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RESEARCH ARTICLE

REVISED Effects of Ashwagandha (*Withania somnifera*) standardized root extract on physical endurance and VO_{2max} in healthy adults performing resistance training: An eight-week, prospective, randomized, double-blind, placebo-controlled study [version 2; peer review: 1 approved, 2 approved with reservations]

Previously titled: Effects of Ashwagandha (*Withania somnifera*) standardized root extract on physical endurance and VO_{2max} in healthy adults performing resistance training: An eight-week, prospective, randomized, double-blind, placebo-controlled study

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Abstract

Background

Ashwagandha is a well-known Ayurvedic herb used for youthful vigor and wellbeing. This study investigated the effects of 600 mg standardized root extract (>5% withanolides) of Ashwagandha (*Withania somnifera*) on muscle size, strength and cardiorespiratory endurance following resistance training.

Methods

In this eight-week, parallel-group, multicenter, randomized, double-

Open Peer Review

Approval Status   

	1	2	3
version 2 (revision) 08 Apr 2024	 view		 view
version 1 24 Mar 2023	 view	 view	

1. Mark E. T. Willems , University of Chichester, Chichester, UK

2. Nawab John Dar , The University of Texas

blind, placebo-controlled clinical study, 80 healthy male and female participants aged 18-45 years, who engaged in regular physical activity were randomly allocated in a 1:1 ratio to receive Ashwagandha (AG, n=40) 300 mg capsules twice daily for eight weeks, or identical placebo (PB, n=40). Seven (3 AG, 4 PB) participants were excluded due to poor compliance. All participants conducted eight-week resistance training. Study outcomes included muscle strength (1RM bench press and leg extension), muscle size (circumference of arm, chest and upper thigh) and cardio-respiratory endurance (VO₂max) assessed at baseline and at eight weeks. Analysis of covariance (ANCOVA) was used to estimate adjusted differences based on sex, BMI and chest circumference at baseline.

Results

AG caused greater improvement in bench press (males: $p = 0.0084$; females: $p = 0.0005$), leg press (males: $p = 0.0049$; females: $p = 0.018$) and endurance (males: $p < 0.0001$; females: $p < 0.0001$) as compared to PB. Also, greater improvements in muscle girth for arm, chest and thigh were seen in both male and female participants with AG. No adverse events were reported in the study.

Conclusions

Eight weeks of AG root extract supplementation along with resistance training is effective in improving muscle strength, growth and endurance in both male and female participants. AG root extract could be a safer, effective and low-cost alternative for athletes to improve muscle endurance.

Keywords

Muscle strength, Cardiorespiratory endurance, Muscle girth, Resistance training



This article is included in the **Agriculture, Food and Nutrition** gateway.

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Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: **Verma N:** Supervision; **Gupta SK:** Supervision; **Patil S:** Writing – Review & Editing; **Tiwari S:** Data Curation, Formal Analysis; **Mishra AK:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft Preparation

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REVISED Amendments from Version 1

Kindly note the following changes:

Title: Change from 'preforming' to 'performing' has been done.

Abstract: Background (ashwagandha dose corrected as 600 mg); Conclusion (removed the term 'recovery' as there were no measures for it).

Introduction: Sentence referring to citation number 6 has been reframed as "Serum creatine kinase is raised in individuals who regularly participate in endurance exercise, with peak levels seen 48 hours after the exercise."

Sentence referring to citation number 18 is reframed as "A previously published study evaluating muscle strength focused only on male participants." and rationale behind including both sexes in our study has been added.

Methods: Due to repetition, details of ethical approval are removed (Study design section).

A statement regarding the personal history and habits of both study groups has been added (Subjects section).

Number of participants in each group who consumed the capsules with milk has been added (Interventions section).

Under "Outcomes" names of tests done for safety assessment are added.

Results: In all the 4 tables, decimal point of one place has been maintained for mean and standard deviation.

Figures 3 and 4 have been removed to avoid duplication of data.

A statement for the outcomes of clinical tests done for safety and tolerability of Ashwagandha has been added in the "Adverse events and safety parameters" section. Data regarding the same has been made available on figshare repository as extended data.

Discussion: A statement regarding milk consumption is added as a limitation. Failure to anticipate lost to follow up in the sample size calculation and study planning has been added.

Data availability: Updated URL of figshare repository having the extended data is added.

"Extended data" has data related to the results of clinical tests done for safety evaluation.

Throughout the article 'muscle mass' is replaced with 'muscle size'; 'chest press' with 'bench press'.

Any further responses from the reviewers can be found at the end of the article

Introduction

Resistance training is a specific conditioning technique in which an 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. The repetition of actions allows the human body to adapt to such resistance and induces strength and endurance over time.²⁻⁴ In addition to strength development, resistance training improves immunity, bone and muscle health and overall function of all organs.⁵ Furthermore, regular exercise alters biochemical and physiological processes. Serum creatine kinase is raised in individuals who regularly participate in endurance exercise, with peak levels seen 48 hours after the exercise.⁶

A major component of physical fitness and resistance training is cardiorespiratory endurance. Maximal oxygen consumption (VO_{2max}) refers to the maximum amount of oxygen that an individual can utilize during an intense exercise session and is considered a valid measure to estimate cardiovascular fitness.⁷ Studies suggest that resistance training enhances VO_{2max} by improving heart-muscle function, increased blood volume and increased oxygen-carrying capacity.⁸

Alongside resistance training and exercises, diet plays a key role in attaining physical and mental fitness and improving overall health. Traditionally, herbs and their extracts have been considered important dietary supplements with multiple health benefits.⁹ Several studies have shown multifaceted efficacy of herbs as they can diminish recovery time, improve performance, enhance muscle size and reduce fat levels.^{10,11} This has resulted in a growing demand for safe and effective herbal supplements to improve endurance and strength performance among athletes and those with an active lifestyle.¹² *Withania somnifera*, popularly known as Ashwagandha (AG), is a plant that belongs to the Solanaceae family and grows in arid or semi-arid regions. AG 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.^{13–15} Pharmacologically, AG has been explored extensively for its potential as a versatile health supplement.¹³

Sandhu *et al.* (2010) studied the impact of 500 mg of AG supplementation for eight weeks on aerobic exercise performance and the activity of associated muscles in healthy young adults. There was a significant increase in VO_{2max} and muscular power.¹⁶ In another study, Raut *et al.* (2012) explored the safety, tolerability and activity of 750 mg to 1250 mg of AG in healthy volunteers for 30 days and found that AG supplementation increased muscle strength, improved sleep quality and reduced lipid concentrations.¹⁷ Wankhede *et al.* (2012) also demonstrated that as in adjunction to a resistance training program, 600 mg of AG supplementation for eight weeks increased muscle strength in untrained male adults.¹⁸ Looking at these previous studies, the current study was based on a hypothesis that AG will lead to a significant improvement in muscle strength, muscle growth and cardio-respiratory endurance in the study participants.

