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Original Article

ANTI-AGING EFFICACY AND SAFETY OF GLYCOSIDES-BASED STANDARDIZED FENUGREEK SEED EXTRACT SUPPLEMENTATION IN HEALTHY AGING ADULTS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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ABSTRACT

Objective: The efficacy and safety evaluation of oral "glycosides-based standardized fenugreek seed extract" (SFSE-G) supplementation to a healthy aging adult population.

Methods: A total of 112 healthy individual's aged ≥ 45 y (determined by chronological age and Levine's phenotypic age) were randomized using a computer-generated list (1:1 ratio) to receive 300 mg capsules of either SFSE-G or placebo daily for 12 w in a double-blind design. The outcome measures were changes in plasma "nicotinamide adenine dinucleotide" (NAD^+) levels, plasma "adenosine triphosphate" (ATP) levels, phenotypic age, physical performance, fatigue, and quality of life, along with safety and tolerability.

Results: Out of 112 participants enrolled, 103 completed the study (SFSE-G: 50; placebo: 53). Plasma NAD^+concentrations were significantly ($P<0.05$) higher when age correlated in SFSE-G-treated groups (vs. placebo). No serious adverse events or safety concerns were reported, indicating good tolerability of treatments. In addition, the study found that 12 w of 300 mg SFSE-G oral supplementation significantly ($P<0.05$) increased ATP levels, improved physical fatigue, and showed trends in reducing phenotypic age and improving quality of life in correlation with age.

Conclusion: SFSE-G supplementation showed potential to mitigate age-related declines in energy metabolism and physical fatigue, with a robust safety profile.

Keywords: Anti-aging, Fatigue, Fenugreek seed, Glycosides, Nad^+, Quality of life

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INTRODUCTION

According to the "World Health Organization" (WHO), the global life expectancy has increased to 73 y and is anticipated to reach 78 y by 2050, highlighting the need for healthy aging interventions [1, 2]. Aging involves chronic inflammation, macromolecular damage, loss of proteostasis, epigenetic alterations, and cellular senescence [3]. The WHO describes healthy aging as sustaining the functional abilities that contribute to well-being in older adulthood [4]. Evidence shows a 40% reduction in pulmonary and aerobic capacity between the ages of 25 and 80 y, with dysfunction in secretory organs such as the liver, kidney, and pancreas [5].

A primary factor contributing to age-associated decline in function is the reduction of "Nicotinamide Adenine Dinucleotide" (NAD^+), which is critical for cellular energy metabolism, redox homeostasis, and longevity pathways [6, 7]. The decrease in NAD^+ levels with age is predominantly driven by elevated "Cluster of Differentiation 38" (CD38) expression, an NADase that depletes NAD^+ and facilitates the accumulation of proinflammatory immune cells in tissues [8]. Consequently, the overexpression of CD38 is a significant contributor to age-related metabolic and mitochondrial dysfunction [9], thereby positioning CD38 inhibition as a promising strategy to mitigate age-related physiological decline [10].

Current anti-aging strategies, while beneficial in combating single aspects of anti-aging process, often fail to provide comprehensive solutions against age-related complications. Synthetic supplements, such as Nicotinamide Riboside (NR) and Nicotinamide Mononucleotide (NMN), have shown efficacy in enhancing NAD^+ levels in humans [11, 12]. However, they are limited in their scope and effectiveness when faced with complex age-induced complications [13]. In addition, their long-term side effects, such as nausea, headache, gastrointestinal discomfort [14, 15], biochemical enzyme disturbances [14], and photosensitivity [16], which reduce the quality of life of consumers necessitates the development of safer, natural alternatives.

In contrast, natural products, rich in phenolic and flavonoid compounds, are emerging as safer alternatives with antioxidant and anti-inflammatory activities that combat two key players in aging processes to prevent age-related complications [17]. Flavonoid glycosides modulate NAD^+ levels through CD38 inhibition to enhance cardiovascular and metabolic health [18]. Flavonoid glycosides, such as vitexin and quercetin (found in fenugreek seeds), have demonstrated free radical-scavenging effects that may prolong cellular aging [18]. Recently, glycoside-rich fenugreek (*Trigonella foenum-graecum* L.) seed extract has exhibited CD38-inhibitory activity *in vitro* [19]. Additionally, glycosides-based standardized fenugreek seed extract (SFSE-G) demonstrated improved lung function in bleomycin-induced fibrosis in rats [20] and is considered safe at a human-equivalent dose of up to 9.7 g/day [21]. Therefore, SFSE-G can help restore NAD^+ levels, combat inflammation and oxidative stress, enhance mitochondrial ATP production, and alleviate age-related physiological decline in skeletal muscle. However, its anti-aging properties need to be evaluated in human populations. The aim of the present study was to evaluate clinical efficacy and safety of oral supplementation of SFSE-G in healthy adults.

