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Efficacy of Ashwagandha (withania somnifera [l.] Dunal) in improving cardiorespiratory endurance (VO2 max test) in healthy subjects

Bargale Sushant Sukumar*[1], Tripathy T B[1], Shashirekha H K[2], Suhas Kumar Shetty[3]

[1]Department of Swasthavritta, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan 573201, Karnataka, India
[2]Department of Samhita Sanskrit, Faculty of Ayurveda, Institute of medical science, Banaras Hindu University, Varanasi, Uttar Pradesh 221005, India
[3]Department of Manasa Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan 573201, Karnataka, India

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ABSTRACT

In Ayurveda, certain herbal formulas are considered to be Rasayana and they are typically taken over periods of time to regenerate both brain and body tissue. Ashwagandha (Withania Somnifera) is used as an adaptogen, antioxidant, immune modulator, free radical scavenger, anti stress, anti arthritic, antispasmodic, anti inflammatory, nervous tonic, nerve soothing and anticancer agent. Ashwagandha (WS) as a nutritional supplement is yet too established. Maximum oxygen uptake (VO2 max) is a gold standard of cardiopulmonary and muscle cell fitness is considered. The study evaluated the efficacy of Ashwagandha to improve cardiorespiratory endurance (VO2 max) in healthy subjects. They randomized single blind controlled comparative clinical study. 54 health volunteers in each group, study group received Ashwagandha Choorna 12gm with milk (200ml) empty stomach in the morning and the control group only milk (200ml). Maximal capacity of oxygen intake in ml/kg/min (VO2 max) with Rockport fitness walking test of both study and control group were measured before intervention (0^{th} day), after the intervention (60^{th} day) and follow up (90^{th} day). A significant improvement in the VO2 max (F=20.675, P <0.0001) and Hemoglobin (X^2=74.150 P <0.0001) in the study group was found. Supplementation of Ashwagandha (Withania Somnifera) with milk improve hemoglobin and VO2 max (maximum aerobic capacity).

*Corresponding Author

Name: Bargale Sushant Sukumar
Phone: +91 9036524877
Email: dr.sushant99@gmail.com

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INTRODUCTION

Ayurveda is widely used in India as a primary health care system and is flourishing in the promotion of health worldwide. Ayurveda aims to make society happy, healthy and peaceful. In general, the simple regimes mentioned in Ayurveda texts are of immense use faced by the present world today regarding health promotion (Sharma, 1999). As a society, our physical appearance is of growing importance to us. In developing countries, for example, as many as 24 percent of individuals admittedly exercise to enhance their performance. Professional men and amateurs are constantly searching for new ways that will help them to produce better results

in less time. Among means, dietary supplements belong to a prominent position. However, manufacturers also market goods which are neither evidence based nor safe for use in sport. This makes the use of herbal supplements to be irrational, which often leads to unwanted side effects but is more frequently of no use (Končić, 2018; Sangwan *et al.*, 2004). In traditional Indian systems of medicine, one of the most common plants is Ashwagandha (Withania Somnifera Dunal), also referred to as winter cherry (Bhandari, 1970).

Ashwagandha (WS) has the property of Rasayana as mentioned in literature. It not only maintains the equilibrium of Dosha and Dhatu of the body but also promotes health. Ashwagandha is Vatakaphahara, Jara Vyadhi Nashaka, Balya and Dhatu Vriddhikara properties. Because of these properties, it shows not only preventive but also promotive and curative effects as well as slowdown Jara by breaking the pathogenesis. Ashwagandha (WS) maintains the positive health, enhance the quality of life, cures morbid sleep, drowsiness, physical and mental fatigue, laziness weakness, and preserves youthness. In Ayurveda, Unani and Siddha, this plant is used in more than 200 formulations. One of the prime drugs of Ashwagandha is Medica Ayurveda Material (Chunekar and Pandey, 2010; Sharma *et al.*, 2001; Mishra *et al.*, 2000).

Physical fitness is defined as a degree to execute a physical activity under different environmental conditions. It has five components aerobic capacity, muscular endurance, flexibility and body composition. The gold standard of cardiopulmonary and muscle cell fitness is considered to be maximum oxygen intake. The maximum oxygen uptake (VO2 max) is the highest oxygen consumption rate that can be reached during maximum or exhaustive exercises (Setty *et al.*, 2013).

