STUDY PROTOCOL

A proof-of-concept clinical study to evaluate the impact of a combination nutraceutical on muscle function, cognitive performance, and immune resilience in older adults.

Study Code: GP/CT/25-26/001
Protocol Version No.: 1.00
Date: 19/11/2025
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SPONSOR DETAILS
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CONFIDENTIALITY STATEMENT

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1. STUDY APPROVAL AND MANAGEMENT TEAM

1.1. SPONSOR'S APPROVAL

I, the undersigned, have read and understood this protocol, and hereby agree to abide by the pertinent responsibilities of the sponsor for the conduct of the study in accordance with the WMA Declaration of Helsinki, current Protocol, ICH-GCP, ICH guidelines for Good Clinical Practice and local regulatory guidelines.

No changes in the protocol will be implemented without formal authorization by the ethics committee (IRB) and regulatory authority unless there are logistic or administrative changes.

I further assure that the investigational products to be used in this study are manufactured as per the current Good Manufacturing Practices.

_____ _____

Sponsor's Representative Date

2. TABLE OF CONTENT

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4. LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Full Form
ICH	International Council For Harmonisation Of Technical Requirements For Pharmaceuticals For Human Use
GCP	Good Clinical Practice
WMA	World Medical Association
IRB	Institutional Review Board
AE	Adverse Event
SAE	Serious Adverse Event
SPSS	Statistical Package For The Social Sciences
PP	Per Protocol
IP	Investigational Product
CRF	Case Report Form
ICF	Informed Consent Form
ADL	Activities Of Daily Living
MedDRA	Medical Dictionary For Regulatory Activities
LAR	Legally Acceptable Representative
PIS	Participant Information Sheet
QA	Quality Assurance
IPIB	Investigational Product Information Brochure
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
SPIRIT	Standard Protocol Items: Recommendations For Interventional Trials
IPR	Intellectual Property Rights

5. STUDY SYNOPSIS

Table 2: Study Synopsis

Study Title	A proof-of-concept clinical study to evaluate the impact of a combination nutraceutical kit on muscle function, cognitive performance, and immune resilience in older adults.			
Study Design	A single-arm, open-label, exploratory pilot clinical study.			
Clinical Phase	Proof of concept			
Treatment Arm	Single arm (Interventional nutraceutical formulation)			
Dosage	30 g sachet content to be mixed with water and consumed twice daily before meals.			
Objectives	The objectives of the study will be to: 1. To evaluate the impact of the combination nutraceutical kit on **muscle function**, including strength, endurance, and muscle mass. 2. To assess effects on **cognitive performance**, including executive function, memory, and processing speed. 3. To evaluate **immune resilience and inflammation modulation** through relevant immune biomarkers and immune cell measurements. 4. To assess **safety, tolerability, and compliance** of the combination nutraceutical kit.			
Efficacy Endpoints	Assessment of changes in:			
	Domain	**Test and Method**	**Outcome Measure**	**Time frame**
	Primary Endpoint 1	6-minute walk test	6-minute walk distance (6MWD) (meters)	Screening, and Day 60.
	Secondary Endpoint 1	1-RM strength test (knee extension)	1 RM (kg) by leg press	Screening, and Day 60.
	Secondary Endpoint 2	DXA scan	Muscle volume	Screening, and Day 60.

	Secondary Endpoint 3	NIH Toolbox Fluid Composite	Fluid Composite score executive function, attention and processing speed, working memory	Screening, and Day 60.
	Secondary Endpoint 4	Cognitive Biomarkers	p-tau181	Screening, and Day 60.
	Secondary Endpoint 5	Immunological markers	CD4/CD8 ratio, CD45, CD3, CD4, CD8, T cells, B cells, NK cells, lymphocyte/neutrophil ratio	Screening, and Day 60.
	Secondary Endpoint 6	Inflammatory markers	hs-CRP, TNF-alpha, IL-6	
	Secondary Endpoint 7	Longevity and Putative Energy Markers	MDA, SOD, NAD+	Screening, and Day 60.
	Secondary Endpoint 8	Anthropometric parameters	Body Weight (kg), BMI (Kg/m^2) and hip, waist, chest circumferences (cm)	Screening, and Day 60.
	Secondary Endpoint 9	Hematological & biochemical investigations	Complete blood count (CBC), Renal Function Test (RFT). Liver Function Test (LFT), Thyroid profile, lipid profile, electrolytes.	Screening, and Day 60.
	Secondary Endpoint 10	AE/SAE monitoring	Adverse events,	Continuously, from baseline to end of the study.
	Secondary Endpoint 11	Tolerability	-	
	Secondary Endpoint 12	Compliance	-	
Sample Size	A total of 29 participants will be enrolled, allowing for an anticipated 20% dropout rate to ensure at least 23 evaluable participants complete the study. Participant recruitment targets should strive for balance in sex (ideally, 50%			

	female, but 40-60% balance is acceptable with accommodation for intersex individuals)
Inclusion criteria	Participants meeting all of the following criteria will be eligible for the study: 1. Participants with • Generally good health with life expectance ≥5 years; • Male and female participants aged between 50-80 years having a BMI between 25–35 kg/m² (both inclusive); • Health not severely compromised (i.e. no life-threatening illness or disability) 2. Participants with • Intrinsic Capacity score showing mild-to-moderate decline, and/or • Short Physical Performance Battery (SPPB) score between 9–11 and/or • Montreal Cognitive ≥ Assessment (MoCA) >20 and ≤25; 3. Participants with or without comorbidity. If comorbidity is present, it should be on a stable regimen (i.e., same drug and same dose) for at least 12 weeks before the screening visit; 4. Participants are able and willing to provide written informed consent, comply with the study protocol requirements, and can read and write in English.
Exclusion criteria	Participants meeting any of the following criteria will not be eligible for the study: 1. Severe or uncontrolled chronic disease (osteoarthritis, advanced cardiovascular disease, kidney failure, uncontrolled diabetes, severe COPD, terminal cancer); 2. Physical disability requiring a walker or wheelchair; 3. Diagnosed dementia or cognitive impairment preventing protocol compliance; 4. Acute infection or illness within 3 months before enrolment; 5. Unstable medical conditions (e.g., recent MI, unstable angina, uncontrolled hypertension); 6. Major surgery in the past 6 months or planned during study; 7. Severe psychiatric disorders unless well controlled; 8. Substance abuse within past 6 months; 9. Participation in another clinical trial within the last 6 months; 10. Known allergy to any component of the nutraceutical kit; 11. Participants with sedentary lifestyles are not able or not advised to undergo the resistance training as per protocol; 12. Any clinically relevant macro or micro-nutrient deficiency as per investigator discretion;

	13. Pregnant women, lactating women, women of child bearing potential not following adequate contraceptive measures, women who were found positive for urine pregnancy test; 14. Participants who are currently taking any herbal products, nutraceuticals, Ayurvedic formulations, dietary supplements, or vitamin/mineral supplements and are unwilling to discontinue their use for the duration of the study; 15. According to the investigator, any other illness or abnormal laboratory investigations would interfere directly with the study results or jeopardize the participant's health.
Study Duration	Each patient will participate in this study for approximately 60 days (minimum).
Study Visits	Scheduled study visits: 1. Screening visit (-7 to day 0) 2. Visit 1: Baseline Visit: Enrolment (Day 1) 3. Visit 2: Day 30 ± 5 days 4. Visit 3: Day 60 ± 5 days (End of the study)
End of the Study	The end of the study will be defined if a patient meets the following criteria: 1. Completer of the study 2. Progression of disease, non-compliant patient, and as per discretion of investigator (Termination from the study) 3. Incidence of SAE (Termination from the study) 4. Patient withdraws consent 5. Development of a new comorbidity during the study period, leading to discontinuation based on the investigator's clinical judgment 6. Depends upon the investigator's discretion
Interim Analysis	There would be at least one interim analysis. It will be documented in the interim analysis record.
Statistics	All statistical analyses will be performed using IBM SPSS Statistics (Version 30) or an equivalent validated statistical software package. All statistical tests will be two-sided with a significance level (α) of 0.05. Given the exploratory, proof-of-concept nature of this single-arm pilot study, the emphasis will be on estimation of effect sizes and confidence intervals, with hypothesis testing considered supportive and descriptive. Continuous variables will be summarized using descriptive statistics: number of observations (n), mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages.
Patient Population for Analyses	**Enrolled Population** All participants who sign informed consent and are enrolled into the study.

