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Original Article

Effect of Ashwagandha Root Extract on Serum Testosterone and Muscle Recovery in Strength Training

Narsingh Verma[1], Sandeep Kumar Gupta[2], Shashank Tiwari[2], Ashok Kumar Mishra[2], Vaishali Thakare[3], Shirish Patil[3]*

[1]King George's Medical University, Chowk, Lucknow, 226003, Uttar Pradesh, India
[2] M V Hospital and Research Centre, Chowk, Lucknow-226003, Uttar Pradesh, India
[3] D Y Patil University School of Medicine, Navi Mumbai, Maharashtra, India

ABSTRACT

Background: Ashwagandha (Withania somnifera also known as Indian Ginseng or Winter Cherry) is a prominent herb in Ayurveda medicine, commonly used to promote youthful vigor, longevity, and overall wellbeing. This paper focuses on ashwagandha as an ergogenic aid and presents the results of a randomized, double-blind, placebo-controlled clinical study on the effects of ashwagandha administered as an adjuvant to a resistance training program, on serum testosterone and inflammatory markers in healthy adults.

Aim: To investigate the effects of Ashwagandha root extract on serum testosterone and inflammatory markers in healthy adults following resistance training.

Methods: This was a prospective, randomized, double-blind, placebo-controlled study in 80 healthy male and female adults between 18–45yearsof age after obtaining informed consent. Enrolled participants were randomly allocated to receive capsule Ashwagandha root extract 300 mg, or an identical placebo capsule containing starch. Both treatments were given twice daily for with milk or water for 8 weeks. Study assessments included serum testosterone (total and free), CD4 cell counts, and serum levels of inflammatory markers (IL-6, TNF-Alpha), done at baseline and after 8 weeks.

Results: Four participants in the placebo and 3 from ashwagandha group did not complete the study, and analyses were done on data of 73 (36 ashwagandha, 37 placebo) participants. Both free and total serum testosterone levels increased ($p<0.0001$) in males (8.00% versus 1.95%) and females (7.05% versus 2.52%) receiving Ashwagandha compared to placebo respectively. The levels of IL-6 and TNF-alpha were less in the Ashwagandha group, but these were statistically insignificant ($p>0.05$).

Conclusion: Ashwagandha root extract supplementation improves muscle recoveryand serum testosteronelevels in adults undergoing resistance training.

Key Words: *Ashwagandha root extract, resistance training, testosterone*

***Corresponding Author**
Shirish Patil*
D Y Patil University School of Medicine, Navi Mumbai, Maharashtra, India

INTRODUCTION

Resistance training is a specific conditioning technique in which individual works against a broad spectrum of resistive loads to enhance general health, fitness, and performance [1]. A resistance training program comprises a set of well-organized exercises and aids in rhythmic muscular contraction and relaxation against external resistance. Repetition of actions allows the human body to adapt to such resistance and induces strength and endurance over time [2,3]. In addition to strength development, resistance exercise improves immunity, bone, and muscle health, reduces inflammation and the overall function of all organs [4]. Furthermore, regular exercise alters biochemical and physiological processes.

Reductions in steroid hormones in men occur as age advances. Males experience reduction in testosterone levels at the rate of 1%–2% per annum after the age of 40 years [5,6]. Testosterone influences sexual health, lean body mass, mental health, cognition, bone density, cardiovascular function, and metabolic activity [7].

Low serum testosterone levels in men are strongly associated with increased morbidity and reduced quality of life [8,9].

Withania somnifera, popularly known as Ashwagandha, is a plant that belongs to the Solanaceae family and that grows in the arid or semi-arid regions. Ashwagandha is one of the most frequently used medicinal plants in the Ayurvedic system of complementary medicine for a varied range of ailments. It has been used to treat musculoskeletal conditions and improve general vitality and quality of life [10].It has been demonstrated in several animal studies that ashwagandha can influence the hypothalamic–pituitary–gonadal hormonal axis and increase testosterone concentrations [11,12].

The present study aims to examine the effects of a standardized Ashwagandha extract (as an adjunct to resistance training) on exercise recovery, concentrations of testosterone and levels of inflammatory/ immune markers in healthy, active participants of either sex.

MATERIALS AND METHODS

80 participants met inclusion criteria and were enrolled to participate. All of the munderwent randomization and were allocated respective medicinal packs of either treatment or placebo in a ratio of 1:1. The clinical study protocol followed in this study is provided in detailin the following section.

Study Design

An 8 week-long randomized, double-blind, placebo-controlled, prospective clinical study was designed to assess the efficacy and safety of the Ashwagandha root extract on muscle mass, muscle strength, cardio-respiratory endurance and muscle recovery in healthy individuals. Following the international mandate, the study protocol was designed as per the Declaration of Helsinki developed by the World Medical Association, the 'New Drugs and Clinical Trials Rules - 2019'. The study was approved by the respective Institutional Ethics Committee (IEC) from both the study sites(Ref. #202/IEC/R.Cell-18, #IEC/01/28/18). The study was conducted in accordance with the Good Clinical Practice (GCP) guidelines, issued by the Indian Council of Medical Research (ICMR), Government of India. The entire study was conducted and reported following the Consolidated Standard of Reporting Trials (CONSORT) statement. It was also prospectively registered with the Clinical Trial Registry of India(Reg.# CTRI/2018/07/014969).

