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EFFECTS OF ASHWAGANDHA (WITHANIA SOMNIFERA) SUPPLEMENTATION ON BODY COMPOSITION AND BLOOD HEALTH INDICES IN PROFESSIONAL WRESTLERS

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Abstract

Introduction. The purpose of this randomized, double-blind, placebo-controlled study was to evaluate the effect of ashwagandha supplementation on the body composition, blood health and recovery indices, as well as the safety of ashwagandha supplementation in wrestling. ***Material and Methods.*** To investigate this, body composition (using dual energy X-ray absorptiometry; DEXA) and blood parameters as indicators of athletes' health status (i.e., hematology, liver, kidney and muscle tissue function, lipid profile) were measured before and after 8-week supplementation with ashwagandha extract (600 mg/day) in professional national team wrestlers following an intensive training plan (as part of an annual training cycle). ***Results.*** At the end of the study, there was a significant increase in fat-free mass and muscle mass, with a significant decrease in fat mass (both in absolute and relative terms) in both ashwagandha and placebo groups (main effect of time, $p < 0.05$). However, ashwagandha supplementation did not affect any of the body composition parameters (main effect of group or interaction of time and group, $p > 0.05$). Moreover, intense wrestling training induced an increase in blood creatine kinase activity as an indicator of muscle tissue damage (main effect of time, $p < 0.05$). Ashwagandha supplementation did not significantly affect any hematological or biochemical blood parameters ($p > 0.05$ for main effect of group and interaction of time and group). However, the increase in creatine kinase activity during the study seemed to be more pronounced in the placebo group (post-hoc; $p < 0.05$) than in the ashwagandha group (post-hoc; $p > 0.05$). ***Conclusions.*** In conclusion, ashwagandha supplementation at 600 mg daily for 8 weeks is well tolerated and safe for professional wrestlers. This strategy does not appear to offer additional body composition benefits beyond the effect of intense wrestling training. On the other hand, our results indicate some potential for ashwagandha in improving muscle tissue recovery, which needs to be confirmed on a larger population of athletes.

Key words: herbal supplement, athletes, fat free mass, fat mass, biochemical parameters

Introduction

Wrestling belongs to the mixed sport disciplines (power/endurance sports), in which the determinants of sports success include, among other things, body composition, which affects motor skills of an athlete [1]. Unfortunately, the period of rapid weight reduction of athletes before competition may induce less favourable changes in body composition (reduction not only in fat mass, but also in lean body mass), which can adversely affect athletes' physical performance [2]. Hence the search for plant-based supplements that promote training adaptations, and which can be useful in mitigating muscle mass loss and promoting fat mass reduction during periods of weight loss.

Ashwagandha (Withania somnifera) is a plant with potential health properties. The roots of this plant are widely used in traditional Eastern medicine to increase the body's resistance to physical and mental stress. Active components of ashwagandha extract include, among others, steroid lactones (withanolides) and saponins. These substances are thought to be responsible for the "adaptogenic" properties of ashwagandha, with the potential to improve mental and physical performance [3] by affecting many biological functions, including regulation of adrenal and gonadal function [3-4]. Indeed, Wankhede et al. [5] reported that ashwagandha supplementation may increase adaptations to chronic (8-week) resistance training program in untrained men. In that study, significantly greater gains in muscle strength, muscle size, as well as an increase in basal testosterone levels and reductions in body fat percentage were found in the ashwagandha group, as compared to the placebo group [5]. Also, in another study [6], ashwagandha extract promoted favourable weight distribution in young, recreationally active men undergoing 12 weeks of resistance training program. On the other hand, scientific evidence regarding the effectiveness of ashwagandha supplementation in professional sports is still scarce. In the available scientific literature, there is a lack of studies evaluating the effect of ashwagandha administration on body composition in professional athletes, so it seems reasonable to conduct a study involving professional wrestlers, who often experience changes in weight and body composition during the training cycle.

It must also be emphasized that consumption of herbal preparations has increased in last decades [7, 8]. The problem of potential dangers of using plant preparations is also too often overlooked. Plant products are widely considered safe for the body. Several factors may be responsible for this phenomenon, including the belief that herbs are safe because they are "natural" and a harmless alternative to conventional medicine. Meanwhile, according to recent data, increased consumption of herbs

has been accompanied by an increase in cases of herb-induced liver damage [8, 9 , 10].

