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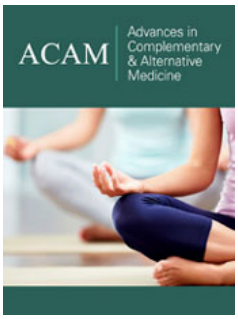
Research Article

Preclinical Assessment of NS1902F01, a Nitrate-Standardized Beetroot Extract, on Exercise Capacity and Muscular Endurance in Mice

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Abstract

Dietary nitrate has emerged as a promising nutritional strategy for improving exercise capacity through enhanced nitric oxide bioavailability, muscle efficiency, and contractile function. This study evaluated the effects of NS1902F01, a hydroalcoholic *Beta vulgaris* root extract standardized to 10% nitrate, on exercise performance, muscle related outcomes, biochemical parameters, and acute oral safety. Male Swiss albino mice were allocated to five groups comprising a negative control, exercise-control, arginine reference group, and NS1902F01 low and high dose treatment groups. Exercise-trained groups underwent treadmill training for 28 days, following which endurance performance, forelimb grip endurance, muscle weights, serum biochemical parameters, lipid profile, and nitric oxide levels were assessed. NS1902F01 supplementation significantly improved treadmill exercise capacity and forelimb grip endurance compared with the exercise-control group.

Treatment also reduced time spent in the shock zone and the number of shocks received during the endurance test, consistent with improved exercise tolerance under the experimental conditions. NS1902F01 increased gastrocnemius and soleus muscle weights and significantly elevated serum nitric oxide levels. No treatment-related adverse changes were observed in body weight, blood glucose, measured biochemical parameters, or liver and heart weights. In a separate acute oral toxicity study, administration of NS1902F01 at a high dose produced no mortality, clinical signs of toxicity, or treatment-related gross pathological abnormalities. Overall, NS1902F01 improved exercise performance and muscle-related functional outcomes while supporting preliminary evidence of tolerability under the tested conditions, supporting its further investigation as a nitrate-standardized botanical preparation for exercise-performance applications.

Keywords: *Beta vulgaris*; Beetroot extract; Dietary nitrate; Nitric oxide; Exercise performance; Endurance; Acute oral toxicity

Abbreviations: ALP: Alkaline Phosphatase; ANOVA: Analysis of Variance; BW: Body Weight; CCSEA: Committee for Control and Supervision of Experiments on Animals; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals (now CCSEA); CM: Centimeter; HDL: High-Density Lipoprotein; IAEC: Institutional Animal Ethics Committee; LDL: Low-Density Lipoprotein; Min: Minute; OECD: Organisation for Economic Co-operation and Development; S: Second; SGOT: Serum Glutamic-Oxaloacetic Transaminase; SGPT: Serum Glutamic-Pyruvic Transaminase; TC: Total Cholesterol

Introduction

Exercise-induced fatigue results from interactions among metabolic, vascular, and neuromuscular factors that limit the ability of muscle to sustain force production. Adequate oxygen and substrate delivery, efficient energy generation, and neuromuscular function are therefore essential for exercise performance. Nutritional interventions targeting these processes have attracted increasing interest as complementary approaches to exercise training and adaptation [1,2]. Inorganic nitrate has received particular attention because it can contribute to nitric

oxide formation independently of nitric oxide synthase. Following ingestion, nitrate is reduced to nitrite through the entero-salivary pathway, with oral bacteria contributing to this conversion. Nitrite can subsequently be reduced to nitric oxide, particularly under hypoxic, acidic, and metabolically demanding conditions. Through this pathway, dietary nitrate may influence vascular regulation and muscle function during exercise [2,3].

Nitrate supplementation may improve muscle oxygen utilization and exercise economy. Studies have shown that nitrate can reduce oxygen requirements at a given submaximal workload and improve exercise efficiency, potentially through effects on mitochondrial function and oxidative phosphorylation [4,5]. These effects may be particularly relevant during prolonged or progressively intensified exercise, when inefficient energy utilization contributes to fatigue. Nitrate-derived nitric oxide may also influence muscle contractile function through effects on calcium handling, excitation-contraction coupling, myofilament sensitivity, and force generation. Accordingly, nitrate supplementation has been investigated for its effects on muscle force, power, and fatigue resistance. However, responses vary according to exercise modality and supplementation protocol [6,7].

Beetroot (*Beta vulgaris*) is an important natural source of inorganic nitrate and has been extensively investigated in endurance, high-intensity, intermittent, and resistance exercise. Evidence suggests that nitrate supplementation may improve selected measures of exercise capacity, muscular endurance, and power, although responses vary with dose, supplementation duration, exercise intensity, training status, and individual characteristics [1,8-10]. These findings support further evaluation of standardized nitrate-rich botanical preparations. Animal models enable controlled assessment of exercise performance alongside muscular and biochemical responses. Progressive treadmill exercise provides a model for evaluating endurance and fatigue-related performance, while forelimb grip testing provides a complementary measure of muscle function. Muscle weights, serum nitric oxide levels, biochemical markers, and organ weights can further characterize physiological responses and preliminary tolerability.

