



Assessment of Antioxidant Activity of *Withania somnifera* in Equine

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2023/v29i81775

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/103617>

Original Research Article

Received: 18/05/2023

Accepted: 21/07/2023

Published: 28/07/2023

ABSTRACT

Aims: The present research was done to evaluate the antioxidant activity of aqueous extract of *Withania somnifera* (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 *Withania somnifera* (Ashwagandha) and was continuously administered orally for 15 days to treatment group animals @ 100 mg/kg body weight. Blood samples were collected on 0th, 8th, and 16th 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).

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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 *Withania somnifera*.

Conclusion: Administration of aqueous extract of *Withania somnifera* not significantly modulated the activity of enzymatic and non-enzymatic antioxidants.

Keywords: Horse; aqueous extract; *Withania somnifera*; 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 and effective than synthetic drugs. 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” [1]. “Physical activities are more 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” [2]. “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” [3]. “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 plays a central role in enhancing antioxidant defenses by regenerating crucial exogenous antioxidants such as Vitamin C and E from their oxidized form” [4].

“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, peroxy, radicals, and hydroperoxy radicals” [5]. *Withania somnifera* (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 *Withania somnifera*, 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 [6]. “*Withania somnifera* has been known for its potent antioxidant and free radical quenching properties in various conditions. The antioxidant effects of *Withania somnifera* depend on the presence of steroidal lactones and withanolides, which are the main active components (2.8%)” [7]. As an antioxidant, *Withania somnifera* and its active constituents

(sitoindoside vii-x and withaferin A) have been proven to increase enzymatic antioxidants i.e. catalase and superoxide dismutase [8]. The root of *Withania somnifera* is a good source of nonenzymatic and enzymatic antioxidant components. *Withania somnifera* 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) [9].

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

2. MATERIALS 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, Rajasthan from 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 *Withania somnifera* (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. [10]. For administration, an aqueous extract prepared from roots of *Withania somnifera* (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 0th, 8th, and 16th day after daily administration of aqueous extract of *Withania somnifera*(Ashwagandha).

The plasma was used for the determination of enzymatic oxidants such as Catalase (CAT), and superoxide 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 Arbor, MI 48108, USA. Estimation of reduced glutathione and Vitamin C was conducted by the method developed by Beutler et al. [11] and Denson and Bowers [12] respectively.

The statistical analysis of collected observations will be done as per methods described by Snedecor and Cochran [13].

3. RESULTS AND DISCUSSION

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

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

Non-significant effect on CAT and SOD activity was observed on treatment with *Withania somnifera* root extract as compared to untreated animals [14] supported the present study. However, a significant increase in the plasma CAT and SOD activity was observed by Das et al. [15] and El-Sabbagh et al. [16] reporting that the herb possesses antioxidant properties, and administration of an aqueous root extract of *Withania somnifera* 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 and Varalaxmi [17] reported no effect on glutathione and vitamin C values in the control group administered with *Withania somnifera* as compared to control group without administration. After treatment with *Withania somnifera*, values of catalase, superoxide dismutase, reduced glutathione, and Vit C modulated near to the control group values in the present study.

Table 1. Mean \pm SE values of biomarkers of oxidative stress on 0th, 8th and 16th 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 \pm SE (Range)	Mean \pm SE (Range)	Mean \pm SE (Range)
Enzymatic antioxidant				
CAT (nmol/min/ml)	Treatment	32.77 \pm 0.88 (26.98-35.6)	33.18 \pm 1.01 (27.30-36.48)	33.39 \pm 0.10 (27.84-38.01)
	Control	31.41 \pm 1.11 (25.11-35.98)	31.98 \pm 0.80 (28.34-35.91)	32.05 \pm 1.25 (25.51-38.73)
SOD (U/ml)	Treatment	3.10 \pm 0.36 (1.25-4.98)	3.21 \pm 0.35 (1.58-5.74)	3.64 \pm 0.42 (1.25-6.15)
	Control	3.37 \pm 0.27 (2.34-5.17)	3.20 \pm 0.25 (1.66-4.43)	3.07 \pm 0.32 (1.09-5.14)
Non-Enzymatic antioxidant				
GSH (mg/dl)	Treatment	2.40 \pm 0.10 (2.03-3.17)	2.44 \pm 0.13 (1.98-3.45)	2.54 \pm 0.16 (2.14-3.8)
	Control	2.53 \pm 0.091 (2.21-3.04)	2.48 \pm 0.09 (2.16-2.98)	2.43 \pm 0.08 (2.12-2.89)
Vit C (mg/l)	Treatment	2.39 \pm 0.26 (1.08-3.52)	2.51 \pm 0.28 (1.09-3.81)	2.64 \pm 0.26 (1.57-4.04)
	Control	2.45 \pm 0.23 (1.48-3.36)	2.34 \pm 0.22 (1.06-3.26)	2.32 \pm 0.25 (1.25-3.33)

4. CONCLUSION

Administration of aqueous extract of *Withania somnifera* 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 *Withania somnifera* by using its other extract (ethanolic and methanolic) on body tissues along with plasma of already stressed or diseased horses to observe its effect on enzymatic and non-enzymatic antioxidants.

ACKNOWLEDGEMENT

We all are highly thankful to Department of Veterinary Medicine, Post Graduate Institute of Veterinary Education and Research, Jaipur, National Research Centre on Equines, Bikaner, and Department of Animal Nutrition, Post Graduate Institute of Veterinary Education and Research, Jaipur, Rajasthan for conducting trial and research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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