

RESEARCH ARTICLE

Efficacy and Safety of Low-Dose Ashwagandha Supplementation on Exercise Endurance: A Randomized, Placebo-Controlled, Double-Blind, Clinical Trial

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Received: 4 July 2025 | **Revised:** 25 December 2025 | **Accepted:** 30 December 2025

Keywords: ashwagandha | fatigue | low-dose | safety | VO₂max | *Withania somnifera*

ABSTRACT

Ashwagandha (*Withania somnifera*) is a well-known adaptogen with established roles in stress modulation and physical performance enhancement traditionally. Low dose Ashwagandha (LDA/Ashwa 0.30) is a bioactive-optimized root-only extract standardized to withanolides (> 15% w/w) and an ATP-active fraction (> 15% w/w). This study investigated the efficacy and safety of LDA supplementation on cardiorespiratory fitness and exercise-induced fatigue in healthy individuals. In this eight-week, randomized, double-blind, placebo-controlled study, healthy adults received either LDA (30 mg/day) or placebo. VO₂max was assessed as primary endpoint using an indirect method via the Modified Bruce Protocol Treadmill Test (MBPTT). Secondary endpoints included lactic acid, creatine phosphokinase (CPK), Rating of Perceived Exertion (RPE), fatigue scores (VAS), and safety biomarkers. LDA significantly improved VO₂max and maximal heart rate compared to the placebo. Participants in the treatment group showed lower lactic acid levels at exercise, indicating prolonged aerobic metabolism. CPK levels were significantly reduced, suggesting mitigation of muscle energy stress. Borg RPE and fatigue scores improved in the LDA group. LDA was well tolerated, with no treatment-related adverse events. Hematological, hepatic, renal, metabolic, and vital sign parameters remained within normal clinical ranges following intake of LDA. LDA supplementation for 8 weeks significantly enhanced cardiorespiratory fitness, delayed fatigue onset, and was safe for use in healthy adults. These findings support its potential as a safe and effective low-dose aid for endurance enhancement and overall exercise performance.

1 | Introduction

Herbal products derived from various plant parts—such as roots, seeds, leaves, and stems—have long been recognized for their broad-spectrum health benefits. In recent years, there has been a notable increase in the consumption of herbal supplements among both athletic and non-athletic/general populations to

enhance endurance and energy levels (Amir et al. 2023). During moderate to high-intensity exercise, the human body primarily relies on key energy substrates such as glucose stored in hepatic and muscular tissues. However, intense physical activity is also associated with the accumulation of metabolic byproducts, such as lactic acid and ammonia, which contribute to intracellular acidosis and thereby lead to fatigue.

Abbreviations: AE, adverse event; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; CDSCO, Central Drugs Standard Control Organization; CONSORT, consolidated standards of reporting trials; CPK, creatine phosphokinase; CRF, case report form; CRO, clinical research organisation; CTRI-India, clinical trial registry-India; DBP, diastolic blood pressure; EMG, electromyogram; GCP, good clinical practice; GMP, good manufacturing practice; HRmax, maximum heart rate; HRrest, heart rate at rest; ICH, international conference on harmonisation; IP, investigational product; LDH, lactate dehydrogenase; MBPTT, modified bruce protocol treadmill test; N, number of subjects; NDCT, new drugs and clinical trials; PP, per protocol; RBC, red blood cell; RBG, random Blood Glucose; RPE, rating of perceived exertion; SBP, systolic blood pressure; SpO₂, peripheral oxygen saturation; TSH, thyroid stimulating hormone; USP, United States Pharmacopeia; VAS, visual analogue scale; VO₂max, maximal oxygen uptake; WBC, white blood cell.

Addressing physical endurance and exercise-induced fatigue through herbal interventions has become a growing area of scientific interest (Chen et al. 2022). Adaptogenic herbal extracts, known to enhance the organism's nonspecific resistance to a variety of stressors, have demonstrated the ability to improve mitochondrial ATP production efficiency. This leads to enhanced cardiovascular endurance, increased energy availability, and reduced fatigue (Panossian and Wikman 2009).

Among the various adaptogens, *Withania somnifera* (commonly known as Ashwagandha) is one of the most widely studied and traditionally used herbs worldwide.

W. somnifera is cultivated in hot and arid regions of tropical and subtropical climates. It has been utilized for over 3000 years in the Indian traditional system of medicine (Ayurveda) as a *rasayana*—a rejuvenative tonic believed to promote physical and mental well-being and support longevity (Mandlik and Namdeo 2021; Lopresti and Smith 2021). Classical Ayurvedic texts such as *Charaka Samhita* and *Sushruta Samhita* (circa 1000 BCE) specifically emphasize the use of the root (*Mula*) of the plant for its therapeutic effects (Joshi and Joshi 2021).

The global surge in demand for Ashwagandha-based products has resulted in the development of various formulations, most commonly derived from the root or root-stem combinations. Despite traditional recommendations favoring root use, many commercially available root-based formulations either require high daily doses (typically in the range of 300–600 mg) to demonstrate clinical efficacy or lack globally harmonized standardization protocols for active constituents. A recent review by Zellner et al. (2025) critically evaluated 19 commercial Ashwagandha products, highlighting the absence of validated, universally accepted standardization techniques. This underscores a significant unmet need for low-dose, standardized Ashwagandha formulations with proven efficacy and safety.

The present study was designed to evaluate the effects of a low-dose Ashwagandha (LDA) formulation administered over an 8-week period in healthy adults aged 18–45 years. The primary objective was to assess the influence of LDA supplementation on cardiovascular endurance, as measured by maximal oxygen consumption (VO_2max)—a gold standard parameter for aerobic fitness. Secondary objectives included evaluating the effects of LDA on lactic acid accumulation, a biomarker of anaerobic metabolism during high-intensity exercise, as well as assessing perceived exertion and fatigue experienced by study participants.

2 | Methods

2.1 | Study Design

A randomized, prospective, double-blind, placebo-controlled clinical study was conducted at a clinical study site in India between November 2024 and February 2025, involving healthy exercising subjects. The study was initiated following approval by an independent ethics committee and was prospectively registered with the Clinical Trial Registry-India (CTRI/2024/10/075637). It was conducted in accordance with the study protocol, the applicable requirements of the Central Drugs Standard

Control Organization (CDSCO), the International Council for Harmonisation Good Clinical Practice (ICH-GCP) Guidelines, the Declaration of Helsinki, and the New Drugs and Clinical Trials (NDCT) Rules 2019 of India.