Although preliminary studies mentioned above have been conducted, robust human clinical trials evaluating the performance-enhancing effects of standardized AG root extract are still limited. A previously published study evaluating muscle strength focused only on male participants.¹⁸ However, data on muscle strength and endurance in female participants is scarce. Since gender is an important factor affecting serum hormone levels (and subsequent response to effects of exercise) due to physiological factors, this study focussed on testing the responses in both the genders who are regularly active exercising. There is scarcity of data in previously published literature. Therefore, this randomized, double-blind, placebo-controlled study aims to examine the effects of a standardized AG root extract (as a complement to a resistance training program) on changes in muscle strength, muscle growth and cardio-respiratory endurance in active, healthy participants of either sex.

Methods

Ethical approval

The study was approved by the Institutional Ethics Committees of both King George Medical University (Reference number. #202/IEC/R.Cell-18) and M. V. Hospital and Research (Reference number #IEC/01/28/18) and it was prospectively registered with the Clinical Trials Registry of India (Registration# CTRI/2018/07/014969).

Study design

This was a parallel-group=eight-week, multicenter, randomized, double-blind, PB-controlled study. It was conducted in compliance with the Declaration of Helsinki guidelines, the New Drugs and Clinical trials rules 2019 and the Indian Council of Medical Research (ICMR) ethical guidelines for biomedical research on human subjects.

Recruitment and randomization

The recruitment of participants was performed by circulating printed fliers in the purlieu of a gymnasium which served as the site for the training program. This brochure was approved by both institutional ethics committees. Recruitment of participants started on 11 September, 2018 (first visit of first participant) and ended on 28 March, 2019 (last visit of last participant). Subjects were randomly and equally allocated to two groups in 1:1 ratio using stratified randomization (male and female) to receive either an AG capsule standardized root extract (n=40), or PB which looked identical to AG capsule (n=40). The randomization was computer-generated through randomly permuted blocks of 20. Within each block, the number of participants assigned to each of the two treatment arms was equal. The AG and PB capsules were manufactured and packed in identical containers and labeled equivalently, along with unique serial numbers to ensure blinding. The study centers received numbered and sealed envelopes that contained no information about treatment allocation.

Subjects

All participants read and signed an informed consent form before final selection and enrollment in this study. Healthy adults of either sex aged between 18 to 45 years engaging in regular physical activity (gymnasium/strength training exercise at least three months before screening for this study) were considered eligible. Sex of the participants was defined based on self-reporting. Personal history and habits were found similar in both AG and PB groups. Participants agreed to engage in the same exercise schedule and diet regime for the study period as prescribed in the study protocol and if of child-bearing age, agreed to use barrier birth control measures. Participants were excluded if they were taking any nutritional supplements, medications, or steroids used to enhance physical performance. Those with a history of drug abuse, who smoked more than 10 cigarettes a day, or were habitually consuming more than 14 grams of alcohol per day were also excluded. Other exclusion criteria were: any planned participation in any sporting event during the study period; a weight loss of >5 kg in the previous three months; a history of any orthopedic injury or surgery in the past six months; any known hypersensitivity to AG; participation in any clinical study in the past three months; a history of heart disease, diabetes, depression, stroke or neurological disorder. Participants were also excluded if they were taking

anti-hypertensive drugs, beta-blockers, beta-agonists, hormonal contraceptives, corticosteroids, or psychotropic substances over the previous three months.

Interventions

Capsules containing either 300 mg of AG standardized root extract (KSM-66, Ixoreal Biomed, CA, US) or 300 mg starch (Shri Kartikeya Pharma, Hyderabad, India) were used for AG and PB groups respectively. Both the AG and PB formulations were cellulose-based hard capsules and identical in appearance, weight, texture and color. Participants were required to take one capsule twice daily with milk or water. Two males and one female consumed capsules with milk in AG group, whereas 3 males and one female in PB group consumed capsules with milk.

The AG root extract is a light yellowish powder extracted using a proprietary process. The green extraction process is devoid of any alcohol or similar solvents, hence maintaining the standards as recommended by traditional Ayurveda. The product, KSM-66 Ashwagandha, contains a high concentration of root extract of this herb and more than 5% of total withanolides as standardized by high-performance liquid chromatography. The batch number of product used was KSM/VG/18/1020. The chemical profile of the study product was confirmed by Shri Kartikeya Pharma, Hyderabad, India.

Sample size

The target 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 AG root extract on muscle strength and recovery.¹⁸ A recently published systematic review on AG also supported this kind of improvement (effect size of 0.67) in physical performance.¹⁹ Considering the previous study, we hypothesized that AG treatment is better than PB by an effect size of 0.6 with regards to change in the one-repetition maximum (1 RM) bench press exercise after eight weeks. To detect an effect size of 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 were required while considering a 10% drop-out rate. Thus, 80 healthy male and female subjects were recruited in the study.

Resistance training exercise program

The resistance training regimen used in this study was focused on improving muscle strength, increasing muscle size and enhancing cardiorespiratory endurance. 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.²⁰ Each participant was required to complete the training session every alternate day with a one-day complete rest per week. Therefore, participants were training three days per week. Each training session started with a warm-up of low intensity aerobic exercise. Participants were instructed to perform the maximum repetitions possible for each set until exhaustion. The exercise program is detailed in [Figure 1](#), comprising exercises for week 1, week 2 and remainder of the study (weeks 3 to 8).