Recruitment and Randomization

The recruitment of participants was performed by circulating printed fliers in the purlieu of the gymnasium which served as the site of the training program. Subjects were randomly and equally allocated into two groups using stratified randomization (male and female) to receive either an Ashwagandha root extract or a placebo. The randomization code was computer-generated through randomly permuted blocks. Within each block, the number of participants allocated to each of the two treatment arms were equal. The test and placebo capsules were manufactured and packed in identical containers and labeled equivalently to ensure blinding. The study centers received numbered and sealed randomization envelopes that contained no information about treatment allocation.

Study participants

All the participants read and signed the informed consent form prior to final selection and enrollment in the study. Healthy adults of either sex aged between 18 and 45 years engaged in regular physical activity (gymnasium/ strength training exercise at least 3 months before screening for this study) were included. Another inclusion mandate was those willing to comply with the protocol and likely to be compliant with the prescribed product. Females of child-bearing age, agreed to use appropriate birth control measures were eligible for the study. Those who were using any supplements, medications, steroids, or hormonal contraceptive were excluded from study. Smokers (more than 10 cigarettes a day) alcoholics (consuming more than 14 grams of alcohol per day) were not eligible for study. Exclusion was also made if participants had history of any orthopedic injury or surgery in the past 6 months. Those with weight loss of >5kg in the previous 3 months, history of heart disease, bronchial asthma, diabetes, depression, stroke, or other neurological disorder were not considered. Participants with known hypersensitivity to Ashwagandha were excluded.

Investigational products

High-concentration ashwagandha root extract, KSM-66, manufactured by Ixoreal BioMed, Los Angeles, California, USA and placebo were used for the treatment in capsulated form. This extract was produced using a water-based process that does not use alcohol or solvents and was standardized to a 5 % concentration of with anolides as measured by HPLC. Both types of capsules, i.e., Ashwagandha root extract and the placebo weighed 300 mg and were produced in a Good Manufacturing Practice (GMP) certified facility. All the capsules used in this study were identical in appearance, shape, color, and packaging. Both the intervention and placebo were cellulose-based vegetarian capsules. The batch number of the test product used was KSM/VG/18/1020 and the chemo profile of the study product was confirmed by a third party, an independent laboratory. Participants were also instructed to store the capsules at room temperature.

Interventions

The treatment group received 300 mg of Ashwagandha root extract twice daily, once shortly after awakening, and next before bedtime with milk or water for 8 weeks in capsule form. The control or placebo group received an identical dose of placebo capsules. Both the control and ashwagandha groups received a bottle of 60 pills at the start of study and at 4 weeks. The pill count at 4 weeks allowed for a compliance check. All the participants were evaluated at baseline and after eight weeks with the outcome measures as described below. Data on safety and adverse effects were collected at the end of 8weeks.

Study procedure

Informed consent was obtained from the participants during screening and enrollment. The participants were screened for brief medical history, general physical examination, and vital parameters. Once enrolled, Muscle strength, size measurements, Cardio respiratory endurance in form of VO_2 max were conducted at baseline (first day of training) and the end of the study after the completion of 8-weeks of training and supplementation. The Resistance training exercise program used in this study was focused on improving muscle strength, enhancing cardio respiratory endurance, and increasing muscle mass. The various resistance exercises were chosen with the objective of training targeted muscle groups. Such training programs target the upper and lower body and consist of a series of exercises with suitable resistance, grouped into multiple sets and repetitions as per the National Strength and Conditioning Association's (NSCA) regulations and guidelines [13]. Each participant was required to complete the training session every alternate day with a one-day complete rest per week. Therefore, participants were training 3 days per week. Each training session started with a warm-up of aerobic exercise of low intensity. Participants were instructed to perform maximum repetitions possible for each set until exhaustion. The exercise program is detailed in Figure 1, comprising exercises for week 1, week 2, and then the remainder of the study (weeks 3 to 8).

