Am J Transl Res 2026;18(6):5316-5327
www.ajtr.org /ISSN:1943-8141/AJTR0170534

Original Article

NS2128E03 improves testosterone, performance, and recovery: translational insight from animal models to human trials

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Received December 5, 2025; Accepted May 26, 2026; Epub June 15, 2026; Published June 30, 2026

Abstract: Objectives: Nutraceutical formulations with adaptogenic and androgen-supportive properties are increasingly being explored for improving endurance, muscle strength, and reproductive health. NS2128E03, a proprietary herbal blend, was evaluated through pre-clinical and clinical studies to establish its safety and efficacy. Methods: Pre-clinical studies in Sprague Dawley rats assessed toxicity, sexual behavior, and hormonal modulation following oral administration of NS2128E03 at low and high doses for 50 days. A randomized, double-blind, placebo-controlled clinical trial was conducted in healthy adult males receiving NS2128E03 or placebo for 60 days alongside resistance training. Outcomes included muscle performance (cable pulldown, Mid Upper Arm Circumference [MUAC]), treadmill endurance, serum testosterone, and safety considerations. Results: Pre-clinical findings showed that NS2128E03 significantly increased total and free testosterone in male rats, improved sexual behavior indices (mount frequency, intromission latency, ejaculatory latency), and enhanced physical endurance without adverse effects. Histopathologic examination of reproductive organs confirmed normal architecture, supporting safety. In the clinical trial, NS2128E03 supplementation produced statistically significant improvements in muscle strength (bench press, leg press, handgrip), treadmill endurance, and both total and free testosterone levels compared to placebo. Significant changes ($P < 0.01$) were also observed in recovery biomarkers [increased mechanistic Target of Rapamycin (mTOR), reduced lactate dehydrogenase (LDH), and Creatine kinase (CK)]. No adverse events or clinically meaningful laboratory abnormalities were reported. Conclusion: NS2128E03 is a safe and effective nutraceutical that enhances testosterone, improves reproductive indices in animals, and translates into improved strength, endurance, and recovery in humans.

Keywords: NS2128E03, testosterone, muscle strength, endurance, mTOR, randomized controlled trial

Introduction

The maintenance of optimal muscle strength and hormonal balance is fundamental to male health, physical function, and quality of life. Beginning in the third decade of life, men experience a gradual decline in serum testosterone levels at a rate of approximately 1% per year, a condition commonly referred to as late onset hypogonadism or age related androgen decline [1]. This hormonal reduction is associated with decreased muscle mass, strength, endurance, libido, and overall vitality [2, 3]. Testosterone is the primary anabolic hormone regulating skeletal muscle protein synthesis, lean mass development, and post-exercise recovery [4]. Its deficiency is linked to sarcopenia, fatigue, insulin resistance, dyslipidemia, and visceral adiposity [5]. Although testosterone replacement therapy (TRT) can alleviate hypogonadal symptoms, long-term safety concerns--particularly cardiovascular and prostate risks--have prompted interest in safe, natural alternatives [6, 7].

In parallel, muscle protein synthesis and hypertrophy are known to be regulated by the mechanistic Target of Rapamycin (mTOR) pathway, a central signaling node activated by resistance training and nutritional stimuli [8]. Nutritional supplementation aimed at upregulating anabolic pathways such as mTOR, increasing testosterone, and enhancing recovery holds promise as a safe strategy to support performance and longevity in healthy, active individuals.

https://doi.org/10.62347/JBIB2641

NS2128E03 is a proprietary blend formulated to support anabolic physiology through botanical and nutraceutical components. Though the precise composition is confidential, preliminary laboratory evidence suggest it may influence testosterone levels and exercise performance by endocrine and metabolic modulation. Published reports suggest that no clinical trials to date have comprehensively evaluated the efficacy of NS2128E03, a standardized combination of *Tamarindus indica* and *Moringa oleifera* extract that was developed to support modulation of muscle strength, endurance, hormonal elevation, and safety in a healthy male population. Therefore, our study aimed to assess the effect of 60-day oral supplementation with NS2128E03 on validated measures of strength (1-repetition maximum tests), endurance (treadmill exhaustion time), serum testosterone (total and free), and muscle recovery biomarkers (Creatine kinase - CK, Lactate dehydrogenase - LDH and mTOR) in a randomized, double-blind, and placebo-controlled clinical trial design. We hypothesized that NS2128E03 would result in statistically and clinically significant improvements in these outcomes compared to placebo, without adverse safety signals.

Materials and methods

Plant material

NS2128E03, a proprietary natural herbal blend used in this study, was prepared using *Tamarindus indica* seed and *Moringa oleifera* leaves. It is a hydroalcoholic extract, developed and named by Natural and Essential oils Pvt Ltd., Mysore, India. NS2128E03 was phytochemically standardized to procyanidin B2 > 5% w/w by using the High-Performance Liquid Chromatography (HPLC) method [9, 10]. Briefly, Procyanidin B2 was analyzed using an HPLC system equipped with an Inertsil ODS-3 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid, applied under a gradient elution program. The flow rate was maintained at 1.0 mL/min, with a detection wavelength set at 280 nm and an injection volume of 5 µL. The total run time was 30 minutes, and the column temperature was maintained at 25 ± 2°C. The reference standard used for quantification was Procyanidin B2 (≥ 97% purity) procured from Sigma-Aldrich (Product No. SML3965, CAS No. 29106-49-8). Standard and test solutions were prepared in 0.1% orthophosphoric acid as diluent, followed by sonication and filtration prior to injection.