	Safety Population (mITT) All enrolled participants who receive at least one dose of the investigational product. This population will be used for all safety and tolerability analyses, and for compliance assessments. **Per-Protocol (PP) Population** All participants in the Safety Population who: o complete the Day 60 visit (within the allowed window), o have primary endpoint data at both baseline and Day 60, and o have no major protocol deviations that, in the opinion of the investigator and/or statistician, may significantly impact primary efficacy outcomes, and o demonstrate treatment compliance of **≥90%** (see compliance definition in Section X.7). The **primary efficacy analyses** will be based on the **PP Population**, with sensitivity analyses performed on the **Safety (mITT) Population** where applicable. Safety analyses will be based on the Safety Population. Participants who are enrolled but do not receive any dose of the investigational product will be listed separately under the category **"Treatment not given"** and will not be included in efficacy or safety summaries.
Efficacy Analyses	The efficacy endpoints will be analyzed using appropriate statistical tests, including the student's paired or unpaired t-test, Wilcoxon signed-rank test, Mann–Whitney U test, or Chi-square test, depending on the nature of the data (parametric or non-parametric). The final statistical analysis plan (SAP) will be drafted after the locking of the database and will be documented and notified to the sponsor as well as ethics committees (IRBs).
Safety Analysis	Adverse events (AEs) and serious adverse events (SAEs) will be summarized by reporting both the total number of events and the number of participants experiencing at least one event during the study period. Additionally, these summaries will be stratified based on seriousness, severity, and their relationship to the study medication. The percentage of participants experiencing events and overall tolerability will be analyzed and compared using the Chi-square test.

6. INTRODUCTION

6.1. Background information

Humanity is experiencing a demographic shift unprecedented in history. By 2050, the number of people aged ≥60 years will surpass 2 billion, reshaping the landscape of healthcare, productivity, and social stability [1]. Although modern medicine has successfully extended lifespan, it has achieved far less in extending healthspan—the period of life lived with autonomy, mobility, cognitive vitality, and immune resilience. Aging is increasingly understood as a biological phenomenon characterized by metabolic inefficiency, mitochondrial decline, chronic inflammation, loss of neuromuscular mass, and immune dysregulation [2,3]. These interconnected processes erode the capacity of older adults to live actively and independently long before the onset of overt disease.

Sarcopenia is a central contributor to late-life frailty, affecting 10–20% of individuals over 60 years [4]. Progressive decreases in mitochondrial ATP output, muscle fiber density, and motor unit recruitment drive reductions in physical capability and gait endurance [3,6]. Functional performance measures such as the six-minute walk distance (6MWD) provide a holistic assessment of biological capacity—capturing cardiopulmonary endurance, neuromotor synchronization, musculoskeletal output, and motivation [7]. Clinically meaningful improvements in 6MWD (20–50 meters) are associated with reduced hospitalization, better survival, and delayed disability in older adults [7]. Thus, 6MWD embodies the real-world essence of healthspan better than isolated laboratory biomarkers.

The European Working Group on Sarcopenia in Older People (EWGSOP2) specifically recommends DXA for confirming sarcopenia diagnosis and utilizes standardized cut-off points, such as ASM/height^2 <7.0kg/m^2 for men and <5.5kg/m^2 for women, to identify clinically significant low muscle quantity [4]. The Dual-energy X-ray absorptiometry (DXA) system provides appendicular Lean Mass (ALM), which is widely accepted in research as a validated surrogate for appendicular skeletal muscle (ASM) mass and forms the foundation in all major sarcopenia consensus definitions as a clinically recognized research tool [4,5].

Cognitive decline represents a second pillar of aging. Mild cognitive impairment and executive dysfunction emerge gradually, shaped by neuroinflammation, oxidative stress, and impaired neurogenesis [8]. Multifactorial nutraceuticals targeting mitochondrial function, synaptic plasticity, and antioxidative defense have demonstrated potential to improve cognitive performance in domains such as processing speed, working memory, and executive decision-making [8]. Tools like the NIH Toolbox Fluid Composite enable comprehensive assessment of multiple functional domains over short time frames, reducing bias inherent in single-domain tests.

Aging also drives profound immune remodeling. Immunosenescence—a hallmark of aging—encompasses reductions in naïve T-cell pools, impaired antigen presentation, and chronic low-grade inflammation ("inflammaging") [9]. Dysregulated cytokine networks such as elevated IL-6 and TNF-α further propagate oxidative stress and tissue degeneration [9]. Nutritional and phytochemical interventions have demonstrated immunomodulatory activity, increasing NK cell surveillance and supporting adaptive immune balance [10].

The proposed nutraceutical combination aims to simultaneously enhance muscle function, cognitive performance, and immune resilience using synergistic bioactive components. Unlike single-target pharmaceuticals, nutraceutical combinations may modulate multiple biological axes—mitochondrial respiration, oxidative stress pathways, neurotrophic signaling, and immune regulation—providing versatility and scalability [2,10,11]. Most importantly, the intervention is accessible, non-invasive, and feasible for mass deployment across diverse socioeconomic contexts.

If a short-term intervention can produce measurable improvements in functional mobility, cognitive performance, and immune resilience, it will demonstrate that aging is not a passive, unidirectional decline but a modifiable biological state. This work directly advances to extend the period of life lived in capability, autonomy, and human dignity.

6.2. Details of Investigational Product

6.2.1 Ingredients of Investigational Products

Table 3: Active Ingredients of Investigational product

S/N	Ingredient	Composition (g)
1	Whey isolate 90%	17.50
2	Vegan Collagen peptide	4.00
3	Mango freeze dried	5.00
4	Creatine monohydrate	1.50
5	Magnesium glycinate	0.50
6	Resveratrol 98%	0.25
7	Quercetin 95%	0.25
8	Cordyceps extract	0.14
9	Marine algae (B12 + D3)	0.10
10	Acetyl-L-Carnitine	0.10
11	Phosphatidylserine	0.10
12	AKBA	0.12
13	Brahmi extract	0.19

14	Ginkgo biloba extract	0.10
15	Ashwagandha extract	0.07
16	Seaweed extract	0.05
17	Curcumin 95%	0.03
	TOTAL	**30.00**

- **Other ingredients:** Q.S.

6.2.2 Pharmacology of Product ingredients:

The investigational product includes scientifically validated bioactive ingredients targeting key determinants of health span: skeletal muscle function, cognitive performance, immune resilience, and inflammatory regulation. These compounds act across mitochondrial metabolism, synaptic neurotransmission, antioxidant activity, neuromuscular repair, and immune signalling pathways.

- **Whey Protein Isolate (90%)**

Whey isolate is a rapidly absorbed complete protein rich in leucine, which activates the mTOR pathway and increases skeletal muscle protein synthesis in aging populations [12,13]. Supplementation promotes recovery from activity-induced microtrauma and supports preservation of lean muscle mass relevant to sarcopenia mitigation [12].

- **Creatine Monohydrate**

Creatine increases intramuscular phosphocreatine levels, enabling faster ATP regeneration during muscular contraction [14]. In older adults, creatine supplementation combined with resistance exercise improves muscle strength, lean mass, and functional performance including walking capacity and chair-rise time [15].

- **Vegan Collagen Peptides**

Collagen peptides stimulate fibroblast activity and extracellular matrix synthesis, improving tendon integrity and muscle–tendon force transmission [16]. Supplementation increases collagen turnover, reduces joint discomfort, and supports exercise recovery, indirectly improving mobility and gait performance [17].

- **Cordyceps Extract**

Cordyceps sinensis bioactives upregulate mitochondrial ATP production, enhancing endurance capacity and oxygen utilization [18]. The polysaccharide fraction also enhances macrophage and NK-cell responses, contributing to immune resilience in aging cohorts [19].

- **Marine Algae (Vitamin D3 + Vitamin B12)**

Vitamin D3 modulates calcium handling and skeletal muscle contractility and is essential for immune homeostasis and T-cell activation [20]. Vitamin B12 supports neuronal myelination and

neurotransmitter synthesis; deficiencies are associated with cognitive impairment, fatigue, and reduced neuroperformance [21].

- **Acetyl-L-Carnitine (ALCAR)**

ALCAR facilitates mitochondrial transport of long-chain fatty acids, improving ATP production in both neurons and skeletal muscle [22]. Clinically, it demonstrates cognitive benefits such as improved mental energy, memory, and executive function in aging adults [23].

- **Phosphatidylserine**

Phosphatidylserine is a core neuronal membrane phospholipid that enhances synaptic plasticity, promotes acetylcholine turnover, and reduces stress-induced cortisol elevations [24]. Supplementation improves accuracy, attention, and working memory performance [25].

- **AKBA (Boswellia serrata)**

Acetyl-11-keto-β-boswellic acid (AKBA) is a selective 5-lipoxygenase inhibitor reducing leukotriene-mediated inflammation and oxidative tissue stress [26]. Clinical data demonstrates improved joint function and reduced inflammatory symptoms without NSAID-related gastrointestinal toxicity [27].

- **Brahmi (Bacopa monnieri)**

Bacosides increase dendritic branching density, promote synaptic communication, and enhance retention of newly acquired information [28]. Chronic supplementation improves memory recall and reduces cognitive latency in aging cohorts [29].

- **Ginkgo biloba**

Ginkgo flavone glycosides enhance cerebral blood flow, reduce oxidative neuronal damage, and modulate neurotransmitter activity [30]. It demonstrates clinically significant benefits in attention, psychometric performance, and age-related cognitive decline [31].