NS1902F01 is a proprietary hydroalcoholic extract of *Beta vulgaris* roots standardized to 10% nitrate by HPLC. The roots are sometimes also referred to as tubers. The present study evaluated the effects of 28-day NS1902F01 supplementation at 118.36 and 205.50mg/kg Body Weight (bw) in male Swiss albino mice subjected to regular treadmill exercise. Exercise performance was assessed using treadmill distance, shock-zone time, and number of shocks, together with forelimb grip endurance and muscle weights. Serum nitric oxide levels, lipid profile, liver and kidney biochemical markers, blood glucose, body weight, and food intake were also evaluated. An acute oral toxicity study at 5000 mg/kg bw was conducted to provide preliminary safety information. The study investigated whether repeated administration of nitrate-standardized *Beta vulgaris* extract could improve exercise capacity and muscular performance while producing favourable or non-adverse changes in selected biochemical and physiological parameters. The findings may provide preclinical evidence for the potential use of NS1902F01 as a standardized dietary nitrate preparation for exercise-performance support.

Materials and Methods

Plant material

NS1902F01, a proprietary natural herbal preparation used in this study, was prepared from *Beta vulgaris* roots. It is a hydroalcoholic extract, developed and named by Natural and Essential Oils Private Limited, Mysuru, India. NS1902F01 is phytochemically standardized to 10% nitrate by HPLC method [11].

Study 1: Endurance-Efficacy study

Animals: Male Swiss albino mice (7-8 weeks old; 24-26g bw), bred in-house, were used for the study. The animals were housed in standard polycarbonate cages under controlled environmental conditions, with a temperature of 22±3 °C, relative humidity of 30-70%, and a 12h light/12h dark cycle. The mice were provided with a standard laboratory diet (PURINA 5L79, PMI Nutritional, USA) and water ad libitum throughout the study. All experimental procedures were conducted following approval from the Institutional Animal Ethics Committee (IAEC) of Radiant Research Services Pvt. Ltd., Bengaluru, India (Approval No. RR/IAEC/75-2020). The study was conducted in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA; Registration No. 1803/PO/RcBi/S/2015/CPCSEA).

Study design and experimental procedure: Animals were divided into five groups, with nine animals in each group. Group 1 served as the negative control, whereas Group 2 served as the exercise-control (no treatment). Group 3 was treated with the reference standard, Arginine (411mg/kg bw; Avantor, India). Groups 4 and 5 received low (118.36mg/kg bw) and high (205.50mg/kg bw) doses of NS1902F01, respectively, for 28 days (Table 1). Animals in Groups 2-5 were subjected to treadmill training 5 days per week for 28 days. Forelimb grip endurance was evaluated on Days 0 and 28. Body weight was recorded weekly, and feed intake was measured daily. Blood glucose levels were measured once weekly during the treatment period. On Day 29, blood samples were collected via the retro-orbital route for biochemical analysis, following which the animals were euthanized under anesthesia.

Table 1: Study design.

Groups	Description	Dose	No. of Animals (n)
G1	Negative control	0.5%CMC	9
G2	Exercise-control	0.5%CMC	9
G3	Reference Standard-Arginine	411mg/kg bw	9
G4	NS1901F01-low dose	118.36mg/kg bw	9
G5	NS1901F01-high dose	205.50mg/kg bw	9

The weighable organs, including the liver, heart, gastrocnemius, and soleus muscles, were isolated, collected, and weighed. Serum was separated from the collected blood samples and used for the estimation of various biochemical parameters. Serum samples were analyzed for biochemical parameters, including liver

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and renal function markers such as SGOT (Cat. No. 31090172), SGPT (Cat. No. 31090216), ALP (Cat. No. 31070321), total protein (Cat. No. 30020037), urea (Cat. No. 31080381), and creatinine (Cat. No. 30020458), as well as lipid profile parameters, including triglycerides (Cat. No. 31100111), total cholesterol (Cat. No. 31070231), HDL (Cat. No. 32010207), and LDL (Cat. No. 31070139). All assay kits were procured from Agappe Diagnostics, India, and the analyses were performed according to the manufacturer's instructions.

Fore limb grip endurance: Forelimb grip endurance was measured on days 0 and 28 using a stainless-steel grid to assess muscle endurance. Grip endurance was assessed 1h after treatment. Each mouse was placed on the grid, and the grid was inverted to allow the animal to grip the steel bars. The time for which each animal maintained its grip on the inverted grid was recorded. The maximum duration for each trial was 6 min. Three trials were performed for each animal at 6 min intervals, and the mean duration of the three independent trials was calculated for each animal [12].

