25 days increased the values of CAT and SOD in dehydration-induced oxidative stress-related uremia in male rats.The results observed in the present study for the values of Reduced Glutathione (GSH), and Vitamin C was found similar to Rasool andVaralaxmi (2008) reported no effect on glutathioneand vitamin C values in the control group administered with *Withaniasomnifera* as compared to control group without administration.After treatment with *Withaniasomnifera*, values of catalase, superoxide dismutase, reduced glutathione, and Vit C modulatednear to the control group values in the present study.

4. CONCLUSION

Administration of aqueous extract of *Withaniasomnifera*modulated the activity of enzymatic and non-enzymatic antioxidants, but not significantly. It is suggested that there is a further scope of studying the antioxidative and ergogenic effect of *Withaniasomnifera* by using its other extract (ethanolic and methanolic) on body tissues along with plasma of already stressed or diseased horses on to observe its effect on enzymatic and non-enzymatic antioxidants.

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COMPETING INTEREST

The author declares that they have no known competing interest.

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