- **Ashwagandha (Withania somnifera)**

Ashwagandha regulates the hypothalamic-pituitary-adrenal (HPA) axis and reduces cortisol, improving resilience, sleep, and cognitive performance [32]. It also enhances muscle strength and immune activation via NK-cell and macrophage pathways [33].

- **Seaweed Extract (Fucoidans)**

Fucoidan polysaccharides exhibit immunomodulatory actions through macrophage stimulation, enhanced NK cytotoxicity, and antioxidant protection [34]. They have demonstrated anti-fatigue, anti-glycation, and metabolic stability effects relevant to aging [35].

- **Curcumin (95%)**

Curcumin suppresses NF-κB, COX-2, and pro-inflammatory cytokine activity (TNF-α/IL-6), reducing systemic inflammation and oxidative stress [36]. It also demonstrates neuroprotective properties, supporting mitochondrial function and attenuating amyloid pathology [37].

- **Mango Freeze-Dried (Polyphenols, Mangiferin)**

Mango pulp delivers mangiferin, xanthones, and flavonoids with potent antioxidant and anti-inflammatory effects, reducing lipid peroxidation and reactive oxygen species burden relevant to

aging physiology [38]. Mangiferin modulates glucose homeostasis, protects neuronal membranes, and demonstrates neurocognitive benefits by attenuating pro-oxidant cytokine cascades in preclinical and early translational models [39].

- **Magnesium Glycinate**

Magnesium acts as a cofactor for more than 300 enzymatic reactions, including ATP synthesis, nerve conduction, and muscle contraction, and deficiency contributes to fatigue, impaired performance, and neuromuscular instability [40]. The chelated glycinate form offers superior gastrointestinal tolerance and supports sleep regulation, immune homeostasis, and neurocognitive stability through modulation of NMDA receptor excitability [41].

- **Resveratrol 98%**

Resveratrol activates SIRT1 and AMPK signalling, promoting mitochondrial biogenesis, metabolic resilience, and longevity-associated pathways [42]. It exerts anti-inflammatory and antioxidant effects by downregulating NF-κB and inhibiting TNF-α/IL-6 expression, supporting immune and neurovascular integrity in aging populations [43].

- **Quercetin 95%**

Quercetin is a flavonol with strong free-radical scavenging capacity, inhibiting xanthine oxidase and suppressing NF-κB–driven inflammatory cascades [44]. It modulates innate immunity through mast-cell stabilization and enhances antiviral and NK-cell responses, while also providing neuroprotection against oxidative injury [45].

6.2.3 Indications

Improvement in muscle domain, immunity parameter and cognitive parameters in aging population.

6.2.4 Dosage

30 g sachet content to be mixed in water and consumed twice daily before meals.

6.2.5 Packaging for Clinical Trial Supply

30g sachet for each serving

6.2.6 Storage Condition

Store in a cool & dry place away from sunlight. Once opened the sachet needs to be consumed at once, no storage after opening of sachet.

6.2.7 Shelf Life

18 months from the date of manufacturing

6.3. Preclinical and Clinical Study Data

The investigational nutraceutical formulation integrates bioactive compounds with validated molecular actions on muscle physiology, cognitive performance, immune modulation, and systemic inflammation. The combination approach is grounded in the principle that aging is a multisystem decline requiring coordinated biological modulation rather than single-pathway intervention. Selected ingredients within the formula possess established clinical evidence across performance, neurocognitive, and immunological domains, which collectively support the rationale for conducting this proof-of-concept study.

Muscle Function and Physical Performance

Creatine monohydrate has undergone extensive clinical evaluation across aging and sarcopenic populations. Supplementation increases intramuscular phosphocreatine, enhances ATP regeneration, and augments resistance training outcomes, particularly lower-limb power and gait endurance [46]. Older adults receiving creatine in combination with strength training demonstrated superior improvements in lean mass and muscle performance compared to training alone [47]. Similarly, whey protein isolate, rich in leucine, stimulates mTORC1-dependent muscle protein synthesis and supports post-exercise recovery and anabolic maintenance in aging adults [48]. A synergistic interaction has been described whereby creatine enhances the anabolic sensitivity of muscle protein synthesis under protein-supplemented conditions [49].

Cognitive Performance and Neuroprotection

Bacopa monnieri (Brahmi) has been repeatedly evaluated in aging populations; standardized extracts have shown improvements in memory acquisition, retention, and recall latency following chronic supplementation periods of 8–12 weeks [50]. The bacoside fraction promotes dendritic arborization and cholinergic neurotransmission, mechanisms associated with enhancement of working memory and attention. Ginkgo biloba has demonstrated benefits in cognitive processing speed and attention, particularly in subjects with age-related cognitive decline. Randomized trials suggest vascular-neuroprotective effects mediated through antioxidant flavonol fractions and improved cerebral perfusion [51]. Acetyl-L-Carnitine also exhibits neurotrophic activity, enhancing mitochondrial fatty acid transport and acetylcholine synthesis, with demonstrated improvements in cognitive symptoms in elderly cohorts [52].

Immune Function and Inflammation Modulation

Cordyceps sinensis polysaccharides increase innate immune activation, promoting NK-cell cytotoxicity and macrophage functional responsiveness [53]. Clinical and preclinical findings describe improvements in exercise tolerance and VO_2max, likely mediated by mitochondrial ATP enhancement and oxidative stress reduction. Ashwagandha (Withania somnifera) exerts immunomodulatory and anti-stress properties; standardized extract administration reduces cortisol, enhances NK-cell activity, and improves subjective resilience scores in stressed adult populations [54]. Polyphenolic compounds, including quercetin and resveratrol, demonstrate potent NF-κB inhibition and attenuation of IL-6 and TNF-α expression, supporting mechanisms of inflammation reduction and mitochondrial protection in aging tissues [55,56].

6.4. Risk-Benefit Assessment

The investigational nutraceutical consists of widely used dietary ingredients with established human safety at recommended doses. Expected risks are low and generally limited to mild gastrointestinal discomfort, headache, or hypersensitivity in susceptible individuals [57,58]. Potential benefits include improvements in physical function, cognitive performance, immune balance, and inflammatory control, all of which are clinically relevant to older adults. With appropriate eligibility screening and ongoing safety monitoring, the anticipated benefits to participants are considered to outweigh the potential risks.

7. STUDY RATIONALE

The collective evidence indicates that the key constituents target distinct yet interconnected physiologic domains—mitochondrial ATP generation, neuromuscular anabolism, immune regulation, and oxidative stress. Unlike pharmaceutical monotherapies, dietary bioactives exhibit complementary mechanisms, enabling multi-domain functional improvement through moderate biological modulation. While each ingredient has been individually studied, no prior clinical investigation has systematically tested a combined formulation that concurrently addresses muscle endurance, cognition, immune resilience, and inflammation within a short-duration intervention window. The current trial is therefore intended as a proof-of-concept to assess functional responses and biomarker trajectories in older adults.

8. STUDY OBJECTIVES AND ENDPOINTS

8.1. Study Objectives

The primary objective of this study is to evaluate the impact of the combination nutraceutical on muscle function, as reflected by changes in performance and functional capacity. Secondary objectives include assessing its effects on cognitive performance, immune resilience, and inflammatory biomarkers, along with evaluating tolerability, safety, and participant compliance over the intervention period. This proof-of-concept trial aims to generate early clinical evidence supporting the formulation's multi-domain benefits in older adults.

8.2. Endpoints

8.2.1. Efficacy endpoints

Table 4: Efficacy Endpoints

Domain	Test and Method	Outcome Measure	Time frame
Primary Endpoint 1	6-minute walk test	6-minute walk distance (6MWD) (meters)	Screening, and Day 60.
Secondary Endpoint 1	1-RM strength test (knee extension)	1 RM (kg) by leg press	Screening, and Day 60.
Secondary Endpoint 2	DXA scan	Muscle volume	Screening, and Day 60.
Secondary Endpoint 3	NIH Toolbox Fluid Composite	Fluid Composite score executive function, attention and processing speed, working memory	Screening, and Day 60.
Secondary Endpoint 4	Cognitive Biomarkers	p-tau181	Screening, and Day 60.
Secondary Endpoint 5	Immunological markers	CD4/CD8 ratio, CD45, CD3, CD4, CD8, T cells, B cells, NK cells, lymphocyte/neutrophil ratio	Screening, and Day 60.
Secondary Endpoint 6	Inflammatory markers	hs-CRP, TNF-alpha, IL-6	Screening, and Day 60.
Secondary Endpoint 7	Longevity and Putative Energy Markers	MDA, SOD, NAD+	Screening, and Day 60.
Secondary Endpoint 8	Anthropometric parameters	Body Weight (kg), BMI (Kg/m^2) and hip, waist, chest circumferences (cm)	Screening, and Day 60.
Secondary Endpoint 9	Hematological & biochemical investigations	Complete blood count (CBC), Renal Function Test (RFT). Liver Function Test (LFT), Thyroid profile, lipid profile, electrolytes.	Screening, and Day 60.

