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## The efficacy and safety of aqueous extract of *Withania somnifera* (Ashwagandha) on biochemical parameters in horses

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### Abstract

Ashwagandha was widely used as health benefactor since ancient times. The present research was undertaken to study the effect of an aqueous extract of *Withania somnifera* on biochemical parameters of apparently healthy horses. Twenty apparently healthy non-pregnant females of approximately equal age group (8-10 years) were divided into control and treatment group. The treatment group was daily administered orally with an aqueous extract of *Withania somnifera* @ 100 mg/kg body weight for 15 days. Blood samples from both groups of animals were collected on 0<sup>th</sup>, 8<sup>th</sup> and 16<sup>th</sup> day. Biochemical parameters (ALT, AST, GGT, total protein, albumin, creatinine, BUN, total bilirubin, and direct bilirubin) were recorded. Non-significant change in the values of biochemical parameters was observed on 8<sup>th</sup> and 16<sup>th</sup> days in the treatment group after administration of an aqueous extract of *Withania somnifera*, with respect to control group. There was no effect of administration of an aqueous extract of *Withania somnifera* for 15 days on biochemical parameters determined in the present study. Aqueous extract of *Withania somnifera* did not alter the normal level of biochemical parameters in the horses proving that it is non-toxic and fit for administration.

**Keywords:** Aqueous extract, *withania somnifera*, biochemical parameters

### Introduction

Plants are one of the most important sources of medicines in the world (Dasgupta and De, 2004) [2]. *Withania somnifera* (WS) belongs to the Solanaceae family, commonly known as Ashwagandha, Indian ginseng, and Winter cherry. Ashwagandha in Sanskrit means "horse's smell" probably originated from the odor of its root, which resembles the smell of horse urine. The traditional uses of 'Ashwagandha' are to increase energy, youthful vigor, endurance, strength, health, nurture the time elements of the body, increase vital fluids, muscle fat, blood, lymph, semen and cell production. *Withania somnifera* shows anti-inflammatory potential in the treatment of joint diseases and an appropriate remedy for asthma and bronchitis. It wildy grows in all drier parts of subtropical India and occurs in Madhya Pradesh, Uttar Pradesh, Punjab plains and northwestern parts of India like Gujarat and Rajasthan. Roots are 20-30 cm long and 6-12 mm in diameter, straight and are unbranched (John, 2014) [5]. It is in use for a very long time for all age groups, both sexes, and even during pregnancy without any side effects (Sharma *et al.*, 1985) [12].

*Withania somnifera* (Ashwagandha) possesses anti-inflammatory, adaptogenic, antitumor, antistress, antibacterial, liver tonic, antioxidative, immunomodulatory, hemopoietic, and astringent properties (Mishra *et al.*, 2000) [7]. Medicinal plant extracts possess antioxidant activities with a relatively high content of flavonoids. *Withania somnifera* possesses saponins, cardenolide, glycosides, polyphenol compounds, flavanoids, tannins, and reducing substances in its aqueous extract. Proteins and alkaloids were found absent in the extract (Attanayake *et al.*, 2016) [1]. Herbal medicine is usually considered safe and used widely in India for both human and veterinary medicine for the treatment of various diseases. Ashwagandha is one of the widely used shrubs preferred for its therapeutic efficacy, as it possesses an active chemical constituent as "withaferin" which has many biological activities. The present study was designed in order to investigate the safety and potency of aqueous extract of *Withania somnifera* (Ashwagandha) on various physiological and biochemical functions.

## Materials and Methods

This study was conducted on equines at a private equine farm and all laboratory work was performed in the Department of Veterinary Medicine, PGIVER, Jaipur and the National Research Centre on Equine (NRCE), Bikaner, Rajasthan. Deworming and clinical examination of all animals were done before commencement of research and twenty apparently healthy non-pregnant females of 8-10 years of age were selected. These horses were divided into control and treatment comprising 10 animals in each and kept under similar feeding, housing and environmental conditions.

To study the efficacy of *Withania somnifera*, an aqueous extract was prepared using the Soxhlet apparatus (hot extraction method) as proposed by Handa *et al.*, (2008) [13]. The aqueous extract prepared from the roots of *Withania somnifera* was dried and ground into powder form and stored into airtight polythene bags for the purpose of daily usage in the treatment trial. An aqueous extract of *Withania somnifera* @ 100 mg/kg body weight was diluted in sterile water and administered orally daily for 15 days in treatment group horses. Blood samples were collected from both groups on 0, 8 and 16<sup>th</sup> day. Plasma was separated from whole heparinized blood and was aliquoted into different micro-centrifuge tubes, which were then stored in a deep freezer at -20°C until the time of biochemical parameter analysis.

The blood samples were analyzed to estimate Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total Protein, Albumin, Blood Urea Nitrogen (BUN), Creatinine, and Bilirubin (total and direct). Estimation of ALT, AST, Total Protein, Albumin, and Bilirubin was done by using kits manufactured by SPINREACT, S.A./S.A.U. Ctra. Santa Coloma, 7-E-17176 SANT ESTEVE DE BAS-(GI) SPAIN. Estimation of Blood Urea Nitrogen (BUN) and Creatinine was done using kits manufactured by TRANSASIA BIO-MEDICALS LTD., Nalagarh Road, Village Malpur, Baddi, Dist. Salon, HP.