Taking into account the issues mentioned above, the aim of the study was to evaluate the effect of 8-week ashwagandha supplementation on the body composition of wrestlers following an intense training plan (as part of an annual training cycle) and the safety of ashwagandha supplementation based on the assessment of biochemical parameters in the blood as indicators of athletes' health status, i.e. the function of liver, kidney and muscle tissue (alanine and aspartate aminotransferase activity, creatine kinase activity, protein indices/nitrogenous compound metabolism: total protein, albumin, uric acid, urea, creatinine), as well as cardiovascular risk factors (lipid profile) and hematological indices.

Material and Methods

Subjects

The randomized, double-blind study involved professional athletes – highly qualified national team wrestlers (juniors training in Greco-Roman style wrestling). Exclusion criteria were as follows: taking any medications or steroids that increase physical performance, as well as drugs/medications that are sedative, anti-anxiety or sleep-inducing; orthopedic injury or past surgery within the last 6 months; chronic diseases; known hypersensitivity to ashwagandha or other herbal supplements of similar composition; taking any herbal preparations or supplements containing antioxidant and anti-inflammatory substances within the last 3 months.

Before the experiment, athletes were randomly assigned, in a double-blind fashion, to 2 groups. Initially, 26 wrestlers were recruited and randomly assigned to the ashwagandha (A, n = 12) or the placebo (PL, n = 14) group (through simple randomisation: tossing a coin). Four participants (1 from A and 3 from PL group) dropped out of the study for personal reasons, and one participant from A group dropped out due to poor tolerance of the preparation ashwagandha extract (diarrhoea). Finally, 21 wrestlers (10 from the A and 11 from the PL group) were included in the analysis. Wrestlers were asked to refrain from modification of their diet during the study period.

The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants gave their consent to participate in the study and the research protocol was approved by the Local Ethics Committee of Józef Piłsudski University of Physical Education in Warsaw (SKE 01-43/2022).

Wrestling training program

During the eight-week experiment, the subjects participated in a planned training process (the second and third parts of the preparatory period) aimed at optimal preparation for the Polish Senior Championships in Greco-Roman style, according to the specifics of the sport [11, 12]. The wrestlers implemented 10 training units per week, and each training unit lasted 120 minutes. In total, the volume of training work amounted to 160 hours, the specificity, intensity and complexity of which were varied to prevent overtraining [13, 14, 15].

In the first 6-week part of the preparatory period (before the experiment), as recommended in the literature, the athletes mainly focused on developing general endurance [16]. In the second part of the preparatory period (5 weeks), individual technique (35%), speed (15%), strength (static – 5% and dynamic – 15%), strength endurance (25%) and special fitness (5%) were improved. In the third part of the preparatory period (3 weeks), training was aimed at improving technical-tactical skills (30%), speed (20%), special endurance (40%) and special fitness (10%) according to modern concepts of periodization [17, 18].

Supplementation

Ashwagandha supplement was administered at a dose of 2 x 300 mg per day (2 capsules a day) for a period of 8 weeks. One capsule contained 300 mg of ashwagandha root extract, KSM-66, standardized to a 5 % concentration of withanolides as measured by HPLC. The dosage of the A was based on the recommendations of the producer, being within the lower range of the dose recommended for physically active, strength and endurance-trained individuals [3].

Placebo capsules were administered in the same form as A supplement. Both the A and PL capsules were identical in appearance (size, shape, and color), and they both were manufactured by the same producer. The subjects were instructed to take the capsules twice a day after meals (breakfast and dinner).

The capsules were supplied to the athletes in dark nontransparent bottles. Wrestlers were asked weekly about the number of capsules they skipped. Respondents (3 from the PL group and 2 from the A group) declared skipping single capsules not exceeding 10% of the total number of capsules.

Research staff were in constant contact with participants, who were asked weekly about their wellbeing and any side effects of supplementation (abdominal pain, headaches and dizziness, lethargy, diarrhoea, vomiting, etc.). Generally, the participants (from both PL and A group) declared good tolerability of capsules, with no adverse events. As mentioned above, only one participant from A group dropped out of the study due to intolerance of ashwagandha capsules.