Secondary Endpoint 10	AE/SAE monitoring	Adverse events,	Continuously, from baseline to end of the study.
Secondary Endpoint 11	Tolerability	-	
Secondary Endpoint 12	Compliance	-	

9. INVESTIGATIONAL PLAN

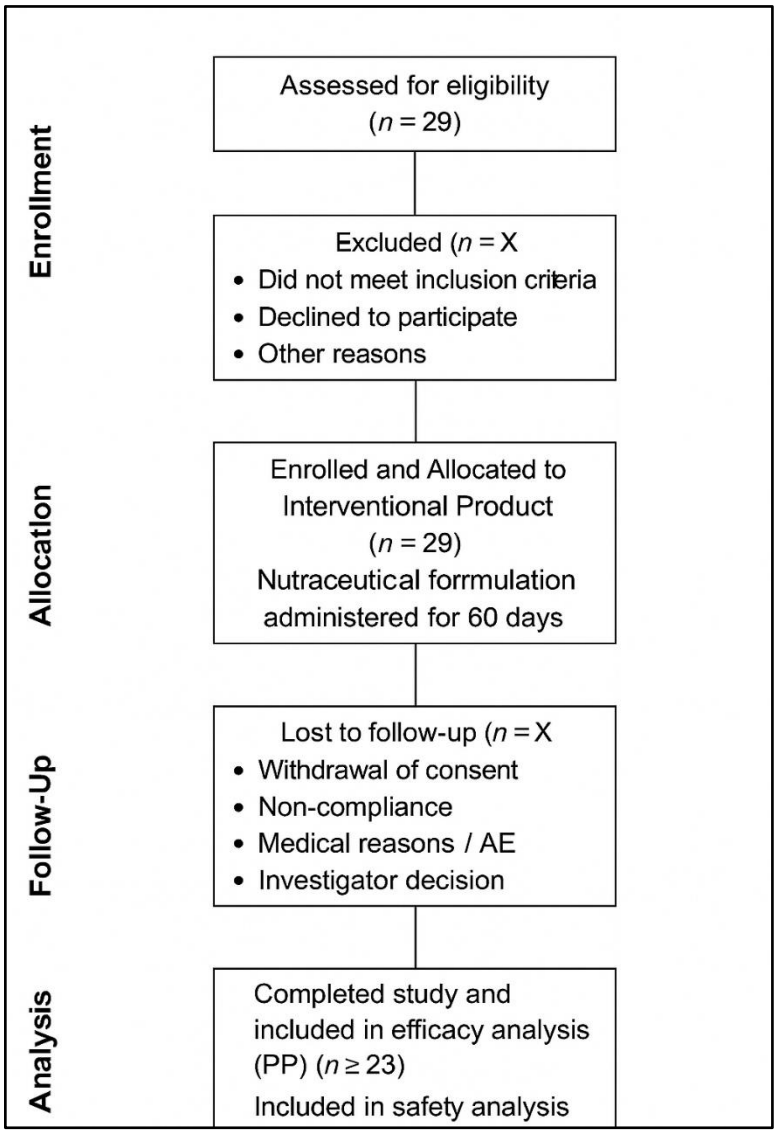
9.1. Study Design

This open-label, single arm interventional clinical study aims to evaluate the efficacy and safety of nutraceutical formulation (Interventional product) in older aduts. A total of 29 participants will be screened and enrolled to ensure at least 23 evaluable cases. The total treatment period will be 60 days.

9.2. Overall Study Plan

This is an open-label, single-arm interventional clinical study designed to evaluate the efficacy, safety, and tolerability of the investigational nutraceutical formulation in older adults over a 60-day treatment period. A total of 29 participants will be screened, with the intent to obtain at least 23 evaluable subjects. Eligible participants will complete baseline assessments at screening and day 1, followed by administration of the study product for 60 days. The primary efficacy endpoint is improvement in functional capacity as assessed by change in 6-minute walk distance from baseline to Day 60. Secondary endpoints include changes in muscle strength (1-RM knee extension), muscle volume (DXA), cognitive performance (NIH Toolbox Fluid Composite and p-tau181), immune cell profiles, inflammatory biomarkers (hs-CRP, TNF-α, IL-6), oxidative and longevity-related markers (MDA, SOD, NAD+), anthropometry. Hematological and biochemical safety laboratories (CBC, RFT, LFT, thyroid profile, lipid profile, electrolytes) will be evaluated at baseline and Day 60. Adverse events, serious adverse events, tolerability, and compliance will be monitored continuously from enrollment to end of study. Participants will attend a screening visit (−7 to 0 days), baseline/enrollment visit (Day 1), and an end-of-study visit on Day 60 ± 5 days.

Diagram depicting the overall study plan:



9.3. Selection and Handling of Study Population

The research team will recruit more than 23 eligible adult participants (both men and women) at the study site.

9.3.1. Inclusion Criteria

Participants will be eligible for the study if they meet all of the following conditions:

1. Participants with
    - Generally good health with life expectance ≥5 years;

- Male and female participants aged between 50-80 years having a BMI between 25–35 kg/m² (both inclusive);
- Health not severely compromised (i.e. no life-threatening illness or disability)

2. Participants with
   - Intrinsic Capacity score showing mild-to-moderate decline, and/or
   - Short Physical Performance Battery (SPPB) score between 9–11 and/or
   - Montreal Cognitive ≥ Assessment (MoCA) >20 and ≤25;
3. Participants with or without comorbidity. If comorbidity is present, it should be on a stable regimen (i.e., same drug and same dose) for at least 12 weeks before the screening visit;
4. Participants are able and willing to provide written informed consent, comply with the study protocol requirements, and can read and write in English.

9.3.2. Exclusion criteria

Participants meeting any of the following criteria will not be eligible for the study:

1. Severe or uncontrolled chronic disease (osteoarthritis, advanced cardiovascular disease, kidney failure, uncontrolled diabetes, severe COPD, terminal cancer);
2. Physical disability requiring a walker or wheelchair;
3. Diagnosed dementia or cognitive impairment preventing protocol compliance;
4. Acute infection or illness within 3 months before enrolment;
5. Unstable medical conditions (e.g., recent MI, unstable angina, uncontrolled hypertension);
6. Major surgery in the past 6 months or planned during study;
7. Severe psychiatric disorders unless well controlled;
8. Substance abuse within past 6 months;
9. Participation in another clinical trial within the last 6 months;
10. Known allergy to any component of the nutraceutical kit;
11. Participants with sedentary lifestyles are not able or not advised to undergo the resistance training as per protocol;
12. Any clinically relevant macro or micro-nutrient deficiency as per investigator discretion;
13. Pregnant women, lactating women, women of child bearing potential not following adequate contraceptive measures, women who were found positive for urine pregnancy test;
14. Participants who are currently taking any herbal products, nutraceuticals, Ayurvedic formulations, dietary supplements, or vitamin/mineral supplements and are unwilling to discontinue their use for the duration of the study;
15. According to the investigator, any other illness or abnormal laboratory investigations would interfere directly with the study results or jeopardize the participant's health.

9.3.3. Discontinuation Criteria

Participants will be considered as discontinued based on the following criteria:

- Withdrawal of consent by the participant to continue in the study;
- The appearance of non-tolerable adverse events;
- The appearance of exclusion criteria, which concern the safety of the participant;
- Any situation judged by the investigator to be harmful to the participant;
- At the discretion of the investigator, where the clinical condition of the participant worsens;
- At the discretion of the investigator, poor compliance of the participant to study procedures;
- A positive pregnancy test at any time during the study period;
- Protocol deviation that in the opinion of the sponsor and investigator warrants discontinuation from the study;
- The participant suffered from inter-current illness during the study.

The reason for participant discontinuation will be documented in the participant's case report form (CRF). Discontinued number of participants will not be replaced. In case of premature discontinuation, all follow-up assessments will be conducted at early withdrawal. Participants discontinued from the study at any stage will be considered for safety analysis.

9.4. End of Study

The end of the study will be defined if a participant meets the following criteria:

1. Completer of the study.
2. Progression of disease, non-compliant participant, and as per discretion of investigator.
3. Incidence of SAE (Termination from the study).
4. The participant withdraws consent.
5. Depends upon the investigator's discretion.

9.5. Blinding

The study is open label study, no need of blinding process.

9.6. Handling of Investigational Product

Supply and Receipt

The study IPs will be labeled according to the participant number before the start of the study. The investigator will be overall responsible for ensuring that IP is stored in a safe, limited access location, as per the condition noted on the label.

The study IP will be labeled and packaged such that the label should include but not be limited to the Sponsor name/address, dosage, route of administration, name/strength, batch number/code,

study reference or protocol number, directions for use, the statement 'for clinical trial use only', storage conditions, expiry/use by date.