Objective

The study evaluated the efficacy of Ashwagandha to improve cardiorespiratory endurance (VO2 max test) in healthy subjects.

MATERIALS AND METHODS

This is randomized, single blind controlled comparative study with pretest and post test design was approved by Institutional Ethics committee Sri Dharmasthala Manjunatheshwara College of Ayurveda and hospital, Hassan, Karnataka, India (IEC No: SDM/IEC/92/2016-2017 dt.25 June 2016) and the study was conducted in compliance with good clinical practice guidelines, declaration of Helsinki and all other applicable regulations. CTRI no- 2019/05/019009.

Source of data

Apparently healthy subjects from OPD of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan either gender who are fulfilling the criteria of inclusion are selected irrespective of gender and caste etc.

Method of collection of data (including sampling procedure, if any) Survey

408 apparently healthy subjects were screened for the features of Arogya (health). 108 apparently healthy subjects were selected by considering the inclusion criteria.

Criteria for selection of subjects

Inclusion criteria

Apparently Healthy subjects who were interested and willing to take Ashwagandha (Withania Somnifera) and subjects of age group 18-40 yrs.

Exclusion criteria

Pregnant women, lactating women etc.

Duration of the study

60 days for both study and control groups.

Follow up

After one month of completion on intervention.

Intervention

Randomly divided into two groups, Group 1 (control group) and Group 2 (study group).

Group 1

In 54 subjects administered 2 Haritaki tablets for Kostha Shuddhi (proper cleansing of stool) with hot water for a minimum of 3 days and 200 ml milk (Dugdha) for 60 days in empty stomach early in the morning kept as a control.

Group 2

In 54 subjects administered 2 Haritaki tablets for Kostha Shuddhi (proper cleansing of stool) with hot water for a minimum of 3 days and 12gm of Ashwagandha Choorna with 200 ml milk (Dugdha) for 60 days in empty stomach early in the morning.

Medicine Name

Ashwagandha Choorna (Withania Somnifera powder)

Study Design

108 apparently healthy subjects were selected for a clinical study. During the study periods, the subjects visited the study center at screening and enrollment and after 60^{th} day at the completion of the trial.

Table 1: Enrollment total of 408 screened subjects and 114 subjects included in the study.

Patient Enrollment	
Screening for eligibility = Total 408 Subjects	
Total subjects selected = 114 subjects	
Randomized (n=114)	
Allocated in Group 1 (n=57)	Allocated in Group 2 (n=57)
Baseline evaluation (n=57)	Baseline evaluation (n=57)
Evaluation after intervention 60 days (n=54)	Evaluation after intervention 60 days (n=54)
Dropout (3 subjects)	Dropout (3 subjects)
Follow up 90 day (n=54)	Follow up 90 day (n=54)
Efficacy Analysis of Ashwagandha (n=54)	Efficacy Analysis of Ashwagandha (n=54)

Table 2: Descriptive statistics in Haemoglobin (Group 1).

Haemoglobin	N	Mean	Std. Deviation
Before intervention (0^{th} day)	54	13.4315	.66696
After intervention (60^{th}day)	54	13.5481	.46974
Follow up (90^{th} day)	54	13.2019	.51487

Estimation of VO2 max - Rock port fitness walking test

VO2 max is a measure of the maximum amount of oxygen that we use during intense physical exercise. To test VO2 max, there are many methods you can use, but several require equipment like a treadmill or a specially calibrated exercise cycle. It can be difficult to perform these tests and are not appropriate for all levels of fitness. Using a basic calculation or a walking/jogging test is the easiest way to calculate VO2 max. Calculate VO_2 max using the following equation-

VO_2 = 132.853 - (0.0769 x weight in-lb) - (0.3877 x age) + (6.315 x gender) - (3.2649 x walk time in minutes) - (0.156 x heart rate).

If you are male, use the number 1, if you are female, use the number 0 for the calculation (Kline *et al.*, 1987; McSwegin *et al.*, 1998).

Statistical Analysis

ANOVA with repeated measures, Friedman Test, Wilcox on test using appropriate statistics software was used for the statistical analysis.

OBSERVATION

Total 408 healthy subjects were screened, 114 subjects were enrolled for the study, 108 subjects completed the clinical trial and there were 6 subjects was drop out Table 1.