Data for this study is available in the online repository 'Figshare' under the dataset named 'Ashwagandha_Muscle study' (*Underlying data*).³⁴

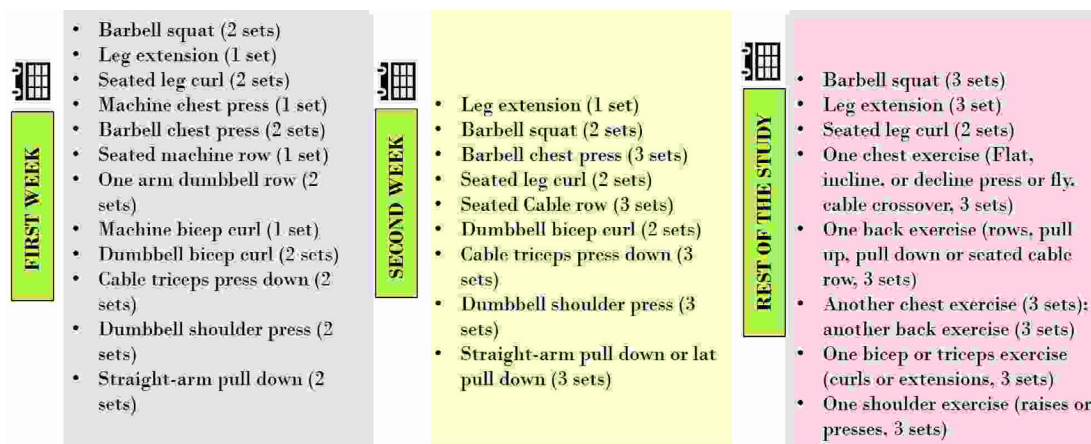


Figure 1. Exercise protocol.

Study outcomes

Primary outcome measures

Muscle strength

Changes in upper and lower body muscular strength were the primary efficacy assessment of this study. As per common practice in sports medicine, muscle strength was assessed by one-repetition maximum (1RM) for bench press and leg press. 1RM testing was performed using NSCA's protocol.²⁰ 1 RM refers to the maximal load lifted by a participant for one cycle of the exercise. Muscle strength measurements were conducted at baseline (first day of training) and end of the study (after the completion of eight weeks of training and supplementation). The equipment used was a Bench Press (SL7028) manufactured by Impulse and a Leg extension (GCEC340) manufactured by Body Solid Inc.

Secondary outcome measures

Muscle girth

Muscle size was measured at three sites - arm (flexed mid-upper arm), chest (sternum at mid-tidal volume) and upper thigh (just inferior to gluteal fold) of each participant. Measurements were undertaken on the first day of training (baseline) and at end of the study (day 56). Maximal cross-sectional area (CSA) was measured for thigh and arm using the Moritani-DeVries method,²¹ whereas, for chest measurement, girth was measured at the level of middle of the sternum by passing a measuring tape under both arms at the end of normal expiration.

Cardiorespiratory endurance (maximum rate of oxygen consumption)

Resistance training and endurance are highly correlated.^{22,23} Cardiorespiratory endurance measures overall body performance during high-intensity exercises. Studies indicate that resistance training induces an increase in the maximal rate of oxygen consumption (VO_{2max}).^{22,23} VO_{2max} is measured during incremental exercise, most typically on a motorized treadmill. Bruce protocol was used to assess the treadmill test outcomes of all participants. Test score was considered as time taken for the test in minutes and it was converted to estimate a VO_{2max} score as per the Bruce protocol.²²

Safety assessment and adverse events

Clinical safety was assessed based on the frequency of adverse events reported by the participants. In addition to this subjective report, standard biochemistry tests (hematology, renal function, liver function and thyroid function tests) were also performed along with measurement of vital signs.

Statistical analysis

Data was entered in Microsoft Excel spreadsheet using manual double-entry method to ensure data accuracy. Statistical analyses were done using MedCalc (version 20.011). Efficacy analysis was done on the modified intention to treat (ITT) dataset (n=73), and safety analysis was done on the whole dataset (n=80). Data for continuous variables are presented as means with standard deviation (SD), whereas categorical and discrete data are presented as counts with percentages. Unpaired t-test was used to assess between-group differences. Change in values from baseline to eight weeks were computed from and compared between the two groups using unpaired t-test. Effects of sex, BMI and chest circumference on different parameters were analyzed using analysis of covariance (ANCOVA). Adjusted means with 95% confidence intervals (CI) and effect size (Cohen's d) were presented for change from baseline. Criteria for effect sizes were 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).²⁴

Normality assumptions were checked on all variables using a one-sample Shapiro-Wilk test. A p-value lower than 0.05 was considered the threshold to claim statistical significance.

Results

Participant demographics

A total of 92 people were screened for participation in the study, of which 80 met the inclusion criteria and were enrolled in the study (Figure 2). All enrolled participants completed the eight-week follow-up. However, four subjects (3 males and 1 female) in the PB and three subjects (3 males) in the AG group did not consume supplementation after their second visit. These participants were documented as having poor compliance to treatment and were excluded from the efficacy