Study IP will be handled by designated personnel at the site. The study personnel involved in IP handling will not participate in any activity pertaining to participant safety and efficacy assessment that may impact the study outcome.

It is the responsibility of the investigator to designate independent dispensers or designated personnel to ensure that the current disposition of the IP is maintained at each study site where IP is inventoried and dispensed. When investigational product shipment is received at the study site, independent dispensers or designated personnel must acknowledge receipt of IP (An attempt would be made to store the IP at the required temperature as early as possible).

Accountability and Retentions of Investigational Product

Independent dispensers or designated study personnel will maintain accurate records in an IP accountability log of receipt of all IP, inventory at the site, dispensing of IP, disposition of used products, and return of unused products to the sponsor. The study IP (used/unused) will be disposed of at the site with proper investigational product accountability maintained. The IP disposition report will be generated separately at the close out of the site.

Dosage and Administration of IPs

30g sachet content will be mixed in water and consumed twice a day before meals.

Treatment Compliance

Treatment compliance of each participant concerning the administration of the IP will be recorded in the respective section of the CRF. The site will maintain the accountability of all used and unused IP. The cut-off for non-compliance will be a maximum of up to 10% per month. If any participant misses doses for more than permissible, it would be considered a dropout.

9.7. Clinical Procedures and Assessments

The following are the clinical procedures and assessments during the study period.

Screening Assessments (Day -7 to Day 0)

Screening procedures will be performed within 7 days before randomization. All aspects of the study will be explained to the participants at screening. The investigator must ensure that the participant meets all of the inclusion and none of the exclusion criteria.

The following activities will be performed before randomization to screen the participants:

- Presentation of participant information and obtaining participant's written informed consent
- Recording of demographic data (age, gender)
- Concomitant diseases/ concomitant medication
- Inclusion/Exclusion criteria review
- Vital parameters:

- blood pressure, heart rate, body temperature, and respiratory rate

- Physical examination
- Clinical examination
- Assessment of 6-minute walk distance (6MWD) (meters)
- Assessment of 1RM (kg) with the leg press
- Assessment of Muscle volume
- Assessment of Fluid Composite score and assessment of executive function, attention and processing speed, working memory
- Assessment of plasma p-tau181
- Assessment of CD4/CD8 ratio, CD45, CD3, CD4, CD8, T cells, B cells, NK cells, lymphocyte/neutrophil ratio
- Assessment of serum hs-CRP, TNF-alpha, IL-6
- Assessment of plasma MDA and SOD, NAD+ from whole blood
- Assessment of Body Weight (kg), BMI (Kg/m^2) and hip, waist, chest circumferences (cm)
- Assessment of complete blood count (CBC), Renal Function Test (RFT). Liver Function Test (LFT), Thyroid profile, lipid profile, electrolytes.

Visit 1: Baseline/Randomization (Day 1)

- Clinical examination
- Physical examination
- Randomization
- Dispensing of IP
- Vital Parameters

- blood pressure, heart rate, body temperature, and respiratory rate.

- Recording and assessment of adverse events
- Assessment of rescue medications

Visit 2: Day 30±5 days

- Clinical examination
- Physical examination
- Vital Parameters
- blood pressure, heart rate, body temperature and respiratory rate
- Dispensing of IP
- Assessment of tolerability of investigational product
- Assessment of compliance
- Recording and assessment of adverse events
- Assessment of rescue medications

Visit 3: Day 60±5 days (End of the study)

- Clinical examination
- Physical examination
- Vital Parameters
- blood pressure, heart rate, body temperature and respiratory rate
- Assessment of tolerability of investigational product
- Assessment of compliance
- Recording and assessment of adverse events
- Assessment of rescue medications
- Assessment of 6-minute walk distance (6MWD) (meters)
- Assessment of 1RM (kg) with the leg press
- Assessment of Muscle volume
- Assessment of Fluid Composite score and assessment of executive function, attention and processing speed, working memory
- Assessment of plasma p-tau181
- Assessment of CD4/CD8 ratio, CD45, CD3, CD4, CD8, T cells, B cells, NK cells, lymphocyte/neutrophil ratio
- Assessment of serum hs-CRP, TNF-alpha, IL-6
- Assessment of plasma MDA and SOD, NAD+ from whole blood
- Assessment of Body Weight (kg), BMI (Kg/m^2) and hip, waist, chest circumferences (cm)
- Assessment of complete blood count (CBC), Renal Function Test (RFT). Liver Function Test (LFT), Thyroid profile, lipid profile, electrolytes.

9.8. Prohibited Concomitant Therapy

Participants will be instructed to refrain from the use of products that may confound the interpretation of efficacy, biomarker, or safety outcomes during the study period. The following therapies are prohibited for the duration of participation unless otherwise approved by the Investigator:

- Nutraceuticals, dietary supplements, herbal preparations, or Ayurvedic formulations (including multivitamins, antioxidants, adaptogens, anti-inflammatory extracts, amino acids, or energy boosters).
- Investigational medicinal products or participation in another clinical trial within the last 6 months.
- Anabolic agents or muscle-enhancing therapies, including testosterone boosters, or growth hormone derivatives.
- Cognitive enhancement agents such as prescription nootropics, cholinesterase inhibitors, stimulants, or memory enhancement blends.
- Immunomodulatory agents, including biologics, corticosteroids, or disease-modifying anti-rheumatic drugs (DMARDs), except when medically necessary and stable for ≥12 weeks prior to screening.
- High-dose vitamin or mineral supplementation (e.g., vitamin D >2000 IU/day, Vitamin B12 injection, NAD+ infusions, IV antioxidant cocktails, etc.).
- Special diets or extreme caloric restriction protocols (ketogenic, intermittent fasting ≥18 hours/day, very low-calorie diets, medical detox regimens).

The Investigator may discontinue a participant if prohibited therapies are initiated during the trial or if continued use could compromise safety, study integrity, or endpoint assessment.

9.9. Permitted Concomitant and Rescue Therapy

Stable and medically necessary therapies that are not expected to interfere with study endpoints may be continued during the study. These include:

- Chronic medications for pre-existing stable conditions (e.g., hypertension, diabetes, dyslipidemia) provided that the drug and dose have remained unchanged for ≥12 weeks prior to screening.
- Occasional use of standard analgesics (e.g., acetaminophen/paracetamol up to 2 g/day) for mild to moderate pain not related to study intervention.
- Routine dietary intake, including normal balanced meals, caffeine intake, and hydration practices.
- Short-term symptomatic treatment, such as local antiseptics or mild topical medications for non-study-related conditions.
- Rescue therapy, at Investigator discretion, for clinically significant deterioration, intolerable symptoms, or medical emergencies. In such cases, the intervention type, dosage, and duration will be documented in the source data and CRF.

All permitted concomitant medications, rescue therapies, and dose changes must be recorded, including start/stop dates and medical indications. The Principal Investigator will assess whether

continuation or withdrawal from the study is appropriate if any therapy is expected to confound efficacy or safety outcomes.

9.10. Protocol Deviations and Violations

The investigator shall strictly adhere to the protocol approved by the regulatory authority and IRB. All protocol 'violations' (for inclusion/exclusion) and protocol 'deviations' (related to study procedures) must be reported to the study monitor and a protocol deviation/violation log should be filled for every such case.

9.11. Study Duration

Each participant will participate in this study for approximately 60 days.

10. SAFETY CONSIDERATIONS

Safety and tolerability of study medication will be assessed by reporting and analyzing the adverse events (AEs) that occurred, vital signs, etc. The investigator asks the participant generally about adverse events without indicating special symptoms.

10.1 Risks and Benefits to Study Participants

In this planned clinical study, the participants will be informed about the expected risks and the inconveniences associated with the clinical trial. In addition, in compliance with ICH-GCP, participants are free to leave the clinical trial at any time they wish and are treated according to standard supportive care. To follow the risks adequately the investigator will see the participants frequently according to the clinical trial scheme provided in the protocol.

The clinical study protocol includes discontinuation criteria on participants and a clinical trial basis to reduce individual risks.

10.2 Definitions Related to Safety

10.2.1 Adverse Event

An AE (or adverse experience) is any untoward medical occurrence in a participant or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. It can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Also, abnormal results of diagnostic procedures are considered to be AE, if the abnormality results in participant withdrawal, is associated with SAE, leads to additional treatment or to further diagnostic tests, or is considered to be of clinical significance by the investigator.

Any “untoward medical occurrence” occurring before the administration of the study medication, does not represent an adverse event, but is reported as a “baseline complaint”.

Pre-existing medical conditions or symptoms occurring before the initiation of the study will not be reported as AEs. A worsening of a pre-existing medical condition or symptom will be reported as an AE.

10.2.2 Adverse Drug Reaction

In the pre-approval clinical experience with a new medicinal product or its new usages, [particularly as the therapeutic dose(s) may not be established], all noxious and unintended responses to a medicinal product related to any dose will be considered adverse drug reactions. The phrase "responses to medicinal products" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Regarding marketed medicinal products, an adverse drug reaction in the post-marketing setting is a response to a drug that is noxious and unintended and which occurs at doses normally used in humans for prophylaxis, diagnosis, or therapy of disease or modification of physiological function.