Exercise training: During the treatment period, the animals underwent treadmill exercise at a moderate intensity. The treadmill was operated at a maximum running speed of 20cm/s, with an electrical stimulus of 0.2mA and an inclination of 10°, for a total duration of 10min. The treadmill speed was manually increased by 5cm/s every 2min throughout the 10min exercise session. The animals were habituated to the treadmill procedure daily, 1h after dosing, approximately between 11:30AM and 2:30PM, for 5 days per week throughout the treatment period. The negative control group was not subjected to treadmill exercise [13].

Muscle endurance treadmill test: On the 28th day of treatment, all animals were subjected to a muscle endurance test using a motorized treadmill. The test was performed at a low-to-moderate intensity, with an initial speed of 5cm/s and a maximum running speed of 50cm/s. The treadmill was maintained at an inclination of 10°, and an electrical stimulus of 0.2mA was applied as an aversive stimulus. The treadmill speed was increased by 5cm/s every 2min until a maximum speed of 50cm/s was reached. Animals were subjected to the treadmill test until exhaustion. The distance traveled (cm), time spent in the shock zone (s), number of shocks received, and total running time (min) were recorded for each animal in the respective treatment groups [14].

Study 2: Acute oral toxicity study

Animals: Female Sprague Dawley rats (9-10 weeks old; 167-199g) were procured from Hylasco Biotechnology (Telangana, India). The animals were housed in standard polycarbonate cages under controlled environmental conditions, with a temperature of 20 °C -23 °C, relative humidity of 48-68%, and a 12h light/12h dark cycle. The rats were provided with a standard laboratory diet and water ad libitum throughout the study. All experimental procedures were conducted following approval from the Institutional Animal Ethics Committee (IAEC) of Vipragen Biosciences Pvt. Ltd., Mysuru, India (Approval No. VIP/IAEC/163/2019). The study was conducted in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA; Registration No. 1683/PO/RcBiBt/S/13/CCSEA).

Study design and experimental procedure: The animals received a single dose of NS1902F01 by oral gavage following an overnight fasting period (approximately 17 and 16h for the initial and confirmatory tests, respectively), with free access to water. Food was provided approximately 3h after dosing. As testing at a dose level of 5000mg/kg bw requires only one step involving three animals (Annex 2, OECD 423) [15], the first animal was initially administered NS1902F01 at 5000mg/kg bw. As no mortality or signs of acute toxicity were observed, two additional naïve animals were subsequently administered the same dose as part of the confirmatory test. The dose volume was 10 mL/kg bw (Table 2).

Table 2: Study design.

Groups	Number of Animals	Animal Identification Number
Initial test (5000mg/kg bw)	1	01
Confirmatory test (5000mg/kg bw)	2	02-03

Morbidity/mortality, clinical signs and necropsy: All animals were observed twice daily for morbidity and mortality throughout the study period. Individual animals were monitored for clinical signs of toxicity at 30 min and 1, 2, 3, and 4h post-dose on Day 0, and subsequently once daily throughout the 14day observation period. At the end of the observation period, all rats were subjected to necropsy on Day 14. The animals were euthanized by CO_2 asphyxiation in a designated euthanasia chamber. A detailed gross pathological examination of the external and internal organs was performed, and all observations were recorded.

Statistical analysis: The values were expressed as mean±SEM. Statistical differences between the NS1902F01 treated groups, reference standard and the exercise-control group were evaluated using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test. $P<0.05$ was considered statistically significant.

Result

Effect of NS1902F01 on treadmill exercise performance

NS1902F01 supplementation significantly increased treadmill distance during the endurance test compared with the exercise-control group (Figure 1). No significant differences were observed among the groups at baseline (Day 0), indicating comparable initial exercise performance. Treadmill distance increased markedly from Day 0 to Day 28 in all exercise trained groups. On Day 28, the exercise-control group travelled 1483.33±37.68 cm, whereas the reference standard group travelled 2183.33±17.57cm, representing a 47.2% increase compared with the exercise-control group. NS1902F01 administration significantly increased the distance travelled to 2097.22±28.18 cm and 2158.33±29.13 cm in the low and high dose groups, respectively ($P<0.0001$). These values corresponded to 41.4% and 45.5% increases, respectively, relative to the exercise-control group. Thus, both doses of NS1902F01 markedly enhanced treadmill exercise capacity.

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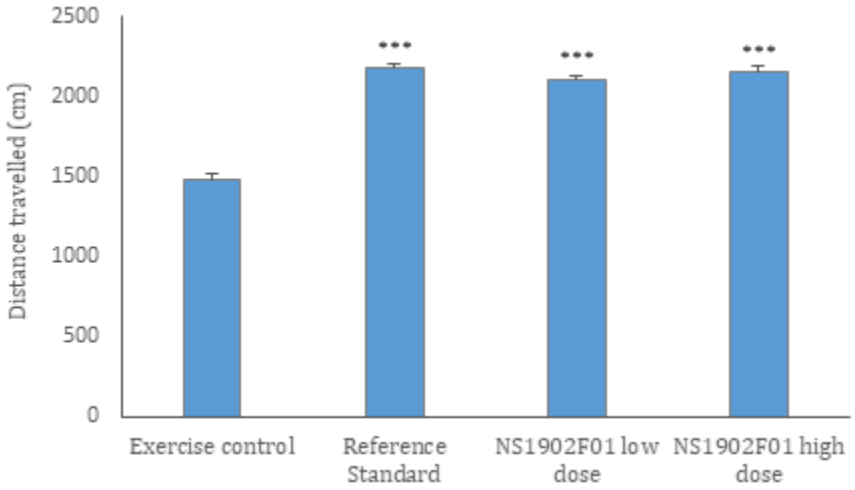