Animals

Male and female Sprague Dawley rats of age 6-8 weeks were used in the study. The temperature of the experimental room was maintained at 22°C ± 3°C and the relative humidity between 30-70% and the photoperiod was about 12 h light and 12 h dark cycles. Normal chow diet (Purina lab diet 5L79 - 18%) and RO water were provided *ad libitum* to all the animals throughout the experiment. All the experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of Radiant Research Services Pvt. Ltd., Bangalore, India (Approval no.: RR/IAEC/116-2023) and experiments were conducted in accordance with the guidelines of Committee for the Control and Supervision of Experiments on Animals (CCSEA Registration Number-1803/PO/RcBi/S/2015/CCSEA).

Subchronic oral toxicity study

In the 28-day repeated-dose oral toxicity study (RR240496), male and female Sprague Dawley rats aged 8-10 weeks were randomized into six groups, including a vehicle control, three treatment groups receiving 250, 500, or 1000 mg/kg/day of the test formulation, and two recovery groups. The test material was administered once daily by oral gavage for 28 consecutive days, while recovery groups were observed for an additional 14 days to assess the reversibility of any potential effects. Animals were monitored daily for clinical signs and mortality, with weekly assessments of body weight and food intake. Ophthalmic examinations, hematology, serum biochemistry, urinalysis, organ weight measurements, gross necropsy, and histopathologic examinations were conducted to evaluate systemic safety.

Sexual behavior and hormonal activity

A sexual behavior and hormonal activity study (RR230988) was conducted in male Sprague Dawley rats divided into three groups: a control group, a low-dose group receiving 41.13 mg/kg/day, and a high-dose group receiving 82.26

mg/kg/day of the test formulation. The treatment was administered orally for 50 consecutive days. Male rats (n = 8 per group) were individually paired with sexually receptive female rats in a 1:1 ratio (one male per one female) for 24 h period in a separate observation cage. Sexual behavior findings including mount frequency, intromission latency, ejaculation latency, and post-ejaculatory interval were assessed at baseline and again on day 50. Serum testosterone and free testosterone concentrations were measured at days 0, 25, and 50 to evaluate endocrine effects. Throughout the study, animals were also monitored for body weight changes, feeding consumption, and general health status to ensure safety.

At the end of the experimental period, blood samples were collected from animals under mild inhalational anaesthesia using isoflurane to minimize pain and distress during the procedure. Animals were placed in an induction chamber and exposed to a low concentration of isoflurane (typically 2-3%) until adequate sedation was achieved, as evidenced by reduced movement and loss of response to external stimuli. Blood collection was then performed using an appropriate technique under aseptic conditions. Following completion of blood collection, euthanasia was carried out by administering a high concentration of isoflurane (≥ 5%) via the nasal route or within a closed chamber, ensuring rapid induction of deep anaesthesia leading to respiratory arrest. Animals were continuously monitored throughout the procedure to confirm progression from anaesthesia to death. To ensure complete euthanasia, exposure to isoflurane was maintained for an adequate duration even after cessation of respiration. Death was confirmed by the absence of respiratory movements, heartbeat, and reflexes. Immediately after confirmation of death, eye specimens were carefully enucleated and collected using sterile instruments. The collected tissues were then preserved in appropriate fixative (such as 10% neutral buffered formalin) for subsequent evaluations. All procedures were performed in accordance with internationally accepted animal welfare guidelines and ethical standards, including those outlined by CPCSEA and the AVMA Guidelines for the Euthanasia of Animals, which recommend the use of inhalational aesthetic overdose as an acceptable method for humane euthanasia in small laboratory animals [11].

Prospective, randomized, double-blind, placebo-controlled clinical trial

The trial was conducted at Medstar Specialty Hospital, Bengaluru, in accordance with International Council for Harmonisation - Good Clinical Practice (ICH-GCP) guidelines and Central Drugs Standard Control Organization (CDSCO) regulations. The study was registered on the Clinical Trials Registry-India (CTRI/2024/03/063586) and received approval from the institutional ethics committee on 28 January 2024, vide approval number - RRS/CL/NSMS/2023. Fifty (50) healthy males aged 25-45 years, with a minimum of four months of resistance training experience (≥ 4 sessions/week), were enrolled. Key exclusion criteria included systemic illness, prior hormonal therapy, or recent corticosteroid use in addition to other eligibility criteria (**Table 1**). The participants were advised not to take ergogenic dietary supplements, and also they were instructed to maintain their diet throughout this study.

Blinding technique: The research employed a double-blind design method, indicating that participants and investigators remained unaware of group assignments. The placebo and NS2128E03 formulations looked the same, having the same hue, scent, and wrapping, and were marked with hidden labels made by third party. Grouping information was revealed only when the review analysis of data was finished.