1. Among 408 screened subjects and 114 subjects were included in the study. Though the majority of 246 (60.30%) subjects were males and 162 (39.70%) subjects were females.
2. The age group of subjects showed that among 114, the majority of 112 (98.22%) subjects belonged to the age group of 18 to 25 completed years followed by 02 (1.75%) subjects belonged to 26-35 years.
3. Among 114 subjects, the majority of 102 (89.45%) had completed the schooling till PUC and 12 (10.52%) subjects were graduates.
4. Among 114 subjects, a majority of 84 (73.67%) subjects having joint family and 30 (26.33%) subjects having a nuclear family.
5. Among 114 subjects, majority of 70 (61.39%) subjects having poor housing condition, 32 (28.09%) subjects having moderate housing condition, 10 (8.77%) subjects having good housing condition and 02 (1.75%) having very good housing condition.
6. Among 114 subjects, all the subject having mixed food (vegetarian and non-vegetarian).
7. Among 114 subjects, majority 86 (75.44%) consume the food timely and 28 (24.56) consume untimely.
8. Among 114 subjects, majority 78 (68.40%) were of Vata Pitta Kapha Prakruti. A total of 15 (13.15%) belonged to Pitta Vata Kapha Prakruti, 02 (1.75%) each of Pitta Vata Kapha, Pitta Kapha Vata, Vata Kapha Pitta

and 15 (13.15%) belonged to Kapha Vata Pitta Prakruti.

RESULTS AND DISCUSSION

In the parameter, N was fixed to 54 in each group. The initial significance level considered in this study is 0.05. The subjects were analyzed at an interval of BT (Before intervention- i.e. on the baseline), AT (After the intervention, i.e. on 60^{th} day) and FU (Follow up, i.e. 90 days).

Haemoglobin in the control group

Repeated measure ANOVA with Green house Geisser correction determined that mean hemoglobin differed statistically significantly between time points (F (1.503, 79.685) = 11.94, p<0.0001). Post hoc tests using Bonferroni correction revealed that administration of milk slight increase in Hemoglobin from before intervention to after intervention (13.43± 0.68gm/dl vs 13.54± 0.47 gm/dl respectively) in the control group, which was statistically significant (p< 0.0001) but no significant differences between after intervention and follow up (p=0.079).

However, after treatment had been reduced to 13.20± 0.51 gm/dl, which was not statistically significant to after intervention status of Hemoglobin (p=0.079) and to follow up Hemoglobin (p=0.036) (Tables 2, 3 and 4).

Therefore we can conclude that oral administration of milk (60 days) statistically significant in Hemoglobin from before intervention status to after intervention status, but not after follow up in the control group.

Hemoglobin in the study group

Repeated measure ANOVA with Green house Geisser correction determined that mean hemoglobin differed statistically significantly between time points (F (1.116, 59.134) = 20.675, p<0.0001). Post hoc tests using Bonferroni correction revealed that administration of Ashwagandha Churna with milk elicited an increase in Hemoglobin from before intervention to after intervention (13.24 ± 1.38 gm/dl vs 14.01 ± 0.49 gm/dl), which was statistically significant (p< 0.0001). Also, the Hemoglobin during the follow up increased to 14.18± 0.59 gm/dl, which was statistically significant to after intervention status of Hemoglobin (p<0.0001) and to before intervention (p<0.0001) (Tables 5, 6 and 7). Therefore we can conclude that oral administration of Ashwagandha Churna (12gm) in a single dose with milk (60 days) elicits a statistically significant in Hemoglobin from before intervention status to after intervention status and follow up in study group.

VO2 max test in the control group

There was a statistically significant difference in VO2 max test from BT (0^{th} day) to AT (60^{th} day) and FU (90^{th} day), $\chi 2$ = 33.091, p< 0.0001. Post hoc analysis with Wilcoxon signed rank tests was applied to result and it also significantly changes. There was significant differences between BT (0^{th} day) to AT (60^{th} day) (Z = -4.264, p <0.0001), in between BT (0^{th} day) to FU (90^{th} day) (Z = -4.025, p <0.0001). There was no significant differences between AT (60^{th} day) to FU (90^{th} day) (*Z* = -1.414, p = 0.157) (Tables 8, 9 and 10)

VO2 max test in the study group

There was a statistically significant difference in VO2 max test from BT (0^{th} day) to AT (60^{th} day) and FU (90^{th} day), $\chi 2$ = 74.150, p< 0.0001. Post hoc analysis with Wilcoxon signed rank tests was applied to result and it also significantly changes. There was significant differences between BT (0^{th} day) to AT (60^{th} day) (Z = -6.245, p <0.0001), in between BT (0^{th} day) to FU (90^{th} day) (Z = -6.164, p <0.000). There was no significant differences between AT (60^{th} day) to FU (90^{th} day) (Z = -0.577, p = 0.564) (Tables 11, 12 and 13).