Figure 1: Effect of NS1902F01 on treadmill endurance in mice.
Treadmill endurance was assessed as the distance travelled until exhaustion on Day 28 following the treatment period. Data are presented as mean±SEM (n=9 per group). Statistical significance was determined using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple-comparison test. Statistical comparisons were performed between the exercise-control group and the reference standard, NS1902F01 low and high dose groups. ***P<0.0001.

The effect of NS1902F01 on time spent in the shock zone is presented in Figure 2. On Day 28, the exercise-control group spent 60.67±1.51s in the shock zone. This was reduced to 32.67±0.70 s in the reference standard group, corresponding to a 46.2% reduction compared with the exercise-control group. The NS1902F01 low and high dose groups showed values of 36.11±1.13s and 33.67±1.17s, respectively, representing 40.5% and 44.5% reductions, respectively. All reductions were statistically significant compared with the exercise-control group (P<0.0001). Similarly, NS1902F01 significantly reduced the number of shocks received during treadmill exercise (Figure 3). On Day 28, the exercise-control group received 12.00±0.63 shocks, compared with 4.11±0.29 shocks in the reference standard group, corresponding to a 65.8% reduction. The number of shocks was reduced to 7.11±0.48 and 5.00±0.38 in the NS1902F01 low and high dose groups, representing 40.8% and 58.3% reductions, respectively, compared with the exercise-control group (P<0.0001).

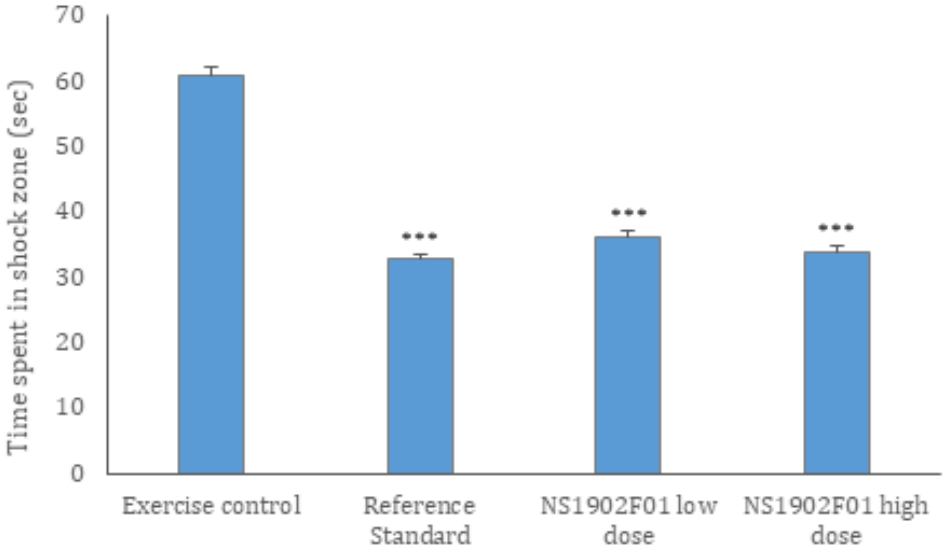


Figure 2: Effect of NS1902F01 on time spent in the shock zone following the 28-day exercise training period.
Data are presented as mean±SEM (n=9 per group). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test. Statistical comparisons were performed between the exercise-control group and the reference standard, NS1902F01 low and high dose groups. ***P<0.0001.

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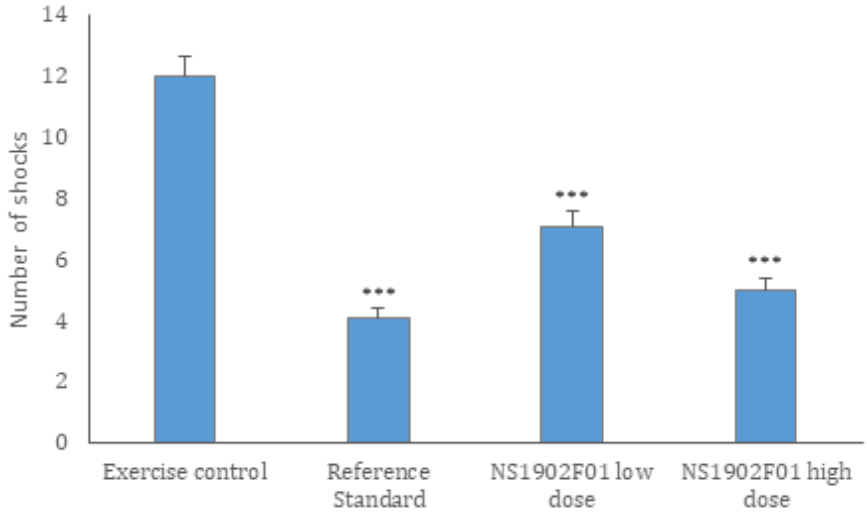