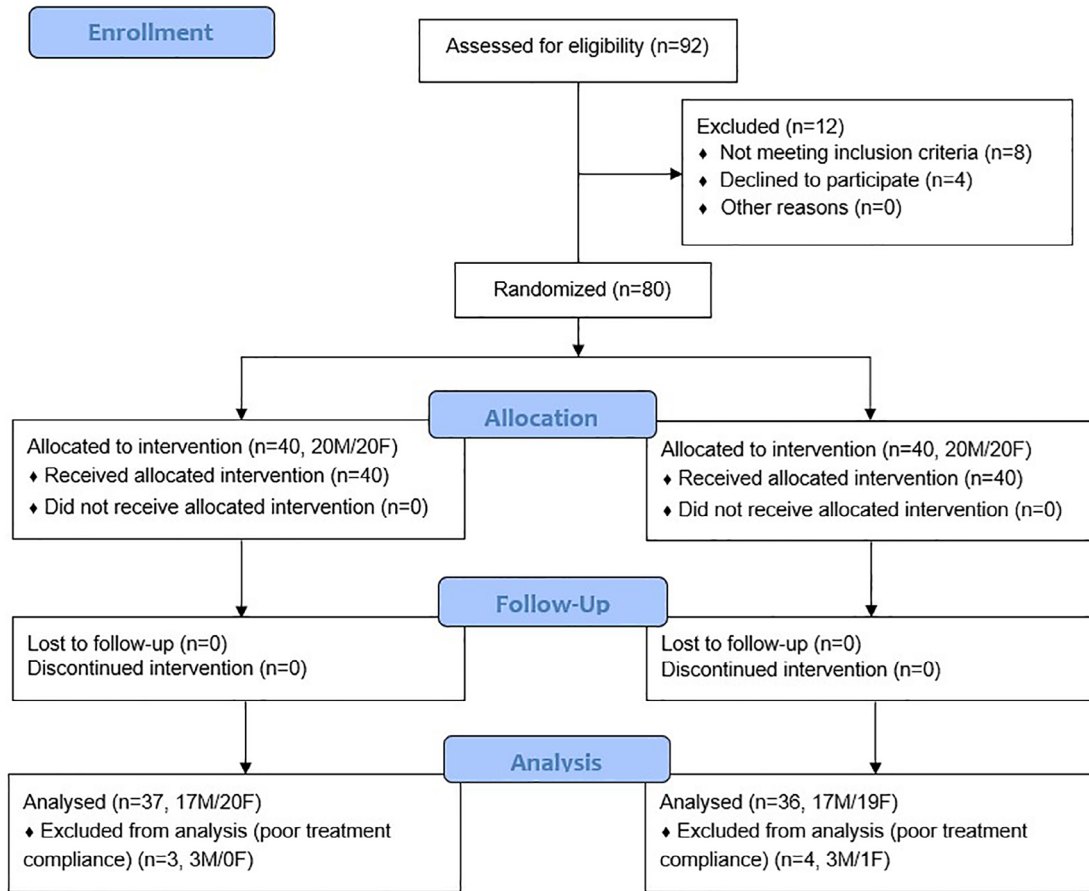


Figure 2. CONSORT flow chart.

analysis. Reasons for premature medication discontinuation were reported as unintentional forgetfulness due to travel and other personal issues by these seven subjects. Baseline demographic characteristics and vital signs are detailed in [Table 1](#) and indicate that the study population was homogenous with no significant differences between the treatment and control groups. Baseline parameters across both the groups are displayed in [Table 2](#).

Table 1. Baseline demography and vital parameters in two groups.

		AG (n=37) M=17/F=20 Mean (SD)	PB (n=36) M=17/F=19 Mean (SD)	Unpaired t-test p
Age (yrs.)	Male	25.2 (4.4)	23.3 (3.9)	0.165
	Female	25.2 (4.5)	23.9 (4.8)	0.528
	Total	25.2 (4.4)	23.7 (4.3)	0.155
Pulse (per min)	Male	78.4 (3.4)	77.6 (5.1)	0.484
	Female	79.0 (4.5)	76.9 (2.1)	0.264
	Total	78.8 (4.0)	77.3 (3.8)	0.213
Systolic BP (mm Hg)	Male	126.8 (5.9)	124.6 (4.5)	0.589
	Female	126.0 (5.2)	127.1 (5.5)	0.476
	Total	126.3 (5.5)	125.9 (5.2)	0.321
Diastolic BP (mm Hg)	Male	79.2 (3.5)	78.2 (4.3)	0.936
	Female	78.3 (4.6)	79.6 (4.4)	0.439
	Total	78.7 (4.1)	78.9 (4.3)	0.165

AG: Ashwagandha; PB: Placebo.

Table 2. Baseline parameters in two groups.

		AG (n=37)	PB (n=36)	Unpaired t-test
		M=17/F=20	M=17/F=19	
		Mean (SD)	Mean (SD)	p
Weight (kg)	Male	62.1 (6.7)	60.3 (4.7)	0.351
	Female	54.9 (4.0)	54.3 (4.3)	0.706
	Total	58.2 (6.4)	57.1 (5.4)	0.451
BMI (kg/sq.m)	Male	23.5 (1.0)	22.5 (1.9)	0.059
	Female	23.0 (0.9)	22.7 (1.4)	0.382
	Total	23.2 (1.0)	22.6 (1.6)	0.049
Body fat (%)	Male	23.8 (2.7)	25.0 (2.3)	0.157
	Female	29.0 (5.0)	28.3 (3.4)	0.771
	Total	26.6 (4.8)	26.7 (3.3)	0.718
1-RM Bench Press (kg)	Male	51.8 (7.8)	54.8 (7.5)	0.261
	Female	49.0 (5.0)	51.4 (6.4)	0.221
	Total	50.3 (6.5)	53.0 (7.0)	0.270
1-RM Leg Press (kg)	Male	60.1 (6.4)	59.5 (9.5)	0.848
	Female	60.0 (6.7)	59.7 (8.4)	0.907
	Total	60.0 (6.4)	59.6 (8.8)	0.553
Max. VO ₂ (ml/kg/min)	Male	38.2 (1.4)	37.8 (2.4)	0.804
	Female	28.8 (1.4)	28.7 (1.4)	0.817
	Total	33.1 (5.0)	33.0 (5.0)	0.982
Mid-arm circumference (cm)	Male	31.1 (2.9)	30.2 (2.1)	0.185
	Female	29.2 (3.0)	27.3 (3.1)	0.063
	Total	30.1 (3.1)	28.6 (3.0)	0.029
Thigh circumference (cm)	Male	58.5 (4.2)	55.8 (10.0)	0.307
	Female	57.8 (6.1)	57.3 (7.4)	0.894
	Total	58.1 (5.3)	56.6 (8.6)	0.396
Chest circumference (cm)	Male	92.2 (6.0)	88.2 (7.5)	0.019
	Female	88.2 (3.5)	84.8 (4.2)	0.031
	Total	90.0 (5.2)	86.4 (6.2)	0.002

AG: Ashwagandha; PB: Placebo.