The study comprised screening visits (Day-7 to Day 1), randomization visit (Day 1), follow-up visits (Day 7, Day 28 and Day 54), and a final visit (Day 56), with a treatment duration of 8 weeks for each subject. During the screening period (Day-7 to Day 1), subjects were assessed for eligibility based on the inclusion and exclusion criteria. Eligible subjects underwent baseline assessments including demographic data collection, personal and medical history, prior medication review, vital signs measurement, physical examination, and laboratory investigations. Additionally, baseline clinical evaluations for perceived exertion (using the Borg rating of perceived exertion (RPE) scale), fatigue (using a Visual Analog Scale (VAS)), and neuromuscular activation via electromyogram (EMG) following a 20-Meter Shuttle Run Test were carried out during the screening visits). A Modified Bruce Protocol Treadmill Test (MBPTT) was carried out on the randomization visit (Day 1), and it included determination of $\text{VO}_{2\text{max}}$, blood lactate, and blood creatine phosphokinase (CPK) measurements during the MBPTT. Perceived exertion (using the Borg RPE scale) and fatigue (using a Visual Analog Scale (VAS)) were also assessed following the MBPTT.

Following baseline evaluations, eligible subjects were randomized into two groups (LDA group or placebo group) and instructed to take one capsule of either LDA or placebo orally each morning either before or after food for 56 days. Each subject was provided with a diary with instructions to record date and time of investigational product (IP) consumption. Medication compliance was done by site personnel, from subject diary during their follow-up visit and end of study visit. All subjects were instructed to bring the diary to each subsequent study visits. At each post-randomization visit, vital signs, physical examinations, and compliance with study procedures were assessed. Clinical evaluations including perceived exertion (using Borg RPE scale), fatigue (using VAS), neuromuscular activation (by EMG) following the 20-Meter Shuttle Run Test and $\text{VO}_{2\text{max}}$, lactic acid threshold and blood CPK levels during the MBPTT along with the laboratory investigations and IP accountability were assessed during the final study visit.

2.2 | Participants

Healthy adult male and female (non-pregnant female subjects if they were of child-bearing potential) subjects who provided written informed consent to participate in the study, performing regular physical exercise almost 5 days a week over the last 2 years, aged between 18 and 45 years, with a body mass index (BMI) between 18.5 and 24.9 kg/m^2 , systolic blood pressure (SBP) ≤ 130 mmHg, and diastolic blood pressure (DBP) ≤ 89 mmHg, and who agreed to abstain from alcohol, caffeine, and vigorous physical activity for 24 h prior to each study visit were included.

Subjects were excluded if they met any of the following criteria: known hypersensitivity to the study product; blood donation within 1 month prior to screening; hemoglobin level < 12.0 g/dL;

history of any pulmonary disorder, peripheral oxygen saturation (SpO_2) < 96%, random blood glucose (RBG) levels ≥ 140 mg/dL; abnormal thyroid stimulating hormone (TSH) level (> 4.2 $\mu\text{IU/mL}$); history of any medical condition where exercise was contraindicated; inability to run due to any joint disorder; history of smoking or active smokers using any form of tobacco; history/symptoms of any cardiovascular disorder such as coronary artery disease or myocardial infarction; history of any significant neurological and psychiatric condition that could affect the participation or inference of the study endpoints; participation in other clinical trials in the last 90 days before screening; use of nutritional supplements, medications, or steroids intended to enhance physical performance; history of any orthopedic injury or surgery that could limit exercise capacity; or plans to participate in any sporting event during the study period were excluded from the study.

2.3 | Randomization and Blinding

Eligible subjects were randomized into Arm 1 (LDA group) or Arm 2 (Placebo group) in a 2:1 ratio according to the randomization schedule. Subjects deemed eligible for randomization were assigned a unique treatment assignment number, which was appropriately recorded in the source documents and case report forms (CRFs). Study medication was dispensed based on the next available treatment assignment number, which was distinct from the subject number.

Randomization documentation, including sealed, blinded envelopes and pharmacy records, was securely stored in the site pharmacy, separate from participant study files. This information was not accessible to the study staff responsible for conducting study procedures with participants. Blinding was maintained until all study data had been entered into the study database, all endpoint and other data included in the final analysis had been collected, verified, and finalized, and the database was ready for analysis. The blinding process and conditions under which unblinding might occur were explained to participants during the informed consent process. Only the designated study pharmacist from the Contract Research Organization (CRO) had full access to the randomization list/schedule. Site personnel, including the investigator and study team, had only access to the treatment assignment number.

2.4 | Plant Material & Intervention

Withania somnifera, also known as Ashwagandha (Family: Solanaceae), cultivated roots were sourced from Andhra Pradesh, India. The authentication was done, and voucher specimen number 128101 is kept in the Herbarium of the Foundation for Revitalisation of Local Health Traditions (FRLHT), Bengaluru.

Test substance used was a proprietary (Pending patent: 202541047928) extract derived from the roots of *Withania somnifera*. It was extracted with ethanol and water to achieve a standardization of withanolides ($> 15\%$ w/w determined using a modified USP HPLC method). In addition, the extract was also standardized to ATP active fraction ($> 15\%$ w/w determined

using gravimetric method). Notably, the ATP-active fraction in LDA is devoid of withanolides yet plays a pivotal role in promoting endurance. Chemically, this fraction is enriched in arabinogalactans and contains a complex matrix of secondary metabolites, including saponins, micronutrients (vitamins, minerals, and amino acids), phenolic compounds, and other minor bioactive constituents, which together are hypothesized to contribute synergistically to the endurance-enhancing effects of LDA (Patent Pending).

LDA capsules containing 30 mg of Ashwagandha extract (Brand name: Ashwa 0.30) were used as the test product and capsules containing microcrystalline cellulose and syloid 244 FP excipients were used as the placebo. Both LDA and placebo capsules were manufactured in a Good Manufacturing Practice (GMP)-certified facility and the test products complied with the United States Pharmacopeia (USP) requirements for dietary supplements, including limits for microbial contaminants, heavy metals, residual pesticides, aflatoxins and residual solvents. Each white bottle dispensed to subjects contained 34 capsules of either LDA or placebo. Each product bottle was labelled with a unique randomization number. Subjects were instructed to take one capsule daily in the morning, either before or after food, for a duration of 56 days. The study products (LDA capsules and placebo capsules) were identical in appearance and were packaged in a manner designed to maintain the study blinding.

2.5 | Outcomes

The primary outcome of the study was the mean change in cardiovascular endurance (by maximal oxygen uptake ($\text{VO}_{2\text{max}}$)) from baseline to the end of the study compared to placebo. The secondary outcomes included changes in lactic acid levels, blood CPK levels, subjective rate of perceived exertion, fatigue level, and neuromuscular activation from baseline to Day 54/Day 56 compared to placebo. Additionally, safety assessments including the documentation of adverse events were also considered as secondary outcomes.

