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A double blind, randomized, placebo-controlled trial on effect of Ashwagandha (Withania somnifera Dunal.) extract in improving cardiorespiratory endurance and recovery in healthy athletic adults

Shashank Tiwari1*, Sandeep Kumar Gupta2 and Anklesh Kumar Pathak3,

1 OM Research Centre, SA-17/3, P-4, Sri Krishna Nagar, Paharia Ghazipur Road, Varanasi-221002, Uttar Pradesh, India; drs.tiwari@yahoo.in
2 M V Hospital and Research Centre, 314/30 Mirza Mandi, Post Office, Chowk, Lucknow-226003, Uttar Pradesh, India; mvhrclko@gmail.com
3 Rajajipuram, Lucknow-226017, Uttar Pradesh, India; pathananklesh@gmail.com

* Correspondence: drtiwari.bds@gmail.com ; Tel.: +91-9838172269 (S.T.)

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Abstract:

Background and objectives: Improved cardiorespiratory endurance can aid in attaining better health through altering the physiological, biochemical, molecular, structural and functional abilities in human. Athletes are seeking a better scientific way to improve their capacity through various ergogenic aids. Herbal supplements have the potential to induce cardiorespiratory health. According to Ayurveda, Ashwagandha is having such potential. The aim of this study was to evaluate the efficacy and safety of Ashwagandha root extract in enhancing cardiorespiratory endurance and improving quality of life (QOL) in healthy athletic adults. *Materials and Methods*: Fifty healthy athletic adults were chosen randomly and equally divided into experimental and placebo groups. The experimental group received 300 mg capsule containing Ashwagandha root extract twice daily for 8 weeks. Cardiorespiratory endurance was assessed by measuring the VO2 max. Estimation of stress management was done through Total Quality Recovery Scores (TQR), Recovery-Stress (RESTQ) and Daily Analysis of Life Demands for Athletes (DALDA) questionnaires. The antioxidant level was assessed to understand the management of the oxidative stress in the subjects. *Results*: Significant improvement of VO2 max was observed in the experimental group (P value <0.0001) compared to the baseline parameters and the placebo group. The mean change was 6.60 ml/kg/min with 95% CI in the experimental group and 2.02 ml/kg/min in the placebo group. Improved TQR scores were observed in the experimental group members compared to their placebo counterparts. DALDA questionnaire analysis in the experimental group was found statistically significant (P value <0.0001). RESTQ assessment also hinted better outcome, especially for fatigue recovery, lack of energy, and fitness analysis. The improved antioxidant level in the subjects was noted for the experimental group. *Conclusions*: The findings suggest that Ashwagandha root extract successfully enhanced the cardiorespiratory endurance and improved the quality of life in healthy athletic adults. Further analysis with a larger population and different ethnic group may provide more insight.

Keywords: Ashwagandha; Cardiorespiratory endurance; Vo2 Max, TQR, RESTQ-sport, DALDA, Antioxidant assay

1. Introduction

Cardiovascular health is of immense importance to lead a healthy life. The alarming growth of cardiovascular patients globally warrants additional attention from the medical community and public health management authorities [1-3]. Advanced research and analysis in cardioprotective and cardiorespiratory endurance will aid tremendously to the younger and future generation. Maintaining physical fitness and overall health is the need of the hours that have long been prescribed in every healthcare system globally [4]. Cardiorespiratory endurance training is being embraced by several national programs to ensure better health [5]. Such practice can lead to a better state of the cardiovascular system in healthy person and athletes [6]. A plethora of scientific investigations have been conducted on cardiorespiratory endurance and attempts were made to enhance the outcome [7-10]. Yet, mature and output-oriented healthcare policies in many developing countries still remain a challenge [11-12].

Nutrients and supplements are regularly used to increase the cardiorespiratory endurance in healthy being and in athletes [13]. Effects of different nutrients and diet pattern on cardiorespiratory endurance have been exercised [14]. In this context, herbal supplements are proven to be effective in improving cardiorespiratory endurance and attaining better health condition. Popular Ayurveda based supplements and herbal extracts have reflected a positive impact on human health. *Elwendia persica* or *Bunium persicum* aqueous extract showed increased cardiorespiratory capacity through improving the lipid profile in animals having hypercholesterolemia [15]. Panax ginseng (PG) was also tested in endurance runner in humid environmental condition [16]. Quercetin was found effective in improving exercise performance and muscle mitochondrial biogenesis [17]. Often, supplement combination such as Rhodiola and Gingko also demonstrated appreciable outcome for endurance related performances [18].

Ashwagandha (*Withania somnifera* [L.] Dunal), an adaptogen, is a valued herb in Ayurveda having ample medicinal use in traditional Indian Ayurvedic medicine system and in modern treatment regime [19-20]. Interestingly, Ashwagandha is used simultaneously for various disease conditions and in healthy being. Acute and chronic ailments including neurodegenerative disorders [21], cardiovascular diseases (CVD) [22], degenerative musculoskeletal problems [23], gastrointestinal conditions [24] can be treated with Ashwagandha. In healthy persons, this plant extract is used as immune modulator [25], neuroprotector [26], cardiorespiratory endurance enhancer [27].