Muscle strength

Changes in 1 RM bench press and leg press across both treatment groups throughout the eight-week trial period are detailed in [Table 3](#). There were significant differences between the groups in both 1 RM bench press (males: effect size, 0.91; $p=0.0084$; females: effect size, 1.56; $p=0.0005$; total: effect size, 1.14; $p<0.0001$) and 1 RM Leg press (males: effect

Table 3. Change from baseline at 8 weeks in two groups (Univariate tests).

		AG (n=37)	PB (n=36)	Unpaired t-test
		M=17/F=20	M=17/F=19	
		Mean (SD)	Mean (SD)	'p'
Weight (kg)	Male	1.4 (1.1)	1.2 (1.2)	0.605
	Female	1.2 (0.9)	0.8 (0.8)	0.114
	Total	1.3 (1.0)	1.0 (1.0)	0.177

Table 3. *Continued*

		AG (n=37)	PB (n=36)	Unpaired t-test
		M=17/F=20	M=17/F=19	
		Mean (SD)	Mean (SD)	'p'
BMI (kg/sq.m)	Male	0.5 (0.5)	0.4 (0.5)	0.636
	Female	0.5 (0.4)	0.3 (0.3)	0.079
	Total	0.5 (0.4)	0.4 (0.4)	0.140
Body fat (%)	Male	-1.6 (0.3)	-1.7 (0.3)	0.413
	Female	-1.7 (0.2)	-1.7 (0.2)	0.839
	Total	-1.7 (0.3)	-1.7 (0.3)	0.428
1RM Bench Press (kg)	Male	10.7 (8.5)	8.8 (7.4)	0.440
	Female	11.2 (6.1)	9.0 (5.2)	0.226
	Total	11.0 (7.3)	8.9 (6.3)	0.176
1RM Leg Press (kg)	Male	8.0 (7.4)	6.2 (5.9)	0.382
	Female	5.9 (10.5)	2.8 (9.9)	0.330
	Total	7.0 (9.03)	4.5 (8.2)	0.194
Max. VO ₂ (ml/kg/min)	Male	3.6 (1.6)	1.4 (0.5)	<0.0001
	Female	2.0 (0.9)	1.0 (0.6)	<0.0001
	Total	2.8 (1.5)	1.2 (0.6)	<0.0001
Mid-arm circumference (cm)	Male	1.6 (0.4)	1.2 (0.4)	0.009
	Female	1.5 (0.7)	1.2 (0.6)	0.226
	Total	1.5 (0.5)	1.2 (0.5)	0.014
Thigh circumference (cm)	Male	3.4 (3.3)	2.9 (1.1)	0.540
	Female	0.8 (0.1)	0.6 (0.2)	0.009
	Total	2.1 (2.6)	1.8 (1.4)	0.519
Chest circumference (cm)	Male	3.7 (1.7)	2.9 (1.5)	0.099
	Female	3.5 (2.1)	2.0 (0.8)	0.006
	Total	3.6 (1.9)	2.5 (1.3)	0.002

Within group changes (baseline versus week 8) significant at $p < 0.05$ (paired t-test); AG: Ashwagandha; PB: Placebo.

size, 1.22; $p = 0.0049$; females: effect size, 0.63; $p = 0.018$; total: effect size, 1.11; $p = 0.0005$). A within-group analysis in AG group demonstrated a significant ($p < 0.0001$) increase of 23.5 % and 22.8% in 1 RM bench press and an increase of 15% and 9.9% in 1 RM leg press over time and in both male and female participants respectively. Table 4 presents the comparison of AG and PB for change (unadjusted and adjusted for baseline values) in 1 RM leg press and 1 RM bench press from baseline to eight weeks.

Muscle size

Changes in muscle size across the two treatment groups during the study period are detailed in Table 3. At the end of the study, there was a significant increase in both chest circumference (Total participants: $p = 0.019$) and arm circumference (Total participants: $p < 0.0001$), among the participants in AG group as compared to the PB group. However, no improvement was seen in thigh circumference when compared to PB group. A within-group analysis in AG group demonstrated a significant increase in the chest circumference (males: $p < 0.0001$; females: $p < 0.0001$), mid-arm circumference (males: $p < 0.0001$; females: $p < 0.0001$) and thigh circumference (males: $p = 0.0003$; females: $p < 0.0001$). Table 4 presents the comparison of AG and PB for change (unadjusted and adjusted for baseline values) for mid-arm, thigh and chest circumference from baseline to eight weeks.

Endurance

Changes in the VO_{2max} across the two treatment groups throughout the eight-week trial period are detailed in Table 3. At the end of the study, there was a statistically significant between-group difference in VO_{2max} values (males: $p < 0.0001$; females: $p = 0.0001$), when compared with the PB group. In the AG group—a statistically significant 9.5% and 7% increase

Table 4. Comparison between AG and PB for change from baseline at 8 weeks in two groups.

	Difference (AG – PB)		Effect size		'p'	Power
	Unadjusted Mean (95% C.I.)	Adjusted Mean (95% C.I.)	Unadjusted	Adjusted		
Weight (kg)	0.4 (-0.01 to 0.91)	0.7 (0.22 to 1.19)	0.324	0.483	0.005	0.695
BMI (kg/sq.m)	0.2 (0.01 to 0.38)	0.3 (0.10 to 0.49)	0.343	0.497	0.004	0.733
1RM Bench Press (kg)	2.1 (-1.08 to 5.20)	2.5 (-0.96 to 6.04)	0.218	0.241	0.152	0.214
1RM Leg Press (kg)	2.7 (-1.52 to 6.87)	4.4 (-0.02 to 8.85)	0.212	0.332	0.051	0.418
Max. VO ₂ (ml/kg/min)	1.5 (0.99 to 2.10)	1.6 (1.08 to 2.10)	0.930	1.034	<0.0001	1.000
Body fat (%)	0.1 (-0.04 to 0.18)	0.1 (-0.05 to 0.20)	0.209	0.193	0.251	0.238
Mid-arm circumference (cm)	0.3 (0.07 to 0.58)	0.4 (0.16 to 0.70)	0.420	0.526	0.002	0.865
Thigh circumference (cm)	0.3 (-0.73 to 1.34)	0.2 (-0.65 to 1.11)	0.097	0.086	0.607	0.063
Chest circumference (cm)	1.3 (0.49 to 2.06)	1.3 (0.59 to 2.08)	0.538	0.596	0.001	0.910