MATERIALS AND METHODS

Study plan

The study was conducted using "randomized controlled clinical study" design in accordance with Indian ethical guidelines [22] and "declaration of Helsinki" [23], and other applicable regulatory guidelines with approved protocols from institutional ethics committees of the center namely "Poona medical research foundation", "Noble hospital", "Ojas multispecialty hospital", "LPR ethics committee", and "Vedant multispecialty hospital". The research was listed in the "Clinical Trial Registry of India" (CTRI/2023/07/055305).

Participants

The study participants comprised males and females aged ≥ 45 y, as determined by both chronological age and 'phenotypic age (by Levine's calculator) [24], who provided written informed consent. The exclusion criteria were recent participation in other trials, substance abuse, pregnancy, lactation, use of fenugreek supplements, relevant allergies, abnormal laboratory results or uncontrolled medical conditions, cognitive impairment, and any condition deemed unsuitable by the investigator.

The sample size was determined using software, using R statistical software (v4.3) with a 80% power (5% type I and a 20% type II error), based on published data on NAD^+ levels [25]. Utilizing the data from baseline to Day 60 (mean change = 0.0 ± 11.2 (µg/ml), estimated effect size = 0.6), and with possible 20% dropout rate, 56 per group, i. e. total 112 participants were deemed necessary for the study.

Randomization and blinding

Following the screening evaluations, 112 participants who provided informed consent and performed 1:1 randomization towards 12-weeks SFSE-G or a matching placebo (identical color, size etc.) supplementation based on computer-generated randomization list. The capsules were filled in two bottle containers (50 capsules each), which were then pasted with preprinted unique numbered diaper labels (for blinding purpose to ensure allocation concealment). Both the participants and investigators were blinded to the randomization process, ensuring that neither party was aware of the allocation, in accordance with the double-blind design.

Interventions

Each SFSE-G capsule contained 300 mg of SFSE-G powder and an inert excipient. Both SFSE-G capsules and matching placebo capsules (containing everything other than SFSE-G) were manufactured at Syndy Pharma (Hyderabad, India) and supplied by Indus Biotech Limited (Pune, India). The products were stored in a controlled-access room, maintained in a cool and dry environment, and protected from sunlight. The test compound, SFSE-G, marketed as Testosurge, is a standardized extract of fenugreek seeds (containing 81.19% glycoside content), standardized by "high-performance liquid chromatography" method. The dosage of SFSE-G, set at 300 mg once daily, was established based on effective and safe doses reported in animal studies [20, 21]. Oral administration of SFSE-G at 20 and 40 mg/kg demonstrated efficacy in mitigating bleomycin-induced pulmonary fibrosis, such as lung index and oxygenation, in rats [20]. The human equivalent dose (HED) corresponding to the midpoint (30 mg/kg) was calculated to be 300 mg/day for a 60 kg individual, which exceeds the safe HED derived from oral toxicity data by over 30 times [21].

Procedure

The participants underwent screening assessments of eligible participants seven days before baseline (Week-0), which included evaluations of demographics, medical and medication history, vital signs, and phenotypic age biomarkers, namely albumin, glucose, lymphocytes, "mean cell volume" (MCV), creatinine, "alkaline phosphatase" (ALP), c-reactive protein (CRP), "white blood cells" (WBC), and "RBC distribution width" (RDW), for eligibility criteria. Randomized participants (Week-0, W0) received capsules according to the randomization list in a blinded manner, with dosing instructions for 12 w. They were instructed to record concomitant medications, study product compliance, and "adverse events" (AEs) or "serious AEs" in a diary. Follow-up visits were conducted at the end of week-6 (W6), week-12 (W12), and one-week post-treatment telephonic follow-up at week-13 (W13), during which compliance, diaries, and remaining study products were reviewed, and study assessments were repeated.

Outcome measures

All assessments were conducted at baseline, W6, and W12. The efficacy outcomes were plasma NAD^+(ng/ml) levels, energy and fatigue parameters (plasma ATP, aerobic capacity and endurance, strength of the lower body, fatigue severity), phenotypic age, and quality of life. The safety parameters included vital parameters, physical examination, hematology, biochemistry, liver function, kidney function, lipid profile, urinalysis, organ function (heart, lungs, liver, kidney, pancreas, and brain), and AE monitoring (including tolerability and acceptability, and treatment compliance), as shown in Table 1.