Blood sampling and biochemical analyses

Blood samples from the ulnar vein were obtained in the morning (at 7:00 a.m.), after an overnight fast, prior to (term 1) and after (term 2) the 8-week wrestling training. The blood samples were collected to a tube (6 mL) without an anticoagulant (to separate serum) and to a tube (2 ml) with anticoagulant (EDTA) to hematological measurements. To separate serum, blood samples were centrifuged at 3000× g for 10 min (4^{0}C) and serum samples were frozen and stored at – 80^{0}C until analysis.

Hematological parameters, i.e. hemoglobin (Hb), hematocrit (Ht), red blood cells (RBC), leukocyte (LEU) and subset counts: lymphocytes (LYMPH), monocytes (MONO), total granulocytes (GR), eosinophils (EOS), basophils (BASO), were assessed by using an automated method at a local commercial diagnostic laboratory (hematology analyzer; BIOMAXIMA BM HEM 5 TS; Poland).

The following serum levels of biochemical parameters were determined with commercially available kits (Alpha Diagnostics, Poland): albumin (Alb; cat. no. A6502-100), total proteins (TP; cat. no. B6528-125); uric acid (UA; cat. no. K6681-100); urea (U; cat. no. M6652-075); creatinine (Cr; cat. noTR35121/125); total cholesterol (TC; cat. no. C6608-100), HDL-cholesterol (HDL-C; direct liquid two-component reagent, cat. no. H6421-080), LDL-cholesterol (LDL-C; direct liquid two-component reagent, cat. no. L6410-080), triglycerides (TG; cat. no. T6630-100), alanine aminotransferase (ALT) activity (cat. no. A6424-075), aspartate aminotransferase (AST) activity (cat. no. A6461-075) and creatine kinase (CK) activity (cat. no. C6412-060). During analyses, multicalibrator (cat. no. K6504–03), as well as normal and pathological control serum (cat. nos. S6590–05 and S6591-05, respectively; Alpha Diagnostics, Poland) were used. These biochemical parameters were measured spectrophoto-

metrically at 37°C using an automatic biochemical analyzer A15 (Bio-Systems S.A., Montcada I Reixac, Spain).

Body composition

Body composition and bone mineral content BMC (g) were obtained from a whole-body scan with the use of dual-energy X-ray absorptiometry (DEXA) on a HORIZON Ci device (USA). For the total-body scan, participants were asked to lie in the supine position, centered within the scan field. The hands were placed on the sides of the legs in the prone position, while the legs were straight and strapped together. Measurements were collected in the morning after an overnight fast at the beginning and end of the treatment period. Subjects were to refrain from any physical activity for 12 h, without the intake of any fluids for 4 h, and urinate 30 min before the measurements.

During the examination, apart from body mass (BM), fat free mass (FFM), muscle mass (MM) and fat mass (FM) were determined (in kilograms and as percentage content). Additionally, BMC of total body (BMC_{total}) was registered.

Statistical analysis

Statistical analysis was conducted with Statistica version 13.3 (StatSoft, Krakow, Poland) with the Bonferroni post-hoc test for multiple comparisons. Normality assumptions were checked on all variables using Shapiro-Wilk test and visual inspection (quantile distribution plots). Non-normal distributions were transformed using log10. All values were reported as mean ± standard deviation (SD). The level of statistical significance was set at $p < 0.05$. Statistical significance of intergroup (A vs. PL) differences in anthropometric characteristics (age and height) were verified with unpaired Student t-test. Data regarding body composition, as well as blood biochemical and hematological parameters were analysed with 2 (groups: PL and A) x 2 (time points: term 1 and term 2) mixed factorial ANOVA, with the Bonferroni post-hoc test for multiple comparisons.