The statistical analysis of collected observations will be done as per the methods described by Snedecor and Cochran (1994) [14].

## Results and Discussion

Mean  $\pm$  SE values of biochemical parameters in the treatment and control group 0<sup>th</sup>, 8<sup>th</sup> and 16<sup>th</sup> days are presented in Table 1 and Figures i and ii. Mean values of biochemical parameters observed in the present study were found within the normal

range as given by Radostits *et al.* (2009) and Simenew *et al.* (2011) [10, 13].

A non-significant decrease in the values of ALT, AST, GGT, BUN and creatinine were observed on 8<sup>th</sup> and 16<sup>th</sup> days in the treatment group after administration of an aqueous extract of *Withania somnifera*, with respect to control group. Similarly, the non-significant decrease was observed within the treatment group on 16<sup>th</sup> day. The findings of this study are also augmented by the earlier researcher (Kumari *et al.*, 2015; Raut *et al.*, 2012 and Patel *et al.*, 2016) [6, 11, 8] reported no significant effect of *Withania somnifera*. Harikrishnan *et al.* (2008) [4] described hepatoprotective nature of root powder of *W. somnifera* and the study revealed that rats treated with *W. somnifera* @500 mg/kg thrice in a week for 8 consecutive weeks, showed significantly low levels of circulatory ammonia, urea, TBARS, AST, ALT, and ALP when compared with the corresponding group in which hyperammonemia condition was induced. Hepatoprotective nature of the herb is due to the presence of alkaloids, phenolic compounds withanolides, and flavonoids. A non-significant increase in the values of total protein and albumin was observed on 8<sup>th</sup> and 16<sup>th</sup> days in the treatment group after administration of an aqueous extract of *Withania somnifera*, with respect to the control group. Similarly, a non-significant increase was observed within the treatment group on 8<sup>th</sup> and 16<sup>th</sup> days. The findings of our study agree with the results of Prabhu *et al.* (2013) and Patel *et al.* (2016) [8] reported non-significant change in these values after administration of ashwagandha. Values of total bilirubin and direct bilirubin did not vary significantly on the 8<sup>th</sup> and 16<sup>th</sup> days in the treatment group, with respect to the control group. Similarly, a non-significant change was observed within the treatment group on 8<sup>th</sup> and 16<sup>th</sup> days. Non-significant change in total bilirubin after administration was found similar to Patel *et al.* (2016) [8]. Dose-related tolerability, safety and activity of *Withania somnifera*. In apparently healthy volunteers was done by giving *W. somnifera* capsules (aqueous extract 8:1) daily in two divided dose with increase in daily dosage every 10 days for 30 days. Clinical, haematological and biochemical examination of all volunteers revealed no clinical abnormality in clinical examination and all haematological and biochemical parameters revealed no significant change and all the parameters were found within the normal range. Thus, the formulation was found to be safe on biochemical organ function tests (Raut *et al.*, 2012) [11].

**Table 1:** Mean  $\pm$  SE values of biochemical parameters before and after administration of an aqueous extract of *Withania somnifera*

Biochemical parameters	Group	Days of Sampling		
		0 <sup>th</sup> day	8 <sup>th</sup> Day	16 <sup>th</sup> Day
		Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
		(Range)	(Range)	(Range)
ALT(U/L)	Treatment	276.05 $\pm$ 15.90 (231.45-367.27)	273.89 $\pm$ 14.57 (230.41-351.44)	268.91 $\pm$ 14.06 (224.87-338.02)
	Control	283.51 $\pm$ 15.91 (214.18-354.28)	285.32 $\pm$ 17.85 (208.76-366.16)	287.36 $\pm$ 19.15 (215.89-373.92)
AST(U/L)	Treatment	16.70 $\pm$ 0.989 (12.55-22.12)	16.71 $\pm$ 1.10 (11.58-24.18)	15.97 $\pm$ 0.10 (11.65-22.05)
	Control	16.35 $\pm$ 1.44 (10.31-24.06)	16.84 $\pm$ 1.32 (12.12-25.44)	17.56 $\pm$ 1.31 (12.54-26.12)
GGT(U/L)	Treatment	22.89 $\pm$ 1.47 (16.61-29.76)	22.04 $\pm$ 1.18 (17.78-27.67)	20.85 $\pm$ 1.11 (17.41-27.89)
	Control	20.50 $\pm$ 1.29 (15.12-28.49)	21.98 $\pm$ 1.09 (16.60-28.99)	21.77 $\pm$ 1.02 (16.98-28.98)
Total Protein (g/dl)	Treatment	5.97 $\pm$ 0.288 (4.88-7.43)	5.98 $\pm$ 0.31 (5.02-7.63)	6.00 $\pm$ 0.29 (5.10-7.81)
	Control	5.82 $\pm$ 0.29	5.92 $\pm$ 0.27	5.87 $\pm$ 0.27