Table 1: Details of efficacy and safety outcome measures

Outcome measures	Method and parameters	Equipment and reference
Efficacy outcome measures		
NAD+	Method: "enzyme-linked immunosorbent assay" (ELISA) on plasma	ELISA kit (MyBioSource Inc. California, USA, Catalogue No: MBS2700640) ELISA plate reader (Bio-Rad Laboratories, Inc., India) (Model: 4100 absorbance microplate reader software-Magellan software)
Energy and fatigue: ATP	Method: ELISA on plasma	ELISA kit (MyBioSource Inc., California, USA, catalogue no: MBS166236) ELISA plate reader (Bio-Rad Laboratories, Inc. India) (Model: 4100 absorbance microplate reader software-Magellan software)
Energy and fatigue: Aerobic capacity and endurance	Parameters: distance covered	"6 min walk test"(6MWT) [26, 27]
Energy and fatigue: Strength of the lower body	Parameters: Total score	"30 sec chair stand test"(30s CST) [28]
Energy and fatigue: Fatigue	Method: self-administered → 9 questions, → rate their level of	"Fatigue severity scale" (FSS [29]

severity	fatigue. Parameters: Total score	
Phenotypic age	Parameters: glucose, lymphocytes, creatinine, "mean corpuscular volume" (MCV), RDW, alkaline phosphatase (ALP), WBC, albumin, CRP and chronological age	Levine's phenotypic age calculator [24, 30]
Quality of life	Parameters: Physical and mental component summary respectively (PCS and MCS)	SF-36 version 1.0 [31, 32]
Safety outcome measures		
Vital parameters	Parameters: Blood pressure, and body temperature	-
Physical examination	Parameters: Height, weight, "body mass index" and general appearance	-
Hematology	Blood related parameters	Autoanalyzer (HORIBA Ltd., Japan, New Yumizen H550)
Biochemistry	Parameters: Fasting insulin, fasting glucose, electrolytes (sodium, potassium, calcium, chloride, magnesium, phosphate, bicarbonate)	Autoanalyzer (Roche Diagnostics, North America, Model: cobas c311)
Liver function, kidney function, lipid profile	Serum biochemical parameters	Autoanalyzer (Roche Diagnostics, North America, Model: cobas c311)
Urinalysis	Parameters: color, appearance, dipstick tests: specific gravity, acidity, protein, glucose, leukocyte esterase, ketones, bilirubin, microscopic urine examination	Urine Chemistry Autoanalyzer (Siemens Healthineers, India, Model: CLINITEK Advantus)
Heart function	Parameters: Blood pressure, oxygen saturation, and heart rate	Digital Arm Blood Pressure Monitor (OMRON Healthcare India Pvt Ltd, India, model: HEM 7121J) Pulse oximeter (Yobekan, China, model: YBK303)
	"two-dimensional echocardiography"	Model: Vivid cardiac T8 ultrasound systems, (Wipro GE Healthcare Pvt Ltd, Bangalore, India)
	"Treadmill Stress Test"-time (minutes: seconds)	Bruce protocol [33] on Treadmill 594 XL (Schiller, AG, Baar, Switzerland)
Lungs function	Parameters: Lung volumes	Spirometry (model: Helios 401, RMS India Ltd, Panchkula, India)
Brain function	Method: Mini-Cog test-cognitive and executive function, visual-motor skills. Parameters: Total score	The Mini-Cog test [34]
AEs and SAEs	Method: AEs reported by participants, principal investigator, or participants' diaries, and case report forms	"common terminology criteria for adverse events" (CTCAE) version 5.0 [35]
Tolerability and acceptability	Parameters: Convenience (inconvenient/neither convenient nor inconvenient/convenient), taste (tastes bad/leaves an aftertaste/taste neither good, nor bad/tastes good), perceived effectiveness (has no effect/works well/works very well), side effects (no/cannot tell), and overall satisfaction of treatment (neither happy nor unhappy/happy/very happy)	5-items-self-reported questionnaire measurement of parameter response to assessment criteria
Treatment compliance	Method: % of treatment compliance	Number and % of capsules consumed versus dispensed

Statistical analysis

The analysis was performed on the modified intention-to-treat (mITT) population using IBM SPSS for Windows ("v26, NY, USA") with $P<0.05$. Data is expressed as mean±standard deviation (continuous variables) or numbers and (%) (categorical variables). Non-continuous variables are expressed as counts and percentages. Continuous variables were analyzed between groups (SFSE-G vs. placebo) using "independent samples t-tests" after verifying the normality assumption. Categorical variables were assessed using Pearson's chi-square test. In addition, changes in each variable were calculated for two intervals: baseline to W6 and baseline to W12. Age-related physiological changes may vary between individuals [36], and the boundary for the elderly population with respect to NAD+was defined as 70 y in an earlier study [37]. Therefore, we performed subgroup analysis with one subgroup between 45 and 70 y and another with ≥ 70 y of age. Within each group, linear correlations were computed between age and the different variables at both pre-and post-treatment intervals using Fisher's z-score based on the test for the difference between correlations, as previously reported [38]. A positive correlation indicates an increase, whereas a negative correlation indicates a decrease in the variable value with age.