Cardiovascular endurance (by $\text{VO}_{2\text{max}}$) was assessed during the Modified Bruce protocol Treadmill Test at baseline (Day 1) and on day 56. The Modified Bruce protocol Treadmill Test (McDermott et al. 2022) commenced with a warm-up phase, during which subjects performed a 5 to 10-min light exercise prior to the test. Resting heart rate was measured prior to the initiation of the test. The test began with subjects walking on a treadmill at a speed of 2.7 km/h (1.7 mph) with no incline (Level 0) for 3 min. In the second stage, the incline was increased to Level 1 while maintaining the same speed for another 3 min. The third stage involved raising the incline to Level 2, with the speed remaining constant at 2.7 km/h (1.7 mph) for an additional 3 min. Subsequent stages involved increases in both treadmill speed and incline at three-minute intervals as detailed in Supplementary Table S1. The test was continued until the subject reached volitional exhaustion, defined as either achieving their maximum heart rate or being unable to continue exercise. Blood samples were collected at three-minute intervals during the exercise to assess the lactic acid threshold, which was measured using a Lacto Spark instrument (Manufactured by Sensa Core Medical Instrumentation PVT. LTD, India).

The cardiovascular endurance (by VO_2max) was calculated using the formula:

$$\text{VO}_2\text{max} = 15 \times (\text{HRmax} / \text{HRrest})$$

where HRmax represents the maximal heart rate recorded at volitional exhaustion during the Modified Bruce Protocol Treadmill Test and HRrest is the resting heart rate measured prior to the test.

Blood samples collected during the Modified Bruce Protocol Treadmill Test at baseline (Day 1) and on day 56 were used to determine the lactate threshold using the Lacto Spark instrument (Sensa Core Medical Instrumentation Pvt. Ltd., India). The device measures the blood lactate from 0.5 μL of capillary blood, providing results within 5 s. Serum CPK levels were measured at baseline (Day 1) and on Day 56 using the NAC (N-acetyl-L-cysteine) kit method, Alinity CI module instrument (Abbott, USA).

For the 20-Meter Shuttle Run Test (Léger and Lambert 1982; Ozmen et al. 2017; Sletdaløkken and Rønnestad 2014), subjects began with a 5–10-min warm-up exercise, followed by practice trials to familiarize themselves with the procedure. During the test, subjects were instructed to run back and forth between two markers placed 20 m apart, in synchrony with pre-recorded beep signals. The initial running speed was set at 8.5 km/h, increasing by 0.5 km/h every minute. Subjects were required to reach one end of the 20-m course at the sound of each beep. The test was terminated when a participant could no longer continue or failed to complete two consecutive laps within the designated time frame, as outlined in Supplementary Table S2.

Perceived exertion was assessed at baseline (Day-2 and Day 1), Day 7, Day 54, and Day 56 by Borg Rating of Perceived Exertion (RPE) scale while performing the 20-Meter Shuttle Run Test and Modified Bruce Protocol Treadmill Test. The Borg RPE scale measures an individual's subjective perception of effort during physical activity and the scale ranges from 6 (no exertion) to 20 (maximal exertion). Neuromuscular activation was measured by electromyogram (EMG) of four lower limb muscle groups: the Quadriceps, Calf muscles (Gastrocnemius), Hamstring, and Adductor muscles. Assessments were conducted at baseline (Day-2), Day 7, and Day 54 after Modified Bruce Protocol Treadmill Test. Fatigue levels were evaluated using the VAS at baseline (Day-2 and Day 1), Day 7, Day 54, and Day 56 after completion of both the Modified Bruce Protocol Treadmill Test and 20-Meter Shuttle Run Test. The VAS ranged from 0 (no fatigue) to 100 (complete exhaustion).

2.6 | Safety Assessments

Safety assessments included monitoring of adverse events (AEs), vital signs (SpO_2 , blood pressure, respiratory rate, pulse rate, and body temperature), physical examinations, and laboratory investigations. AEs were monitored continuously throughout the study. Vital signs and physical examinations were performed at each study visit. The physical examination included assessment of general appearance, respiratory, skin, head, eyes, ears, nose, throat, heart, abdomen, reflexes, lymph nodes, and

extremities, neurological, and mental status. Laboratory assessments included hematology (hemoglobin, RBC count, platelet count, WBC count, hematocrit, neutrophils, eosinophils, lymphocytes, basophils, and monocytes) and clinical biochemistry (LDH, ALT, AST, RBG, serum creatinine, total cholesterol, triglycerides, and BUN). Laboratory investigations were conducted at baseline and at the end of the study except LDH, which was additionally assessed on Day 7 and Day 28.

2.7 | Sample Size

The sample size for this clinical study was calculated based on a standard deviation of 2.86 and a difference to be detected of 3.22, as reported in the literature (Malik et al. 2013). The calculation followed the method described by Dell et al. (2002), with a significance level (alpha) of 0.05 and a statistical power of 90%. Allowing for a 20% dropout rate, the estimated total sample size required was 44 participants. However, keeping in view the differences in the experimental methods for endurance, the sample size was increased to 66.

2.8 | Statistical Methods

Descriptive statistics were used for demographic and baseline clinical characteristics, including laboratory safety parameters. Quantitative variables were reported as mean $\pm$ standard deviation, while categorical variables were presented as counts or percentages. Within-group comparisons were performed using a paired *t* test or Wilcoxon signed-rank test, depending on data distribution. Between-group comparisons were conducted using an unpaired *t* test or Mann-Whitney *U* test. Normality tests (Kolmogorov-Smirnov or Shapiro-Wilk) were conducted to assess data distribution assumptions. All statistical tests were two-sided, with a significance threshold set at $p \leq 0.05$. Per Protocol (PP) population was used for the analysis. PP population included all randomized subjects who completed the study as per the protocol without any major protocol deviation/protocol violation.

3 | Results

3.1 | Demography (Baseline Characteristics) and Participants Flow

Overall, 86 potential subjects were assessed for participation in the study after providing informed consent. Of these, 20 subjects did not meet the inclusion criteria, and a total of 66 subjects were subsequently enrolled. The enrolled subjects were randomized to two groups, namely the LDA group ($n=44$) and the placebo group ($n=22$). The flow of participants through the study, as per CONSORT guideline is illustrated in Figure 1.

The mean (SD) age of the subjects in the LDA and placebo groups was 34.0 (6.28) years and 37.1 (5.20) years, respectively. No notable differences were observed in demographic characteristics between the two groups, except for concomitant medication use, where one subject (4.55%) in the placebo group reported concomitant medication use, compared to none (0%) in the LDA