Statistically, a significant increase in the hemoglobin study group compared to the control group. In the control group, hemoglobin means before intervention (13.43%), after the intervention (13.54%) and follow up (13.20). In the study group, hemoglobin means before intervention (13.24%), after the intervention (14.01%) and follow up (14.18%). Ashwagandha (WS) increased both the RBC and Hemoglobin count increase in RBC mass leads to increase in capacity of blood transport oxygen directly to exercising muscles, thereby enhancing the aerobic capacity (Ziauddin *et al.*, 1996). Results indicate a significant (p< 0.05) improvement in the Maximal oxygen intake (VO2 max) test in the study group (Ashwagandha Choorna). Ashwagandha (WS) reduced oxidative stress, as various oxidants and improved level of diverse antioxidants (Choudhary *et al.*, 2015).

VO2 max represent long term aerobic and cardio vascular endurance. Endurance training combined with a favorable genetic disposition, results in a series of physiological adaptations, designed to maximize endurance performance by increasing the amount of oxygen, which can be delivered to utilized by working muscle. Exercises that cause damage to the amount of different human body systems are long term strenuous free radical release. Increased lipid peroxidation may be caused by stress, whereas catalase and glutathione peroxidase enzymes minimize antioxidant levels.

Table 3: Tests of within subjects effects in Haemoglobin (Group 1).

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	3.353	2	1.676	11.936	<0.0001	.184
	Greenhouse-Geisser	3.353	1.503	2.230	11.936	<0.0001	.184
	Huynh-Feldt	3.353	1.538	2.180	11.936	<0.0001	.184
	Lower-bound	3.353	1.000	3.353	11.936	0.001	.184
Error (time)	Sphericity Assumed	14.887	106	.140	-	-	-
	Greenhouse-Geisser	14.887	79.685	.187	-	-	-
	Huynh-Feldt	14.887	81.501	.183	-	-	-
	Lower-bound	14.887	53.000	.281	-	-	-

Table 4: Pairwise comparisons in Haemoglobin (Group 1).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
1	2	-.117	.051	0.079	-.243	.010
	3	.230*	.088	0.036	.012	.448
2	1	.117	.051	0.079	-.010	.243
	3	.346*	.072	<0.0001	.168	.525
3	1	-.230*	.088	0.036	-.448	-.012
	2	-.346*	.072	<0.0001	-.525	-.168

Based on estimated marginal means *. The mean difference is significant at the .05 level. b. Adjustment for multiple comparisons: Bonferroni.

Table 5: Descriptive statistics in Haemoglobin (Group 2).

Haemoglobin	N	Mean	Std. Deviation
Before intervention (0^{th} day)	54	13.2407	1.37601
After intervention (60^{th} day)	54	14.0130	.48605
Follow up (90^{th} day)	54	14.1852	.58902

All the parameters of free harm are standardized in a dose dependent manner when researchers administered Ashwagandha (WS) one hour prior to daily stress inducing therapy (Bhattacharya and Muruganandam, 2003).

Ashwagandha (Withania Somnifera) chemical constituents such as flavonoids, alkaloids, and steroidal lactones (withanolides) or antioxidants (superoxide dismutase, catalase and glutathione peroxidase) may be behind VO2 max improvement. In healthy adults and also in athletes, Ashwagandha also enhances cardiovascular fitness, providing an additional alternative as a dietary supplement to boost the VO2 max measure (Choudhary *et al.*, 2015; Dhuley, 1998a).

The active principles of Ashwagandha (WS), VII-X sitondosides and withaferin A (glycowwithanolides) have been evaluated for antioxidant activity using the major free radical scavenging enzymes in the frontal cortex and striatum of the rat brain, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Dhuley, 1998b).