Standardized resistance training program: Subjects followed a training routine of 4 workouts per week during this research, with 25-30 minutes for every exercise period. The resistance exercise plan involved 17 chest/shoulder, back, leg, and arm movements. The participants executed exercises focusing on specific muscle areas every day:

i. Chest/shoulder (incline dumbbell press, flat barbell press, dumbbell shoulder press, and lateral raise); ii. Back (bent rows, cable pulldowns, one arm dumbbell, and seated rear deltoid raises); iii. Legs (calf raises, leg extension, lying leg curls, and leg press); iv. Arms (EZ-bar curl, alternating dumbbell curls, overhead dumbbell extensions, hammer curls, and kickback/cable pushdown). Throughout the practice times, attendees completed warm-up movements at 50% of 1-Repetition Maximum (1-RM) across two sets of 8-10 repetitions.

Table 1. Eligibility criteria

#	Inclusion Criteria
1	Male volunteers between the age group of 25-45 years.
2	Subjects who are familiar with weight training and had at least 4 months of experience in the gym (defined as 4 times per week for a minimum of 3 to 4 hours per week).
3	Subjects who are willing and able to understand and follow the protocol and provide informed consent.
4	Agree not to use any medication (prescription and over the counter), including vitamins and minerals, and any alternative medications during this study.
5	Subjects whose blood chemistries are within a normal range or not considered clinically significant if outside the normal range.
#	Exclusion Criteria
1	Concomitant severe decompensated systemic disease (cardiovascular, renal, hepatic, endocrine, hematological, neurological, immunological).
2	Patients with pre-existing severe systemic disease necessitating long-term medication.
3	Subjects suffering from any serious medical or surgical illness.
4	History of cancer, including solid tumors, hematologic malignancies and carcinoma in situ.
5	Any neurological (congenital or acquired), musculoskeletal, joint health conditions, psychological, vascular or systemic disorder which could affect any of the efficacy assessments.
6	Significant abnormal findings as determined by baseline history, physical examination, vital signs (blood pressure, pulse rate, respiration rate), ECG, hematology, serum chemistry.
7	More than 4 short courses of oral corticosteroids within the year preceding the screening visit, or any oral corticosteroids in the preceding 4 weeks.
8	Use of Astemizole, Nedocromil, Cromolyn, long acting β agonists, Ketotifen, Theophylline or testosterone within 4 weeks before.
9	Participation in the current or previous clinical trial with any approved or investigational products during the past 1 month.

Following this, the individuals completed 2-3 sets of 10 repetitions at 70% of baseline 1-RM. The participants rested for 2 min between any two sets of one specific exercise. Afterward, the resistance steadily increased to 90% of the starting 1-RM, using 8-10 repetitions until exhaustion. In between any pair of exercises, participants were permitted to rest for 10 min. The practice schedules and performance evaluations occurred under the observation of skilled coaches and research staff. During the evaluation appointments of the research, only the effectiveness evaluation movements such as bench press, leg press, cable pull-down, handgrip strength, and treadmill test were executed. Participants were randomized in a 1:1 ratio to receive either NS2128E03 (500 mg capsule) or placebo (maltodextrin), administered once daily for 60 days (**Figure 1**). All exercise sessions were scheduled and conducted in the morning. All the subjects had breakfast (egg+bread+banana) 60 to 90 minutes before exercise and NS2128E03 or Placebo capsules were administered to subjects 30-45 minutes before exercise.

The primary outcomes included: a) Muscle strength (cable pull down, mid-upper arm circumference - MUAC): 1-RM bench press, leg press, handgrip.

1-RM strength: The 1-RM strength of bench and leg presses were assessed according to the National Strength and Conditioning Association. Participants got ready by completing two rounds of 8-10 movements at roughly 50% of what they expected to lift maximum. The people then carried out sequential exertions, beginning near 70% of their expected 1-RM and going up by 5 kg until they achieved the 1-RM.

Handgrip strength: A grip measuring device that was previously adjusted was employed to gauge the handgrip strength of their primary hands following the established procedure. The individuals were instructed to remain standing while having their shoulders drawn inward, arms alongside their bodies with complete elbow straightening. The individuals gripped the measuring device with the greatest power for 3 sec. Among three attempts made one