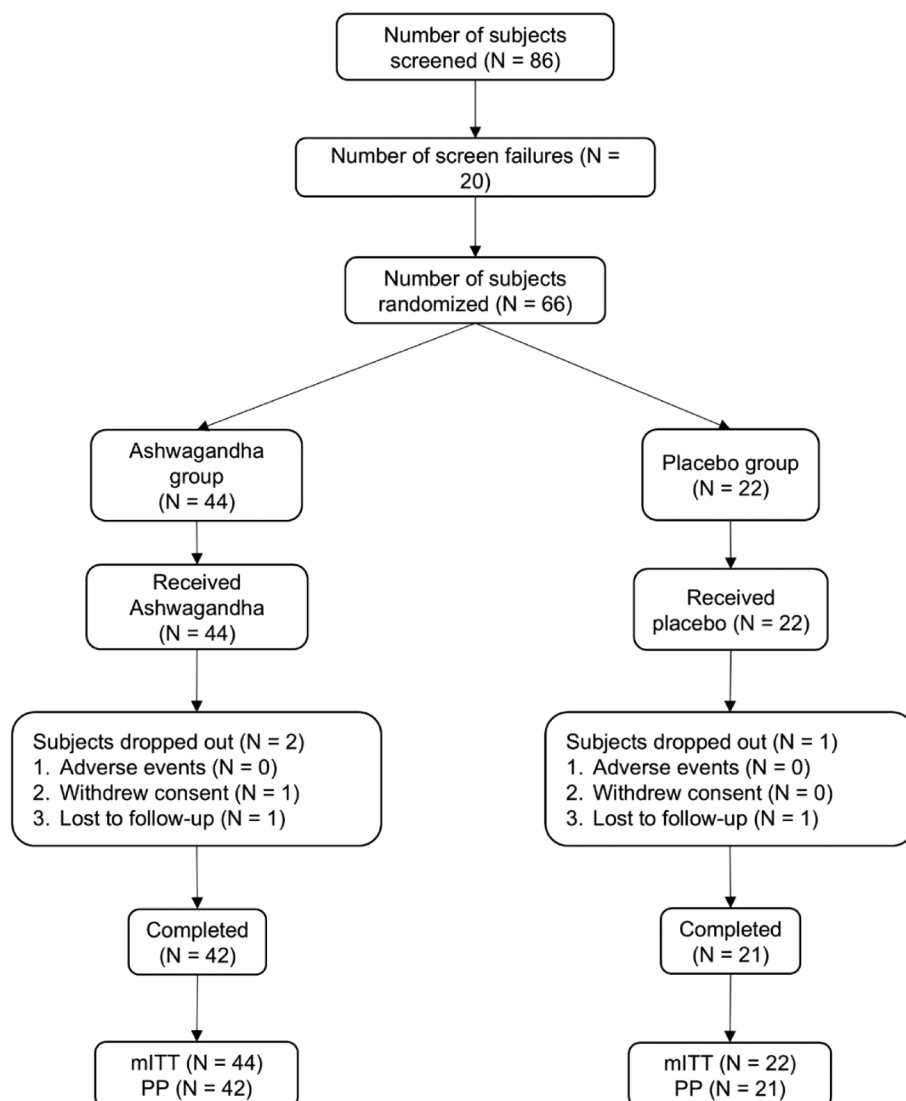


FIGURE 1 | Participants flow in the study (CONSORT Chart). mITT, analyzed modified Intention-to-treat population; PP: analyzed per protocol population.

TABLE 1 | Baseline demographic characteristics of participants.

	LDA (N = 44)	Placebo (N = 22)
Male, <i>n</i> (%)	39 (88.6)	20 (90.9)
Female, <i>n</i> (%)	5 (11.4)	2 (9.1)
Age (Years)*	34 ± 6.28	37.1 ± 5.2
Height (cm)*	164.4 ± 5.62	164.0 ± 5.3
Weight (kg)*	60.1 ± 3.9	58.8 ± 2.1
BMI (kg/m ²)*	22.24 ± 1.2	21.9 ± 1.6
Concomitant medication, <i>n</i> (%)	0	1 (4.55)

*Each value is represented as mean ± SD.

group. Baseline demographic characteristics of both groups are presented in Table 1. A total of three subjects discontinued from the study, with two subjects from the LDA group and one

from the placebo group. Investigational product compliance was found to be acceptable, with rates of 95.90% in the LDA group and 95.05% in the placebo group.

3.2 | Effect of LDA on Maximal Oxygen Uptake by (VO₂max) & Heart rate (HR) During the Modified Bruce protocol Treadmill Test in the PP Population

In the PP population, at baseline, VO₂max levels (Table 2) assessed during the Modified Bruce protocol Treadmill Test were comparable between the LDA (29.29 ± 1.71 mL/kg/min) and placebo (29.86 ± 1.42 mL/kg/min) groups. Following 56 days of intervention, the LDA group demonstrated a significant increase ($p \leq 0.05$) in VO₂max to 32.20 ± 1.82 mL/kg/min (increased by 10.11%), whereas the increase observed in the placebo group to 30.21 ± 1.31 mL/kg/min (increased by 1.23%) was not statistically significant compared to its baseline. Furthermore, the improvement in VO₂max in the LDA group was significantly ($p \leq 0.05$) greater than that of the placebo group. These findings

indicate that LDA supplementation enhances cardiorespiratory endurance and aerobic capacity compared to placebo.

A significant ($p \leq 0.05$) increase in maximal heart rate (HRmax) was observed in the LDA group following 56 days of intervention ($p \leq 0.05$), compared to both its baseline value and the placebo group. This suggests improved cardiovascular response and exercise tolerance with LDA supplementation during the Modified Bruce Protocol Treadmill Test. Results of the heart rate at rest (HRrest) and maximum heart rate (HRmax) of the PP population used to calculate the $VO_{2\max}$ are shown in Table 2.

3.3 | Effect of LDA on Exercise Stage Completion

The proportion of subjects achieving higher exercise stages improved greatly in the LDA group compared to placebo over 56 days (Table 2). Notably, 100% of LDA subjects reached Stage 7 on Day 56 compared to 92.86% of people at baseline, and the number of subjects reaching Stage 8 increased from 30.95% at baseline to 61.90% on Day 56, while the placebo group showed minimal improvement at Stage 7 (90.48% at baseline to 95.24% on Day 56) and no improvement at Stage 8 (9.52% at baseline and on Day 56). This indicates a clear enhancement in exercise tolerance and endurance capacity with LDA supplementation.

3.4 | Effect of LDA on Blood Lactate Concentration

At baseline, the mean blood lactate concentration of the PP population at Stage 6 (6.7 km/h, Level 5 incline) of the Modified Bruce protocol Treadmill Test was 4.88 ± 1.23 mmol/L in the LDA group and 5.03 ± 1.17 mmol/L in the placebo group. Following 56 days of supplementation, lactate levels at this stage decreased ($p \leq 0.05$) to 4.49 ± 0.50 mmol/L in the LDA group compared to 4.80 ± 0.73 mmol/L in the placebo group (Table 3). The lactate concentrations at Stage 1 remained stable in both groups over the study period. These findings suggest an improvement in lactate handling capacity in both groups, with a ($p \leq 0.05$) greater reduction observed in the LDA group compared.

3.5 | Effect of LDA Muscle Stress evident from the Blood CPK Levels

After 56 days of supplementation, blood CPK levels decreased significantly ($p \leq 0.05$) in the LDA group, from 138.04 ± 63.99 U/L at baseline to 116.32 ± 47.88 U/L, indicating a 15.7% reduction. In comparison, the placebo group showed a non-significant reduction, from 126.82 ± 47.53 U/L to 117.95 ± 45.02 U/L (7% decrease) (Table 3). This significantly greater decline in CPK levels in the LDA group suggests enhanced muscle protection and reduced exercise-induced muscle stress, supporting its role in improving recovery and endurance performance.