In the ancient medical system, Ashwagandha is referred as "Rasayana", a rejuvenator and described as having "balavardhan" (vital energy increment) and "mamsavardhan" (muscle mass increment) properties [28]. A study conducted on healthy volunteers revealed that the Ashwagandha extract is well accepted in healthy adults with quality of sleep induction. The extract was effective even with varying dosage. It was potent in the reduction of total cholesterol and LDL, and increment in muscle strength [28]. A combined clinical study of *Withania somnifera* and *Terminalia arjuna* extract reported that Ashwagandha could be effective in neuromuscular coordination improvement and lower limb muscle strength enhancement. On the other hand, Arjuna extract also might have a role in cardiovascular endurance management and diminishing the systolic blood pressure [29].

In an earlier study, the substantial increment of maximal aerobic capacity (VO_2 max) and quality of life (QOL) have been reported without any adverse effect [27]. Improvement of VO_2 max and the metabolic equivalent of task (MET) was also reported when the Indian cyclists were considered as participants in a clinical study [30]. Clinical investigation on Ashwagandha in recovering exercise-induced muscle damage provided an output with increased muscle mass and supported the use of such supplement for resistance training program [31].

All these prior attempts on effectiveness, safety, and efficacy of *Withania somnifera* extract in healthy human being and sports persons inspired us to conduct further clinical studies. A double-blind, randomized, placebo-controlled trial experiment was designed to understand the impact of Ashwagandha root extract (*Withania somnifera* Dunal.) on cardiorespiratory endurance in a

healthy population. We have also considered the recovery of fatigue, antioxidant level maintenance in the bloodstream during this study.

The popular endurance and psychometric analyses such as maximum oxygen consumption (VO_2 max), Total Quality Recovery Scores (TQR), Recovery-Stress Questionnaire for Athletes (RESTQ), and Daily Analysis of Life Demands for Athletes (DALDA) questionnaire were considered to document the outcome of this study.

2. Materials and Methods

2.1 Study design:

This study was a prospective, double-blind, randomized, placebo-controlled, single-centered program where all the subjects were randomized in a 1:1 ratio. Declaration of Helsinki (1989) was followed during this study program. The guidelines for clinical trials on pharmaceutical products in India, issued by the Central Drugs Standard Control Organization (CDSCO), Ministry of Health, Government of India, was followed during the study.

2.2 Ethical consideration:

Ethics committee approval was obtained from the Institutional Ethics Committee (IEC) of MV Hospital and Research Center, Mirza Mandi, Chowk, Lucknow-226003, Uttar Pradesh, India (Reference No. MV/02/17/16). The ethical committee followed the Good Clinical Practice (GCP) guidelines issued by the Central Drugs Standard Control Organization (CDSCO) and ethical guidelines for biomedical research on human subjects, issued by the Indian Council of Medical Research (ICMR).

2.3 Participant enrollment:

The study was an exploratory study in nature. Enrollment of 50 healthy, athletic volunteers was done for this purpose and all the 50 subjects were randomized in 1:1 ratio. Therefore, 25 subjects were given KSM-66 Ashwagandha (*Withania somnifera*) and another 25 subjects received the placebo capsules. Patients of both gender who were aged between 18 and 45 years, and who had a normal body mass index (BMI) range of 18.5 to 24.9 kg/m^2 were included in the study.

2.4 Eligibility criteria for participants:

Following the study objectives, inclusion and exclusion of the subjects were done carefully. Strict criteria were followed during the inclusion of the subjects. Subjects diagnosed with heart disease, diabetes, stroke, depression, and other neurological disorders were excluded. Moreover, subjects who were already consuming nutritional or energy supplements, using medications, especially related to blood pressure, beta-blockers, beta agonists, hormonal contraceptives, were also barred from participation. Similarly, those who were taking corticosteroid at least three months prior to the study program, and those who were considering psychotropic medication at least two months prior to the study, were excluded. Subjects those who were having any kind of substance dependence, at least within the last one year, alcohol abuse, known hypersensitivity to Ashwagandha (WS) or any other herbal products, were not considered for participation. Moreover, breastfeeding women and patients unwilling to provide informed consent were excluded from the study.

2.5 Study settings, location, and participant recruitment:

This specific study took place at MV Hospital and Research Centre, Lucknow, Uttar Pradesh, India. Recruitment of the prospective subjects was conducted from the nearby community through poster advertisement and telephonic calls. All the plausible subjects were supplied with information on the consent form, background information on the purpose of the study, a description of the

intended care as per study protocol. An information sheet regarding study participation and contact information for study enrolment was also supplied to each person.

2.6 Randomization, sample allotment, and data collection:

The randomization was performed using SAS version 9.2 software applying predetermined randomization block design. The study investigational product (IP) was packed in such a manner that the study and control medication packs seemed identical. The packs were coded to conceal the nature of drugs inside and the label contained the subject serial number (study ID). After enrollment, the subject was provided with the medication (IP) pack having the corresponding serial number. The randomization codes were provided in separate sealed envelopes for each subject and were opened by the investigator after the subject was enrolled and received a serial number. The unblinding was allowed only after completion of the entire data collection or in case of any serious adverse event. The data analysts and the persons in charge of reporting the study results were unaware of the identity of the study groups. The data details were blinded to the statisticians as well. The enrolled subjects were segregated into two groups, the experimental or test group, and the control or placebo group. The members of the test group were given KSM-66 Ashwagandha capsule (300 mg) daily twice, one in the morning and one in the evening, orally with water. The control group received a placebo product as a capsule which was identical to KSM-66 Ashwagandha devoid of the active ingredients. The dosage of the placebo was identical to the test group.