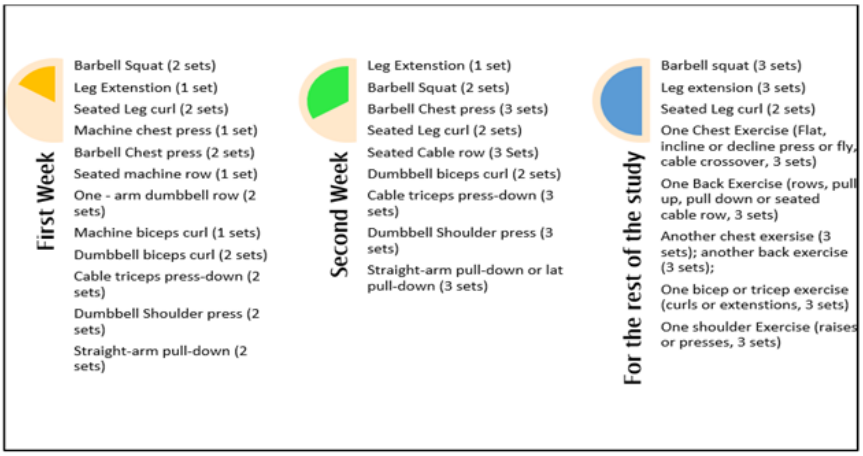

Figure 1: Exercise protocol

Clinical safety was assessed based on the frequency of adverse events reported by the participants /researcher and the PGATT (Physicians Global Assessment of Tolerability to Therapy) form. In addition to the subjective report, standard biochemistry tests were also performed along with measurement of vital signs.

Sample Size

The sample size was estimated using G*Power (Version 3.1.9.3). It was based on a previously published randomized controlled clinical study evaluating the effect of an Ashwagandha root extract on muscle strength

and recovery [14].A recently published systematic review on Ashwagandha also supports this kind of improvement (effect size of 0.67) in physical performance [15]. Considering the previous study, we hypothesized that Ashwagandha treatment is better than placebo by an effect size of 0.6 with regards to change in the one-repetition maximum (1 RM) Chest press exercise after eight weeks. To detect an effect size = 0.6, in a two parallel group design (1:1), using independent Student t-test, with a 5 % risk of type 1 error (alpha) and 80% power, 40 subjects per group are required considering also a 10% drop-out rate. Thus, 80 healthy male and female subjects were recruited in the study.

Randomization and blinding

Following the screening, research coordinators randomized the eligible participants through computer-based predetermined randomization (Rando version 1.2) in a 1:1 ratio to receive either Ashwagandha-root extract or placebo. The randomization list had non-stratified blocks of the same length. The study was a double-blind one, i.e., doctors and participants were unaware of participants receiving the treatment and the placebo. The treatment and placebo medication packs were made tamper-proof and identical in appearance and weight. All the packs were coded to conceal their contents along with proper labeling that contained the subject serial number (ID of the study). After the participants were enrolled, they were provided with a medication pack having the corresponding serial number only. During data collection, the research coordinators, the study investigators, and the attending care personnel were not allowed to access the randomization codes and allocations. Unblinding was allowed only after completion of the entire data collection or in case of any serious adverse event. The randomization codes were covered in aluminum foil and placed in a separate sealed envelope for each patient. Data analysts and responsible personnel for reporting study results were also unaware of the identity of the study groups. The data were double-entered and blinded to the statisticians as well.

Study outcomes

Serum Testosterone

Total and free blood testosterone serum levels were measured twice: at the start of study and at the end of study. The blood withdrawal was timed to be between two hours and three hours of each subject's regular waking time, and prior to any substantial physical activity, in order to minimize the effects of the natural diurnal variation in testosterone level. The 20 ml blood withdrawn from an antecubital vein, punctured with a 20-gauge disposable needle connected to a Vacutainer tube. The blood serum samples were analyzed by an ELISA (enzyme-linked immunosorbent assay).

Immune markers

Resistance training exercise can increase in CD3,CD4, CD8 cells count. Test drug can enhance the effect of exercise.

Inflammatory markers

Resistance training can cause decrease inflammatory markers like IL-6 and TNF-Alpha. Test drug can enhance the effect of exercise.

Muscle recovery

Muscle recovery refers to the reduction in exercise-induced muscle damage over time. The level of creatine kinase, a protein, in the blood is a commonly used measure of muscle damage because this protein is specific to muscle tissue. The body on its own repairs such damage over 1 to 10 days and serum creatine kinase return to baseline levels. Serum creatine kinase was measured at 24 h and at 48 h after the end of the first exercise session, and the last exercise session approximately 8 weeks later, from 20 ml blood draws using a 20-gauge disposable needle and a Vacutainer setup. The creatine kinase level was determined in a commercial laboratory using enzymatic analysis tracking nicotinamide adenine diphosphopyridine (NADPH). The increase in creatine kinase from the 24-h point to the 48-h point can be taken as a biomarker of recovery in that a smaller increase corresponds to faster stabilization of creatine kinase level and hence faster recovery of muscle tissue from exercise-induced damage.

Tolerability

The subjects were asked to report any adverse events experienced at any point in the study. We used the Physicians Global Assessment of Tolerability to Therapy (PGATT) form [16,17]. Subjects used a five-point scale to assess tolerability from "worst tolerability" (which corresponds to patients not being able to tolerate

the drug at all) to "excellent tolerability" (which corresponds to no adverse effects and the patient being able to tolerate the drug excellently).