RESULTS

Demographics

Among 218 screened potential participants, 112 (females: males = 31:81) satisfied the inclusion criteria and were randomized into the SFSE-G (n = 56) or placebo (n = 56) groups. A total of 103 participants (91.96%) completed the treatment, comprising 50 (48.54%) and 53 (51.46%) participants in the SFSE-G and placebo groups, respectively. None of the demographic parameters showed statistical significance (vs. placebo) (Table 2). Nine participants (7.1%) discontinued the study after randomization. All items of the study design were conformed as per the consolidated standards of reporting trials (CONSORT)-2025 statement [39] with a flow chart presented in 1.

Table 2: Demographic characteristics

	Mean±standard deviation	P[1 r2]

Characteristics	Placebo	SFSE-G	
Chronological age (years)[a]	58.80±8.44	58.38±8.19	0.786
Gender [n (%)][b]			
Female	16 (28.6%)	15 (26.8%)	0.833
Male	40 (71.4%)	41 (73.2%)	
Weight (Kg)[a]	66.01±11.84	64.72±10.8	0.547
Height (cm)[a]	161.52±8.59	160.28±7.98	0.429
"Body mass index" (Kg/m^2)[a]	25.2±4.06	25.23±3.75	0.966
Education [n (%)][b]			0.558
Primary	9 (16.1%)	13 (23.2%)	
Middle school	12 (21.4%)	14 (25.0%)	
High school	18 (32.1%)	13 (23.2%)	
Intermediate/diploma	10 (17.9%)	6 (10.7%)	
Graduate	7 (12.5%)	9 (16.1%)	
Occupation [n (%)][b]			0.699
Service	15 (26.8%)	19 (33.9%)	
Self-employed	16 (28.5%)	16 (28.5%)	
Housewife	14 (25.0%)	13 (23.2%)	
Retired	11 (19.6%)	8 (14.2%)	

"n = 56, sample size", (% participants). [1]Parametric, "t-test for independent samples". [2]Nonparametric, "Chi-square test."

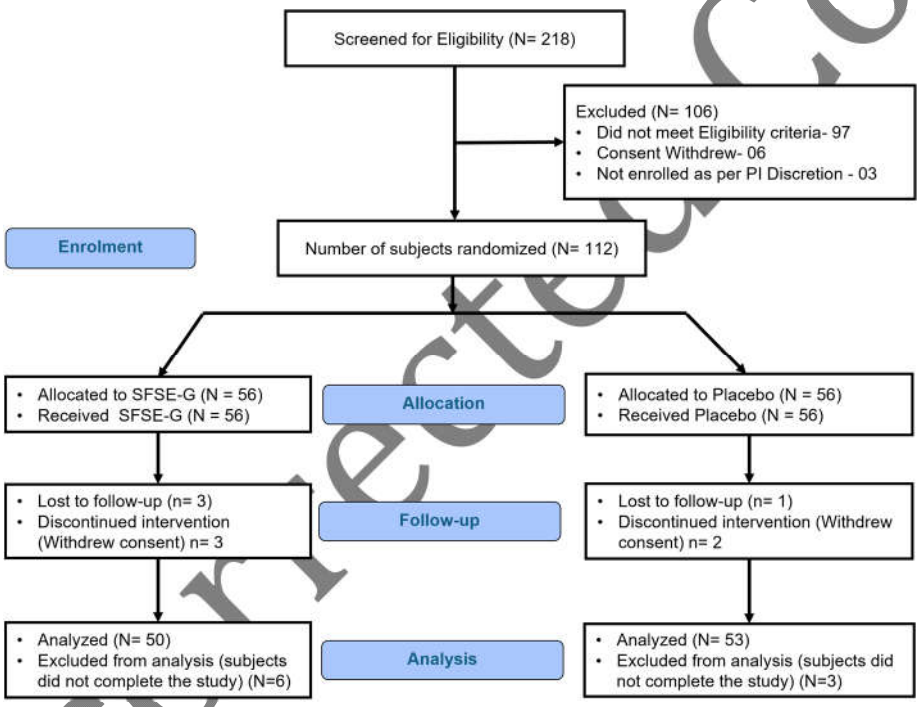

Fig. 1: Study design flow chart in CONSORT format

Effect on plasma NAD$^+$

For SFSE-G, the plasma NAD$^+$levels significantly increased between the groups at W6 (P<0.05), with changes in plasma NAD$^+$values from baseline not being significant. In placebo, a W12, significant (P<0.050 increase in NAD$^+$levels (between the groups) was found.