TABLE 2 | Cardiorespiratory endurance and exercise performance parameters of the PP population.

Test parameters	LDA (N=42)		Placebo (N=21)	
	Baseline	Day 56	Baseline	Day 56
HRrest (bpm)	83.83 ± 5.30	82.38 ± 4.54	83.67 ± 4.70	83.62 ± 3.07
HRmax (bpm)	163.33 ± 8.93	$176.50 \pm 8.16^{*,\#}$	166.33 ± 8.99	168.24 ± 5.98
$VO_{2\max}$ (ml/kg/min)	29.29 ± 1.71	$32.20 \pm 1.82^{*,\#}$	29.86 ± 1.42	30.21 ± 1.31
Stage 7 subjects (%)	92.86	100	90.48	95.24
Stage 8 subjects (%)	30.95	61.9	9.52	9.52

Note: Unless otherwise specified, each value represents mean $\pm$ SD; N=42 for LDA group and N=21 for placebo group; Stage 7 and Stage 8 represent the stages of the Modified Bruce Protocol Treadmill Test as outlined in Supplementary Table S1.

Abbreviations: HRmax, maximum heart rate; HRrest, heart rate at rest; PP, per protocol; $VO_{2\max}$, maximal oxygen uptake.

* $p \leq 0.05$ compared to respective baseline.

$p \leq 0.05$ compared to placebo.

TABLE 3 | Cardiorespiratory endurance and exercise performance parameters of the PP population.

Test parameters	LDA (N=42)		Placebo (N=21)	
	Baseline	Day 56	Baseline	Day 56
Stage 1 Blood lactate (mmol/L)	1.47 ± 0.63	1.56 ± 0.40	1.56 ± 0.55	1.47 ± 0.38
Stage 6 Blood lactate (mmol/L)	4.88 ± 1.23	$4.49 \pm 0.50^{\#}$	5.03 ± 1.17	4.80 ± 0.73
Blood CPK (U/L)	138.04 ± 63.99	$116.32 \pm 47.88^{*}$	126.82 ± 47.53	117.95 ± 45.02

Note: Unless otherwise specified, each value represents mean $\pm$ SD; N=42 for LDA group and N=21 for placebo group.

Abbreviation: CPK, creatine phosphokinase.

* $p \leq 0.05$ compared to respective baseline.

$p \leq 0.05$ compared to placebo.

3.6 | Effect of LDA on Subjective Rate of Perceived Exertion by Borg Rating of Perceived Exertion (RPE) Scale Post Modified Bruce Protocol Treadmill Test

Following the Modified Bruce Protocol Treadmill Test, the mean Borg RPE significantly ($p \leq 0.05$) decreased in the LDA group from 16.95 ± 0.99 at baseline to 16.60 ± 1.01 on Day 56. In contrast, the placebo group showed a slight non-significant increase in perceived exertion, from 17.33 ± 1.02 at baseline to 17.43 ± 0.81 on Day 56. This reduction in perceived exertion in the LDA group is significantly ($p \leq 0.05$) greater compared to the placebo group and indicates improved exercise tolerance and reduced fatigue perception following endurance effort (Figure 2a).

3.7 | Effect of LDA on Subjective Rate of Perceived Exertion by Borg Rating of Perceived Exertion (RPE) Scale Post 20-Meter Shuttle Run Test

The mean Borg RPE following the 20-m shuttle run test decreased significantly ($p \leq 0.05$) in the LDA group from 16.86 ± 1.24 at baseline to 16.38 ± 1.23 on Day 54. In contrast, the placebo group showed no change, with RPE values of 16.62 ± 1.16 at baseline and 16.62 ± 0.86 on Day 54. This reduction in perceived exertion in the LDA group suggests improved exercise tolerance and reduced subjective fatigue with supplementation over the study period. Results of the Borg RPE following the 20-m shuttle run test are shown in Figure 2b.

3.8 | Effect of LDA on Fatigue Level Post Modified Bruce Protocol Treadmill Test

The LDA group demonstrated a significant reduction ($p \leq 0.05$) in fatigue post-treadmill test over 56 days from its baseline, whereas the placebo group showed a significant increase ($p \leq 0.05$). Nevertheless, there are no between group significance (Figure 3a).

3.9 | Effect of LDA on Fatigue Level post Post 20-Meter Shuttle Run Test

There were no statistically significant differences in post-exercise fatigue levels, measured immediately after the 20-m shuttle run test, either within the LDA and placebo groups over time or between the groups at any of the assessment points (Baseline, Day 7, and Day 54). However, a numerical reduction in perceived fatigue scores post 20-m shuttle run test was observed in the LDA group over 54 days, whereas a slight increase was noted in the placebo group (Figure 3b).

3.10 | Electromyogram

The EMG findings suggest that there were no significant changes indicative of exercise-induced muscle damage in either group.