10.2.3 Unexpected Adverse Drug Reaction

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

10.2.4 Change of Laboratory Parameters

If, after administration of study medication, changes in pathological laboratory values occur, which were not present before the treatment started, further clinical or laboratory tests must be carried out until the values return to the normal range or until a plausible explanation is found (e.g. concomitant disease) for the change of the laboratory values.

A change in laboratory parameters during the study represents an adverse event:

- if the change is clinically relevant.

Pathological values still present at end of the study, which were not present before the start of the study, have to be followed up by further appropriate investigations until the values have returned to normal range or until an explanation (e.g. concomitant disease) has been found.

10.2.5 Serious Adverse Event or Adverse Drug Reaction

A SAE is any untoward medical occurrence that at any dose:

- results in death;
- permanent disability;
- is life-threatening [The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe;
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity, or;
- is a congenital anomaly/birth defect.

Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or prolongation of hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above. These will also usually be considered “serious” as well.

10.3 Adverse Event Documentation and Reporting

The documentation of AEs starts with randomization for each participant and ends with the discharge of the participant from the hospital. If an AE occurs after the termination of the clinical trial of the individual participant and if the investigator judges the AE to be at least possibly related to the clinical trial, the investigator has to inform the sponsor.

All AE related, signs or symptoms that are reported will be recorded in the AE form as part of CRF. In particular, the information will include details of the administration of the investigational product and details of AE with its date, onset time, causality, frequency, severity, outcome, and if any treatment or diagnostic steps were taken in relation to it. All AEs will be followed until resolution. All the AEs will be evaluated based on severity, causality, and outcome as given in the below table.

Table 5: AE evaluation based on severity, causality, and outcome.

Parameter	Description
Severity	
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Parameter	Description
Grade 2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.
Causality	
Certain	▪ Event or laboratory test abnormality, with plausible time relationship to drug intake. ▪ Cannot be explained by disease or other drugs. ▪ Response to withdrawal plausible (pharmacologically, pathologically). ▪ Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon). ▪ Rechallenge satisfactory, if necessary.
Probable / Likely	▪ Event or laboratory test abnormality, with reasonable time relationship to drug intake. ▪ Unlikely to be attributed to disease or other drugs. ▪ Response to withdrawal is clinically reasonable. ▪ Rechallenge is not required.
Possible	▪ Event or laboratory test abnormality, with reasonable time relationship to drug intake. ▪ Could also be explained by disease or other drugs. ▪ Information on drug withdrawal may be lacking or unclear.
Unlikely	▪ Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible). ▪ Disease or other drugs provide plausible explanations.
Conditional/ Unclassified	▪ Event or laboratory test abnormality. ▪ More data for proper assessment is needed, or; ▪ Additional data under examination.
Unassessable/ Unclassifiable	▪ A report suggesting an adverse reaction. ▪ Cannot be judged because information is insufficient or contradictory. ▪ Data cannot be supplemented or verified.
Outcome	
Resolved / Resolved with sequel / Ongoing / Stable chronic condition / Continuing at death / Death	

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refers to bathing, dressing, undressing, feeding self, using the toilet, taking medications, and not being bedridden.

Any AE regardless of casualty relationship will be recorded and reported to the IRB.

AEs will be coded as per the MedDRA current version.

10.4 Reporting of Serious Adverse Event

10.4.1 Central Licensing Authority Requirements for Expedited Reporting of SAE

- SAE will be reported by the clinical investigator to the sponsor, chairperson of EC, safety review board chairman, and central licensing authority within 24 hours of the knowledge of its occurrence.
- The clinical investigator and sponsor will submit a detailed report of SAE to the chairperson of EC, the head of the clinical trial site, and the central licensing authority within 14 days of the knowledge of its occurrence.
- IEC will send its review report to the central licensing authority for SAE within 30 days of its occurrence.
- In case the clinical investigator fails to report any SAE within the stipulated period, he shall furnish the reason for the delay to the satisfaction of the licensing authority along with the report of SAE.
- If applicable, the central licensing authority and safety review board recommended compensation by order will be paid by the sponsor within 30 days of receipt of such order from the central licensing authority for the SAE that occurred.

All SAEs will be followed until a satisfactory resolution or until the investigator deems the event to be chronic or the participant to be stable. Participants /LAR will inform the investigator regarding the SAEs (whether related or unrelated) within 24 hours of their occurrence.

The details of the sponsor's contact personnel for SAE reporting are given below:

Dr. Shridhar Pandya

gplifehealthcare@gmail.com

10.4.2 Reporting of Pregnancy

Pregnancy is not expected due to age range of participants (50–80 years). However, if pregnancy is identified at any time during the study or within 30 days after last administration of the investigational product, it will be reported to the Sponsor and the Ethics Committee as a safety event. The participant will be withdrawn from the study, and appropriate medical follow-up will be arranged. Pregnancy outcomes and fetal/neonatal health will be documented in accordance with institutional and regulatory requirements.

11. STATISTICAL CONSIDERATIONS AND EFFICACY EVALUATION

11.1 Sample Size Consideration

Base sample size (evaluable subjects)
From G*Power:

- **Effect size (Cohen's *d*)** = 0.70
- **Power (1–β)** = 90%
- **α** = 0.05
- **Design**: Paired t-test (pre–post change)
- ➤ **Required = 23 evaluable subjects**

Adjust for 20% dropout

Approximate Formula for Sample Size in Paired t-Test

For a **two-tailed paired t-test**, the approximate sample size n required is:

$$n = \left(\frac{(Z_{1-\alpha/2} + Z_{1-\beta}) \cdot \sigma_d}{\Delta} \right)^2$$

Where:

- $Z_{1-\alpha/2}$ = Z-value for the desired significance level (e.g., 1.96 for α = 0.05)
- $Z_{1-\beta}$ = Z-value for the desired power (e.g., 1.28 for 90% power)
- σ_d = Standard deviation of the **difference** between paired measurements
- Δ = Expected mean difference (effect size × standard deviation)
- $\frac{\Delta}{\sigma_d} = d$ → This is **Cohen's *d*** (standardized effect size)

Simplified version using Cohen's d:

$$n = \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{d} \right)^2$$

For example:

- $\alpha = 0.05 \rightarrow Z_{1-\alpha/2} = 1.96$
- $\beta = 0.10 \rightarrow Z_{1-\beta} = 1.28$
- $d = 0.70$

$$n = \left(\frac{1.96 + 1.28}{0.70} \right)^2 = \left(\frac{3.24}{0.70} \right)^2 \approx (4.63)^2 \approx 21.4$$

A**pproximately 22–23 participants** are required.

This single-arm pilot study aims to evaluate the effect of the investigational nutraceutical on muscle strength, measured as changes in one-repetition maximum (1RM) in elderly male participants. A relevant reference is the randomized trial conducted by Kim et al. (2023), which assessed resistance training intensity in elderly adults and reported statistically significant improvements in lower-limb strength and functional performance after 12 weeks. In that study, differences in 1RM gains across intervention arms corresponded to **standardized effect sizes ranging from *d* = 0.60 to 0.75**, depending on training intensity and muscle group.
Based on this precedent, and in consideration of our study's nutraceutical-based intervention without formal resistance training, a **conservative within-subject effect size of *d* = 0.70** is considered appropriate for this pilot. Using G*Power 3.1.9.7 software for a **paired-sample t-test** with **two-tailed α = 0.05**, **90% power**, and **Cohen's *d* = 0.70**, the estimated **sample size is 23 evaluable participants**.
To accommodate an expected **20% dropout rate**, the study will enrol **29 participants**.

Reference:
Kim S, et al. *Effects of Resistance Training Intensity on Muscle Strength and Functional Performance in Elderly Adults: A Randomized Trial.* Appl Sci. 2023;15(2):757. https://www.mdpi.com/2076-3417/15/2/757

11.2 Statistical analysis plan

General Principles

All statistical analyses will be performed using IBM SPSS Statistics (Version 30) or an equivalent validated statistical software package. All statistical tests will be two-sided with a significance level (α) of 0.05. Given the exploratory, proof-of-concept nature of this single-arm pilot study, the emphasis will be on estimation of effect sizes and confidence intervals, with hypothesis testing considered supportive and descriptive.
Continuous variables will be summarized using descriptive statistics: number of observations (n), mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages.

Analysis Populations

- **Enrolled Population**
- All participants who sign informed consent and are enrolled into the study.
- **Safety Population (mITT)**
- All enrolled participants who receive at least one dose of the investigational product. This population will be used for all safety and tolerability analyses, and for compliance assessments.
- **Per-Protocol (PP) Population**
- All participants in the Safety Population who:
    - complete the Day 60 visit (within the allowed window),
    - have primary endpoint data at both baseline and Day 60, and
    - have no major protocol deviations that, in the opinion of the investigator and/or statistician, may significantly impact primary efficacy outcomes, and
    - demonstrate treatment compliance of **≥90%** (see compliance definition in Section X.7).