Adjusted values based on Analysis of Covariance for change from baseline (Covariates appearing in the model are evaluated at the following values: Sex=1.50, baseline BMI=22.83 kg/sq.m, baseline chest circumference=88.18 cm); AG: Ashwagandha; PB: Placebo.

were observed over time in both males ($p<0.0001$) and females ($p<0.0001$), respectively. Table 4 presents the comparison of AG and PB for change (unadjusted and adjusted for baseline values) in VO_{2max} from baseline to eight weeks. The analysis for VO_{2max} yielded 100% power and greater changes were observed with AG as compared to placebo (effect size, 1.034, $p<0.0001$).

Adverse events and safety parameters

The participants did not report any adverse events during the study period. No significant changes were observed from baseline to week 8 in any of the laboratory tests conducted for hematology, renal function, liver function and thyroid function tests with both AG and PP treated participants (supplementary data). Similarly, no changes in any of the vital parameters was observed at week 8 in both treatment groups.³⁴

Discussion

The present study assessed the impact of AG root extract supplementation on resistance training adaptations such as muscle strength and endurance in healthy and active adults. The primary findings of the study are that significantly greater improvements in muscle strength for both lower body (1 RM Leg press) and upper body (1 RM Bench press) occurred in participants consuming AG as compared to PB. In addition, AG supplementation significantly increased muscle size and endurance. However, there were no differences in thigh muscle size (male) and body composition (body fat) between the two groups.

The impact of resistance exercise training on increasing muscular strength was better ($p<0.05$) with AG (23%) versus PB (8%). Similarly, a previous study by Wankhede *et al.* (2012) reported significant improvements in both bench press and leg extension after eight weeks of the AG supplement in healthy adult males.¹⁸ However, these improvements were greater in comparison to the current study, which could be due to the fact that experiment was conducted on inexperienced individuals in the previous study versus resistance-trained adults in the current investigation. Earlier studies recommended that along with strength and muscle growth, factors such as human growth hormone and testosterone were found higher in males compared to females.²⁵ Our study also measured the muscular strength of male and female participants (1 RM bench press and leg press), and a significantly greater outcome was found in males than female participants with an exception of 1 RM bench press in females, both before and after the resistance training program. Furthermore, due to resistance training, general development from the baseline parameters was observed for all the participants. This suggests that the improvement was of clinical relevance (effect size, 0.9, indicating larger effect) which is an improvement over the meta-analysis conducted by Bonilla *et al.* (2021)¹⁹ that analyzed twelve clinical trials of AG on physical performance; however, only two trials were relevant for muscular strength.

The percentage of adults whose chest, thigh and mid-arm muscle size increased differed significantly between the two study groups, although men's thigh size remained similar in both groups. Furthermore, endurance measured by VO_{2max} also increased significantly in AG group which suggests that the effect, although small in magnitude (8%), might be clinically relevant. This observation is lower compared to a previous study by Tiwari *et al.*,²⁶ which reported a 16% improvement in VO_{2max}. Similarly, another study by Choudhary *et al.*²⁷ observed 13% increase in VO_{2max} after eight weeks of AG supplementation. Therefore, the current improvement is comparable and consistent with literature review.

No significant benefit was seen with respect to body fat, as assessed by the body composition monitor. Slight improvement was seen in AG group, but there was no significant difference between the two groups. However, the study was not powered enough to assess the change in body fat.

AG was well tolerated by the subjects and no serious side effects were reported. This finding is consistent with previously published studies on AG (*Withania somnifera*) root extract in healthy volunteers, which also advocated the herb's tolerance.^{17,18,28,29} Thus, it could be a safer alternative to improve and maintain physical performance.

Increased levels of anabolic hormone (serum testosterone) may be the cause of improved muscular strength, which could be associated with its structural similarities to withanolides (major constituents of AG root extract).³⁰ Most of the studies included in the meta-analysis by Bonilla *et al.*, which involved muscle strength, endurance and recovery might be owed to the antioxidant properties of this plant.¹⁹ In addition, as previously shown, AG alleviates stress, improves sleep and physical performance,^{14,31–33} even though these were not measured in this study.

Our trial had certain strengths, including enrollment of resistance-trained adult men and women, adequate sample size, a double-blind, PB-controlled design, excellent participant retention and use of standardized AG extract. Since it was expected that the study participants were highly motivated adults performing regular exercises, we had not anticipated data loss due to lost to follow-up. However, we had data loss not due to lost to follow-up, but due to poor compliance (3 participants in AG and 4 in PB group). We failed to anticipate this possibility during study planning. However, we estimated the effect size (1.14 and 1.11 for 1-RM bench press and 1-RM leg press respectively) for the primary outcomes, to derive our conclusions. Another noteworthy drawback was the lack of quantitative data on the subjects' food intake. They were, however, instructed not to change and follow the specified diet for entire length of the trial. Although the subjects claimed to have followed the instructions (as per subject's diary), we were unable to determine if these factors influenced the effect of AG due to a lack of reliable detailed quantitative information on daily calorie and protein intake. Also, as some participants in both groups consumed the capsules with milk our study lacked to establish the effects of milk consumption on the study parameters. Another limitation is that our research focused on resistance-trained adults for eight 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' daily calorie and protein consumption, as well as a longer-term follow-up with diverse populations.

Conclusions

In conclusion, eight weeks of AG root extract supplementation in combination with resistance training is effective in promoting muscle strength, muscle growth and can also improve endurance in both male and female participants. The study also indicated that the participants have tolerated the AG root extract well. Thus, AG root extract could probably represent a safer low-cost alternative for athletes.

Author 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. SP worked on review and editing of the article. ST helped in data collection and analysis. AKM conceptualized the study, helped in data analysis and writing 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.