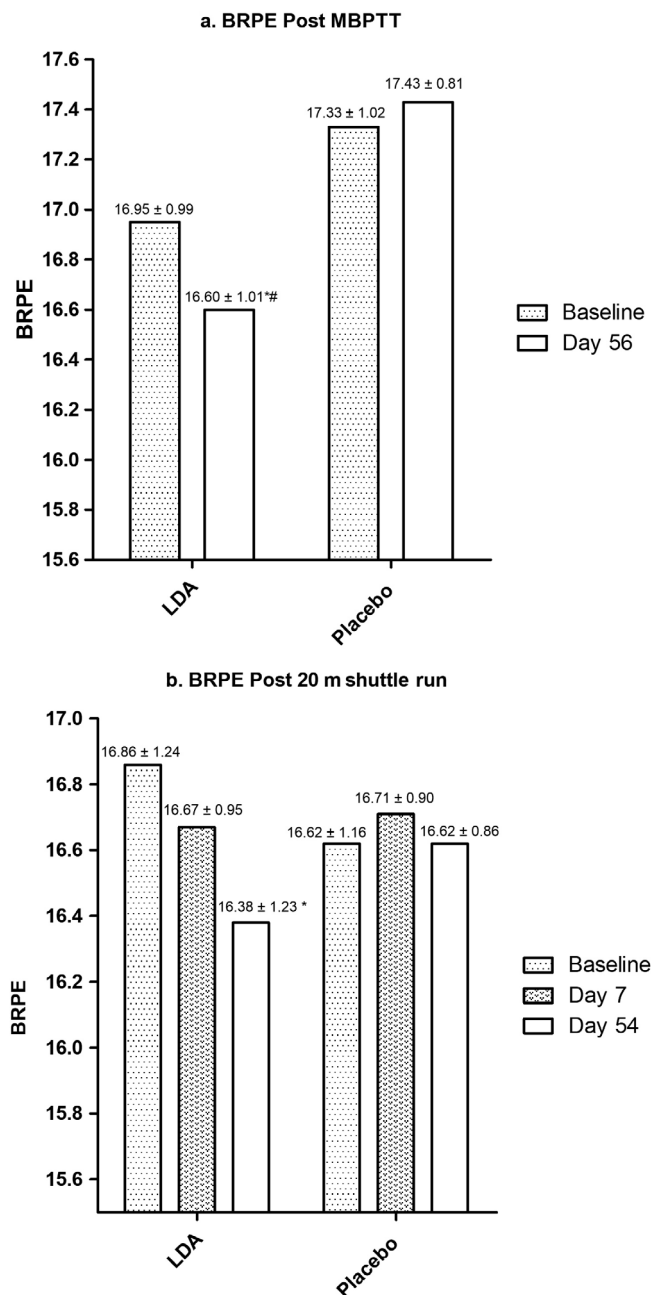


FIGURE 2 | (a) BRPE post MBPTT and (b) BRPE post 20 m shuttle run. Figure 2a $*p \leq 0.05$ compared to respective baseline; $\#p \leq 0.05$ compared to placebo; $N=42$ for LDA group and $N=21$ for placebo group. Figure 2b $*p \leq 0.05$ compared to respective baseline; $N=42$ for LDA group and $N=21$ for placebo group.

3.11 | Safety Assessments

A total of three adverse events (AEs) were reported during the study with one in the test group and two in the placebo group. The reported AEs included headache in the test group, and headache and nasopharyngitis in the placebo group. All events were mild in severity, non-serious, and assessed as unrelated to the study intervention. All subjects with AEs recovered, and no subject discontinued the study due to adverse events.

No clinically significant changes were observed in vital signs and upon physical examinations throughout the study period.

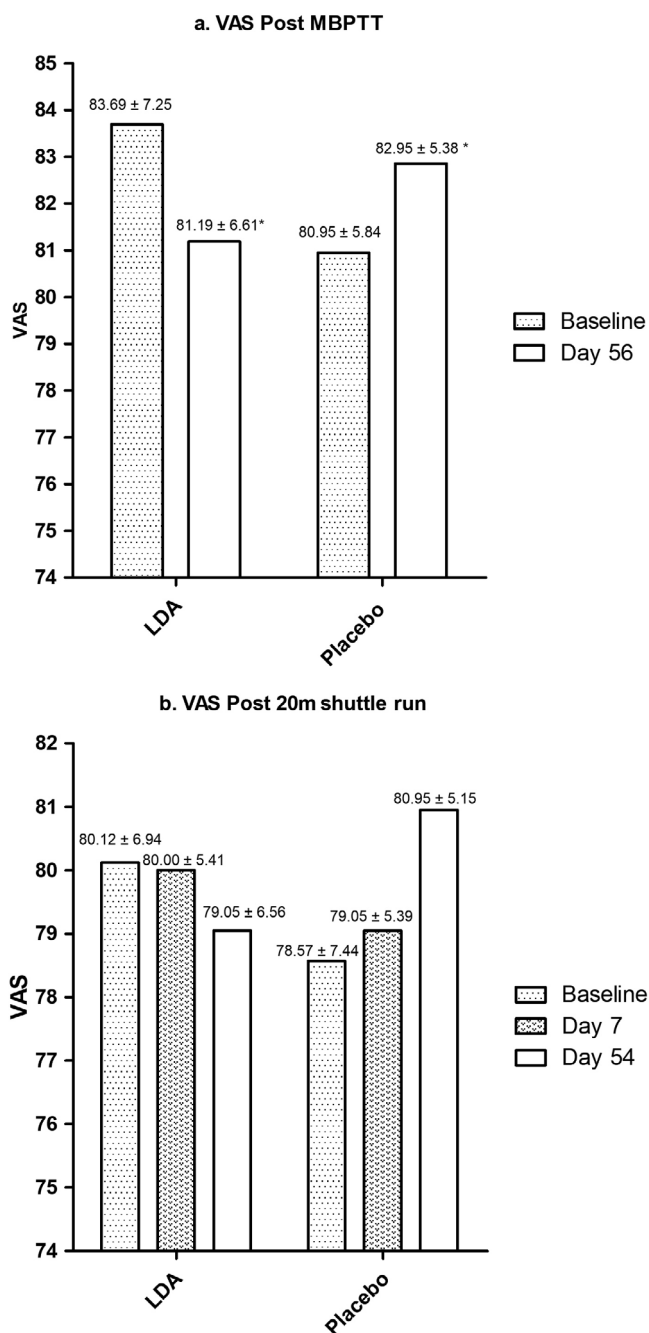


FIGURE 3 | (a) VAS post MBPTT and (b) VAS post 20 m shuttle run. Figure 3a * $p \leq 0.05$ compared to respective baseline; $N=42$ for LDA group and $N=21$ for placebo group. Figure 3b $N=42$ for LDA group and $N=21$ for placebo group.

The results of the laboratory investigations are presented in Supplementary Table S3. No safety concerns were identified, with all laboratory values within normal physiological ranges.

4 | Discussion

This 8-week, randomized, double-blind, placebo-controlled study demonstrated that daily supplementation with LDA extract resulted in a statistically significant increase in maximal oxygen uptake (VO_{2max}), along with a reduction in exercise-induced

lactic acid accumulation, perceived exertion, and fatigue levels among healthy, physically active individuals. The LDA intervention was well tolerated, with no major treatment-related adverse events reported, and no participant discontinuations attributable to adverse effects. Pre- and post-treatment evaluations of vital signs, physical examination findings, hematological indices, blood biochemistry, lipid profile, and glucose levels remained within clinically acceptable ranges, indicating that LDA supplementation is safe for human consumption under the conditions studied.

Cardiorespiratory fitness, or endurance capacity, is a critical determinant of overall health and physical performance. It reflects the integrated functional capacity of the pulmonary, cardiovascular, and musculoskeletal systems to support sustained aerobic activity during daily life or exercise. Maximal oxygen uptake (VO_{2max}) is widely recognized as the gold standard metric for assessing cardiorespiratory fitness. Both direct and indirect methods of VO_{2max} estimation, using treadmill or cycle ergometer protocols, are well established and broadly accepted in clinical and research settings (Neshitov et al. 2023).

In the present study, VO_{2max} was assessed using a validated indirect method—based on heart rate response—employing the Modified Bruce Protocol Treadmill Test (MBPTT). The results demonstrated a significant improvement in VO_{2max} among participants receiving LDA compared to the placebo group over the eight-week intervention period. Multiple studies on Ashwagandha extracts have reported within-group improvements in VO_{2max} following Ashwagandha supplementation in athletes and healthy individuals (Sandhu et al. 2010; Tripathi et al. 2016; Malik et al. 2013; Shenoy et al. 2012). A systematic review and meta-analysis encompassing randomized controlled trials reported that Ashwagandha supplementation significantly enhanced VO_{2max} in healthy adults and athletes, with a pooled mean difference of approximately 3.00 mL/kg/min compared with control conditions ($p = 0.04$) across a range of participant profiles and dosages (300–1000 mg/day) over intervention periods of 2–12 weeks. Comparatively, the magnitude of VO_{2max} improvement observed with LDA in our cohort is consistent with or exceeds the effects reported for other standardized root extracts.