During the age correlation analysis, at baseline, plasma NAD$^+$levels were negatively correlated with age in the SFSE-G and positively correlated in the placebo group (P<0.05). Following treatment, the SFSE-G exhibited a shift towards a positive correlation, whereas the placebo showed no significant change in NAD$^+$. Changes in NAD$^+$ levels from baseline were positively associated with age at W6 (P<0.05) and W12 (P<0.05) in the SFSE-G and negatively in the placebo group, with significant between-group differences in the correlation coefficients at W6 and W12 from the baseline (P<0.05). Subgroup analysis (age 45-70 y) showed consistent trends, although the post-treatment differences were not statistically significant (

Table 3).

Table 3: Plasma NAD+(ng/ml)

Age	Time	Mean±standard deviation			Age correlation coefficients		
		Placebo	SFSE-G	P[1]	Placebo	SFSE-G	P[2]
≥ 45 y	W0	3355.29±1512.54	3384.29±1385.52	0.759	0.230	-0.218	0.019*

	W6	4216.79±1573.65	4890.58±1794.29	0.026*	0.044	0.209	0.403
	W12	5211.10±1854.90	4673.35±2314.67	0.043*	0.010	0.163	0.447
	Change W6	912.30±2080.23	1452.04±2000.07	0.323	-0.131	0.334#	0.018*
	Change W12	1949.05±2096.00	1238.29±2481.44	0.070	-0.140	0.274	0.038*
45-70 y	W0	3195.99±1465.23	3490.79±1400.86	0.304	0.057	-0.080	0.504
	W6	4168.55±1604.27	4804.86±1798.57	0.071	-0.026	0.149	0.403
	W12	5122.98±1839.67	4637.40±2367.25	0.269	-0.120	0.165	0.178
	Change W6	1037.91±2042.34	1278.17±1934.73	0.557	-0.044	0.220	0.207
	Change W12	2038.44±2016.42	1111.03±2514.64	0.053	-0.110	0.220	0.119

W= Week; "n = sample size", n = 50 to 56 (≥ 45 y), n = 46 to 51 (45 to 70 y), [1]t-test for independent samples" (Between group, difference of means), [2]Correlation of parameters with age at each visit; #and*-significance (P<0.05) within and between the group, respectively.

Effects on plasma ATP

Plasma ATP levels between and within groups at W6 and W12, or in their respective changes from baseline values, showed no significant differences. In the age correlation analysis, a subgroup of participants aged 45–70 y showed a significant positive correlation between age and ATP levels in the SFSE-G group at W12 (P<0.05) (vs. placebo). In contrast, a negative correlation between age and ATP levels was observed in the placebo group (**Error! Reference source not found.**).

Table 4: Energy-related parameters

Parameter (Units)	Age (Yr)	Visit	Mean±standard deviation			Age correlation coefficients		
			Placebo	SFSE-G	P[1]	Placebo	SFSE-G	P[2]
Plasma ATP (nmol/l)	≥45	W0	1219.50±508.71	1090.77±341.09	0.120	-0.099	0.139	0.220
		W6	1108.71±725.67	1187.55±830.67	0.605	-0.134	-0.139	0.979
		W12	1398.87±977.17	1334.93±852.57	0.725	-0.246	-0.031	0.278
		Change W6	-117.49±815.55	86.39±871.29	0.221	-0.035	-0.187	0.445
		Change W12	161.69±1153.52	245.09±996.81	0.697	-0.158	-0.067	0.651
	45-70	W0	1233.44±530.51	1105.73±344.13	0.154	-0.054	0.304	0.073
		W6	1117.75±755.00	1191.73±838.31	0.651	-0.147	-0.163	0.938
		W12	1396.62±972.51	1385.11±870.58	0.952	-0.344#	0.099	0.032*
		Change W6	-123.82±849.36	75.79±857.75	0.257	-0.074	-0.271	0.336
		Change W12	142.67±1163.38	280.83±1029.91	0.546	-0.262	0.001	0.209
Fatigue Severity Scale-(Total score)	≥45	W0	26.80±8.37	27.20±7.59	0.795	-0.005	0.156	0.403
		W6	23.60±6.83	25.04±7.58	0.310	0.055	0.020	0.862
		W12	23.92±9.21	22.66±8.40	0.469	0.193	0.139	0.782
		Change W6	-3.06±5.59	-2.00±6.77	0.385	0.043	-0.177	0.269
		Change W12	-2.74±7.47	-4.32±7.42	0.283	0.219	-0.038	0.199
	45-70	W0	26.73±8.63	27.35±7.66	0.698	-0.043	0.253	0.139
		W6	23.67±7.09	25.29±7.81	0.289	0.078	0.099	0.920
		W12	23.94±9.67	22.57±8.74	0.473	0.243	0.141	0.619
		Change W6	-2.9±5.83	-1.94±6.67	0.455	0.126	-0.204	0.114
		Change W12	-2.63±7.68	-4.61±7.22	0.201	0.330#	-0.146	0.022*
"6 min walk test"-Distance covered (m)	≥45	W0	200.56±72.19	190.41±62.40	0.255	0.005	-0.171	0.360
		W6	221.31± 80.94	199.66±53.75	0.188	0.048	0.164	0.560
		W12	232.12±78.72	220.00±51.32	0.918	0.004	0.238	0.240
		Change W6	19.96±38.32	13.56±40.17	0.860	0.129	0.278	0.438
		Change W12	30.76±44.31	31.86±46.69#	0.649	0.032	0.286#	0.196
	45-70	W0	199.92±75.59	191.76±64.88	0.467	-0.017	-0.155	0.495
		W6	218.96±84.55	197.88±55.12	0.291	-0.013	0.121	0.523
		W12	228.98±82.02	217.27±52.11	0.919	-0.087	0.167	0.230
		Change W6	18.2±39.69	11.51±41	0.817	0.050	0.219	0.413
		Change W12	28.22±45.68	28.67±46.77	0.639	-0.089	0.194	0.180
"30 sec chair stand test" (Total score)	≥45	W0	7.45±3.62	7.50±3.35	0.909	0.261	0.243	0.921
		W6	7.68±3.65	7.73±3.40	0.972	0.442#	0.407#	0.831
		W12	8.21±3.22	8.66±3.31	0.379	0.428#	0.392#	0.831
		Change W6	0.51±1.00	0.23±0.00	0.172	0.178	0.137	0.834
		Change W12	1.04±1.24	1.26±1.77	0.134	-0.001	0.089	0.657
	45-70	W0	7.39±3.78	7.51±3.48	0.946	0.290#	0.348#	0.752
		W6	7.54±3.79	7.65±3.52	0.891	0.468#	0.430#	0.821
		W12	8.08±3.35	8.54±3.43	0.469	0.451#	0.393#	0.740
		Change W6	0.46±1.34	0.19±1.47	0.210	0.127	0.094	0.874
		Change W12	1.00±1.29	1.20±1.82	0.191	-0.072	0.025	0.649