Figure 3: Effect of NS1902F01 on the number of shocks received during treadmill endurance testing in mice. Data are presented as mean±SEM (n=9 per group). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test. Statistical comparisons were performed between the exercise-control group and the reference standard, NS1902F01 low and high dose groups. ***P<0.0001.

As shown in Figure 4, NS1902F01 also improved grip endurance following 28 days of treatment. The exercise-control group exhibited a Day 28 value of 260.11±3.91 s, whereas the reference standard group showed 331.04±5.46s, corresponding to a 27.3% increase relative to the exercise-control group. NS1902F01 treatment increased grip endurance to 305.56±3.72 s in the low dose group and 319.11±3.14 s in the high dose group, representing 17.5% and 22.7% increases, respectively. These increases were statistically significant compared with the exercise-control group (P<0.0001). Overall, 28 days of NS1902F01 supplementation significantly improved treadmill running distance and grip endurance while reducing time spent in the shock zone and the number of shocks received, demonstrating enhanced exercise performance and fatigue tolerance in treadmill-trained mice consistent with improved exercise tolerance under the experimental conditions.

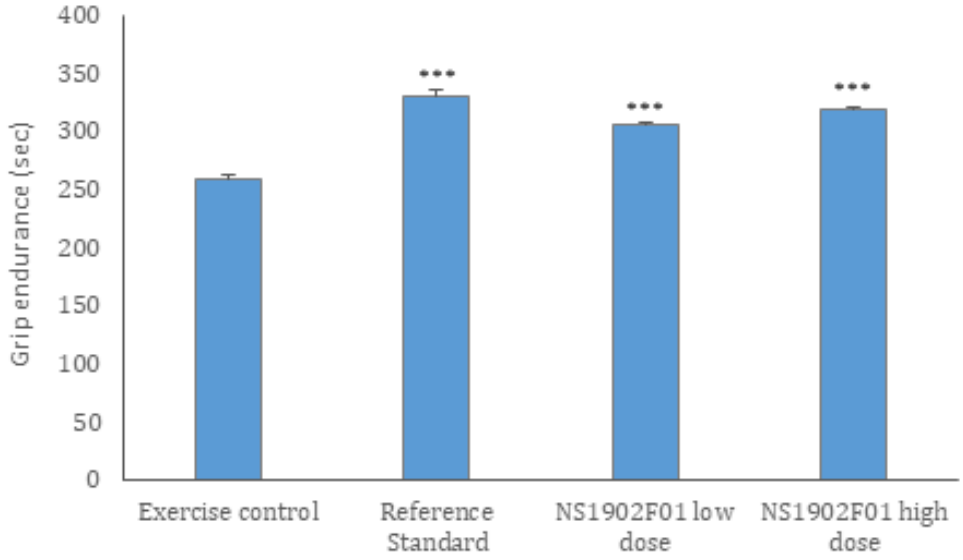


Figure 4: Effect of NS1902F01 on forelimb grip endurance in exercised mice. Data are presented as mean±SEM (n=9 per group). Statistical significance was determined using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple-comparison test. Statistical comparisons were performed between the exercise-control group and the reference standard, NS1902F01 low and high dose groups. ***P<0.0001.

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Effect of NS1902F01 on organ and muscle weights

The effects of NS1902F01 administration on selected organ and muscle weights are presented in Table 3. No significant differences were observed among the treatment groups in liver or heart weights, indicating that 28 days of exercise training and NS1902F01 supplementation did not produce appreciable changes in these organ weights. In contrast, gastrocnemius muscle weight was increased in the reference standard group (175.33±0.94mg) compared with the exercise-control group (164.00±1.65mg; P<0.0001). NS1902F01 administration also increased gastrocnemius weight, with values of 169.11±0.54mg in the low dose group (P<0.05) and 173.11±0.63mg in the high dose group (P<0.0001) compared with the exercise-control group. The effect was more pronounced at the higher dose, with the high dose group approaching the value observed for the reference standard.

Table 3: Effect of NS1902F01 on organ weights in exercised mice.