Further meta-analysis (Pérez-Gómez et al. 2020) indicated that significant VO_{2max} enhancements were typically associated with high cumulative Ashwagandha intake (> 50 g over the intervention period), the current study observed comparable improvements at a markedly lower cumulative dose (< 2 g). This highlights the potential of Ashwa 0.30, a bioactive-optimized extract developed using mathematical and biological modeling, to deliver measurable physiological benefits at low doses.

Given the variability in phytochemical composition, extraction methods, and standardization practices across commercially available Ashwagandha formulations, it is important to interpret the generalizability of these findings with caution. The efficacy observed in this study is specific to LDA, a root-only extract standardized to contain > 15% withanolides and enriched with an ATP-active fraction. Extrapolation to other Ashwagandha preparations should therefore be approached carefully.

As a byproduct of anaerobic glycolysis, lactic acid generation reflects a metabolic shift from aerobic to anaerobic ATP production. While this increase in lactic acid is transient and typically resolves post-exercise, its accumulation during exertion is indicative of the onset of anaerobic metabolism (Chand et al. 2023).

In the present study, lactic acid concentrations measured at Stage 6 of the Modified Bruce Protocol Treadmill Test (MBPTT) were significantly higher in the placebo group compared to the LDA group. These findings suggest that participants receiving LDA were able to sustain aerobic metabolism for a longer duration before transitioning to anaerobic pathways, thereby delaying lactic acid accumulation. This prolonged aerobic efficiency implies a higher ATP yield and improved energy availability during exercise in the LDA group relative to the placebo. These findings are directionally consistent with clinical data from other plant-derived bioactives, such as 2S-hesperidin supplementation (500 mg/day) in amateur cyclists for 8 weeks, which has been shown to reduce blood lactate at ventilatory thresholds following chronic supplementation. While between-group differences were not always statistically established in this study, large effect sizes support the concept that botanical interventions can meaningfully influence lactate handling (Martínez-Noguera et al. 2022).

Creatine phosphokinase (CPK) is an intracellular enzyme predominantly localized in skeletal muscle, cardiac tissue, and the brain, commonly used as a biomarker for muscle stress or injury. Although elevated serum CPK levels are traditionally associated with muscle fiber damage, emerging evidence suggests that increases may also result from transient disruptions in cellular energy homeostasis or non-mechanical biochemical stress (Baird et al. 2012). In the current study, a statistically significant reduction in CPK levels was observed in the LDA group following moderate-intensity exercise, indicating that LDA may help stabilize muscle energy metabolism or mitigate subclinical muscle stress.

Collectively, the observed improvements in VO_2max in conjunction with lower lactic acid and CPK levels in the LDA group support the conclusion that LDA supplementation enhances aerobic efficiency, reduces muscle energy disruption, and improves overall exercise performance and recovery in healthy individuals.

Both the Modified Bruce Protocol Treadmill Test (MBPTT) and the 20-m shuttle run test are recognized as high-intensity exercise assessments that effectively challenge aerobic and anaerobic capacity. The Rating of Perceived Exertion (RPE) measured using the Borg scale is a validated subjective tool for quantifying exercise intensity and has been shown to significantly correlate with physiological parameters such as VO_2max , heart rate, and the overall sensation of fatigue.

In the present study, participants in the LDA group reported a statistically significant reduction in RPE scores compared to the placebo group. This decrease in perceived exertion was observed alongside significant increases in VO_2max and maximal heart rate, suggesting a strong concordance between subjective perceptions of effort and objective physiological improvements. Furthermore, individuals in the LDA group experienced

reduced levels of fatigue during exercise, whereas fatigue scores increased in the placebo group, further supporting the anti-fatigue and endurance-enhancing effects of LDA. The observed reduction in fatigue may be partly mediated through neuroendocrine mechanisms. Ashwagandha has been shown to modulate hypothalamic–pituitary–adrenal (HPA) axis activity, including suppression of corticotropin-releasing hormone (CRH) and attenuation of exercise-induced cortisol responses. Acute supplementation with *Withania* extracts has been reported to reduce post-exercise cortisol by ~17% and lowered subjective fatigue scores (VAS scale) by 21% compared to placebo in recreational runners (Jiao et al. 2025). Such stress-modulating effects may complement peripheral metabolic adaptations, contributing to reduced central fatigue and improved overall exercise experience.

The study also rigorously assessed the safety profile of LDA over the eight-week intervention period. No serious adverse events or discontinuations due to treatment intolerance were reported, and the extract was well tolerated by all participants. Post-supplementation evaluations showed no clinically significant alterations in hepatic function markers (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), renal markers (blood urea nitrogen [BUN], and serum creatinine), muscle stress indicators (lactate dehydrogenase [LDH], electromyography), or any hematological parameters (including hemoglobin, red and white blood cell counts, platelets, hematocrit, and leukocyte differentials such as neutrophils, eosinophils, lymphocytes, basophils, and monocytes). Additionally, lipid profiles (total cholesterol and triglycerides) and fasting blood glucose levels remained within normal physiological ranges. Vital signs including blood pressure and heart rate were also stable throughout the study duration.

These findings are consistent with previous safety evaluations of Ashwagandha supplementation in human populations (Durg et al. 2018; Pratte et al. 2014), supporting the conclusion that LDA is safe for daily use at the administered dose in healthy adults.

In summary, 8 weeks of supplementation with LDA—a root-only Ashwagandha extract standardized to > 15% withanolides and enriched with an ATP-active fraction—resulted in significant improvements in VO_2max and maximal heart rate, alongside reductions in lactic acid accumulation, perceived exertion, and fatigue. These findings suggest that Ashwa 0.30 may serve as a safe and effective low-dose adaptogenic intervention to support cardiorespiratory fitness, endurance performance, and overall exercise tolerance in healthy individuals.

Author Contributions

H.P., M.S., D.S., I.B., and T.S. designed the research, conducted the research, analysed the data, and wrote the paper. All authors read and approved the final manuscript.

Acknowledgments

The authors gratefully acknowledge Natural Remedies Pvt. Ltd. for funding the project and for supplying the capsules used for this study.

Funding

The study received funding from Natural Remedies Pvt. Ltd. The funder was not involved in data collection, analysis, and interpretation of data.