The primary efficacy analyses will be based on the PP Population, with sensitivity analyses performed on the Safety (mITT) Population where applicable. Safety analyses will be based on the Safety Population.

Participants who are enrolled but do not receive any dose of the investigational product will be listed separately under the category "Treatment not given" and will not be included in efficacy or safety summaries.

Definition of Endpoints

Primary Efficacy Endpoint

- **Change in 6-minute walk distance (6MWD, meters)** from baseline (Screening) to Day 60.

Key Secondary Efficacy Endpoints

Changes from baseline to Day 60 in:

1. **Muscle Function**
    - 1-RM knee extension strength: peak torque / power and rate of torque development
    - DXA-based muscle volume (pre-specified muscle group/region of interest)
2. **Cognitive Performance**
    - NIH Toolbox Fluid Composite Score (executive function, attention/processing speed, working memory)
    - Cognitive biomarker: plasma p-tau181
3. **Immune and Inflammatory Function**
    - Immunological markers: CD4/CD8 ratio, CD45, CD3, CD4, CD8, T cells, B cells, NK cells, lymphocyte/neutrophil ratio
    - Inflammatory markers: hs-CRP, TNF-α, IL-6

4. **Longevity / Energy / Oxidative Stress Markers**
    - MDA, SOD, NAD^+
5. **Anthropometric and Body Composition Parameters**
    - Body weight (kg), BMI (kg/m^2), waist, hip, chest circumference (cm)
6. **Clinical Laboratory Parameters**
    - Complete blood count (CBC)
    - Renal function tests (RFT)
    - Liver function tests (LFT)
    - Thyroid profile
    - Lipid profile
    - Electrolytes

Safety and Tolerability Endpoints

- Incidence, frequency, and severity of **adverse events (AEs)** and **serious adverse events (SAEs)**.
- Relationship of AEs/SAEs to study product (related / possibly related / not related).
- Overall tolerability based on investigator and participant assessment (e.g., well tolerated / moderately tolerated / poorly tolerated).

Compliance Endpoint

- **Compliance (%)** will be calculated as:

$$\text{Compliance (\%)} = \frac{\text{Total dose used (or capsules taken)}}{\text{Total expected dose over the treatment period}} \times 100$$

Descriptive statistics (mean, SD, median, range) will be provided for compliance. The number and percentage of participants with Compliance ≥90% will be reported. Only participants with Compliance ≥90% will be included in the PP efficacy analyses.

Baseline Characteristics

Baseline demographic and clinical characteristics (e.g., age, sex, BMI, comorbidities, baseline 6MWD, SPPB, MoCA, intrinsic capacity score) will be summarized for:

- Enrolled Population
- Safety (mITT) Population
- PP Population

No formal statistical testing of baseline differences will be performed, as this is a single-arm study; summaries are descriptive and used to characterize the sample.

Primary Efficacy Analysis

The primary endpoint is the **change in 6MWD (meters) from baseline to Day 60**.

1. **Derivation of Change Score**

- For each participant, change in 6MWD = 6MWD at Day 60 − 6MWD at baseline (Screening).

2. **Descriptive Analysis**
   - The change in 6MWD will be summarized using n, mean, SD, median, minimum, maximum, and **95% confidence interval (CI)** for the mean change.
3. **Inferential Analysis**
   - Normality of the change scores will be assessed using the Shapiro–Wilk test.
   - If approximately normally distributed, a paired Student's t-test will be used to evaluate whether the mean change from baseline to Day 60 differs from zero.
   - If normality assumptions are not met, the Wilcoxon signed-rank test will be used instead.
4. **Responder Analysis (Exploratory Within Primary Endpoint)**
   - A responder may be defined as a participant with an improvement in 6MWD of ≥30 meters from baseline to Day 60.
   - The proportion of responders will be summarized descriptively (n, %) with 95% CIs (e.g., Clopper–Pearson) and may be used for exploratory interpretation of clinically meaningful benefit.

The primary analysis set for this endpoint will be the PP Population.

Secondary and Exploratory Efficacy Analyses

All secondary and exploratory endpoints will be analyzed in a similar manner, focusing on within-subject change from baseline to Day 60.

Continuous Secondary Endpoints

For each continuous secondary endpoint (e.g., 1-RM strength, DXA based muscle volume, NIH Fluid Composite, biomarkers, anthropometrics, laboratory measures):

1. **Change Score**
   - Change = value at Day 60 − value at baseline.
2. **Descriptive Summary**
   - n, mean, SD, median, minimum, maximum, and 95% CI for the mean change.
3. **Inferential Testing**
   - Normality assessed via Shapiro–Wilk test.
   - If normal: **paired t-test** for change from baseline.
   - If non-normal: **Wilcoxon signed-rank test**.
4. **Data Transformation (if needed)**
   - Skewed biomarkers (e.g., hs-CRP, IL-6, TNF-α, MDA) may be **log-transformed** prior to analysis. In such cases, analyses will be based on transformed values, and descriptive statistics may be presented for both raw and transformed data as appropriate.

No multiplicity adjustment will be applied; these analyses are considered **supportive and exploratory**.

Correlation and Exploratory Relationship Analyses

Exploratory analyses may be performed to evaluate associations such as:

- Relationship between change in 6MWD and changes in:
  - NIH Fluid Composite score
  - muscle strength and muscle volume
  - immune and inflammatory markers
  - oxidative stress and NAD^+ levels

Depending on data distribution:

- **Pearson correlation** for approximately normal variables
- **Spearman rank correlation** for non-normal variables

Simple linear regression models may be used exploratorily to assess whether changes in selected biomarkers or strength measures predict change in 6MWD. These analyses will be considered exploratory and hypothesis-generating only.

Safety, Tolerability, and Compliance Analyses

Adverse Events

All AEs and SAEs reported by participants or observed by investigators from first dose until end of study will be coded using a standard terminology (e.g., MedDRA, if applicable).

AEs/SAEs will be summarized by:

- number of events
- number and percentage of participants experiencing at least one event
- severity (mild, moderate, severe)
- seriousness (serious / non-serious)
- relationship to investigational product (related / possibly related / not related)

Summaries will be presented for the Safety (mITT) Population. No formal hypothesis testing is planned; however, descriptive comparisons of proportions (e.g., between tolerability categories) may be conducted using Chi-square test or Fisher's exact test, where appropriate.

Clinical Laboratory Parameters, Vital Signs, and Other Safety Parameters

Continuous safety-related variables (e.g., hematology, biochemistry, thyroid, lipid profile, electrolytes) will be summarized at baseline and Day 60, and change from baseline will be described using descriptive statistics. Potentially clinically significant abnormalities will be flagged and listed.

Compliance

Descriptive statistics (mean, SD, median, range) will be provided for compliance. The number and percentage of participants with Compliance ≥90% will be reported. Only participants with Compliance ≥90% will be included in the PP efficacy analyses.

Handling of Missing Data

No formal imputation of missing data is planned for primary analysis. Analyses will generally be based on **observed cases**:

- Participants with missing baseline or Day 60 values for the primary endpoint will not contribute to the respective change analysis in the PP Population.
- For sensitivity purposes, where appropriate, Last Observation Carried Forward **(LOCF)** may be used for participants who discontinue after at least one post-baseline assessment; such analyses will be clearly labeled as sensitivity analyses.

The extent and pattern of missing data will be summarized descriptively and may be explored to assess whether missingness appears random or associated with baseline characteristics.

Interim Analysis

A single interim review may be conducted after a subset of participants completes the Day 30 visit. The purpose of this interim review will be to evaluate:

- feasibility and recruitment
- protocol adherence and data quality
- safety signals and tolerability
- variability of primary and secondary endpoints endpoint

During the interim analysis, the primary and secondary endpoints may be analyzed using the same descriptive and within-subject change approach (paired t-test or Wilcoxon) to assess trends. These analyses will be exploratory only, without inferential conclusions or decision-making.

Statistical Analysis Plan Finalization

A more detailed internal Statistical Analysis Plan (SAP), if required, may elaborate on:

- data handling conventions
- outlier handling
- definitions of protocol deviations and PP inclusion
- derivation rules for composite or derived variables

This SAP will be finalized prior to database lock and prior to performing the final statistical analyses, and will be archived with the study documentation.

12. DOCUMENTATION

12.1 Quality Control and Quality Assurance

Sponsor through the project management organization has a plan in place for the quality of the research being conducted, which describes: 1) How data will be evaluated for compliance with the protocol and for accuracy in relation to source documents; 2) The documents to be reviewed (e.g. CRFs, source documents, product accountability, final study report), who is responsible, and 3) The frequency for reviews will be identified, either in a formal quality management plan or in-house SOPs. Methods of staff training will be specified. The final report will contain a statement for quality assurance (QA) duly signed by the head or designated person of the quality assurance department.