Table 6: Tests of within subjects effects in haemoglobin (Group 2).

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	27.323	2	13.662	20.675	<0.0001	.281
	Greenhouse-Geisser	27.323	1.116	24.489	20.675	<0.0001	.281
	Huynh-Feldt	27.323	1.123	24.338	20.675	<0.0001	.281
	Lower-bound	27.323	1.000	27.323	20.675	<0.0001	.281
Error (time)	Sphericity Assumed	70.043	106	.661	-	-	-
	Greenhouse-Geisser	70.043	59.134	1.184	-	-	-
	Huynh-Feldt	70.043	59.502	1.177	-	-	-
	Lower-bound	70.043	53.000	1.322	-	-	-

Table 7: Pairwise comparisons in haemoglobin (Group 2).

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.b	95% Confidence Interval for difference	
					Lower Bound	Upper Bound
1	2	-.772*	.180	<0.0001	-1.217	-.328
	3	-.944*	.195	<0.0001	-1.427	-.462
2	1	.772*	.180	<0.0001	.328	1.217
	3	-.172*	.054	<0.0001	-.306	-.038
3	1	.944*	.195	<0.0001	.462	1.427
	2	.172*	.054	0.008	.038	.306

Based on estimated marginal means *. The mean difference is significant at the .05 level. b. Adjustment for multiple comparisons: Bonferroni.

Table 8: Descriptive statistics for Friedman test in VO2 max test (group 1).

VO2 max test	N	Mean	Std. Deviation	Min	Max
Before intervention (0^{th} day)	54	2.6111	.52903	2.00	4.00
After intervention (60^{th} day)	54	2.2407	.43155	2.00	3.00
Follow up (90^{th} day)	54	2.2778	.45211	2.00	3.00

Table 9: Friedman test in VO2 max test (Group1).

VO2 max test	N	Mean rank	X2	df	P	Remarks
Before intervention (0^{th} day)	54	2.35	33.091	2	<0.0001	S
After intervention (60^{th} day)	54	1.80				
Follow up (90^{th} day)	54	1.85				

Table 10: Wilcoxon signed rank test in VO2 max test (Group 1).

VO2 max test	AT – BT	FU – AT	FU – BT
Z value	-4.264[b]	-1.414[c]	-4.025[b]
Asymp. Sig. (2-tailed)(P-value)	<0.0001	0.157	<0.0001
Remarks	S	NS	S

a. Wilcoxon Signed Ranks Test, b. Based on positive ranks and c. Based on negative ranks.

Table 11: Descriptive statistics for Friedman test in VO2 max test (Group 2).

VO2 max test	N	Mean	Std. Deviation	Min	Max
Before intervention (0^{th} day)	54	2.8519	.40782	2.00	4.00
After intervention (60^{th} day)	54	2.1296	.61572	1.00	4.00
Follow up (90^{th} day)	54	2.1481	.56326	1.00	4.00

Table 12: Friedman test in VO2 max test (Group 2).

VO2 max test	N	Mean rank	X^2	Df	P	Remarks
Before intervention (0^{th} day)	54	2.71	74.150	2	<0.0001	S
After intervention (60^{th}day)	54	1.63				
Follow up (90^{th} day)	54	1.66				

Table 13: Wilcoxon signed rank test in VO2 max test (Group 2).

VO2 max test	AT – BT	FU – AT	FU – BT
Z value	-6.245[b]	-.577[c]	-6.164[b]
Asymp. Sig. (2-tailed)(P-value)	<0.0001	0.564	<0.0001
Remarks	S	NS	S

a. Wilcoxon signed ranks test, b. Based on positive ranks and c. Based on negative ranks.

CONCLUSIONS

Ashwagandha (WS) an important herb in Ayurveda, the traditional Indian system medicine is considered to be a Rasayana (rejuvenating). In this study, oral administration of Ashwagandha (Withania Somnifera) with milk enhanced VO2 max (maximum aerobic capacity) and increased hemoglobin in healthy subjects. The finding of this study suggests that Ashwagandha improves cardiovascular dynamics by increasing VO2 max levels, thereby enhancing cardiorespiratory endurance and also increases hemoglobin in healthy subjects.

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Conflict of interest

The authors declare that there is no conflict of interest for this study.

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