Results

Table 1 presents anthropometric data (age, height, weight), as well as body composition and total bone mass of the wrestlers before and after the 8-week training period. There were no significant differences between A and PL groups with regard to the parameters shown in Table 1. In both groups, 8-week wrestling training did not induce significant changes in BM as well as BMCtotal, while significant changes over time were observed in all body composition indices, both in absolute values (in kilograms) and relative values (expressed as a percentage of total body mass). Training contributed to increases in FFM (FFM in kg: time main effect, $p = 0.0003$, with post-hoc $p = 0.0007$ for PL and $p = 0.028$ for A; FFM in %: time main effect, $p = 0.00003$, with post-hoc $p = 0.003$ for PL and $p = 0.0004$ for A) and MM (MM in kg: time main effect, $p = 0.0003$, with post-hoc $p = 0.0009$ for PL and $p = 0.031$ for A; MM in %: time main effect, $p = 0.00001$, with post-hoc $p = 0.001$ for PL and $p = 0.0002$ for A), as well as decreases in FM (FM in kg: time main effect, $p = 0.001$, with post-hoc $p = 0.04$ for PL and $p = 0.006$ for A; FFM in %: time main effect, $p = 0.00007$, with post-hoc $p = 0.004$ for PL and $p = 0.0009$ for A). In contrast, no main effects of group or time and group interaction were observed in the parameters discussed above.

The mean values of hematological parameters in the blood of the wrestlers are shown in Table 2. For most of these parameters (except MONO), there were no significant main effects (i.e., time, group, and the interaction of time and group). In contrast, an 8-week training period in wrestlers induced a significant reduction in MONO (main effect of time for MONO count, $p = 0.00008$, with post-hoc analysis, $p = 0.00001$ for PL and $p = 0.0003$ for A; main effect of time for MONO%; $p = 0.0001$, with post-hoc analysis, $p = 0.003$ for both PL and A). In addition, there was only a trend toward lower WBC and EOS (absolute number values) after 8 weeks of training (main effect of time: WBC, $p = 0.058$; EOS, $p = 0.056$). Regarding MONO, WBC and EOS, as with other hematological parameters, no significant main effects of group or interaction of time and group were observed. Also, in both groups and both terms of the measurements, mean values of all hematological parameters were within the reference range for the population studied.

Serum levels of biochemical parameters before and after the 8-week training and supplementation period are listed in Table 3. Wrestling training increased CK activity (main time effect,

Table 1. Anthropometric parameters, body composition and bone mass of wrestlers before (term 1) and after (term 2) 8-week wrestling training combined with placebo (PL) or ashwagandha (A) supplementation

	Placebo (PL) n = 11		Ashwagandha (A) n = 10		Main effects		
	Term 1	Term 2	Term 1	Term 2	Time	Group	Time x Group
Age [years]	19.1 ± 1.8	-	20.8 ± 1.9	-	-	-	-
Height [cm]	178.7 ± 7.3	-	177.4 ± 7.8	-	-	-	-
BM [kg]	78.2 ± 10.8	78.9 ± 10.6	81.9 ± 19.6	81.9 ± 20.0	0.27	0.63	0.25
FFM [kg]	64.6 ± 7.9	65.8 ± 8.1***	67.9 ± 14.6	68.7 ± 15.1*	0.0003	0.56	0.34
FFM [%]	82.9 ± 3.4	83.6 ± 2.8**	83.3 ± 2.0	84.3 ± 2.1***	0.00003	0.67	0.35
MM [kg]	61.3 ± 7.6	62.5 ± 7.8***	64.4 ± 13.9	65.2 ± 14.6*	0.0003	0.56	0.36
MM [%]	78.6 ± 3.4	79.3 ± 2.9**	79.0 ± 1.9	80.0 ± 1.9***	0.00001	0.68	0.46
FM [kg]	13.6 ± 4.2	13.1 ± 3.5*	14.0 ± 5.1	13.2 ± 4.9**	0.001	0.87	0.40
FM [%]	17.1 ± 3.4	16.4 ± 2.8**	16.7 ± 2.1	15.7 ± 2.1***	0.00007	0.66	0.47
BMCtotal [g]	3333.7 ± 371.5	3341.7 ± 372.1	3498.9 ± 742.8	3483.6 ± 691.6	0.79	0.54	0.40

Values are mean ± standard deviation; *, **, *** – significant difference between term 1 and term 2 (within the same group) ($p < 0.05$; $p < 0.01$; $p < 0.001$, respectively); abbreviations: BM: body mass; FFM: fat free mass; MM: muscle mass; FM: fat mass; BMCtotal: bone mineral content (of total body).

p = 0.007). This increase in CK activity was significant in the PL group (post-hoc, p = 0.008) but not in the A group (post-hoc, p = 0.18). In contrast, serum TP levels showed a tendency to decrease over time (main effect of time: p = 0.06), while differences between term 1 and term 2 were significant in the A group (post hoc, p = 0.026), and showed a trend in the PL group (post-hoc, p = 0.09).