Treatment	Liver (mg)	Heart (mg)	Gastrocnemius (mg)	Soleus (mg)
Negative control	1862.67±15.38	191.11±0.68	160.11±1.44	6.74±0.10*
Exercise control	1860.89±17.14	190.89±0.92	164.00±1.65	7.21±0.20
Reference standard	1859.89±14.88	189.78±1.23	175.33±0.94***	9.04±0.10***
NS1901F01 low dose	1860.11±8.78	189.78±0.97	169.11±0.54*	8.01±0.05*
NS1901F01 high dose	1858.44±20.01	189.67±1.08	173.1±0.63***	8.18±0.09***

Organ weights of the liver, heart, gastrocnemius, and soleus muscles were recorded at the end of the 28-day treatment and exercise period. Data are presented as mean±SEM (n=9 per group). Statistical significance was determined using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple-comparison test. Statistical comparisons were performed between the exercise-control group vs negative control group and exercise-control group vs the reference standard, NS1902F01 low and high dose groups. *P<0.05; ***P<0.0001.

Similarly, soleus muscle weight was significantly increased following NS1902F01 administration. The exercise-control group had a soleus weight of 7.21±0.20mg, whereas the reference standard, low dose and high dose of NS1902F01 groups showed values of 9.04±0.10mg, 8.01±0.05mg, and 8.18±0.09mg, respectively (P<0.0001 vs. exercise-control). The negative control group showed a lower soleus weight than the than the exercise-control group (6.74±0.10 vs 7.21±0.20mg; P<0.05). Overall, NS1902F01 supplementation, particularly at the high dose, was associated with increased gastrocnemius and soleus muscle weights without significant alterations in liver or heart weights, supporting an effect on muscle-related outcomes following exercise training.

Effect of NS1902F01 on serum lipid profile and nitric oxide levels

The effects of NS1902F01 supplementation on serum lipid parameters and nitric oxide levels are presented in Table 4. Compared with the exercise-control group, the reference standard group showed significant reductions in total cholesterol (TC; 87.78±0.60 vs. 99.00±1.31mg/dL, P<0.0001), triglycerides (TG; 76.22±1.47 vs. 84.44±1.08mg/dL, P<0.001), and LDL cholesterol (LDL-C; 52.11±0.77 vs. 57.89±1.52mg/dL, P<0.0001), together with a significant increase in HDL cholesterol (HDL-C; 33.33±0.87 vs. 29.56±0.93mg/dL, P<0.001). NS1902F01 treatment produced a significant reduction in TC compared with the exercise-control group. TC decreased to 93.67±0.83mg/dL (P<0.05) and 91.56±1.30mg/dL (P<0.0001) in the low and high dose groups, respectively. However, neither dose produced a statistically significant change in TG, HDL-C, or LDL-C compared with the exercise-control group.

Table 4: Effect of NS1901F01 on serum lipid profile and nitric oxide levels in exercised mice.

Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Nitric Oxide (ng/ml)
Negative control	107.11±1.56***	89.11±1.49	27.22±0.80	59.67±1.01	6.01±0.09
Exercise control	99.00±1.31	84.44±1.08	29.56±0.93	57.89±1.52	9.47±0.13
Reference standard	87.78±0.60***	76.22±1.47**	33.33±0.87**	52.11±0.77**	12.99±0.16***
NS1901F01 low dose	93.67±0.83*	82.11±1.05	30.78±0.64	55.89±0.45	12.64±0.13***
NS1901F01 high dose	91.56±1.30**	79.78±2.02	30.89±0.48	54.89±0.39	12.90±0.10***

Serum concentrations of Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), and Nitric Oxide (NO) levels were determined at the end of the 28-day treatment period. Data are presented as mean±SEM (n=9 per group). Statistical significance was determined using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple-comparison test. Statistical comparisons were performed between the exercise control group vs Negative control group and exercise control group vs the reference standard, NS1901F01 low and high dose groups. *P value<0.05; **P value<0.001; ***P<0.0001.

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The negative control group showed a higher TC compared with exercise-control group (107.11±1.56 mg/dl; P<0.0001). Serum nitric oxide levels were markedly increased following NS1902F01 supplementation. The exercise-control group showed nitric oxide levels of 9.47±0.13ng/mL, whereas the low and high dose NS1902F01 groups showed significantly higher levels of 12.64±0.13ng/mL and 12.90±0.10ng/mL, respectively (P<0.0001). The reference standard group exhibited a comparable significant increase in nitric oxide levels (12.99±0.16ng/mL, P<0.0001). Thus, both doses of NS1902F01 substantially increased circulating nitric oxide levels, with the high dose producing a slightly greater numerical increase than the low dose.

General health and biochemical parameters

Throughout the 28-day treatment period, animals in the exercise-control, reference standard, and NS1902F01 treated groups exhibited normal growth and maintained progressive body weight gain. Blood glucose levels remained comparable across the experimental groups, with no statistically significant treatment-related changes. Similarly, the biochemical parameters evaluated at the end of the treatment period showed no significant alterations attributable to NS1902F01 administration. In conjunction with the absence of significant changes in liver and heart weights, these findings indicate that 28-day administration of NS1902F01 at the tested doses did not produce evident adverse effects on the measured general health or biochemical parameters. Overall, NS1902F01 administration was associated with increased gastrocnemius and soleus muscle weights and a marked elevation in serum nitric oxide levels, while producing relatively limited changes in the serum lipid profile.