Statistical Analysis

All the data were entered into two separate Microsoft Excel spreadsheets (i.e., manual double-key data entry), and compared to assure data quality before analysis. Then, all relevant statistical calculations were completed using MedCalc® (version 20.011). The efficacy analysis was done on the modified intention to treat (ITT) anonymized dataset (n=73), whereas the safety analysis was done on an intention to treat anonymized dataset (n=80). A summary statistic for all the parameters was performed and the results presented as mean with standard deviation (SD) for continuous variables. Categorical and discrete data are presented as counts with percentages. Changes in values form baseline to 8 weeks are computed and compared between the two groups using unpaired t-test. Since the baseline values were not similar for gender, BMI, chest circumference, CD4 counts, and serum testosterone (total and free), the effect of these parameters on the different parameters with respect to the change from baseline were analysed using analysis of covariance (ANCOVA). The dependent variables were the change from baseline in all parameters, the random variable was the treatment (KSM-66 or placebo), whereas the covariates were gender, BMI, chest circumference, CD4 counts, and serum testosterone (total and free). Adjusted means with 95% confidence intervals, and effect size (Cohen's d) are presented for change from baseline values for the different parameters. The criteria for effect sizes are based on the standard criteria (<0.2, trivial; 0.2–0.6, small; 0.6–1.2, moderate; 1.2–2.0, large; 2.0–4.0, very large; and >4.0, nearly perfect).

Normality assumptions were checked on all variables using a one-sample Shapiro-Wilk test. Non-normal distributions were transformed using $\log_{10}$. An unpaired t-test was used to assess baseline differences and the between-group differences. Within-group effects were compared using a paired t-test. P-values were reported to all parameters with Bonferroni correction, where applicable to adjust for multiple comparisons. A p-value of less than 0.05 was considered the threshold to claim statistical significance.

RESULTS

The study was initiated with 80 participants, 40 in each arm. Later, as the study progressed, 73 participants complied with the necessary treatment requirements, i.e., consumed capsules, for 8 weeks. During the study course, 3 and 4 participants dropped out of the treatment and placebo group, respectively, due to noncompliance. No participant withdrew from the study due to self-reported adverse effects because of the capsule intake (Figure 2). The final per-protocol analysis was done using the data of the 73 participants.

Participant Demographics

The mean age of participants was calculated using a per-protocol analysis of the 73 participants enrolled in the study. The mean (SD) age of the experimental and control group was documented as 25.35 (4.59) and 23.93 (4.28) years respectively. The deviation among the participants' age was less in both the groups; thus, we had homogeneous study participants of almost equivalent age groups. The demographic characteristics and baseline features of the two groups are represented in Table 1. The outcome indicates that the study population was quite homogenous with no significant statistical differences between the groups with reference to the baseline demographic characteristics.

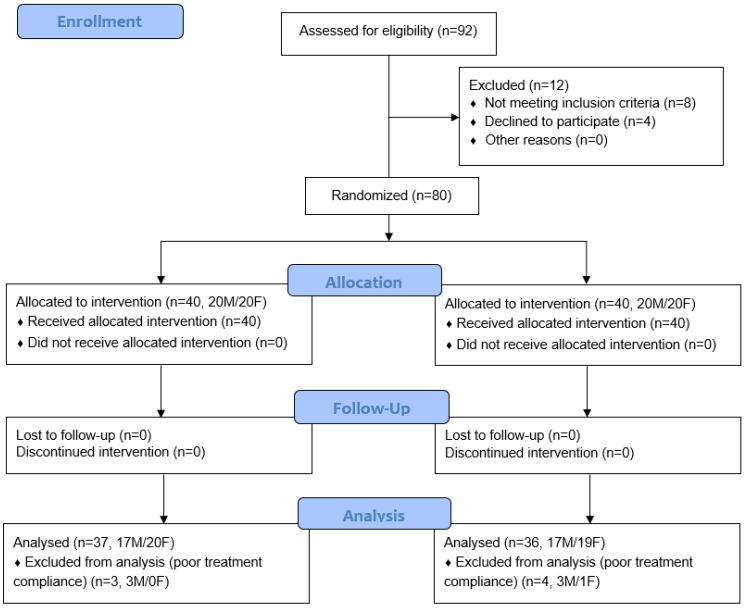

Figure 2: CONSORT flow chart

Serum testosterone

Over the eight weeks, there was a significant increase in free testosterone level in the ashwagandha treatment group in males relative to the placebo group ($p<0.0001$). The level of total testosterone was greater with ashwagandha supplementation than with the placebo, the numbers are not detectable as statistically significantly different (Figure 4).