12.2 Data Confidentiality and Audits

Participant confidentiality along with the information disclosed/provided/produced during study will be deidentified before submitting to sponsor and other regulatory authorities.

The investigator will permit study-related monitoring which is planned, audits, and inspections by the IRB, sponsor or its designated study personnel, licensing authority, and QA groups of all study-related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.).

13. ETHICAL CONSIDERATIONS

13.1 Ethical Conduction and Regulatory Compliance

The present study will be conducted in accordance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Participants, 64th World Medical Association – General Assembly, Fortaleza, Brazil, October 2013), ICH-GCP, New Drugs and Clinical Trials Rules, 14 October 2022, Ministry of Health and Family Welfare, Government of India, Indian Council of Medical Research (Ethical Guidelines for Biomedical Research on Human Participants, 2017) and other applicable regulatory requirements.

13.2 Ethics Committee Approval

The study protocol and related study documents [including English and vernacular informed consent forms (ICFs), translation and back-translation certificates, and drug-related literature] will be submitted to, and approved by an IRB in writing. The study will commence only after obtaining the written approval of the study protocol and its related documents by the IRB, with or without modifications.

13.3 Clinical Trial Registry

After receiving at least one ethics committee approval for the study protocol then the proposed study will be registered with the clinical trial registry of India (www.ctri.nic.in) before enrolment of the first study participant.

13.4 Protocol Amendments

All protocol amendments, interfering with the participant's health interests and involving changes in the design of the study or its scientific significance will be implemented only after written approval of the sponsor and IRB. All such changes will be documented in the amended version of the protocol and a list of changes with reference to the previous version will be generated. Protocol amendments only for logistical or administrative changes may be implemented immediately, and the amendments will be informed/ notified to IRB.

13.5 Informed Consent Process

At screening, all the participants participating in this study will receive study information, verbal and written, in their vernacular/understandable language about the purpose and nature of the study and its procedures as well as potential risks and benefits associated with study drugs as per the participant information sheet (PIS), prior to participation in the study. Participants will be provided enough time and opportunity to read the PIS-ICF. Participants will be encouraged to ask questions and clarify their doubts before signing or agreeing for signing the PIS-ICF in the presence of an investigator or qualified medical personnel.

The legally acceptable representative (LAR) must sign the informed consent form wherever applicable.

Written informed consent will be obtained from each participant according to good clinical practice ICH-GCP, the ethical principles that have their origin in the Declaration of Helsinki, and the regulatory and legal requirements of India.

A copy of the signed and dated written PIS-ICF will be provided to the participant.

13.6 Participant Participation Compensation

- In case of an injury occurring to the participant during the clinical trial, free medical management shall be given as long as required or till such time it is established that the injury is not related to the clinical trial, whichever is earlier.
- The participants will be covered under clinical trial liability insurance for medical management.
- In the event of a trial-related injury or death, the sponsor or its representatives or the investigator or center, as the case may be, in accordance with the "New Drugs and Clinical

Trials Rules, 2022, Department of Health and Family Welfare" issued by the central licensing authority, shall provide financial compensation for the injury or death.

13.7 Compensation to The Participants in Clinical Trial Injury

- Compensation for clinical trial–related injury or death will be provided in accordance with the New Drugs and Clinical Trials (NDCT) Rules, 2019. If a participant suffers any physical injury, permanent disability, or death that is determined to be causally related to the study intervention, procedures, or protocol violations, the Sponsor will provide free medical management and financial compensation as per the prescribed regulatory formula and timelines. The decision regarding causality, eligibility, and compensation will be reviewed by the Ethics Committee and the Sponsor, and reported to the Licensing Authority as required under the NDCT framework.

13.8 Insurance Policy and Finance

The study will be covered by an appropriate insurance contract. Provision will be made for insurance to cover the costs of treatment in the event of trial-related injuries in accordance with the Indian regulatory requirements.
The sponsor should provide all study-related finance as per the approved budget.

14. RECORDING OF DATA, REPORT PREPARATION, AND ARCHIVAL

14.1 Record Keeping

The preparation of the clinical trial protocol is in compliance with ICH-GCP, SPIRIT guidelines. All other EC dossier documents such as ICD, CRF, PIS, IPIB, study logs, and participant files will be prepared as per ICH-GCP guidelines. Data collected on CRFs during the study will be documented and identified by the participant number, the participant's initials, and the study code. The investigator will abide to keep the identity by the participant confidentiality agreement, except if this information is to be disclosed to assess the safety of the participant or for regulatory purposes. The completed CRFs will be checked by the investigator or designee for completeness, accuracy, and legibility, for conformance to the source documents.

14.2 Final Study Report

The final study report will be prepared as per the ICH-GCP guidelines and will be submitted in electronic format.

14.3 Quality Assurance

All the data related to the study will be checked by the QA before archiving.

14.4 Record Retention and Archiving

All the raw and final data generated in connection with this study will be retained in accordance with national legislation or as per the sponsor's requirement, whichever is later. All study-related documents will be archived, which include the following either at sites and/or sponsor as applicable):

- IRB approval for the study protocol and its amendments, if any;
- CRFs;
- Signed Informed Consent forms (deidentified);
- Study Protocol and Study Report;
- Study Master File;
- Any other relevant study documents.

15. TERMINATION OF THE STUDY

The investigator, sponsor or IRB reserve the right to terminate the study at any time on a safety issue. If the investigator terminates or suspends the study without the prior agreement of the sponsor, the investigator will promptly inform the sponsor and IRB and provide a detailed written explanation of the termination or suspension.

If the sponsor prematurely terminates or suspends the study, or one or more investigative sites participating in the study, the investigator must promptly inform the IRB. The justification for the termination will be reported to the IRB within 24 hours and to the central licensing authority within 30 working days of such termination. The reason for study termination will also be provided to the participants. A summary report of the terminated study will be submitted to the central licensing authority within 3 months.

16. PUBLICATION

The sponsor will hold the right to publish the results of the present study at any time. Any information published will not reveal the identity of the participant and their confidentiality will be maintained. Investigators may publish or present the results of this study, in either written or oral format, only after obtaining the written permission of the sponsor.

17. IPR VALUE

The sponsor will hold the right to share IPR rights (if applicable) as per their policy.

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APPENDICES

Appendix-A: Investigator Approval for Protocol

Appendix-B: Schedule of Study Events

APPENDIX-A: INVESTIGATOR APPROVAL FOR PROTOCOL

I, the undersigned, have read and understood this protocol, and hereby agree to conduct the study in accordance with current protocol, Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Participants, 64th World Medical Association – General Assembly, Fortaleza, Brazil, October 2013), Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice, E6(R2), Current Step 4 version dated 9 November 2016, New Drugs and Clinical trials Rules, October 2022, Central Licensing Authority, Ministry of Health and Family Welfare, Government of India, Indian Council of Medical Research (Ethical Guidelines for Biomedical Research on Human Participants, 2017) and applicable regulatory Guidelines.

I agree to comply with all relevant standard operating procedures required for the conduct of this study and to ensure that all associates participating in the conduct of this study are informed regarding their obligations.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical review of the trial, without written authorization from **GP Life Healthcare Pvt. Ltd.**

I understand that the trial can get suspended or prematurely terminated due to valid reasons and will be communicated to all stakeholders. I understand my responsibility to communicate immediately in written, if in case my participation in the trial needs to be discontinued.

Name of the Principal Investigator	Signature and date

APPENDIX-B: SCHEDULE OF STUDY EVENTS

Table 6: Schedule of study events

Parameter / Day	Screening	Day 1	Day 30	Day 60
Informed Consent	√			
Demographics	√			
Medical History / Previous Medication	√			
Concomitant Diseases/ Medication	√			
Inclusion /Exclusion Criteria Review	√			
Randomization		√		
Dispensing of IP		√	√	
Clinical examination	√	√	√	√
Physical Examination	√	√	√	√
Vitals	√	√	√	√
Assessment of adverse events		√	√	√
Assessment of rescue medications		√	√	√
Tolerability			√	√
Compliance of IP			√	√
Assessment of 6-minute walk distance (6MWD) (meters)	√			√
Assessment of 1RM (kg) with the leg press	√			√
Assessment of Muscle volume	√			√
Assessment of Fluid Composite score and assessment of executive function, attention and processing speed, working memory	√			√
Assessment of plasma p-tau181	√			√
Assessment of CD4/CD8 ratio, CD45, CD3, CD4, CD8, T cells, B cells, NK cells, lymphocyte/neutrophil ratio	√			√
Assessment of serum hs-CRP, TNF-alpha, IL-6	√			√
Assessment of plasma MDA and SOD, NAD+ from whole blood	√			√
Assessment of Body Weight (kg), BMI (Kg/m^2) and hip, waist, chest circumferences (cm)	√			√
Assessment of complete blood count (CBC), Renal Function Test (RFT). Liver Function Test (LFT), Thyroid profile, lipid profile, electrolytes.	√			√