2.7 Test product description:

The test product KSM-66 was obtained from the manufacturer Ixoreal Biomed Inc., Los Angeles, California, USA as a gift. The product contains root extract from Ashwagandha with the optimum amount of withanolides precisely estimated by HPLC method.

2.8 Physiological parameters analysis:

Cardiorespiratory fitness was measured through the assessment of maximum oxygen consumption (VO_2 max) through Cooper's 12 minutes run test which is used widely [32]. Total Quality Recovery Scores (TQR), Recovery-Stress Questionnaire for Athletes (RESTQ) [33], and Daily Analysis of Life Demands for Athletes (DALDA) questionnaire [34] were also implemented. All the exercise physiology-based metrics were adopted to ensure the accurate measurement of the outcome. The results of the experimental (KSM-66 supplemented) group was compared with the placebo-supplemented group to understand the obtained results. Moreover, the differences between the baseline readings collected during the program initiation, and the final recordings noted at the end of the study program, were also compared. Estimation of the antioxidant level was done to assess the oxidative stress reduction capability of the subjects. The standard venipuncture procedure was followed to collect the blood samples and each sample was preserved using *Ethylenediaminetetraacetic acid* (EDTA) for further biochemical analyses.

2.9 Statistical Analysis:

All relevant statistical calculations were done employing SAS version 9.2. The analysis was conducted in both intent-to-treat (ITT) and per-protocol (PP) datasets. The obtained analysis outcome of ranking data and scores are represented here as Mean ± SD. All the categorical and discrete data are presented as percentages. Based on the standard statistical requirement, 95% confidence intervals (CI) was accepted for the study. Differences from the baseline are expressed as mean difference and percent change from the baseline. To compare significance of the outcome, t-test was used. Baseline scores of the two groups were compared with the post-treatment group using one-way Analysis of Variance (ANOVA).

3. Results

Ashwagandha has been an excellent medicinal herb since ancient times. Numerous preclinical and clinical studies on the impact of Ashwagandha are being conducted on various diseases recently. Simultaneously, the herb is also considered as a rejuvenator and an outstanding supplement for maintaining general well-being.

This study was aimed to observe the safety and efficacy of Ashwagandha along with exploring its ability to improve cardiorespiratory endurance for athletics. CONSORT guideline (Figure 1) was followed for enrollment, allocation, follow-up, and analyses. A total of 50 subjects were enrolled and were randomized to receive either Ashwagandha extract or placebo in this trial. The obtained data were used for efficacy analysis. The mean age of the participants (50) was well balanced between the two groups (29.28 ± 8.82 years in Ashwagandha treated test group and 28.84 ± 7.47 years in the placebo group).

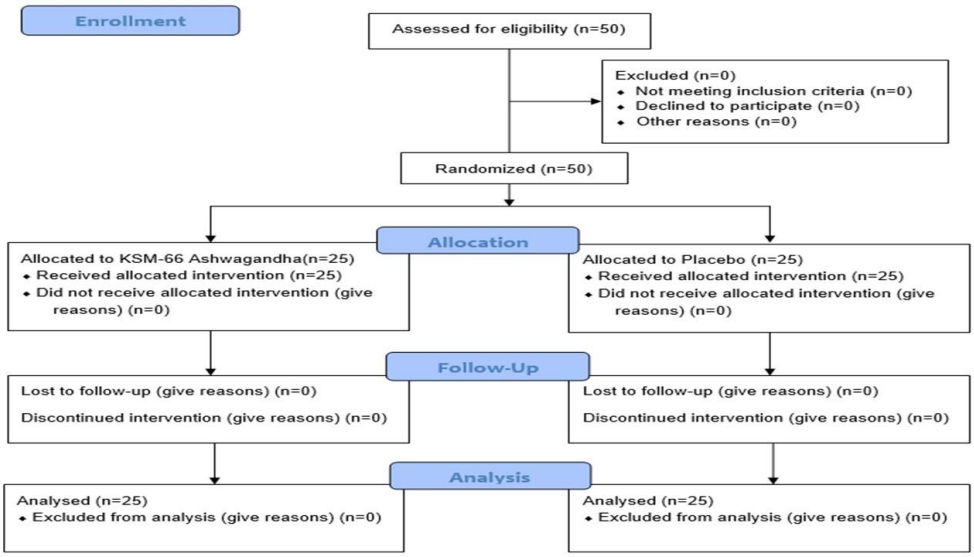

Figure 1. CONSORT flow diagram of the clinical study on efficacy and safety measurement of Ashwagandha in improving cardiorespiratory endurance in athletic human.

3.1. Study conduct:

The overall study was divided into three phases. In phase I, the study initiated with the screening and conducting baseline measurements during the first visit at Day 0 (Supplementary Table 1). In phase II, data collection was done during the second visit for the enrolled study population in 4th week (28 ± 3 Days), and in phase III, the program terminated with final or third visit in the 8th week (56 ± 3 Days) along with final data collection, assembling information and documentation. The data collection system and schedule for each visit along with the respective parameters are represented in Supplementary Table 1.

As mentioned earlier, 50 subjects were equally distributed to the placebo group and the test group. The demographic parameter distribution of the study group is represented in Supplementary Table 2. The mean age for the test group was 29.28 years ± 8.82 and the placebo group was 28.84 ± 7.47 years. Similarly, the mean height and weight in both groups were comparable. The number of male participants was 64% in the test group and 84% in the placebo group (Supplementary Table 2). The distribution of the subjects' demographic parameters was found fairly equivalent.