A significant main effect of time was noted for changes in UA (p = 0.007), with post-hoc analysis revealing a significant increase in serum UA in the PL group (post-hoc, p = 0.016) and non-significant changes in the A group (post-hoc, p = 0.12). The main effect of time was also significant for HDL-C (p = 0.014), with a tendency to decrease in both groups (post-hoc, p = 0.09

Table 2. Hematological blood indices of wrestlers before (term 1) and after (term 2) 8-week wrestling training combined with placebo (PL) or ashwagandha (A) supplementation

Hematological parameter		Placebo (PL) n = 11		Ashwagandha (A) n = 10		Main effects		
	Reference range	Term 1	Term 2	Term 1	Term 2	Time	Group	Time x Group
WBC [10x3/µl]	4.2 - 9.07	6.98 ± 1.32	6.42 ± 1.12	6.79 ± 1.36	6.43 ± 1.06	0.058	0.85	0.67
LYMPH [10x3/µl]	1.0 - 3.7	2.81 ± 0.97	2.76 ± 0.78	3.13 ± 0.71	2.86 ± 0.478	0.24	0.50	0.43
MONO [10x3/µl]	0.0 - 0.9	0.51 ± 0.14	0.36 ± 0.09***	0.50 ± 0.14	0.36 ± 0.11***	0.00008	0.94	0.92
GR [10x3/µl]	1.5 - 7.0	3.40 ± 0.99	3.06 ± 0.77	2.94 ± 0.90	3.02 ± 1.06	0.48	0.49	0.26
EOS [10x3/µl]	0.0 - 0.4	0.23 ± 0.20	0.21 ± 0.15	0.20 ± 0.08	0.16 ± 0.11	0.056	0.48	0.62
BASO [10x3/µl]	0.0 - 0.2	0.04 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.85	0.70	0.06
LYMPH [%]	20.0 - 45.0	39.89 ± 9.04	42.65 ± 8.56	46.11 ± 6.18	45.19 ± 9.25	0.50	0.21	0.18
MONO [%]	3.0 - 12.0	7.55 ± 2.62	5.73 ± 1.55**	7.64 ± 2.58	5.70 ± 2.17**	0.0001	0.97	0.88
GR [%]	45.0 - 70.0	48.42 ± 9.44	47.66 ± 7.50	42.72 ± 6.09	45.86 ± 10.52	0.45	0.28	0.23
EOS [%]	1.0 - 5.0	3.54 ± 3.51	3.43 ± 2.77	2.99 ± 1.25	2.58 ± 1.80	0.28	0.53	0.53
BASO [%]	0.0 - 2.0	0.60 ± 0.27	0.55 ± 0.38	0.54 ± 0.21	0.67 ± 0.22	0.43	0.80	0.11
RBC [10x6/µl]	4.5 - 5.9	5.23 ± 0.30	5.16 ± 0.26	5.17 ± 0.35	5.09 ± 0.26	0.21	0.60	0.88
Hb [g/dl]	13.5 - 17.5	14.94 ± 1.12	14.85 ± 0.85	14.82 ± 0.81	14.68 ± 0.79	0.44	0.71	0.87
HCT [%]	39.0 - 53.0	44.63 ± 3.33	44.08 ± 2.15	44.12 ± 2.31	43.65 ± 2.02	0.30	0.64	0.97
PLT [10x3/µl]	150 - 350	223.7 ± 28.4	216.8 ± 26.3	205.3 ± 28.5	205.8 ± 29.9	0.14	0.19	0.12

Values are mean ± standard deviation; *, **, *** – significant difference between term 1 and term 2 (within the same group) (p < 0.05; p < 0.01; p < 0.001, respectively); abbreviations: WBC: white blood cell count; LYMPH: lymphocytes; MONO: monocytes; GR: granulocytes; EO: eosinophils; BASO: basophils; RBC: red blood cell count; Hb: hemoglobin; Hct: hematocrit; PLT: platelet count.