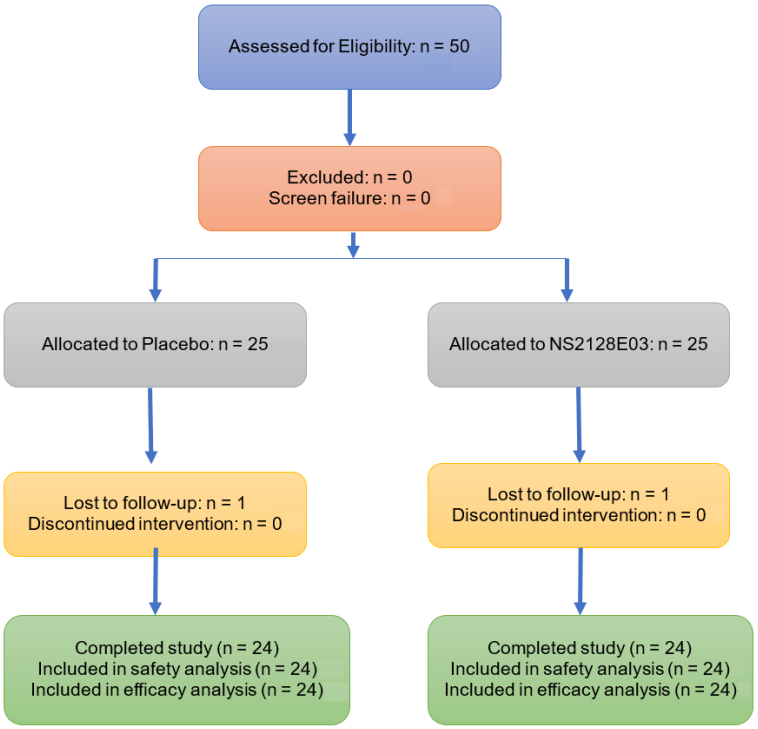

Figure 1. Flow diagram of participants in the two arms.

after another, the greatest measure of power was noted down.

Cable pull-down: Muscle endurance was gauged by how many repetitions subjects managed at 80% of their 1-RM using the cable pull-down with a typical lateral pull-down cable setup. With a regular lateral pull down bar, the individuals executed wide grip anterior pull-down movements with their palms facing forward grips. Every single pull was done from fully stretched arms until the bar touched the chest area.

MUAC: A measuring tape on a centimeter scale was placed around the flexed biceps at the midpoint between the shoulder and the tip of the elbow to measure the MUAC.

b) Endurance - treadmill time to exhaustion: Participants warmed up for 10 min before beginning the time to exhaustion test. The trainer set the treadmill to 6 km/h with a 0% inclination and start a stopwatch. After 3 min, the incline was adjusted to 2% and then increased by 2% every 2 min until the time recorded when subjects were no longer able to continue.

c) Hormonal biomarkers: Samples were collected 24 hours post-exercise for analysis. Serum total and free testosterone were measured (Testosterone ELISA kit, cat. no: EA0009-Ge, detection range: 0.5-150 nmoL/L, BT labs). The Secondary outcomes included: i) Serum biomarkers: mTOR (Human mTOR ELISA kit, cat. no: E359-2Hu, detection range: 0.05-15 ng/ml, BT labs) CK (Human creatine kinase ELISA kit, cat. no: E5124Hu, detection range: 0.3-90 mU/ml, BT labs), LDH (Human lactate dehydrogenase ELISA kit, cat. no: E5591Hu, detection range: 3-900 mU/ml, BT labs), ii) Subject feedback questionnaires, iii) Safety: adverse events, vital signs, hematology, biochemistry.

Statistical analysis

All experimental data including weight of the body, feed consumption, biochemical evaluation and organ weights were subjected to statistical analysis using GraphPad Prism Software, version 5.01. All values were expressed as mean ± SD. The significant variation between the treatment and control group was estimated using one-way ANOVA with Dunnett test. All results of the statistical analysis were summarized in separate tables and graphs. All of the values were considered significant at $P < 0.05$. The data generated from individual CRFs was compared between active and placebo groups from Day 0 until Day 60. Repeated measures ANOVA followed by Bonferroni pairwise comparisons comparing mean change between the active and placebo groups were used to identify significant changes between groups. p value < 0.05 was considered as significant for the study.

Results

Effect of NS2128E03 on rat subchronic oral toxicity

The 28-day repeated dose oral toxicity study of the herbal blend in Sprague Dawley rats demonstrated that the test item was well tolerated at all tested dose levels (250, 500, and 1000 mg/kg/day). No mortality, morbidity, or treatment-related clinical signs were observed throughout the study. Body weight gain, feed

Table 2. Sexual organ weights and quality measures

Item	Control	NS2128E03 (Low dose)		NS2128E03 (High dose)	
	Mean ± SD	Mean ± SD	t-value (df = 46)	Mean ± SD	t-value (df = 46)
Testis weight (g)	3.02 ± 0.251	3.34 ± 0.254*	4.39	3.49 ± 0.292**	5.9
Epididymis (g)	1.41 ± 0.007	1.42 ± 0.008	4.61	1.43 ± 0.011*	7.46
Sperm Count (1 × 10^6/ml)	246.63 ± 16.23	282.13 ± 18.68*	7.03	329.25 ± 37.53***	9.95
Motility (%)	64.33	71.43	-	80.85	-
Abnormal sperm (%)	35.68	28.58	-	20.15	-

Values were expressed as Mean ± SD (n = 8), Statistical significance are compared between Normal Control versus low and high dose of NS2128E03 were estimated using one-way ANOVA with Dunnett's test (*P Value < 0.05; **P Value < 0.001; ***P Value < 0.0001). The larger the t-value, the stronger the evidence that the groups truly differ.