3.2 Outcome of physiological parameter estimation:

The major physiological indicators for cardiorespiratory endurance considered were estimation and assessment of VO_2 max and total recovery capability. Data were collected prior and post use of the supplement. Assessing the stress management capability of the volunteers was done through RESTQ questionnaire. Understanding the participants' improvement in handling stress and

well-being was conducted through biochemical analysis of the blood samples and antioxidant level measurement.

3.3 Outcome of VO_2 max and Total Quality Recovery Scores (TQR):

Several earlier studies have suggested VO_2 max and Total Quality Recovery Scores (TQR) as a suitable measure for estimating cardiorespiratory endurance in test subjects [32-34]. VO_2 max estimation was done through the usual equation rendered by Cooper (Equation 1).

$$VO2\ max \approx (d12 - 504.9)/44.73, \text{ where d12 is the distance covered in 12 mins.} \quad \ldots\ldots \text{Equation 1}$$

The application of VO_2 max in measuring the cardiorespiratory endurance is well recognized in sports physiology and in common healthy being. Different modern methods of this estimation are being explored recently for the athletes [35-36]. A complementary measurement of the stress recovery was done through the Total Quality Recovery Scores (TQR) [39]. The available questionnaire was used for the estimation and ranking of fatigue recovery in athletes [38-40]. The obtained outcome of VO_2 max estimation and Total Quality Recovery Scores (TQR) assessment is represented in Table 1 and Table 2 respectively. The final outcome of recovery analysis was categorized as a neutral, moderate and improved effect. For all the cases, the result is shown for the baseline estimation, visit 2 and visit 3 and observed significant changes along with their respective P values (Table 1 and Table 2). The mean VO_2 max of the test group was recorded as 40.22 ± 5.34 mL/ kg/min during enrollment or baseline calculation. The mean value of the test group increased to 46.82 ±5.01 mL/ kg/min by the end of the study during the 3rd visit. The mean change of 6.60 mL/ kg/min in VO_2 max was found statistically significant within the test group subjects who received Ashwagandha 300 mg capsule ($p<0.0002$). On the contrary, the observed mean change of 2.02 mL/ kg/min in VO_2 max was found not statistically significant within the placebo group subjects. Improvements were observed in the VO_2 max values in healthy athletes of the test group in comparison to the subjects consuming the placebo. This mean change value between the groups was found statistically significant and the consumption of Ashwagandha 300 mg capsule was found effective.

248 **Table 1.** VO_2 MAX (ml/kg/min) at Baseline, Week 4 and Week 8.

Parameters	Ashwagandha (n=25)		Placebo (n=25)		Difference Between the groups		p-values# (KSM66 vs placebo) (ANOVA)
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	40.22 (5.34)	(38.12, 42.31)	40.63 (3.43)	(39.28, 41.97)	-0.41	(-2.71, 1.89)	0.7483
Visit 2 (4 week)	43.72 (5.52)	(41.56, 45.89)	42.45 (4.13)	(40.83, 44.07)	1.26	(-1.82, 4.36)	0.3625
Visit 3 (8 week)	46.82 (5.01)	(44.86, 48.79)	42.66 (5.51)	(40.49, 44.82)	4.16	(0.77, 7.55)	0.0074
Change at 4 week (absolute)	3.50 (4.09)	(1.89, 5.11)	1.82 (4.11)	(0.21, 3.43)	1.68	(-0.37, 3.73)	0.1543
Change at 8 week (absolute)	6.60 (3.27)	(5.31, 7.88)	2.02 (5.15)	(0.01, 4.04)	4.57	(1.98, 7.16)	0.0005
p-values within group* (visit 1 vs visit 2) (t-test)	<0.0002		0.362				
p-values within group* (visit 1 vs visit 3) (t-test)	<0.0001		0.787				

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251 Total Quality Recovery Scores (TQR) is a regularly used evaluation method implemented for
252 estimating the exhaustion recovery rate in athletes [41]. The same measure was used to assess the
253 fatigue recovery of the subjects belonging to both the test and placebo groups. Table 2 represents the
254 outcome of TQR analysis. The statistical comparison of the records suggested a significant
255 improvement in the group that used Ashwagandha capsules. Further, ANOVA results showed
256 significant P values of <0.0001 and <0.0001 during second (4th week) and third visit (8th week)
257 respectively.
258
259

Table 2. Total Quality Recovery (TQR) Score values observed during baseline, visit 1 and final visit along with their respective significances.

Parameters	Ashwagandha (n=25)		Placebo (n=25)		Difference Between the groups		p-values#(KSM66 vs placebo) (ANOVA)
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	16.04 (0.69)	(15.77, 16.31)	15.35 (1.05)	(14.94, 15.76)	0.69	(0.20 , 1.18)	0.0089
Visit 2 (4 week)	16.61 (0.81)	(16.30, 16.93)	15.3 (1.04)	(14.89, 15.70)	1.31	(0.80, 1.83)	<0.0001
Visit 3 (8 week)	17.11 (1.02)	(16.72, 17.52)	15.15 (1.14)	(14.71, 15.59)	1.96	(1.36, 2.56)	<0.0001
Change at 4 week (absolute)	0.57 (0.77)	(0.27, 0.87)	-0.05 (0.90)	(-0.40, 0.30)	0.62	(0.16, 1.09)	0.0118
Change at 8 week (absolute)	1.07 (1.23)	(0.59, 1.55)	-0.2 (1.05)	(-0.61, 0.21)	1.27	(0.64, 1.91)	0.0003
p-values within group* (visit 1 vs visit 2) (t-test)	0.0011		0.7746				
p-values within group* (visit 1 vs visit 3) (t-test)	0.0002		0.3490				