Table 1: Baseline demography and vital parameters in ITT dataset

	KSM66 (n=40) *Mean (SD)*	**Placebo (n=40)** *Mean (SD)*	**Unpaired t-test** *p*
Age (yrs.)	25.35 (4.59)	23.93 (4.28)	0.155
Weight (kg.)	58.13 (6.20)	57.03 (5.23)	0.394
Pulse (per min.)	78.73 (3.87)	77.60 (4.14)	0.213
Respiratory rate (per min.)	17.75 (0.98)	17.53 (1.11)	0.339
Systolic BP (mm Hg)	126.25 (5.37)	126.43 (5.13)	0.882
Diastolic BP (mm Hg)	78.55 (4.30)	79.05 (4.28)	0.604
BMI (kg/sq.m)	23.15 (1.04)	22.52 (1.61)	0.040
Free Testosterone (ng/dL)	15.12 (12.20)	15.42 (12.48)	0.914
Total testosterone (ng/dL)	336.98 (144.36)	334.70 (132.82)	0.931
VO_2 max (L/min.)	33.43 (4.87)	33.45 (5.16)	0.982
CD3 (cell/µL)	1356.53 (70.57)	1344.65 (100.86)	0.544
CD4 (cell/µL)	836.33 (53.94)	798.53 (65.06)	0.006
CD8 (cell/µL)	520.20 (46.66)	514.98 (52.38)	0.639
CD4/CD8 Ratio	2.77 (0.83)	2.80 (1.07)	0.896
IL-6 (pg/ml)	3.16 (0.83)	3.18 (1.07)	0.933
TNF-Alpha (pg/ml)	1.63 (0.19)	1.57 (0.21)	0.180

Table 2: Cardiorespiratory parameters and BMI in two groups in PP (efficacy) dataset

		Baseline values			Change from baseline at week 8		
		ARE (n=37) M=17/F=20 *Mean (SD)*	Placebo (n=36) M=17/F=19 *Mean (SD)*	Unpaired t-test *p*	ARE (n=37) M=17/F=20 *Mean (SD)*	Placebo (n=36) M=17/F=19 *Mean (SD)*	Unpaired t-test *p*
Pulse (per min.)	Male	78.41 (3.37)	77.65 (5.13)	*0.484*	-2.18 (3.09)	0.47 (3.94)	*0.074*
	Female	79.05 (4.52)	76.95 (2.12)	*0.264*	0.80 (6.12)	1.53 (4.72)	*0.774*
	Total	78.76 (4.00)	77.28 (3.81)	*0.213*	-0.57 (5.12)	1.03 (4.34)	*0.227*
Resp. rate (per min.)	Male	17.82 (1.01)	17.35 (1.22)	*0.231*	0.00 (1.22)	0.71 (1.21)	*0.191*
	Female	17.75 (0.97)	17.74 (1.05)	*0.968*	0.20 (1.28)	0.21 (1.62)	*0.913*
	Total	17.78 (0.98)	17.56 (1.13)	*0.359*	0.11 (1.24)	0.44 (1.44)	*0.329*
Systolic BP (mm Hg)	Male	126.76 (5.95)	124.59 (4.49)	*0.589*	-0.06 (5.99)	3.29 (4.63)	*0.317*
	Female	126.00 (5.24)	127.11 (5.54)	*0.476*	0.20 (5.24)	0.37 (3.99)	*0.865*
	Total	126.35 (5.51)	125.92 (5.16)	*0.321*	0.08 (5.52)	1.75 (4.49)	*0.374*
Diastolic BP (mm Hg)	Male	79.18 (3.52)	78.18 (4.30)	*0.936*	-1.41(4.73)	0.71 (4.98)	*0.604*
	Female	78.30 (4.59)	79.63 (4.37)	*0.439*	1.00 (5.37)	1.32 (6.25)	*0.746*
	Total	78.70 (4.10)	78.94 (4.34)	*0.165*	-0.11 (5.16)	1.03 (5.62)	*0.559*
VO_2 max (L/min.)	Male	38.24 (1.44)	37.82 (2.40)	*0.804*	3.65 (1.73)	1.56 (0.43)	*<0.0001*
	Female	28.80 (1.36)	28.74 (1.41)	*0.817*	2.08 (0.92)	0.98 (0.58)	*<0.0001*
	Total	33.14 (4.96)	33.03 (4.98)	*0.982*	2.80 (1.55)	1.26 (0.59)	*<0.0001*
BMI (kg/sq.m)	Male	23.52 (1.05)	22.48 (1.87)	*0.059*	0.53 (0.46)	0.38 (0.46)	*0.636*
	Female	23.02 (0.94)	22.73 (1.43)	*0.382*	0.52 (0.39)	0.29 (0.31)	*0.079*
	Total	23.25 (1.01)	22.62 (1.63)	*0.049*	0.52 (0.42)	0.33 (0.39)	*0.140*

Within group changes (baseline versus week 8) significant only for VO2 maxat p<0.05 (paired t-test). For all other parameters p>0.05 for within group comparisons.