Data availability

Underlying data

Figshare: Ashwagandha_Muscle study, <https://doi.org/10.6084/m9.figshare.22081895.v4>.³⁴

The project contains the following underlying data:

- Ashwagandha_Muscle study_Raw data.csv
- Ashwagandha_Muscle study_Raw data.xlsx
- Filled consort checklist
- Study protocol

Extended data

Figshare: Ashwagandha_Muscle study, <https://doi.org/10.6084/m9.figshare.22081895.v4>.³⁴

This project contains the following extended data:

- Data related to the outcomes of clinical tests done for safety assessment of the participants are available in the Figshare repository.

Reporting guidelines

Figshare: CONSORT checklist for ‘Effects of Ashwagandha (*Withania somnifera*) standardized root extract on physical endurance and $\text{VO}_{2\text{max}}$ in healthy adults performing resistance training: An eight-week, prospective, randomized, double-blind, placebo-controlled study’. <https://doi.org/10.6084/m9.figshare.22081895.v4>.³⁴

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgments

The authors thank Ixoreal BioMed Inc., Los Angeles, California, USA, for supplying the AG high concentration root extract used in this study.

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Open Peer Review

Current Peer Review Status:   

Version 2

Reviewer Report 09 July 2024

<https://doi.org/10.5256/f1000research.163630.r287564>

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Giuseppe Potrick Stefani 

Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brazil

This work is a randomized, double-blind, placebo-controlled clinical trial, in which researchers evaluated the effect of Withania somnifera supplementation on different markers of body composition and maximum strength. The writing of the manuscript is adequate, although it presents some necessary changes to improve the reading agreement. The present study advances the knowledge about phytochemicals, especially about Withania somnifera. However, it presents some limitations that should be highlighted to readers. Likewise, he left some questions with the aim of better understanding the experiment that the authors conducted:

- Inform methodology for evaluating the percentage of body fat;
- Is the evaluator of body composition parameters an ISAK-certified anthropometrist?
- What is the technical intra-evaluator measurement error for perimeters and skinfolds?
- How was the participants' supplementation controlled? This must be described in the methodology and results of adhesion to the capsules.
- Did the method for assessing the perimeter of the lower and upper limbs include only the total perimeter or corrected by skinfold thickness? The entire experimental procedure must be described if it was the second method.
- Did the authors analyse food consumption close to the strength and VO2max tests? The absence of these data is a limiting factor for data interpretation and should be highlighted to readers if quantification control has not been carried out.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Sports Nutrition, Physiology, Metabolism, Body Composition, Dietary Supplements.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 22 April 2024

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Mark E. T. Willems 

Institute of Applied Sciences, College Lane, University of Chichester, Chichester, England, UK

Thanks for addressing all my comments.

I have no further essential comments. The authors may want to change the units for VO₂max to "mL / (kg min)" or mL x kg⁻¹ x min⁻¹.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Exercise Physiology, Training, Sports Nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 27 November 2023

<https://doi.org/10.5256/f1000research.143730.r200814>

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Nawab John Dar

School of Medicine, The University of Texas Health Science Center, San Antonio, Texas, USA

The study entitled " Effects of Ashwagandha (*Withania somnifera*) standardized root extract on physical endurance and VO_{2max} in healthy adults performing resistance training: An eight-week, prospective, randomized, double-blind, placebo-controlled study by Verma *et al*, is well designed and conducted, however, I have few queries before it is approved for indexing:

1. The authors did not mention about the habits of the volunteers, what about their smoking habits and sleep cycle?
2. Who manufactured the AG capsules and how did they made sure Quality of the capsules (QC&QA), why would they buy the control and AG from different companies?
3. I would love to know what happened to the endurance once the study is over and the volunteers stopped taking AG?

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neuropharmacology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Apr 2024

sayali patil

Dear Nawab John Dar,

Thank you for your time and valuable feedback on our manuscript. We have carefully reviewed your comments. As suggested, we provide a point-by-point response as below:

- *The authors did not mention about the habits of the volunteers, what about their smoking habits and sleep cycle?*

The two groups were similar with respect to the personal history and habits. A statement to that effect is added in the "subjects" section of the article (version 2).

- *Who manufactured the AG capsules and how did they made sure Quality of the capsules (QC&QA), why would they buy the control and AG from different companies?*

Control capsules were manufactured specifically for the study purpose and hence, it was outsourced to third party manufacturing. However, the study capsules (AG) were provided to manufacturer to match (identical placebo) the control (PB) capsules with AG.

- *I would love to know what happened to the endurance once the study is over and the volunteers stopped taking AG?*

Follow-up was not a part of the study plan and we did not assess this.

Competing Interests: None

Reviewer Report 13 November 2023

<https://doi.org/10.5256/f1000research.143730.r219543>

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Mark E. T. Willems 

Institute of Applied Sciences, College Lane, University of Chichester, Chichester, England, UK

In the title, change “preforming”.

Please clarify In the background section of the abstract that 600 mg was used.

Please revise in the background of the abstract that muscle mass was not measured. An indirect measurement of cross sectional area seems to have been measured.

Please have specific conclusions. There are no measurements of recovery. Please revise the conclusion in the abstract.

The introduction does not provide justification for the need to examine the effects in females. Please provide that justification.

P3, Does we need the statement supported by reference 6. It is very misleading and has nothing to do with the study.

P3. Just linking physical endurance in males with testosterone is an extreme simplification of all the factors and determinants of physical endurance. Please revise.

There is repeat in the method section regarding ethical approval. Please revise.

The methods indicate that participants could take the capsules with milk. Please provide the number of participants in each group that consumed the capsules with milk. Milk intake may enhance protein synthesis and may have affected some of the parameters of interest. This needs to be discussed and probably acknowledged as a limitation of the study.

The sample size calculation is confusing. It seems 40 participants were required in each group and with consideration of 10% drop-out, 44-45 participants should have been in each group. Please clarify.

On P5 is mentioned “standard biochemistry tests”. Please provide more information.

Table 1. I suggest to provide values without decimal places.

Table 2. Do you need to express values with two decimal places? Please reconsider.

Please be consistent with terminology. Is the bench press the same as the chest press?