Acute oral toxicity study

No treatment-related mortality was observed following oral administration of NS1902F01 at 5000mg/kg bw. Throughout the observation period, none of the animals exhibited clinical signs of toxicity. All animals administered NS1902F01 at 5000mg/kg bw remained clinically normal, with no treatment-related systemic or local signs observed at 0.5, 1, 2, 3, or 4h after dosing on Day 0 or during the subsequent observation periods. At necropsy, no treatment-related external or internal gross abnormalities were observed in any of the animals.

Discussion

The present study demonstrates that 28 days of NS1902F01 supplementation improved exercise performance in treadmill-trained mice. Both doses increased treadmill endurance, forelimb grip endurance, and muscle weights, while reducing shock-zone time and number of shocks during the exhaustion test. These effects were accompanied by increased serum nitric oxide levels, without evident adverse effects on body-weight progression, blood glucose, measured hepatic and renal biochemical parameters, or liver and heart weights. Acute oral administration at 5000mg/kg bw produced no mortality, clinical toxicity, or gross pathological abnormalities. The improvement in treadmill performance was substantial. On Day 28, exercise-control mice travelled 1483.33±37.68cm, compared with 2097.22±28.18 and 2158.33±29.13cm in the low and high dose NS1902F01 groups, representing increases of approximately 41.4% and 45.5%, respectively (P<0.0001). The high-dose response was numerically comparable to the arginine reference group (2183.33±17.57cm).

These findings are consistent with evidence that dietary nitrate can improve exercise tolerance, particularly in endurance and time-to-exhaustion paradigms. Earlier human studies demonstrated reduced oxygen cost and prolonged high-intensity exercise tolerance following beetroot-derived nitrate supplementation [2,4,16,17]. More recently, an umbrella review of 20 systematic reviews and meta-analyses reported improvements in time-to-exhaustion, total distance, muscular endurance, peak power, and time to peak power, although effects differed across exercise domains and methodological quality varied among the underlying reviews [1]. A 2025 umbrella review focused on beetroot juice similarly reported small improvements in selected endurance and muscular outcomes, with responses influenced by population, dose, timing, and exercise modality [18]. A plausible explanation for the improved exercise capacity is enhanced nitrate–nitrite–nitric oxide signalling. NS1902F01 was standardized to 10% nitrate, and treatment increased serum nitric oxide levels from 9.47±0.13ng/mL in exercise controls group to 12.64±0.13 and 12.90±0.10ng/mL in the low and high dose groups, respectively.

Dietary nitrate provides an alternative pathway for nitric oxide generation through reduction to nitrite and subsequently nitric oxide, particularly under conditions of reduced oxygen availability and altered tissue redox status [2,19]. Increased nitric oxide bioavailability may influence vascular regulation, oxygen delivery, mitochondrial efficiency, and muscle contractility, thereby contributing to improved exercise tolerance [2,4,19]. However, the increased serum nitric oxide levels should be considered an association rather than direct evidence of mechanism. Downstream pathways, including soluble guanylate cyclase/cGMP signalling, calcium handling, mitochondrial respiration, and muscle fiber-specific adaptations, were also not evaluated. Therefore, the findings support an association between nitric oxide related changes and improved performance but do not establish causality. Future studies should incorporate nitrate/nitrite pharmacokinetics, nitric oxide metabolites, mitochondrial respiration, muscle oxygenation, and excitation–contraction coupling.

Improved muscle function may represent another contributor to the observed phenotype. NS1902F01 increased forelimb grip endurance from 260.11±3.91s in exercise-control group to 305.56±3.72 and 319.11±3.14s in the low and high dose groups, respectively. Dietary nitrate has been reported to improve muscle contractile properties, including electrically evoked force production, suggesting effects on excitation–contraction coupling [20,21]. A systematic review and meta-analysis of controlled human studies reported beneficial effects of dietary nitrate on selected measures of exercise performance, although responses varied across exercise protocols [22]. Thus, the present improvement in grip endur-

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ance provides complementary preclinical evidence for enhanced muscular fatigue resistance. NS1902F01 also increased gastrocnemius weight from 164.00±1.65mg in exercise-control group to 169.11±0.54 and 173.11±0.63mg at the low and high doses, respectively. Soleus weight increased from 7.21±0.20mg to 8.01±0.05 and 8.18±0.09mg, respectively. The dose-related increase in gastrocnemius weight is notable, with the high dose value approaching that of the arginine reference group.