The pace of exhaustion recovery was found rapid in the subjects who consumed the test product in comparison to those who took the placebo. Analysis of the TQR scores during the third visit revealed the experimental group represented better outcome. It was found that 14 (56%) subjects showed good improved effect and 9 (36%) subjects displayed moderate improvement according to the TQR scores in the Ashwagandha treated group. In contrast, 7 (28%) and 16 (64%) subjects showed improved effect and moderate improvement respectively in the placebo group.

3.4 Analysis of Daily Analysis of Life Demands for Athletes (DALDA):

Responses of the participants against the DALDA questionnaire were recorded to assess the symptoms defining stress for the life demands of the athletes. Based on the received answers of the participants, responses were recorded following "worse than normal" labeling for both the groups. The obtained statistical results are presented in Table 4. The recorded DALDA score displayed declined score values among subjects who received Ashwagandha capsules in the test group compared to their placebo counterparts. The mean change of the DALDA score observed was -3.6 in healthy subjects receiving the test product. This value was noted as significantly lower compared to the observed mean change of -0.4 recorded for the subjects of the placebo group (Table 3). The obtained P values were significant for the experimental group.

280

281 **Table 3.** Obtained DALDA scores at Baseline, Week 4 and Week 8.

Parameters	**Ashwagandha (n=25)**		**Placebo (n=25)**		**Difference Between the groups**		**p-values#(KSM66 vs placebo) (ANOVA)**
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	8.44 (2.04)	(7.64, 23.41)	8.12 (2.17)	(7.27, 22.37)	0.32	(-1.48, 1.48)	0.5935
Visit 2	6.68 (1.41)	(6.13, 18.69)	7.92 (1.61)	(7.29, 22.21)	1.28	(0.45, 2.10)	0.0056
Visit 3	4.84 (1.14)	(4.39, 13.45)	7.72 (1.37)	(7.18, 21.80)	2.88	(2.18, 3.58)	<0.0001
Change at 4th week	-1.76 (1.76)	(-2.45, -6.56)	-0.2 (1.85)	(-0.92, -2.01)	1.6	(0.61, 2.59)	0.0037
Change at 8th week	-3.6 (2.55)	(-4.59, -12.61)	-0.4 (2.10)	(-1.22, -2.80)	3.2	(1.9, 4.50)	<0.0001
p-values within group* (visit 1 vs visit 2) (t-test)	<0.0001		0.5935				
p-values within group* (visit 1 vs visit 3) (t-test)	<0.0001		0.3508				

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283 *3.5 The Recovery-Stress Questionnaire (RESTQ- Sport 76) analysis:*

284 Stress symptoms development frequency and recovery of stress associated parameters can be
285 evaluated through the RESTQ questionnaire. In the present study, all important parameters such as
286 general stress, fatigue, lack of energy, fitness, and injury related issues and general well-being were
287 considered for the assessment. Individual statistical analysis outcome of these RESTQ parameters
288 are represented from Table 4 to Table 8. The RESTQ general stress, fatigue, lack of energy, and
289 fitness/ injury scores showed a considerable amount of declination. In contrast, the RESTQ general
290 well-being scores displayed acceptable improvements in the test group compared to the placebo
291 group (Table 4 to Table 8).
292 Statistically, the mean change of the RESTQ general stress scores was -4.2 in the test group
293 subjects, having significantly lower values compared to the mean change of -0.96 of the placebo
294 group (Table 4).
295

296 **Table 4**. Observed RESTQ (General Stress) scores at Baseline, Week 4 and Week 8.

Parameters	Ashwagandha (n=25)		Placebo (n=25)		Difference Between the groups		p-values[#](KSM66 vs placebo) (ANOVA)
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	11.24 (2.40)	(10.29, 12.18)	10.76 (2.03)	(9.97, 11.55)	0.48	(-0.75, 1.71)	0.4490
Visit 2 (4 week)	8.68 (1.68)	(8.02, 9.34)	10.12 (2.05)	(9.32, 10.92)	-1.44	(-2.48, -0.40)	0.0091
Visit 3 (8 week)	7.04 (1.99)	(6.26, 7.82)	9.8 (2.48)	(8.83, 10.77)	-2.76	(-4.01, 1.51)	<0.0001
Change at 4 week (absolute)	-2.56 (2.38)	(3.37, 11.24)	-0.64 (2.20)	(-1.05, 0.22)	-1.92	(-3.19, -0.64)	0.0047
Change at 8 week (absolute)	-4.2 (3.5)	(3.37, 11.24)	-0.96 (2.96)	(-2.12, 0.20)	-3.24	(-5.03, -1.44)	0.0009
p-values within group* (visit 1 vs visit 2) (t-test)	<0.0001		0.1581				
p-values within group* (visit 1 vs visit 3) (t-test)	0.0044		0.4569				

297

298 Similarly, the estimated mean change of the RESTQ fatigue score was -8.84 in test group
299 subjects and -0.28 in the placebo group (Table 5), clearly representing the significant difference
300 between the experimental and control group.
301

302

303 **Table 5.** Observed RESTQ (Fatigue) scores at Baseline, Week 4 and Week 8.