Table 3: Inflammatory markers and serum testosterone in two groups in PP (efficacy) dataset

		Baseline values			Change from baseline at week 8		
		ARE (n=37) M=17/F=20 *Mean (SD)*	Placebo (n=36) M=17/F=19 *Mean (SD)*	Unpaired t-test *p*	ARE (n=37) M=17/F=20 *Mean (SD)*	Placebo (n=36) M=17/F=19 *Mean (SD)*	Unpaired t-test *p*
Free Testosterone (ng/dL)	Male	13.76 (2.79)	13.84 (2.22)	*0.197*	1.11 (0.55)	0.27 (0.09)	*<0.0001*
	Female	2.41 (0.16)	2.38 (0.15)	*0.550*	0.17 (0.08)	0.06 (0.09)	*0.0003*
	Total	8.09 (0.26)	8.11 (0.25)	*0.959*	0.64 (0.33)	0.17 (0.12)	*0.8283*
Total testosterone (ng/dL)	Male	334.27 (134.36)	334.70 (136.32)	*0.927*	35.14 (6.49)	7.8 (4.08)	*<0.0001*
	Female	15.04 (9.00)	15.49 (10.28)	*0.885*	1.42 (1.52)	0.32 (0.53)	*0.05*
	Total	174.66 (17.13)	177.10 (19.34)	*0.552*	18.28 (2.06)	4.06 (0.95)	*<0.0011*
Sr CK (IU/L)	Male	139.26 (17.38)	136.30 (19.85)	*0.998*	-7.795 (15.76)	3.725 (10.79)	*0.010*
	Female	123.81 (31.15)	132.72 (20.72)	*0.536*	-10.290 (10.29)	4.490 (9.42)	*<0.0001*
	Total	130.91 (26.59)	134.41 (20.11)	*0.610*	-9.043 (13.17)	4.108 (10.01)	*<0.0001*
CD3 (cell/µL)	Male	1384.41 (65.85)	1343.00 (82.02)	*0.844*	-5.250 (14.71)	7.300 (43.92)	*0.233*
	Female	1332.90 (65.22)	1297.89 (79.63)	*0.218*	-6.350 (12.85)	4.000 (26.01)	*0.119*
	Total	1356.57 (69.64)	1319.19 (82.82)	*0.544*	-5.800 (13.64)	5.650 (35.66)	*0.062*
CD4 (cell/µL)	Male	858.18 (62.34)	825.35 (75.01)	*0.042*	-1.950 (12.80)	3.400 (20.09)	*0.322*
	Female	813.15 (27.74)	782.05 (38.76)	*0.021*	-3.050 (7.23)	4.700 (13.95)	*0.034*
	Total	833.84 (51.49)	802.50 (61.85)	*0.006*	-2.500 (10.27)	4.050 (17.08)	*0.041*
CD8 (cell/µL)	Male	526.24 (37.93)	517.65 (36.45)	*0.489*	-3.300 (7.94)	-1.800 (14.90)	*0.693*
	Female	519.75 (54.93)	515.84 (67.08)	*0.907*	-3.300 (7.94)	-0.700 (23.01)	*0.636*
	Total	522.73 (47.35)	516.69 (54.06)	*0.639*	-3.300 (7.84)	-1.250 (19.15)	*0.533*

CD4/CD8 Ratio	Male	1.64 (0.19)	1.60 (0.19)	*0.212*	0.005 (0.04)	0.010 (0.06)	*0.768*
	Female	1.58 (0.18)	1.54 (0.23)	*0.499*	0.000 (0.00)	0.015 (0.06)	*0.324*
	Total	1.61 (0.18)	1.57 (0.21)	*0.372*	0.003 (0.03)	0.013 (0.06)	*0.372*
IL-6 (pg/ml)	Male	3.05 (0.94)	3.18 (1.19)	*0.665*	-0.175 (0.07)	-0.155 (0.17)	*0.631*
	Female	2.40 (0.51)	2.60 (0.92)	*0.379*	-0.160 (0.08)	-0.155 (0.22)	*0.926*
	Total	2.70 (0.80)	2.88 (1.08)	*0.896*	-0.168 (0.08)	-0.155 (0.19)	*0.710*
TNF-Alpha (pg/ml)	Male	3.43 (0.94)	3.56 (1.19)	*0.665*	-0.515 (0.25)	-0.435 (0.13)	*0.218*
	Female	2.80 (0.52)	2.98 (0.92)	*0.429*	-0.740 (0.75)	-0.500 (0.17)	*0.171*
	Total	3.09 (0.80)	3.26 (1.08)	*0.933*	-0.628 (0.56)	-0.468 (0.15)	*0.088*

Within group changes (baseline versus week 8) significant at p<0.05 (paired t-test)