W= Week,), "n = sample size", n = 50 to 56 (≥ 45 y), n = 46 to 51 (45 to 70 y), n = "no. of participants sample size", [1]t-test for independent samples" or "Mann-Whitney U test" (Between group, difference of means),[2]Correlation of parameters with age at each visit; #and*-significance (P<0.05) within and between the group respectively.

Effects on physical fatigue in FSS

The FSS scores between and within groups at W6 and W12, or in their respective changes from baseline values, showed no significant differences. During the age correlation analysis, a statistically significant change was observed in the 45–70 y age subgroup from baseline to W12 ($P<0.05$). Specifically, a negative correlation with age was observed in the SFSE-G, whereas a significant positive correlation was noted in the placebo ($P<0.05$, between the groups) at W12 from baseline, indicating a reduction in fatigue associated with the SFSE-G with respect to age (**Error! Reference source not found.**).

Effects on aerobic capacity in 6MWT

No significant differences were observed in the distance covered during the 6MWT between the groups at W6 and W12 and in their respective changes from baseline values. However, the SFSE-G showed an increase in the distance covered during the 6 MWT at W12 from baseline ($P<0.05$), with no statistical significance in the placebo group. In the age correlation analysis, the distance covered during the 6MWT did not significantly change between the groups. However, a subgroup of participants aged>45 y showed a significant positive correlation within the group in the SFSE-G ($P<0.05$) at W12, with no statistical significance observed in the placebo group. In addition, a subgroup of participants aged 45–70 y showed a positive correlation with age in the distance covered by the SFSE-G, while a negative correlation with age was noted in the placebo group, with no statistical significance, indicating an increase in aerobic capacity associated with SFSE-G with respect to age (**Error! Reference source not found.**).

Effects on leg strength in the 30s CST

At W6 and W12, no significant differences were observed (between or within groups) or their respective changes or age correlation coefficients for the 30s CST scores (**Error! Reference source not found.**). However, SFSE-G and placebo at W6 and W12 from baseline ($P<0.05$, within groups) showed significant differences.

Effects on phenotypic age using Levine's calculator

In phenotypic age, between-and within-group analyses at W6 and W12 and in their respective changes from baseline values did not show any significant differences. The groups showed no significant variations in phenotypic age during the age correlation analysis. However, participants aged>45 and between 45 and 70 y in the SFSE-G showed a significant negative correlation, whereas a positive correlation was observed in the placebo group at W6 and W12 ($P<0.05$), indicating a reduction in phenotypic age (within-group correlation) for the SFSE-G with age (Table 5).