Parameters	Ashwagandha (n=25)		Placebo (n=25)		Difference Between the groups		p-values[#](KSM66 vs placebo) (ANOVA)
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	14.56 (3.03)	(13.37, 15.74)	13.36 (2.74)	(12.29, 14.43)	1.2	(-0.40, 2.80)	0.1482
Visit 2 (4 week)	9.72 (2.61)	(8.70, 10.74)	13.32 (3.28)	(12.04, 14.60)	-3.6	(-5.24, -1.96)	<0.0001
Visit 3 (8 week)	5.72 (1.59)	(5.09, 6.34)	13.08 (3.01)	(11.90, 14.26)	-7.36	(-8.70, -6.02)	<0.0001
Change at 4 week (absolute)	-4.84 (2.52)	(-5.83, -3.85)	-0.04 (2.28)	(-0.93, 0.85)	-4.8	(-6.13, -3.47)	<0.0001
Change at 8 week (absolute)	-8.84 (2.97)	(-10.00, -7.68)	-0.28 (3.81)	(-1.77, 0.49)	-8.56	(-10.45, -6.67)	<0.0001
P-values within group* (visit 1 vs visit 2) (t-test)	<0.0001		0.9309				
P-values within group* (visit 1 vs visit 3) (t-test)	<0.0001		0.7258				

304

305 The energy depletion (lack of energy) and fitness/injury related RESTQ parameters suggested
306 that the test group was having mean change values of -6.08 and -9.16 respectively compared to the
307 mean change value of -0.04 and -0.04 (Table 6 and Table 7) in the placebo counterparts.

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309

310 **Table 6.** Analyzed RESTQ (Lack of Energy) scores at Baseline, Week 4 and Week 8.

Parameters	**Ashwagandha (n=25)**		**Placebo (n=25)**		**Difference Between the groups**		**p-values#(KSM66 vs placebo) (ANOVA)**
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	13.32 (2.56)	(12.32, 14.32)	12.64 (3.73)	(11.17, 14.10)	0.68	(-1.09, 2.45)	0.4564
Visit 2 (4 week)	10.12 (2.15)	(9.28, 10.96)	12 (2.20)	(11.14, 14.10)	-1.88	(-3.08, -0.67)	0.0036
Visit 3 (8 week)	7.24 (1.51)	(6.65, 7.83)	12.6 (2.68)	(11.55, 13.65)	-5.36	(-6.56, -4.16)	<0.0001
Change at 4 week (absolute)	-3.2 (3.90)	(-4.33, -2.06)	-0.64 (2.89)	(-1.77, 0.49)	-2.56	(-4.16, -0.96)	0.0030
Change at 8 week (absolute)	-6.08 (3.20)	(-7.33, -4.83)	-0.04 (2.98)	(-1.21, 1.13)	-6.04	(-7.75, -4.33)	<0.0001
p-values within group* (visit 1 vs visit 2) (t-test)	<0.0001		0.2783				
p-values within group* (visit 1 vs visit 3) (t-test)	<0.0001		0.0786				

311 **Table 7.** Obtained RESTQ (Fitness/ Injury) scores at Baseline, Week 4 and Week 8.

Parameters	Ashwagandha (n=25)		Placebo (n=25)		Difference Between the groups		p-values#(KSM66 vs placebo) (ANOVA)
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	14.84 (2.75)	(13.76, 15.91)	13.32 (2.98)	(12.15, 14.49)	1.52	(-0.06, 3.11)	0.0671
Visit 2 (4 week)	9 (2.58)	(7.99, 10.01)	13.84 (3.00)	(12.67, 15.01)	-4.84	(-6.39, -3.29)	<0.0001
Visit 3 (8 week)	5.68 (2.34)	(4.76, 6.60)	13.28 (3.74)	(11.82, 14.74)	-7.6	(-9.33, -5.87)	<0.0001
Change at 4 week (absolute)	-5.84 (2.97)	(-7.00, -4.67)	0.52 (2.72)	(-0.54, 1.59)	-6.36	(-7.94, -4.78)	<0.0001
Change at 8 week (absolute)	-9.16 (3.47)	(-10.52, -7.79)	-0.04 (4.33)	(-1.73, 1.66)	-9.12	(-11.30, -6.94)	<0.0001
p-values within group* (visit 1 vs visit 2) (t-test)	<0.0001		0.3495				
p-values within group* (visit 1 vs visit 3) (t-test)	<0.0001		0.3034				

On the other hand, the RESTQ general well-being assessment score exhibited improved values. The test group had a mean change score of 5.24 in comparison to 0.76 of the subjects in the placebo group (Table 8).

Table 8. Recorded RESTQ - General well-being scores at Baseline, Week 4 and Week 8.