Table 3. Are you allowed to express the differences with three decimal places? For example, with circumference changes expressed with 3 decimal places indicates a measurement to the nearest 0.001 cm. I am sure that was not done.

The p-values in the text in the result section does not match with the information provided in the Tables. For example "There were significant differences between the groups in both 1 RM bench press (males: effect size, 0.91; $p=0.0084$; females: effect size, 1.56; $p=0.0005$; total: effect size, 1.14; $p<0.0001$)". Please ensure that text information matches table information.

Figs 3 and 4 has data that is communicated as well in Table 3. Please avoid duplication. I suggest to delete Figs 3 and 4 as it does not present new information.

P10. Please provide the outcome of the clinical tests.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Exercise Physiology, Training, Sports Nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 02 Apr 2024

sayali patil

Dear Mark E. T. Willems,

Thank you for your time and valuable feedback on our manuscript. We have carefully reviewed your comments. As suggested, we provide a point-by-point response as below:

- *In the title, change "preforming".*

Correction from "preforming" to "performing" in the title has been made for version 2 of the article.

- *Please clarify in the background section of the abstract that 600 mg was used.*

Correction from 300mg to 600mg of Ashwagandha has been made for version 2 of the article. [page 1, paragraph 1]

- *Please revise in the background of the abstract that muscle mass was not measured. An indirect measurement of cross-sectional area seems to have been measured.*

As suggested "muscle mass" in abstract is replaced with "muscle size" in the version-2 of the article. (page 1, paragraph 1; page 3, paragraph 3; page 4, paragraph 6)

- *Please have specific conclusions. There are no measurements of recovery. Please revise the conclusion in the abstract.*

We acknowledge that there were no measurements of recovery. Revision has been made for version 2 of the article. [page 2, paragraph 2]

- *The introduction does not provide justification for the need to examine the effects in females. Please provide that justification.*

"Since gender is an important factor affecting serum hormone levels (and subsequent response to effects of exercise) due to physiological factors, this study focussed on testing the responses in both the genders who are regularly active exercising. There is scarcity of data in previously published literature."

This above statement has been added in the paragraph 5 on page 3.

- *P3, do we need the statement supported by reference 6. It is very misleading and has nothing to do with the study.*

"Biochemical assessments reveal that individuals who regularly participate in endurance exercise have significantly higher creatine kinase levels, which peak approximately 48 hours after the exercise."

This statement on page-3 is rephrased to improve clarity as below:

"Serum creatine kinase is raised in individuals who regularly participate in endurance exercise, with peak levels seen 48 hours after the exercise."

- *P3. Just linking physical endurance in males with testosterone is an extreme simplification of all the factors and determinants of physical endurance. Please revise.*

"A previously published study evaluating muscle strength focused only on male participants.¹⁸ Physical endurance is considered higher in males owing it to the testosterone hormone."

The sentences are revised as below:

"A previously published study evaluating muscle strength focused only on male participants.¹⁸ However, data on muscle strength and endurance in female participants is scarce."

- *There is repeat in the method section regarding ethical approval. Please revise.*

We have removed the repetition and ethical approval details are mentioned only in the “ethical approval” section under Methods.

- *The methods indicate that participants could take the capsules with milk. Please provide the number of participants in each group that consumed the capsules with milk. Milk intake may enhance protein synthesis and may have affected some of the parameters of interest. This needs to be discussed and probably acknowledged as a limitation of the study.*

Two males and one female consumed capsules with milk in AG group, whereas 3 males and one female in PB group consumed capsules with milk.

This information has been included in the “Interventions” section of the article (version 2). The same has been included as a limitation of the study as suggested.

- *The sample size calculation is confusing. It seems 40 participants were required in each group and with consideration of 10% drop-out, 44-45 participants should have been in each group. Please clarify.*

Since it was expected that the study participants were highly motivated adults performing regular exercises, we had not anticipated data loss due to lost to follow-up. However, we had data loss not due to lost to follow-up, but due to poor compliance (3 participants in AG and 4 in PB group). We failed to anticipate this possibility during study planning. However, we estimated the effect size (1.14 and 1.11 for 1-RM bench press and 1-RM leg press respectively) for the primary outcomes, to derive our conclusions.

- *On P5 is mentioned “standard biochemistry tests”. Please provide more information.*

Please find the outcomes of the “standard biochemistry tests” as clinical tests (hematology, renal function, liver function and thyroid function tests) included in the article’s Data Availability section as “Extended data”.

- *Table 1. I suggest to provide values without decimal places.*

All 3 tables in the article uniformly have values with a single decimal place in the version-2 of this article.

- *Table 2. Do you need to express values with two decimal places? Please reconsider.*

All 3 tables in the article uniformly have values with a single decimal place in the version-2 of this article.

- *Please be consistent with terminology. Is the bench press the same as the chest press?*

We have made changes and kept the terminology as “bench press” throughout the article in the version 2.

- *Table 3. Are you allowed to express the differences with three decimal places? For example, with circumference changes expressed with 3 decimal places indicates a measurement to*

the nearest 0.001 cm. I am sure that was not done.

Thank you for the observation. All 3 tables in the article uniformly have values with a single decimal place in the version-2 of this article.

- 1. The p-values in the text in the result section does not match with the information provided in the Tables. For example, "There were significant differences between the groups in both 1 RM bench press (males: effect size, 0.91; $p=0.0084$; females: effect size, 1.56; $p=0.0005$; total: effect size, 1.14; $p<0.0001$)". Please ensure that text information matches table information.*

Kindly note that in the result section (page 8, paragraph 1), p-values in the text are with respect to the effect size whereas p-values in tables are with respect to the comparison of means (t-test).

- o Figs 3 and 4 has data that is communicated as well in Table 3. Please avoid duplication. I suggest to delete Figs 3 and 4 as it does not present new information.*

Thank you for the observation. We have removed Figures 3 and 4 for the version 2 of the article.

- o P10. Please provide the outcome of the clinical tests.*

Please find the outcomes of the clinical tests (hematology, renal function, liver function and thyroid function tests) included in the article's Data Availability section as "Extended data".

Competing Interests: None

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