Table 3. Serum biochemical parameters of wrestlers before (term 1) and after (term 2) 8-week wrestling training combined with placebo (PL) or ashwagandha (A) supplementation

Biochemical parameter		Placebo (PL) n = 11		Ashwagandha (A) n = 10		Main effects		
	Reference range	Term 1	Term 2	Term 1	Term 2	Time	Group	Time x Group
ALT [U/l]	≤ 41	27.4 ± 17.6	35.9 ± 21.5	30.3 ± 9.8	27.2 ± 16.4	0.40	0.59	0.23
AST [U/l]	≤ 38	33.6 ± 11.5	37.1 ± 16.5	30.3 ± 9.8	32.5 ± 15.1	0.22	0.99	0.55
CK [U/l]	< 170	458.6 ± 374.5	708.6 ± 654.1**	406.5 ± 333.9	536.9 ± 554.9	0.007	0.61	0.35
TP [g/dl]	6.0 - 8.3	7.42 ± 0.26	7.37 ± 0.38§	7.66 ± 0.35	7.38 0.28*	0.06	0.35	0.13
Alb [g/dl]	4.2 - 5.5	5.00 ± 0.21	4.76 ± 0.24	4.96 ± 0.36	4.84 ± 0.32	0.14	0.46	0.20
Cr [mg/dl]	0.9 - 1.3	1.31 ± 0.16	1.29 ± 0.18	1.27 ± 0.15	1.25 ± 0.11	0.55	0.50	0.92
U [mg/dl]	15.0 - 38.0	35.6 ± 7.4	34.9 ± 8.9	36.6 ± 9.4	35.0 ± 9.9	0.71	0.78	0.56
BUN/Cr	< 20	12.3 ± 2.1	12.8 ± 3.1	13.6 ± 3.9	13.2 ± 4.4	0.99	0.51	0.49
UA [mg/dl]	3.4 - 7.0	3.69 ± 0.57	4.21 ± 0.84*	3.59 ± 0.73	3.96 ± 0.65	0.007	0.54	0.61
TC [mg/dl]	< 190	162.4 ± 30.2	165.1 ± 24.9	188.8 ± 29.8	193.3 ± 63.8	0.63	0.11	0.90
HDL-C [mg/dl]	> 40	60.6 ± 10.7	55.7 ± 7.9§	68.4 ± 13.8	61.8 ± 11.5#	0.014	0.14	0.70
LDL-C [mg/dl]	< 130	120.8 ± 30.4	121.7 ± 26.2	135.5 ± 29.4	155.8 ± 79.8	0.29	0.19	0.33
TG [mg/dl]	< 150	87.3 ± 40.6	98.9 ± 71.1	111.2 ± 74.5	71.1 ± 54.6	0.44	0.92	0.17
TC/HDL-C	≤ 4.5	2.7 ± 0.5	3.0 ± 0.6	2.9 ± 0.7	3.3 ± 1.8	0.16	0.54	0.74

Values are mean ± standard deviation; *, ** – significant difference between term 1 and term 2 (within the same group) (p < 0.05; p < 0.01, respectively); § – tendency to significant difference between term 1 and term 2 (within the same group, PL) (p = 0.09), # – tendency to significant difference between term 1 and term 2 (within the same group, A) (p = 0.06); abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase; TP: total proteins; Alb: albumin; Cr: creatinine; U: urea; BUN: blood urea nitrogen; UA: uric acid.TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides.

for PL and p = 0.06 for A). However, no main effects of group or time and group interaction were observed in the above mentioned parameters. Also, other biochemical parameters did not change significantly throughout the study (a lack of significant main effects).

For the A group, mean value of LDL-C was above the reference range in both terms, while mean TC value exceeded the reference range in term 1. For both groups in both terms, mean values of CK activity were elevated indicating exercise-induced muscle damage and uncompleted post-exercise regeneration. Apart from the above mentioned parameters, the mean values of other biochemical parameters were within the reference range for the population studied.

Conclusions

The main finding of our study is that ashwagandha supplementation (8 weeks, 600 mg daily) is well tolerated and safe for professional wrestlers. However, this strategy does not seem to offer additional body composition benefits beyond the effect of wrestling training.