Parameters	**Ashwagandha (n=25)**		**Placebo (n=25)**		**Difference Between the groups**		**p-values#(KSM66 vs placebo) (ANOVA)**
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	8.84 (3.13)	(7.61, 10.06)	8.88 (3.20)	(7.54, 10.06)	0.04	(-1.72, 1.80)	0.9645
Visit 2 (4 week)	11.96 (1.99)	(11.18, 12.74)	9.6 (2.90)	(8.46, 10.74)	2.36	(0.98, 3.74)	0.0017
Visit 3 (8 week)	14.08 (2.78)	(12.99, 15.17)	9.56 (3.32)	(8.26, 10.86)	4.52	(2.82, 6.21)	<0.0001
Change at 4 week (absolute)	3.12 (3.81)	(1.63, 4.61)	0.8 (3.35)	(-0.51, 2.11)	2.32	(0.33, 4.31)	0.0269
Change at 8 week (absolute)	5.24 (5.17)	(3.21, 7.27)	0.76 (2.91)	(-0.38, 1.90)	4.48	(2.15, 6.80)	0.0005
p-values within group* (visit 1 vs visit 2) (t-test)	0.0004		0.2447				
p-values within group* (visit 1 vs visit 3) (t-test)	0.0005		0.9537				

3.6 Comparative assessment of the antioxidant level in the subjects:

Free radicals and reactive oxygen species (ROS) are known to be highly reactive and are cause for multiple adverse conditions at the cellular, tissue and organ level. Moreover, excess free radicals and ROS serve as disease-promoting and accelerating factors. Natural or supplemented antioxidants or antioxidant boosters allow to limit the level of free radicals and promote better health. Therefore, estimation of elevated antioxidant level in the serum aids in understanding the positive impact of a supplement.

Estimation of antioxidants was carried out in all the subjects during baseline estimation at the initial phase of the study (Phase I) and at the end of the trial program by the 8th week (Phase III) of the study. The antioxidant assay was conducted using the Zen-Bio ABTS (2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)) Antioxidant Assay Kit [42] for all the subjects belonging to the test and placebo group. The results suggested that the test group has exhibited statistically significant changes (P <0.0001) at the end of the experiment compared to the placebo (P 0.0037) as depicted in Table 9.

Table 9. Estimation of antioxidant (μm TE) at Baseline and Week 8.

Parameters	Ashwagandha (n=25)		Placebo (n=25)		Difference Between the groups		p-values[#](KSM66 vs placebo) (ANOVA)
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean	95% CI	
Visit 1 (Baseline)	43.8 (6.91)	(41.09, 124.34)	39.08 (15.01)	(33.20, 104.15)	4.72	(-1.75, 11.20)	0.1624
Visit 3 (8 week)	48.44 (5.95)	(46.11, 138.81)	42.24 (13.92)	(36.78, 114.34)	6.2	(0.27, 12.13)	0.0487
Change at 8 week (absolute)	4.64 (3.25)	(3.37, 11.24)	3.16 (4.93)	(1.23, 5.66)	1.48	(-0.80, 3.79)	0.2172
p-values within group* (visit 1 vs visit 3) (t-test)	<0.0001		0.0037				

3.7 Analysis of plausible adverse effect:

During the course of the study and at the end of the trial, no serious adverse events were reported by any of the participants. Vital signs of each subject were within the normal limits and no clinical abnormality was detected. There were no discontinuation or withdrawal due to the treatment associated adverse events. Altogether, four subjects reported non-serious adverse events in this study. One subject of the test group (Ashwagandha capsules) reported mild ear pain. In the placebo group, two subjects reported mild diarrhea and one reported very low-grade fever.

4.0 Discussion

Cardiorespiratory endurance is considered as the major cardiovascular parameter to ensure better health condition in healthy being and in sports personality [7-8]. This study was aimed to evaluate the efficacy of Ashwagandha (supplied in the form of KSM-66 capsules) on cardiorespiratory endurance of athletic healthy humans. Therefore, assessment of essential physiological parameters associated with cardiorespiratory endurance such as VO_2 max, stress recovery analysis, was conducted to have comprehensive information. Moreover, the assessment of oxidative stress reduction was done to estimate and ensure the physiological and hematological changes due to stress and anxiety. The study has provided appreciable evidence in improving the quality of life (QOL) through the safe use of the test compound within the specified dosage.

Our analysis and interpretation supported the outcomes of the earlier relevant studies. A similar study using the same test product suggested that Ashwagandha root extract enhances the stress resistance ability and improves the general well-being for the individuals who are under stress [43]. An earlier report also suggested that WS extract serves as an excellent supplement in healthy human being, it increases the muscle mass along with a reduction in LDL. Interestingly, the supplement was well accepted even with the higher dosage in volunteers [28].

Other clinical studies on the impact of Ashwagandha in various disease conditions such as endocrinological issues [44], neurological disorders such as obsessive-compulsive disorder (OCD) [45] also provided the pragmatic and optimistic outcome. Another study conducted on athletes by Choudhury et al. suggested the improvement of cardiorespiratory endurance as well [27]. Reports on the enhancement of muscle strength and rapid recovery were established through a randomized clinical trial using Ashwagandha supplement [46].

Apart from cardiorespiratory context, Ashwagandha has been found effective in several other health conditions. The efficiency of Ashwagandha root extract in managing spermatogenic activity in oligospermic males [47] and women sexual functionality [48] have been explored through pilot studies. A recent pilot study recommended that Ashwagandha powder along with Sidh Makardhwaj proved to be effective in relieving chronic arthritis [49]. Ashwagandha displayed efficiency in better management of body weight under extensive mental stress [50].