Ethics Statement

The studies involving human participants were reviewed and approved by ACEAS—Independent Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Conflicts of Interest

This study received funding from Natural Remedies Pvt. Ltd. The funder was not involved in the design of the research study, analysis of data, or writing of the report. Dr. Himanshu Prajapati is an investigator at Prajna Health Care, Ahmedabad. Dr. Milan Satia, Ms. Dhara Shah, Dr. Ishita Basera, and Dr. Tanvi Shah are employees of Ethicare Clinical Trial Services (OPC) Pvt. Ltd., a Clinical Research Organisation.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

- Amir, M., M. Vohra, R. G. Raj, I. Osoro, and A. Sharma. 2023. "Adaptogenic Herbs: A Natural Way to Improve Athletic Performance." *Health Sciences Review* 1, no. 7: 100092.
- Baird, M. F., S. M. Graham, J. S. Baker, and G. F. Bickerstaff. 2012. "Creatine-Kinase and Exercise-Related Muscle Damage Implications for Muscle Performance and Recovery." *Journal of Nutrition and Metabolism* 2012, no. 1: 960363.
- Chand, D., A. Kumar, D. N. Singh, and S. Mishra. 2023. "End Result of Physical Exercise With Pathological Increase in Lactic Acid." *Journal of Ayurveda and Integrated Medical Sciences* 8, no. 8: 186–189.
- Chen, Y. J., R. Baskaran, M. A. Shibu, and W. T. Lin. 2022. "Anti-Fatigue and Exercise Performance Improvement Effect of *Glossogyne tenuifolia* Extract in Mice." *Nutrients* 14, no. 5: 1011.
- Dell, R. B., S. Holleran, and R. Ramakrishnan. 2002. "Sample size determination." *ILAR Journal* 43, no. 4: 207–213.
- Durg, S., S. B. Shivaram, and S. Bavage. 2018. "*Withania somnifera* (Indian Ginseng) in Male Infertility: An Evidence-Based Systematic Review and Meta-Analysis." *Phytomedicine* 15, no. 50: 247–256.
- Jiao, X., X. Liu, Q. Cao, and Z. Deng. 2025. "Plant-Based Supplements in Enhancing Exercise Performance and Recovery." *Frontiers in Nutrition* 12: 1699642. <https://doi.org/10.3389/fnut.2025.1699642>.
- Joshi, V. K., and A. Joshi. 2021. "Rational Use of Ashwagandha in Ayurveda (Traditional Indian Medicine) for Health and Healing." *Journal of Ethnopharmacology* 10, no. 276: 114101.
- Léger, L. A., and J. Lambert. 1982. "A Maximal Multistage 20-m Shuttle Run Test to Predict VO₂ Max." *European Journal of Applied Physiology and Occupational Physiology* 49, no. 1: 1–12. <https://doi.org/10.1007/BF00428958>.
- Lopresti, A., and S. Smith. 2021. "Ashwagandha (*Withania somnifera*) for the Treatment and Enhancement of Mental and Physical Conditions: A Systematic Review of Human Trials." *Journal of Herbal Medicine* 18, no. 28: 100434. <https://doi.org/10.1016/j.hermed.2021.100434>.
- Malik, A., V. Mehta, and V. Dahiya. 2013. "Effect of Ashwagandha (*Withania somnifera*) Root Powder Supplementation on the VO₂ Max. And Hemoglobin in Hockey Players." *International Journal of Behavioral Social and Movement Sciences* 2, no. 3: 91–99.
- Mandlik, D. S., and A. G. Namdeo. 2021. "Pharmacological Evaluation of Ashwagandha Highlighting Its Healthcare Claims, Safety, and Toxicity Aspects." *Journal of Dietary Supplements* 18, no. 2: 183–226.
- Martínez-Noguera, F. J., P. E. Alcaraz, J. Carlos-Vivas, and C. Marín-Pagán. 2022. "Chronic Supplementation of 2S-hesperidin Improves Acid-Base Status and Decreases Lactate at FatMax, at Ventilatory Threshold 1 and 2 and After an Incremental Test in Amateur Cyclists." *Biology (Basel)* 11, no. 5: 736. <https://doi.org/10.3390/biology11050736>.
- McDermott, C. E., H. K. Vincent, A. E. Mathews, et al. 2022. "Impact of Probiotic Supplementation on Exercise Endurance Among Non-Elite Athletes: Study Protocol for a Randomized, Placebo-Controlled, Double-Blind, Clinical Trial." *Trials* 23, no. 1: 603.
- Neshitov, A., K. Tyapochkin, M. Kovaleva, et al. 2023. "Estimation of Cardiorespiratory Fitness Using Heart Rate and Step Count Data." *Scientific Reports* 13, no. 1: 15808.
- Ozmen, T., G. Y. Gunes, I. Ucar, H. Dogan, and T. U. Gafuroglu. 2017. "Effect of Respiratory Muscle Training on Pulmonary Function and Aerobic Endurance in Soccer Players." *Journal of Sports Medicine and Physical Fitness* 57, no. 5: 507–513.
- Panossian, A., and G. Wikman. 2009. "Evidence-Based Efficacy of Adaptogens in Fatigue, and Molecular Mechanisms Related to Their Stress-Protective Activity." *Current Clinical Pharmacology* 4, no. 3: 198–219.
- Pérez-Gómez, J., S. Villafaina, J. C. Adsuar, E. Merellano-Navarro, and D. Collado-Mateo. 2020. "Effects of Ashwagandha (*Withania somnifera*) on VO₂max: A Systematic Review and Meta-Analysis." *Nutrients* 12, no. 4: 1119.
- Pratte, M. A., K. B. Nanavati, V. Young, and C. P. Morley. 2014. "An Alternative Treatment for Anxiety: A Systematic Review of Human Trial Results Reported for the Ayurvedic Herb Ashwagandha (*Withania somnifera*)." *Journal of Alternative and Complementary Medicine* 20, no. 12: 901–908.
- Sandhu, J. S., B. Shah, S. Shenoy, S. Chauhan, G. S. Lavekar, and M. M. Padhi. 2010. "Effects of *Withania somnifera* (Ashwagandha) and Terminalia Arjuna (Arjuna) on Physical Performance and Cardiorespiratory Endurance in Healthy Young Adults." *International Journal of Ayurveda Research* 1, no. 3: 144.
- Shenoy, S., U. Chaskar, J. S. Sandhu, and M. M. Paadhi. 2012. "Effects of Eight-Week Supplementation of Ashwagandha on Cardiorespiratory Endurance in Elite Indian Cyclists." *Journal of Ayurveda and Integrative Medicine* 3, no. 4: 209.
- Slettøløyen, G., and B. R. Rønnestad. 2014. "High-Intensity Interval Training Every Second Week Maintains V̇O₂max in Soccer Players During Off-Season." *Journal of Strength & Conditioning Research* 28, no. 7: 1946–1951.
- Tripathi, R. K., B. Salve, A. Petare, A. Raut, and N. N. Rege. 2016. "Effect of *Withania somnifera* on Physical and Cardiovascular Performance Induced by Physical Stress in Healthy Human Volunteers." *International Journal of Basic & Clinical Pharmacology* 5: 2510–2516.
- Zellner, L., M. Wierer, M. Himmelsbach, B. Schatzmann, B. Thalhamer, and C. W. Klampfl. 2025. "Determination of Withanolides and Withanoides in Ashwagandha Based Products Using HPLC-Drift-Tube-Ion-Mobility Quadrupole Time-Of-Flight Mass Spectrometry." *Electrophoresis* 46, no. 5-6: 340–346.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** Supporting Information.