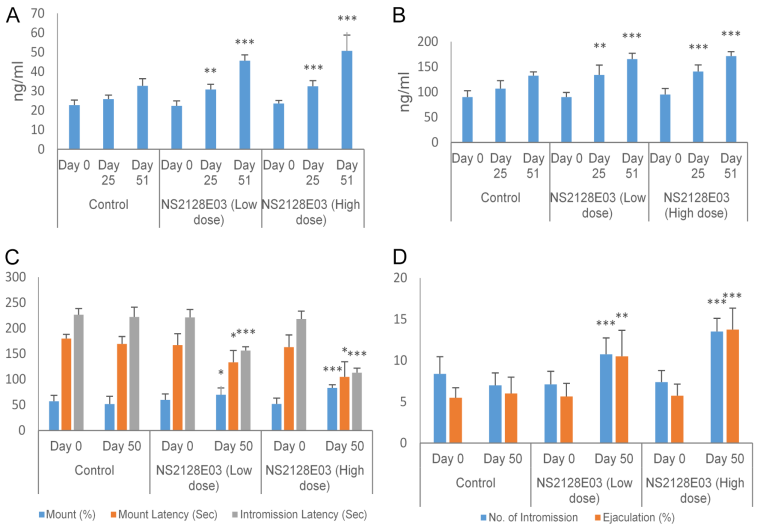

Figure 2. Effect of NS2128E03 on sexual behavior and hormonal activity. A. Serum testosterone levels; B. Free testosterone, (measured at Day 0, Day 25 and Day 51); C. Sexual behavior; D. Intromission and ejaculation behavior (measured at Day 0 and Day 50). Data are expressed as Mean ± SD (n = 8). Statistical significances are compared between normal control versus low and high dose of NS2128E03 were estimated using one-way ANOVA with Dunnett's test (*P Value < 0.05; **P Value < 0.001; ***P Value < 0.0001).

consumption, hematology, and clinical biochemistry values (including liver and kidney function tests, lipid profile, electrolytes, calcium, phosphorus, and glucose) remained within normal limits and were comparable to controls. Organ weights and gross pathology findings showed no abnormalities, and histopathological evaluation confirmed the absence of adverse changes in vital or reproductive organs. Overall, the study suggested the herbal blend did not elicit any toxicologic effects, and the No Observed Adverse Effect Level (NOAEL) was established at 1000 mg/kg body weight/day for both sexes (Results not shown).

Sexual behavior and hormonal activity of NS2128E03

Oral administration of the natural herbal blend, NS2128E03, at both low (41.13 mg/kg) and high (82.26 mg/kg) doses for 50 days produced a clear, dose-dependent improvement in sexual behavior in male Sprague Dawley rats. Treated groups showed significantly higher testosterone and free testosterone levels, increased testis and epididymis weights, improved sperm count, motility, and reduced abnormal sperm percentage compared to controls (**Table 2**). Behavioral findings such as mount frequency, intromission, and ejaculation also improved, while latencies decreased (**Figure 2**). Importantly, no mortality, clinical signs of toxicity, or adverse changes in body weight, feed consumption, biochemical markers, or histopathology of reproductive organs were observed. Overall, the findings support the potential of the herbal blend as a safe and effective pharmacologic agent for enhancing male sexual behavior and reproductive function.

Effect of NS2128E03 on muscle strength, endurance, and hormonal biomarkers in randomized clinical trials

Participant flow and baseline characteristics: A total of 50 participants were enrolled and randomized, with 48 completing the study (24 in each group) and were assessed over a treat-

Table 3. Schedule of assessments

Activity	Screening - Visit 0 (-3 Days)	Day 0	Day 15	Day 30	Day 60
Written Informed consent	X	--	--	--	--
Demographics	X	X	X	X	X
Physical examination	X	--	--	--	--
Medical and medication history	X	--	--	--	--
Vitals	X	X	X	X	X
Hematology & Biochemistry	X	--	--	--	X
Inclusion & Exclusion criteria	X	--	--	--	--
IP dispensing	--	X	--	--	--
Grip Strength (Dynamometer)	--	X	X	X	X
1RM in bench press & leg press	--	X	X	X	X
Cable pull-down	--	X	X	X	X
Mid-Upper arm circumference (MUAC)	--	X	X	X	X
Treadmill	--	X	X	X	X
Biomarkers- Serum CK and Serum LDH, mTOR, Serum lactate, and Insulin	--	X	--	X	X
Testosterone test	--	X	X	X	X
Feedback Questionnaires	--	--	--	--	–
Adverse events	--	X	X	X	X
Concomitant Medication	X	X	X	X	X

Table 4. Demographics

Item	NS2128E03	Placebo	t-value (df = 46)	*P* value
Age (years)	31.28 ± 4.76	28.48 ± 2.80	2.48	< 0.086
Height (Cms)	171.30 ± 4.94	169.63 ± 5.59	1.10	< 0.107
Weight (Kg)	76.21 ± 10.75	73.89 ± 8.91	0.81	< 0.093
BMI (kg/m^2)	26.04 ± 4.05	25.64 ± 2.50	0.41	< 0.201

BMI: Body Mass Index. Both the groups have no statistical significance through student 'p' test, for age, height, weight and BMI parameters.

ment period of 60 days (**Table 3**). Baseline demographic and anthropometric characteristics were comparable between groups, including mean age (NS2128E03: 31.28 ± 4.76 vs. Placebo: 28.48 ± 2.80 years), height, weight, and Body Mass Index (BMI) (**Table 4**).