Table 5: Phenotypic age

Age (Y)	Visit	Mean±standard deviation			Age correlation coefficients		
		Placebo	SFSE-G	P[1]	Placebo	SFSE-G	P[2]
≥ 45	W0	62.26±14.23	62.53±12.17	0.914	0.479	0.537	0.687
	W6	61.49±16.53	61.89±15.78	0.900	0.510#	0.445#	0.675
	W12	60.54±17.45	60.11±15.58	0.897	0.534#	0.398#	0.393
	Change W6	-1.11±12.84	-0.70±14.51	0.878	0.100	0.124	0.903
	Change W12	-1.71±14.55	-2.15±15.66	0.884	0.171	0.066	0.601
45-70	W0	60.51±13.34	61.63±11.70	0.416	0.328	0.503	0.297
	W6	59.41±14.11	61.59±16.35	0.583	0.448#	0.492#	0.789
	W12	59.46±17.35	59.02±15.41	0.454	0.516#	0.338#	0.304
	Change W6	-1.37±11.69	-0.61±14.84	0.927	0.134	0.164	0.884
	Change W12	-1.32±15.02	-2.82±15.61	0.397	0.280	-0.018	0.152

W= Week, n = 50 to 56 (≥ 45 y), n = 46 to 51 (45 to 70 y), "n = sample size",, [1]"Mann-Whitney U test" or "t-test for independent samples"(between-group, difference of means), [2]correlation of parameters with age at each visit #P<0.05: within-group correlation.

Effects on quality of life

The PCS and MCS scores of the SF-36 between and within groups at W6 and W12 or the changes from baseline did not show significant differences. However, participants aged>45 y and those in the 45–70-year age subgroup exhibited a trend towards increased PCS and MCS scores over the 12-week SFSE-G supplementation period. This trend was not observed with the placebo. In the age correlation analysis, significant differences were not observed in either the PCS or MCS scores of the SF-36, either between or within groups.

Effects on vital and laboratory parameters

The evaluated vital parameters did not change significantly between the SFSE-G and placebo groups at baseline, W6, or W12. Furthermore, parameters related to organ function (heart, lung, liver, kidney, pancreas, and brain) and laboratory examinations (hematological, biochemical, liver function, lipid profile, and kidney function) remained within the normal range during the study period.

Effects during AE monitoring

A total of 40 participants (35.71%) reported 59 AEs during the study period. Within SFSE-G, 30 AEs were documented, comprising 19 mild and 11 moderate cases. Placebo reported 29 AEs, of which 19 were classified as mild and 10 as moderate. All AEs were assessed as unlikely to be associated with either treatment, and no participants withdrew from the study due to AEs. Importantly, neither group reported fatalities or SAEs.

Effects on tolerability and acceptance

An analysis of the participants' responses regarding convenience, taste, perceived efficacy, adverse events, and overall satisfaction with SFSE-G indicated no statistically significant differences (vs. Placebo) at either W6 or W12. However, SFSE-G exhibited trends towards enhanced convenience, but without statistical significance. Overall, SFSE-G (and placebo) was generally well tolerated and accepted by participants.

DISCUSSION

This study investigated the efficacy of oral supplementation with SFSE-G on age-related physiological decline in healthy adult participants. A 12-week regimen of daily supplementation with 300 mg SFSE-G was safe and well-tolerated by participants aged 45 y and older. SFSE-G

supplementation significantly elevated plasma $NAD^+$and ATP levels and reduced physical fatigue relative to age. These findings indicate that SFSE-G may serve as a valuable nutraceutical for promoting healthy aging.

The aging process is reported to show a progressive decline in NAD^+ levels, impairing mitochondrial function and ATP synthesis, which are central to tissue maintenance and energy homeostasis [40]. This decline in NAD^+ levels is partially attributed to the increased activity of CD38, a major NAD^+-consuming enzyme, rendering its inhibition a promising therapeutic strategy [41]. SFSE-G is a CD38 enzyme inhibitor [19] that preserves NAD^+ levels, exhibits anti-aging efficacy, and enhances mitochondrial ATP production [42]. The present study demonstrated that a 12-week regimen of SFSE-G supplementation significantly increased plasma NAD^+ and ATP levels. This finding is consistent with previous data, suggesting enhanced mitochondrial efficiency and cellular energy production in the cells. The elevation of NAD^+ is recognized for its role in supporting ATP synthesis, which may consequently mitigate fatigue, a prevalent and debilitating symptom among aging adults [12].

These biochemical alterations (NAD^+ and ATP levels) were associated with improved outcomes in objective outcome efficacy measures of physical fatigue, 6 MWT, and 30s CST. The 6 MWT is indicative of submaximal aerobic capacity, with diminished performance correlating with increased mortality in older populations [43]. The 30s CST assesses lower limb strength, which is a critical factor for mobility and fall prevention [44]. The integration of these objective tests with subjective evaluations, such as the FSS, establishes a comprehensive framework for assessing fatigue in older adults. The 6MWT and 30s CST evaluate physical capacity, while the FSS measures the impact of fatigue on daily life, facilitating an understanding of its burden and evaluation of interventions. Improvements in 6MWT performance after SFSE-G intervention indicate mental health improvement to aging population by preserving mobility [45] and cognitive ability [46]. In addition, our results support the report that herbal products can enhance physical function through skeletal muscle-relaxant effects [47]. Collectively, these findings suggest that NAD^+ restoration through SFSE-G supplementation enhances energy metabolism and mitigates fatigue, consistent with previous evidence of the anti-fatigue and mitochondrial benefits of fenugreek-derived glycosides [48].