		(4.62-7.45)	(4.98-7.47)	(4.88-7.4)
Albumin (g/dl)	Treatment	3.19±0.18 (2.18-4.07)	3.25±0.14 (2.49-4.10)	3.41±0.17 (2.09-3.84)
	Control	3.33±0.17 (2.41-4.21)	3.17±0.17 (2.01-3.84)	3.18±0.19 (2.47-4.38)
BUN (mg/dl)	Treatment	23.14±0.94 (18.57-27.14)	22.93±0.85 (17.14-26.43)	22.79 ±0.94 (17.86-27.14)
	Control	23.43±0.84 (18.57-26.43)	23.14±0.80 (19.29-26.43)	23.36±1.04 (17.14-26.43)
Creatinine (mg/dl)	Treatment	1.67±0.17 (0.91-2.23)	1.64±0.16 (0.84-2.18)	1.61±0.16 (0.89-2.26)
	Control	1.65±0.21 (0.41-2.36)	1.70±0.21 (0.51-2.55)	1.68±0.20 (0.58-2.30)
Total Bilirubin (mg/dl)	Treatment	1.16±0.09 (0.78-1.66)	1.15±0.07 (0.88-1.45)	1.18±0.08 (0.84-1.55)
	Control	1.12±0.09 (0.61-1.49)	1.18±0.08 (0.88-1.70)	1.15±0.08 (0.82-1.64)
Direct Bilirubin (mg/dl)	Treatment	0.53±0.05 (0.32-0.77)	0.48±0.05 (0.32-0.82)	0.55±0.04 (0.35-0.79)
	Control	0.49±0.04 (0.36-0.74)	0.54±0.04 (0.40-0.76)	0.55±0.04 (0.37-0.71)

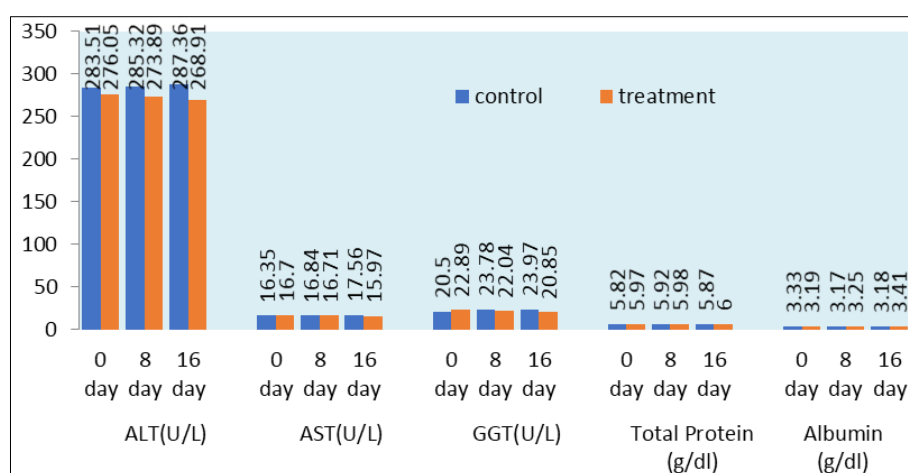


Fig 1: Mean values of biochemical parameters after administration of an aqueous extract of *Withania somnifera*

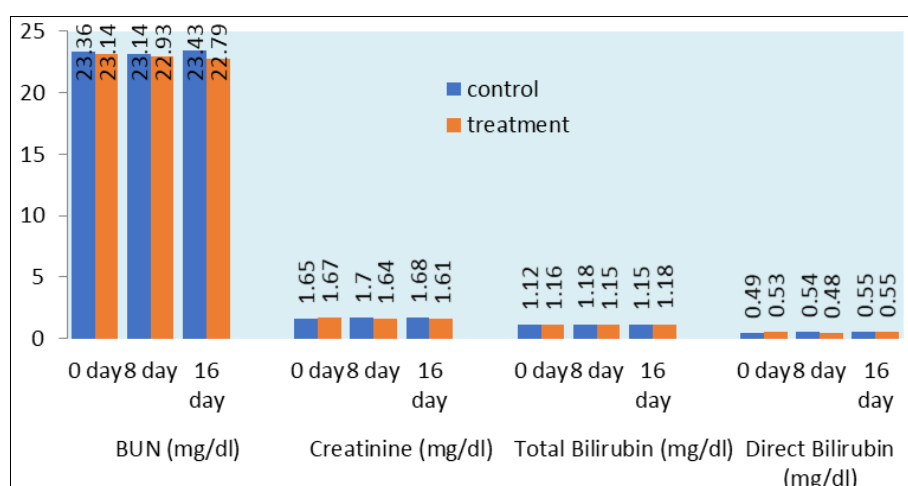


Fig 2: Mean values of biochemical parameters after administration of an aqueous extract of *Withania somnifera*

### Conclusion

An aqueous extract of *Withania somnifera* did not alter the normal limits of biochemical parameters and remained in the normal range after administration. The study concludes that aqueous extract of *Withania somnifera* did not produce any side-effect on the normal physiological functions of the body and the study proves that it is non-toxic and fit for administration based on parameters used in the study.

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