Efficacy outcomes - muscle strength: Significant improvements occurred in the NS2128E03 group from baseline to Day 60, grip strength: +4.52 kg vs. +1.3 kg ($t = 6.16$, $df = 46$, $P < 0.01$) bench press: +9.29 kg vs. +4.4 kg ($t = 2.84$, $df = 46$, $P < 0.01$); leg press: +25.42 kg vs. +11.88 kg ($t = 6.55$, $df = 46$, $P < 0.05$); cable pull down: +11.97 kg vs. +4.83 kg ($t = 4.06$, $df = 46$, $P < 0.01$) (**Table 5**). Physical endurance tests including 'treadmill time to exhaustion' increased by +5.27 min in treatment vs. +2.21 min in placebo ($P < 0.01$) (**Table 6**). Regarding hormonal biomarkers, total testosterone increased up to 139.19 ng/dL ($P < 0.01$), whereas the free testosterone increased up to 9.09 ng/dL ($P < 0.01$) from baseline. mTOR activation could be seen by +12.08 ng/mL in the NS2128E03 group vs. +3.65 ng/mL in the placebo group ($P < 0.01$), while the LDH and CK significantly decreased, supporting improved muscle recovery (**Figure 3**).

Safety outcomes

No adverse or serious adverse events were reported; the vital signs and lab values remained within normal reference ranges with no clinically significant changes.

Participant feedback

All participants in the NS2128E03 group reported subjective improvements in muscle strength and endurance. No discomfort or negative effects were noted in either group throughout the study.

Discussion

This randomized, double-blind, placebo-controlled clinical trial demonstrated that 60 days

Table 5. Resistance exercise values

Evaluation	Grip Strength (in Kgs)			Bench Press (in Kgs)			Leg Press (in Kgs)			Cable pull-down (in Kgs)		
Frequency/Group	Placebo	NS2128E03		Placebo	NS2128E03		Placebo	NS2128E03		Placebo	NS2128E03	
	Mean ± SD	Mean ± SD	t-value (df = 46)	Mean ± SD	Mean ± SD	t-value (df = 46)	Mean ± SD	Mean ± SD	t-value (df = 46)	Mean ± SD	Mean ± SD	t-value (df = 46)
Baseline	44.31 ± 3.35	45.81 ± 5.25	1.18	41.67 ± 5.45	41.67 ± 5.84	0	174.79 ± 14.1	174.58 ± 44.23	0.02	36.12 ± 7.68	35.90 ± 8.32	0.09
Day 15	44.77 ± 3.28	47.03 ± 4.03	2.13	42.71 ± 4.75	44.42 ± 6.42	1.05	177.50 ± 14.45	185.00 ± 36.54	0.94	37.82 ± 7.15	40.33 ± 8.66	1.11
Day 30	45.05 ± 1.94	50.10 ± 5.87	4	43.96 ± 4.53	47.63 ± 6.74	2.21	180.63 ± 14.99	190.42 ± 37.62	1.15	38.27 ± 5.80	43.06 ± 9.39	2.08
Day 60	45.61 ± 2.37	50.33 ± 6.29	3.44	46.21 ± 4.29	50.96 ± 7.9	2.59	186.67 ± 15.08	200.00 ± 39.34	1.55	40.95 ± 7.02	47.88 ± 9.97	2.85
Change from Baseline	1.3 ± 1.77	4.52 ± 1.85*	6.16	4.4 ± 3.05	9.29 ± 7.87*	2.84	11.88 ± 9.42	25.42 ± 3.27#	6.55	4.83 ± 5.66	11.97 ± 6.45*	4.06

All values are Mean ± SD. Repeated measures ANOVA followed by Bonferroni pairwise comparisons comparing mean change between the active and placebo groups were used to identify statistically significant changes between groups. (*P < 0.01. #P < 0.05. placebo arm n = 24 and treatment arm n = 24). The larger the t-value, the stronger the evidence that the groups truly differ.

Table 6. MUAC and treadmill time

Evaluation	MUAC (cm) Left Arm			MUAC (cm) Right Arm			Treadmill Time (min) to Exhaustion		
Frequency/Group	Placebo	NS2128E03	t-value (df = 46)	Placebo	NS2128E03	t-value (df = 46)	Placebo	NS2128E03	t-value (df = 46)
Baseline	34.10 ± 1.07	34.35 ± 3.31	0.36	34.36 ± 1.11	34.60 ± 3.44	0.33	12.70 ± 2.14	11.50 ± 1.91	1.99
Day 15	34.32 ± 1.14	34.86 ± 3.44	0.73	34.56 ± 1.08	35.12 ± 3.39	0.79	12.99 ± 2.00	14.01 ± 1.50	2.03
Day 30	34.57 ± 1.14	35.44 ± 3.42	1.17	34.80 ± 1.15	35.44 ± 3.42	0.89	13.42 ± 1.89	14.73 ± 1.03	2.9
Day 60	35.08 ± 1.18	36.33 ± 3.50	1.64	35.22 ± 1.06	36.55 ± 3.47	1.78	14.91 ± 1.36	16.77 ± 1.47	4.56
Change from Baseline	0.98 ± 1.17	1.98 ± 0.46*	3.89	0.86 ± 1.14	1.95 ± 0.24*	4.98	2.21 ± 1.23	5.27 ± 1.61*	7.27