This study was performed mainly to evaluate potential of ashwagandha supplementation to affect body composition changes in wrestlers during intense training plan (as part of an annual training cycle). Nutrition combined with proper supplementation strategy is considered as one of the fundamental pillars to optimize sports performance. There is some evidence from many reports that ashwagandha is herbal supplement that may provide ergogenic benefits for active individuals and athletes [3]. Indeed, some of the secondary metabolites of ashwagandha could have potential at the level of physical performance improvement, being responsible for various effects at the metabolic and physiological level. Among other things, active substances of ashwagandha extract may accelerate muscle regeneration through the regulation of anti-inflammatory and anti-oxidative pathways [3, 19]. In addition, it has previously been reported that ashwagandha supplementation can regulate adrenal and gonadal functions, including the release of steroid hormones [3, 20].

Consistent with the above, the anabolic effect of ashwagandha supplementation (8 weeks, 300 mg of ashwagandha root extract twice daily) was observed in untrained men who underwent resistance training [5]. In that study, the herbal supplement enhanced training adaptations. Namely, subjects taking ashwagandha, compared to the placebo group, showed significantly greater increases in muscle size in the chest and arms (assessed by measurements of chest circumference and maximum cross-sectional area based on measurements of arm circumference and skinfold). In addition, ashwagandha caused a significantly greater decrease in body fat percentage (as calculated by bioelectrical impedance) than placebo. It should be noted, however, that no significant intergroup differences in the absolute values of muscle size parameters were observed in that study [5].

In our study, favorable changes in the body composition of the wrestlers were observed after eight weeks, which could be attributed to intense wrestling training (10 training sessions per week), since the athletes did not change their dietary habits during the study (also, the groups did not differ in terms of dietary intake; data pending publication). The 8-week wrestling training resulted in a significant increase in FFM and MM and a decrease in FM (both in absolute and relative terms) in both study groups (with a significant main effect of time). As opposed to training, supplementation with ashwagandha (with the same treatment period and dose as in the study by Wankhede et al. [5]) did not affect changes in the body composition of our wrestlers (with non-significant main effects of group or time and group interaction). The reasons for these discrepancies may be different. First, unlike the study of Wankhede et al. [5], the participants in our study were highly trained athletes – national team wrestlers with a high level of adaptation to exercise. It is well known that athletes may need a higher supplement dose to affect exercise metabolism, although we used an ashwagandha supplement dose (i.e., 600 mg per day) designed for physically active individuals and athletes [3, 21]. Secondly, during supplementation, the wrestlers followed a training plan according to an annual schedule. Thus, the wrestlers' training focused on developing strength endurance, dynamic strength, technical-tactical skills, speed and special endurance in consecutive weeks, with little typical strength/resistance training, as in the study performed by Wankhede et al. [5]. Finally, it is worth noting that in our study we used the DEXA method, which is a more rigorous assessment tool that is considered the gold standard for measuring body composition (especially body fat) and its changes over time in trained individuals [22].

It cannot be ruled out that a longer period of ashwagandha supplementation may be needed to exert benefits in a trained population. On the other hand, Ziegenfuss et al. [6], using a longer period of ashwagandha supplementation (12 weeks, 500 mg daily) in combination with strength training in recreationally active men, also reported no significant influence of ashwagandha on body composition (body fat percentage, lean mass or fat mass) measured by DEXA. Interestingly, in that study, only one parameter determining body mass distribution was influenced by ashwagandha (with a significant group x time interaction), i.e. the android/gynoid ratio (as a metric generated by DEXA software), considered a crude marker of visceral fat accumulation. The authors pointed to a possible protective effect of ashwagandha on the body, as ashwagandha-supplemented subjects experienced no change in this ratio, while the placebo group showed it to rise after 12 weeks of training. As the authors explained, the less favorable change in the android/gynoid ratio in the placebo group may be due to the known increase in health risks associated with carrying more weight in the torso compared to the hip/gluteal region (e.g. dyslipidemias). However, the authors themselves acknowledged that the lack of other associated changes with measured body composition variables makes this finding challenging to interpret [6].