Molecular investigations suggested that exercise induces the cardiorespiratory endurance through alteration of multiple molecular and physiological components and parameters. This includes elevation of the heat shock protein expression, reducing the oxidative stress through improved antioxidant quantity, alteration in the arteries, mitochondrial changes according to the cardiorespiratory status, modification of the functioning of the ion channels etc. [51]. Apart from the general anatomical, biochemical and physiological factors mentioned, genetic factors may also play a pivotal role in promoting cardiovascular and cardiorespiratory endurance. The CK/Phosphocreatine (PCr) System and the Creatine Kinase (CK) [52] serve as a major gene with reference to cardiorespiratory endurance maintenance and enhancement. Practically, improvement of these biochemical and physiological conditions can promote cardiorespiratory endurance whereas genetic factors may not be affected directly.

Recovery of fatigue is another vital part of the assessment of such endurance. Multiple factors such as neural connections, proper blood flow, oxygen flow, the balance of different ions, especially calcium, energy production and expenditure, and metabolic factors are of prime importance in fatigue management [53].

Supplement of Ashwagandha was reported to have a role in accelerating the fatigue recovery in healthy human being and sports person. Different research in animal models reported the efficiency of Ashwagandha in maintaining cardiovascular health and fatigue recovery. The results of the present clinical trial are also in accordance with prior reports along with adequate statistical supports.

5.0 Limitations

Even though the considered sample size was adequate to establish the statistical significance and draw a conclusion, a larger study population may yield additional resolution in the outcome. The present study was conducted in a single center. Multi-centered, large subject groups, having diverse background and subsets, may provide minute information about the biochemical, physiological and psychological aspects. This study was restricted to 8 weeks only, a longer duration study and analysis will provide the impact of consuming Ashwagandha on a long-term basis. Analysis of different population and the ethnic groups will also aid in understanding the effect of the product for the individual population group.

6.0 Conclusions

Enhancement of the health condition for better performance in athletes and maintenance of the better health in normal being is our well-cherished expectation, provided we do not encounter any short-term or long-term adverse effect. The ever-growing risk of cardiovascular disease also demands better medication and cardiorespiratory protective medication for a large population.

This extensive requirement can be satisfied through a reliable supplement or formulation that can assure us better health. Ayurveda has its roots in antiquity and Ashwagandha served as a panacea for multiple diseases and as a recommended health supplement. Our quest for natural compounds and for great health can explore Ashwagandha as a major resource to satisfy the global cardiorespiratory health requirement. Extensive preclinical and clinical studies are required to establish the safety and efficacy of the Ashwagandha formulations that may provide better health to the future generation.

The present single site, double-blind, randomized, placebo-controlled clinical study suggested that the considered study population responded well for the test product, a root extract of Ashwagandha. Statistically, significant improvement was observed for the biochemical,

physiological and psychological parameters of the test group. Moreover, the stress management capability of the participants was found improved when the test product was used by the participants. Hence, this study has supported the outcome of the previous clinical studies conducted in this relevance along with establishing the significant improvement in the antioxidant level ($P < 0.0487$) in the study subjects.

7.0 Trial Registration and funding sources

This trial was registered with the registration number CTRI/2016/04/006791 under Clinical Trials Registry - India (CTRI). No research funding was used for this study.

Supplementary Materials:

The associated supplementary Tables are Supplementary Table 1 and Supplementary Table 2. Supplementary Table 1. Representation of the study conduct with the performed activities. Supplementary Table 2. Demography parameters with their respective statistical distribution.

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Author Contributions:

All the authors contributed to this project equally. ST took part in planning, designing and executing the project. SKG coordinated the whole study and overseen the data collection and analysis. AKP took part in data analysis and interpretation. All the authors have carefully evaluated the drafted manuscript and approved the final version of the article.

Conflict of Interest:

The authors declare no conflict of interest in association with this study.

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Supplementary Materials:

Supplementary Table 1. Representation of the study conduct with the performed activities.

Parameter	Visit 1 (Screening & Baseline (Day 0))	Visit 2 (4 Weeks ±3 Days)	Visit 3 (8 Weeks ± 3 Days)
Informed Consent	✓	X	X
Demographics and Medical history	✓	X	X
Inclusion/Exclusion Criteria Review	✓	X	X
Physical examination & vital parameters	✓	✓	✓
Blood sampling for Hematology	✓	X	X
VO_2 Max	✓	✓	✓
Free radical estimation	✓	X	✓
RESTQ- Sport 76	✓	✓	✓
DALDA	✓	✓	✓
Total Recovery Scale	✓	✓	✓
Randomization	✓	X	X
Study Medication Dispensing (Bottle 1)	✓	X	X
Study Medication Acceptability	✓	X	X
Study Medication Dispensing (Bottle 2)	X	✓	X
Study Medication Acceptability	X	✓	✓
Medication Compliance	X	✓	✓
Adverse Events	✓	✓	✓
Concomitant medication	✓	✓	✓
Study Completion/Termination	X	X	✓

The first column presents the parameters, X=procedure not performed, ✓= procedure performed.

582 **Supplementary Table 2.** Demography parameters with their respective statistical distribution.

	KSM-66 Ashwagandha capsule (n=25)	Placebo (n=25)
Age (years)		
n	25	25
Mean	29.28	28.84
SD	8.82	7.47
Median	27	30
Height (cm)		
n	25	25
Mean	158.32	161.48
SD	8.26	9.19
Median	160	161
Weight (kg)		
n	25	25
Mean	56.04	61.08
SD	5.30	9.95
Median	58	59
Gender, n (%)		
Male	16 (64)	21(84)
Female	9 (36)	4 (16)

583 n = number of subjects, SD = standard deviation

584