MUAC: Mid-Upper Arm Circumference. All values are Mean ± SD. Repeated measures ANOVA followed by Bonferroni pairwise comparisons comparing mean change between the active and placebo groups were used to identify significant changes between groups. (*P < 0.01. placebo arm n = 24 and treatment arm n = 24). The larger the t-value, the stronger the evidence that the groups truly differ.

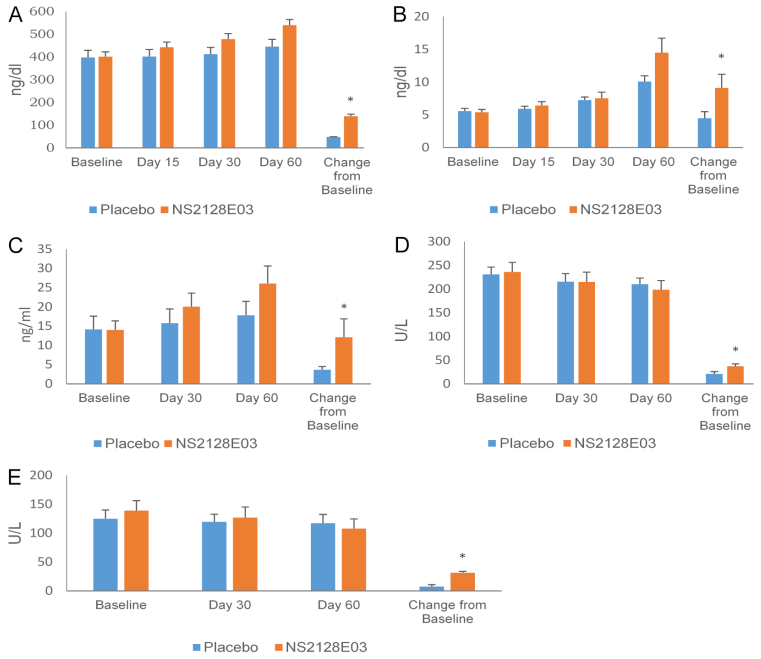

Figure 3. Change in the biomarkers across the visits between the two arms. A. Total testosterone; B. Free testosterone; C. mechanistic Target of Rapamycin: mTOR; D. Lactate Dehydrogenase: LDH; E. Creatine Kinase: CK. All values are Mean ± SD. Repeated measures ANOVA followed by Bonferroni pairwise comparisons comparing mean change between the active and placebo groups were used to identify significant changes between groups. (*P < 0.01. placebo arm n = 24 and treatment arm n = 24).

of supplementation with NS2128E03 significantly improved muscle strength, endurance, testosterone levels, and key biomarkers in healthy male adults compared to placebo. These findings suggest that NS2128E03 may be an effective and safe nutritional supplement for enhancing physical performance and hormonal balance in resistance-trained individuals. The improvements in muscle strength, as measured by bench press (+9.29 kg), leg press (+25.42 kg), and handgrip strength (+4.52 kg), were clinically meaningful and statistically significant. These changes are consistent with previous studies reporting enhanced neuromuscular coordination, increased protein synthesis, and possible androgenic stimulation of muscle fibers [12, 13]. Similarly, Zhang et al., reported an increase in treadmill time to exhaustion (+5.27 minutes) which also supports improved cardiovascular endurance and fatigue resistance, possibly due to better energy use and mitochondrial function [14].

The pronounced increase in both total (+139.19 ng/dl) and free (+9.09 ng/dl) testosterone observed in the treatment group is consistent with prior clinical and nutraceutical intervention studies reporting androgen modulation as a key driver of improved physical performance and body composition. Testosterone is well-established as a central regulator of skeletal muscle hypertrophy through its effects on satellite cell activation, muscle protein synthesis, erythropoiesis, neuromuscular function, mood, and libido, all of which collectively contribute to enhanced exercise capacity and recovery [15, 16]. The magnitude of testosterone elevation observed in the present study exceeded that reported in several lifestyle or botanical-based interventions, suggesting a robust anabolic response associated with NS2128E03 supplementation.

Importantly, the concomitant upregulation of mTOR further supports the presence of an anabolic intracellular environment. Activation of the mTOR signaling pathway is a hallmark of muscle protein synthesis and cellular growth and is known to be stimulated by resistance exercise, amino acid availability, and anabolic hormones such as testosterone [17]. Previous studies have demonstrated that testosterone can directly activate mTOR signaling either through androgen receptor-mediated pathways or by downstream modulation of Insulin-like Growth Factor-1 (IGF-1) signaling, thereby amplifying translational efficiency and myofibrillar protein accretion. The sustained elevation of mTOR observed at Day 60 in this study suggests that NS2128E03 may enhance anabolic signaling not only acutely but also over prolonged supplementation periods.