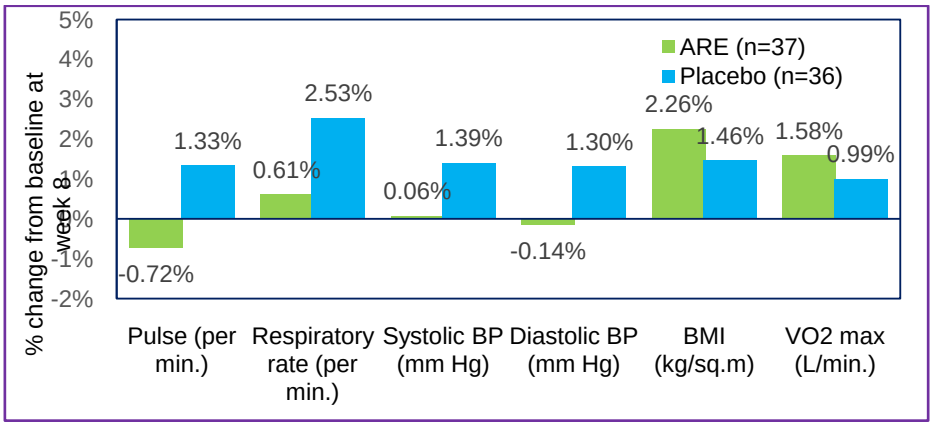

Figure 3: Percent change from baseline in cardiorespiratory parameters in PP dataset

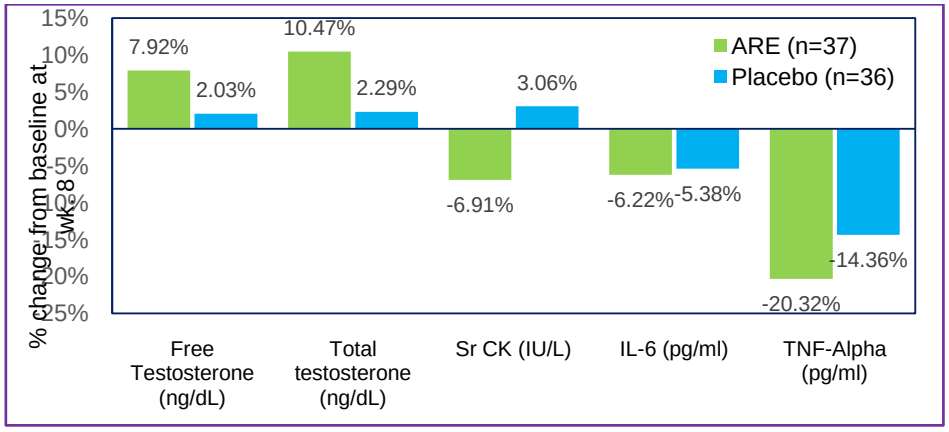

Figure 4: Percent change from baseline in laboratory parameters in PP dataset

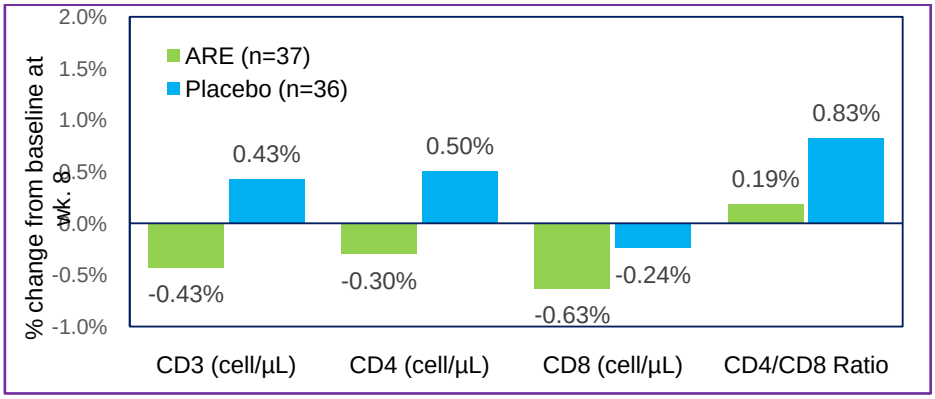

Figure 5: Percent change from baseline in CD4, CD3 and CD8 cell counts in PP dataset

Immune cells & Inflammatory mediators

Reduction in levels of immune cells-CD3, CD4,CD8 cells has been observed in test group but were not statistically significant(Figure 5). Reduction in levels of inflammatory marker (IL-6and TNF-Alpha) were less with Ashwagandha compared to placebo, but these were not statistically significant (Table 3).

Serum creatine phosphokinase(CPK)

CPK levels were low in both the ashwagandha group and the placebo group, likely because of muscle tissue getting accustomed to the training regimen and developing greater integrity to resist any damage. Comparing the ashwagandha group and the placebo group, the results showed that muscle damage was substantially lower in the ashwagandha group. Total Serum creatinine kinase was reduced to significant level in test group ($p<0.0001$) as compared to placebo group(Figure3).

Adverse events and safety parameters

The participants did not report any adverse events during the study period. Additionally, we conducted an array of routine clinical tests such as hematology, renal function, liver function, and thyroid function tests to evaluate the safety and tolerability of Ashwagandha root extract supplementation in participants. None of the parameters in the study samples showed any abnormal changes at the end of the intervention.