Among the various mechanisms of ashwagandha supplementation to enhance training adaptation may be a reduction in fatigue and improved recovery [3]. In fact, in a study by Wankhede et al. [5], favorable changes in body composition after an 8-week study in the ashwagandha group, compared to the placebo group, were, among others, related to accelerated recovery of muscle fibers. This post-exercise recovery was measured as an increase in CK activity from 24 to 48 hours after the last training session, compared to the first training session. Namely, throughout the study period, the ashwagandha group experienced a more pronounced reduction in serum CK activity during recovery in comparison to the placebo group [5]. However, what was interesting in that study [5], 8-week training itself resulted in a huge decline in the mean CK increase during recovery (since a drop of CK activity amounted from 1479 to 16 U/l, and from 1407 to 99 U/l in ashwagandha and placebo groups, respectively). Repeated bout effect is a well-known phenomenon associated with muscle adaptation to exercise, particularly observed in untrained individuals who begin training [23]. In our study, an 8-week period of intense training resulted in

an increase in serum CK activity (with a significant main effect of time). We did not find a significant effect of ashwagandha supplementation on serum CK activity, considering that there was no main effect of group or interaction of time and group. However, it should be noted that the increase in CK after 8-week wrestling training reached significance levels in the PL group, but not in the A group.

Thus, although the protective effects of ashwagandha on muscle tissue appear to be most pronounced in untrained individuals beginning their training, our results may indicate the potential for ashwagandha to achieve some benefit in athletes as well. It is important to emphasize the strengths of our study, which used a randomized, double-blind, placebo-controlled design (the gold standard in clinical trials), with a homogeneous group of high-level professional athletes, which unfortunately limits the ability to involve a large number of subjects. Therefore, further studies involving a more numerous group of athletes are needed to confirm our observations.

Our study also aimed to evaluate the safety of ashwagandha supplementation. It is widely believed that ashwagandha preparations are well tolerated and safe, as previous studies in which ashwagandha preparations were administered at doses of 300-1000 mg/day for 8-12 weeks found no side effects [21, 24]. Meanwhile, there are reports (case studies) in the scientific literature indicating side effects (including hepatotoxicity) of herbal supplements [7, 9], including ashwagandha [8, 10]. Moreover, most of the studies reporting on the safety of ashwagandha administration involve the Indian population, where the use of this herb is part of traditional Ayurvedic medicine, practiced in the population there for thousands of years. Therefore, it cannot be ruled out that the body's bioavailability and tolerance of ashwagandha may vary depending on the ethnicity of consumers.

In our study, a comprehensive analysis of wrestlers' health parameters included hematological blood parameters and markers of liver function (AST, ALT), renal function (Cr, U, UA), protein metabolism (TC, Alb) and lipid profile as an indicator of cardiovascular disease risk. Ashwagandha supplementation did not significantly affect any of the above parameters, indicating that a dose of 600 mg/day for 8 weeks is safe. Negligible changes in the levels of some blood parameters (TC, Alb, UA) may be due to an increase in exercise metabolism induced by intense training. While the changes in hematological parameters (decrease in MONO and a tendency for LEU and EOS to decrease) over time indicate a beneficial effect of training on the immune system, what is concerning is the tendency for HDL-C, known as good cholesterol (albeit within reference values), to decrease in both groups after 8 weeks of training (main time effect). The risk of transient adverse changes in lipid profile in combat sports athletes was described in our previous study with wrestlers [25].

Noteworthy, a possible beneficial effect of ashwagandha on red blood cell indices (RBC, Hb, Ht) and blood lipid profile has been reported in a recent review study [26]. However, these findings are from studies conducted without a placebo group [24] or from studies whose results need to be confirmed due to the lack of significant intergroup differences [27]. Our double-blind placebo-controlled study, given the non-significant main effects of group or time and group interaction, did not confirm the above observations. Furthermore, as in our study, the lack of effect of ashwagandha on the above indicators was reported by Lopresti et al. [4], while in another study [6] even a decrease in Hb levels was observed in the ashwagandha group, while the placebo group experienced an increase in RBC, Hb and Ht, with significant intergroup differences.

Conclusions

Ashwagandha supplementation in a dose of 600 mg daily for 8 weeks is well tolerated and safe for professional wrestlers. This strategy does not seem to offer additional body composition benefits beyond the effect of intense wrestling training. On the other hand, our results indicate some potential for ashwagandha to improve muscle tissue regeneration, which needs to be confirmed on a more numerous population of athletes.

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