In parallel with anabolic signaling, the observed reductions in LDH and CK provided biochemical evidence of improved muscle integrity and recovery. Elevated LDH and CK are widely recognized markers of exercise-induced muscle damage, membrane disruption, and metabolic stress. Consistent with previous reports, the

reduction of these biomarkers indicated attenuation of muscle microtrauma and enhanced post-exercise recovery [18]. Similar effects have been documented with nutraceuticals possessing antioxidant, anti-inflammatory, or mitochondrial-supportive properties, which can reduce oxidative stress, stabilize muscle cell membranes, and limit secondary inflammatory damage following intense physical activity [19, 20].

Our findings are consistent with multiple studies on natural testosterone boosters and muscle-enhancing nutraceuticals. Ashwagandha (*Withania somnifera*) has shown improvements in muscle strength and testosterone levels in resistance-trained males, with one study reporting increased 1-RM strength and serum testosterone after 8 weeks of use [21, 22]. Fenugreek extract, another herbal ingredient, demonstrated significant gains in leg and bench press strength along with increased free testosterone in young male subjects after 8 weeks of supplementation [23, 24]. A meta-analysis of testosterone-boosting supplements concluded that botanical ingredients may provide moderate improvements in serum testosterone and strength metrics in short-term trials, particularly in physically active males [25]. Studies on D-aspartic acid have also shown an increase in testosterone and enhanced performance in male athletes, though long-term benefits remain debated [26].

In addition to the clinical findings, preclinical studies provide supportive evidence regarding the safety and systemic benefits of the investigational formulation. A 28-day repeated-dose oral toxicity study in Sprague Dawley rats demonstrated that the herbal blend was well tolerated at doses up to 1000 mg/kg/day, with no mortality, clinical signs, or histopathologic abnormalities observed. This set up a NOAEL at the greatest amount tested, which showed a large safety allowance. A different trial done with male rats indicated that this specific mixture caused notable jumps in blood serum testosterone and unbound testosterone amounts, plus better sexual conduct measures, while showing no bad outcomes. These findings lead one to think that this preparation brings about helpful effects throughout the body and controls the endocrine system, all the while keeping a good safety record.

NS2128E03 showed similar or larger gains in testosterone and muscle performance during a fairly brief time frame (60 days), backing up its promise as a strong supplement for anabolic support. Taken as a whole, these tests on animals confirm the human trial findings by showing it is safe across different types and suggesting more body-wide anti-oxidant and hormonal pathways that could also help maintain muscle condition. These findings offer good prospects for people looking for non-drug methods to improve output and muscle repair. Employing NS2128E03 might prove especially helpful for older men experiencing slight testosterone decreases, sports participants aiming for genuine physical improvement, or people whose training involves lifting weights who prefer not to use external hormone treatments. Data from our research done previously also suggest that NS2128E03 demonstrated considerable blocking action against both PDE-5 and Aromatase, registering IC50 values less than or equal to 50 μg/ml.

Furthermore, the safety profile and standard safety lab numbers back up the good risk benefit ratio for this specific preparation. Although the research excluded no- or low testosterone men, the steady gains seen in fit healthy males suggest possible uses in larger groups awaiting more tests. Upcoming research needs to look into comparative testing against common testosterone supplements or hormone treatment, studies on the underlying processes (like gene activity, hormone system checking), and trials with patients having low testosterone, muscle wasting, or tiredness.

Conclusions

The integrated pre-clinical and clinical evidence establishes NS2128E03 as a safe and effective nutraceutical formulation with multi-faceted benefits. In animal studies, NS2128E03 greatly boosted testosterone amounts and sexual activity measures without any issues found during tissue examination, proving it works well and is safe. These discoveries moved over to human testing, where a controlled study with healthy grown men revealed clear gains in muscle power, stamina, healing indicators, and testosterone amounts, with zero bad reactions or worries about safety. Although these outcomes look very promising, additional research is needed to broaden the practical medical use of NS2128E03.

Extensive, multi-site research with extended treatment periods are required to verify the lasting nature and broad application of these outcomes. Trials comparing our compound with known nutraceutical and drug treatments, plus basic research clarifying processes such as androgen receptor function, cellular energy production, and mTOR communication, will offer better understanding concerning how it works. Furthermore, specific assessments involving athletes, older males, and those with low-testosterone might assist in improving its role. Overall, the current results establish NS2128E03 as a method for boosting male vigor, bodily function, and hormone stability, possessing the capacity to improve athletic fueling and men's wellness.

Acknowledgements

We thank the participants, clinical staff at Medstar Specialty Hospital, and Radiant Research Services Pvt. Ltd. for their contributions. The study was sponsored by Natural & Essential Oils Pvt. Ltd., Mysuru, India.

Disclosure of conflict of interest

None.

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