DISCUSSION

The present study focused on assessing the impact of an Ashwagandha root extract supplementation on resistance training adaptations such as serum testosterone and inflammation (immune response).In healthy active adults, Ashwagandha supplementation significantly increased testosterone levels. Also, it significantly attenuated the increases in serum creatine kinase levels and inflammatory mediators due to resistance training.

Ashwagandha accelerates muscle recovery that is a collateral effect of resistance training. Compared to the placebo subjects, the subjects receiving ashwagandha also had significantly greater reduction of exercise-induced muscle damage as indicated by the reduction of serum creatinine kinase. It was also assessed by inflammatory markers. These objective and subjective markers reported significant improvement in muscle recovery. In other words, all the trained adults in the ashwagandha group are more likely than those in the placebo group to report that their muscle soreness was alleviated. This kind of benefit will be important for athletes who want to increase the frequency of training without the delayed onset of muscle soreness being the limiting factor.

Some studies have suggested that ashwagandha treatment is associated with increased serum testosterone in men [14,18]. We did observe a statistically significant difference in the free testosterone in the ashwagandha group (male) compared to the placebo group, whereas there was no change in total and free testosterone in females. As far as we are concerned, this is the first study that measured free testosterone improvement with the ashwagandha root extract. The effects of ashwagandha in increasing levels of testosterone have been demonstrated in men undergoing resistance training [14], which leads to muscle growth. Testosterone production by testes is regulated by gonadotropin-releasing hormone (GnRH). It has been demonstrated in-vitro and in animal studies that ashwagandha enhances the activity of GnRH leading to increase in testosterone concentrations [11,19].

The present study demonstrated a decrease in inflammatory markers IL-6 & TNF-α as also stated in a study done by Abudubari Sikandan *et al* with hot water extract of Ashwagandha roots using the human keratinocyte cell line. Probable mechanism is Ashwagandha significantly inhibited mRNA expression of inflammatory cytokines, including interleukin (IL)-8, IL-6, tumor necrosis factor (TNF-α), IL-1β and IL-12, and promoted the mRNA expression of the anti- inflammatory cytokine transforming growth factor (TGF)-β1 [20].

Suppression of inflammation by testosterone were observed in patients with coronary artery disease, prostate cancer and diabetes mellitus through the increase in anti-inflammatory cytokines (IL-10) and the decrease in pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in a study done by Mohamad et al, through its influence on testosterone [21].

The mechanism for improvement in muscular strength appears to be through increase in anabolic hormone (serum testosterone), which could be related to structural similarities with anolides (the major ingredients of

ashwagandha root extract) [22]. However, since the females did not show increased testosterone, it can be hypothesized that other factors such as anti-inflammatory and antioxidant effects also contribute significantly. In addition, as previously shown, reduced stress and improved sleep associated with ashwagandha [16,17&23] does help perform better even though it was not measured in this study. Thus, it could be a safer alternative to improve and maintain physical performance.

The ashwagandha extract was tolerated very well at the study dose with no side effects reported. This good safety profile of ashwagandha is consistent with reports from previous studies [24,25].

Our trial has certain strengths, including enrollment of resistance-trained adult men and women, adequate sample size, a double-blind, placebo-controlled design, excellent participant retention, and use of standardized ashwagandha extract. However, one significant drawback is the lack of quantitative data on the subjects' food intake. They were, however, instructed not to change and follow the specified diet for the length of the trial. Although the subjects claimed to have followed the instructions (as per the subject's diary), we were unable to determine if these factors influenced the effect of Ashwagandha due to a lack of reliable detailed quantitative information on daily calorie and protein intake. Another limitation is that our research focused on resistance-trained adults for 8 weeks, so extrapolating results to other groups for a longer duration should be done with caution. As a result, in future studies, we advocate a comprehensive dietary analysis of participants' calorie and protein consumption, as well as a longer-term follow-up with diverse populations.

CONCLUSIONS

Ashwagandha root extract supplementation for 8 weeks improves muscle recovery and serum testosterone levels in adults undergoing resistance training. The study also indicated that the participants tolerated the Ashwagandha root extract well.

DECLARATIONS:

Authors Contributions:

All the authors of this study contributed to the project equally. NV supervised the study at the KGMU hospital and led the study. SKG supervised the study at MV hospital. ST helped in data collection and analysis. AKM conceptualized the study, helped in data analysis and review of manuscript. SP reviewed the data and prepared the manuscript. All the authors participated in study designing and implementation and all the authors have gone through the final article and approved it for submission. Every author actively participated in drafting the manuscript and approved the final version of the article.

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Declaration of Conflicting Interests:

The authors declare that they have no conflicts of interest with the publication of this study.

Declaration on Data Availability:

The data that supports the finding of this study is available from the corresponding author on reasonable request. The data are not publicly available due to privacy or ethical restrictions.

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