Nevertheless, increased muscle weight alone cannot establish hypertrophy because muscle fiber cross-sectional area, protein synthesis, satellite-cell activity, myosin isoforms, and anabolic signaling were not assessed. Therefore, these findings are more appropriately described as increased muscle weight or muscle adaptation rather than definitive hypertrophy. Enhanced exercise efficiency may also contribute to the observed performance response. Dietary nitrate has been shown to reduce the oxygen cost of submaximal exercise and improve exercise economy, while mechanistic studies suggest potential effects on mitochondrial efficiency and muscle energetics [2,4,17,23]. Nitric oxide related modulation of calcium handling and contractile efficiency may further reduce the energetic cost of force production [19,20]. Although oxygen consumption and mitochondrial function were not directly measured, these mechanisms provide biologically plausible explanations for the improved endurance and fatigue resistance observed with NS1902F01.

The reductions in shock-zone time and number of shocks provide additional evidence of improved treadmill performance. NS1902F01 reduced shock-zone time by approximately 40.5% and 44.5% at the low and high doses, respectively, and reduced number of shocks by approximately 40.8% and 58.3%. These changes were consistent with the increased treadmill distance and grip endurance. Nevertheless, electrical stimulation is an aversive component of forced treadmill testing, and behavioral motivation may influence these measures. Accordingly, shock-related endpoints should be interpreted as supportive rather than independent physiological measures of fatigue. NS1902F01 produced a selective lipid response, significantly reducing total cholesterol from 99.00±1.31mg/dL in exercise-control group to 93.67±0.83 and 91.56±1.30mg/dL at the low and high doses, respectively, without significant changes in triglycerides, HDL-C, or LDL-C. Because exercise performance was the primary objective, these findings should be considered secondary. The absence of consistent changes across the lipid profile does not establish a broad lipid-modifying effect and warrants confirmation in studies specifically designed to investigate lipid metabolism.

The safety findings provide preliminary evidence of tolerability. No treatment-related adverse changes were observed in the 28-day efficacy study with respect to body-weight progression, blood glucose, measured biochemical parameters, or liver and heart weights. In the separate acute toxicity study, no mortality, clinical signs, or gross pathological abnormalities were observed following administration of 5000mg/kg bw. The study followed OECD Test Guideline 423, an acute toxic-class method using a stepwise procedure with three animals per step [15]. These findings support acute oral tolerability but do not establish long-term safety or human safety.

The study has several strengths, including the use of an unexercised negative control, exercise-control, reference standard, and two NS1902F01 doses. Multiple functional endpoints were evaluated, including treadmill distance, shock-zone time, number of shocks and grip endurance, together with muscle weights, biochemical parameters, lipid profile, and nitric oxide levels. The consistent performance response across several endpoints strengthens the conclusion that NS1902F01 improved exercise capacity under the experimental conditions.

Several limitations should be considered. The study included only male mice, limiting assessment of sex-specific responses, and the forced treadmill model does not directly reproduce voluntary human endurance exercise. Mitochondrial efficiency and muscle oxygenation were not assessed. Increased muscle weight cannot establish hypertrophy without histological or molecular confirmation. The anti-aging gene Sirtuin 1 is critical to nitric oxide homeostasis, muscle function and exercise performance Beetroot contains various Sirtuin 1 activators and nitrate is a Sirtuin 1 activator [24-27]. Future experiments may provide Sirtuin 1 effect with relevance to muscle function. In addition, the acute toxicity study used female Sprague Dawley rats rather than the mouse strain used for efficacy testing, and acute tolerability cannot substitute for repeated-dose toxicology or human safety studies.

Conclusion

NS1902F01, a nitrate-standardized *Beta vulgaris* root extract, significantly improved exercise performance in treadmill-trained mice following 28 days of supplementation. Both doses increased treadmill endurance and forelimb grip endurance, reduced shock-zone time during the exhaustion test, and increased gastrocnemius and soleus muscle weights. These functional improvements were accompanied by increased serum nitric oxide levels, supporting a potential role of nitrate-nitrite-nitric oxide-related pathways in the observed response. However, the present study did not directly evaluate nitrate/nitrite metabolism, mitochondrial function, muscle oxygenation, or downstream molecular signalling; therefore, the underlying mechanism remains to be established.

NS1902F01 was well tolerated under the experimental conditions, with no evident treatment-related effects on body weight, blood glucose, measured hepatic and renal biochemical parameters, or liver and heart weights. A single oral dose of 5000 mg/kg bw produced no treatment-related mortality, clinical signs of toxicity, or gross pathological abnormalities during the 14-day observation period. Overall, these findings provide preclinical evidence that NS1902F01 may support exercise endurance and fatigue resistance and warrant further investigation. Future studies should confirm these effects using mechanistic endpoints, repeated-dose safety assessments, and ultimately well-controlled human clinical trials.

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Conflict of Interest

The authors declare that they have no conflicts of interest related to this work.

Author Contributions

Conceptualization and preclinical studies: Sameer Kamalakar Akolkar.

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Competing Interests

Sameer Kamalakar Akolkar, Mohan Gowda C M, Madhukumar M S, Anirudh Ranga, are employed with Natural and Essential Oils Private Limited, Mysuru, Karnataka, India. This does not alter our adherence to Advances in Complementary and Alternative Medicine policies on sharing data and materials.

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