Biological aging exhibits variability among individuals of identical chronological ages, underscoring the necessity for more precise biomarkers [36]. Levine's phenotypic age, which integrates clinical chemistry and chronological age, is a validated predictor of biological age and mortality [24]. This study demonstrated that a 12-week regimen of SFSE-G supplementation significantly reduced phenotypic age relative to chronological age, providing preliminary human evidence of the effect of glycoside-rich SFSE-G on biological age. This finding aligns with the observed restoration of NAD^+ metabolism and supports the potential role of SFSE-G in modulating systemic aging in human cells.

Aging is associated with compromised organ function due to mitochondrial dysfunction and NAD^+ depletion, affecting the heart, lungs, brain,, liver, and kidneys [49]. SFSE-G may alleviate these effects by enhancing cellular energy levels and reducing oxidative stress. Cardiac aging results in diminished energy output and impaired heart and lung function [50]. Although direct evidence regarding its cardiac effects is limited, the metabolic benefits of fenugreek are well documented [51]. Flavonoid glycosides have been reported to preserve lung function by mitigating inflammation and oxidative damage [52]. The role of SFSE-G glycoside content in maintaining cardiac and pulmonary function is supported.

The decline in NAD^+ levels in the aging brain is known to affect cognitive function [53]. Fenugreek flavonoid glycosides, such as isovitexin, offer neuroprotective effects [54] and have been shown to maintain cognitive markers with known antioxidant and mitochondrial benefits [55]. In addition, NAD^+ is crucial for hepatic and renal metabolic activity in the liver and kidney [56]. SFSE-G showed stable liver and kidney biomarkers in this study, indicating preserved liver and kidney function.

Physiological decline, chronic illness, and diminished social interaction are associated with the aging process, all of which adversely impact quality of life [57]. Previous research on fenugreek seed extract indicated benefits for "health-related quality of life" improvement, including well-being and memory enhancement in healthy and cognitively impaired older adults [58, 59]. Consistent with these findings, the present study observed improvements in the overall QoL following supplementation with SFSE-G. These improvements are likely due to the synergistic effects of enhanced energy metabolism, preservation of organ function, and deceleration of the aging process.

While SFSE-G targets similar endpoints regarding metabolic health and longevity as marketed anti-aging products such as NMN and NR, they follow distinct mechanistic pathways. The marketed anti-aging product NMN, which is synthetic in origin, functions primarily as an NAD^+precursor, activating sirtuins while significantly reducing CD38 activity and promoting ATP production [60, 61]. Conversely, the effects of SFSE-G are primarily attributed to $CD38^+$inhibition [19] and its anti-inflammatory properties of bioactive markers and glycosides [20, 62].

NMN has been reported to have dose-limiting serious side effects, such as heightened inflammation and tumorigenesis [63], whereas SFSE-G has been reported to ameliorate inflation and oxidative stress [20, 62], which are important factors for age-associated complications, suggesting a complementary approach to NMN. In the present study, SFSE-G exhibited a favorable safety profile in animals [19] and human participants with a well-tolerated profile, with few AEs mild to moderate in severity, unrelated to the intervention, and no SAE. Vital signs, laboratory tests, and organ function markers remained within the normal physiological range, further confirming the safety of this treatment.

The current findings offer clear evidence of the potential of SFSE-G to promote healthy aging in adults. Some limitations include the relatively short intervention period, modest sample size, and non-significant effects during subgroup analysis (45–70 y). Larger and longer-term studies, such as those investigating dose-response relationships, diverse age groups, and populations with comorbidities, are necessary to enhance generalizability.

CONCLUSION

In conclusion, oral supplementation of SFSE-G for 12-weeks in an aging population offered several benefits with respect to enhanced NAD^+levels, ATP levels, and quality of life with reduced physical fatigue and a reduced trend for phenotypic aging, with a robust safety profile. Additional research on larger and more diverse populations is required to establish it as a comprehensive anti-aging solution.

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AUTHORS CONTRIBUTIONS

PT was involved in the conception and design of the study, project supervision, and manuscript review. PD and ND were involved in the design of the study, project administration, supervision, and manuscript writing and reviewing. DR was involved in data analysis and manuscript reviewing. All authors have significantly and directly contributed intellectually to the project and have approved the manuscript.

CONFLICT OF INTERESTS

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