Research Article

Design of an Effective Nutraceutical (HR15) for Physical Performance and Endurance

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Abstract

The global dietary supplements market has expanded, offering products for athletes that promise enhanced well-being and sports performance. However, standard definitions and regulatory frameworks are lacking, highlighting the need for high-quality scientific research. Rigorous methods are essential to differentiate strong findings from weaker studies. Key considerations in designing quality studies for athlete supplements include power calculations, characterization of supplement components, participant selection, outcome measure justification, error assessment, and blinding techniques. Natural ingredients showing promise in sports performance include *Rhodiola rosea*, which reduces stress and fatigue, and *Rhaponticum carthamoides*, which enhances strength and muscle recovery. Fatty acids like conjugated linoleic acid and medium-chain triglycerides benefit muscle damage reduction, endurance, and body mass regulation. HR15, a novel supplement, was developed with strategies to enhance bioavailability using lipid-based vehicles, addressing solubility, stability, and permeability challenges. Preclinical studies in *Caenorhabditis elegans* indicated increased lifespan, antioxidant effects, and metabolic pathway alterations. Clinical trials showed that HR15 ingredients improved antioxidant defenses, reduced oxidative damage, enhanced emotional well-being, and mitigated oxidative stress, inflammation, muscle damage, and fatigue in active individuals. These findings support HR15's potential to improve sports performance and well-being, advocating for safer and more effective supplements for athletes.

Keywords: *Rhodiola rosea; Raphonticum carthamoides*; Fatty acids; Sport ; Food supplements

Introduction

Although more and more nutritional supplements are sold aimed at "athletes" (global dietary supplements market estimated to be $278 billion by 2024) who have wellness motivations or improvements in sports performance, there is still a lack of a global definition of each type of product and, of course, uniform regulations that define their composition and characteristics [1].

A step forward in scientific evidence will undoubtedly be to increase the publications of high quality scientific studies in this field in order to improve the available evidence to guide health professionals, industry leaders, regulatory agencies and consumers and, at the same time, reduce the risk of exaggerated interpretations of the findings, which will be achieved with well-designed studies to differentiate between findings based on solid evidence and misleading conclusions derived from weak descriptive studies. It is therefore necessary to improve the quality of the growing literature on supplements for athletes in a context where the number of publications can make it difficult to evaluate the quality of the available evidence [2]. The research process on nutritional supplements for athletes begins with the precise formulation of the research question and an exhaustive review of the literature to ensure its uniqueness and relevance. Furthermore, it is essential to identify a specific mechanism of action to avoid misinterpretation of chance findings.

To carry out a high-quality study in the field of supplements for athletes, essential and essential aspects are established, such as calculating the power of the study; characterize the components of the supplement and its preparation, ensuring safety and avoiding contamination; justify the choice of participants and measure their compliance; identify primary and secondary outcome measures, justifying their choice; evaluate specific technical measurement error and consider significant interindividual differences; implement double-blind questionnaires for the intervention, maintaining blinding until statistics are completed, or their absence justified; and, in addition, optional but beneficial aspects are suggested to improve the quality of the study, such as using biochemical markers to verify compliance; study the metabolism of the product, including its metabolites; and explore gender-specific responses to the supplement. Detailed consideration of these essential and optional aspects will contribute to the quality, validity and relevance of studies in this field, providing a more solid basis for decision making in both research and clinical practice [3,4].

All of the above has led to the conclusion, in a consensus statement from the International Olympic Committee (IOC), that randomized controlled trials in high-level athletes with an adequate number of participants, rigorous controls and procedures are urgently needed for sports supplements, appropriate supplementation protocols and clinically meaningful measurement tests [5].

Material and Methods

Natural products for low physical performance and endurance

In recent years, there has been growing interest in the use

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of natural products to enhance physical performance and endurance.

Rhodiola rosea (Rhodiola): *Rhodiola rosea*, as a supplement, has been shown to be beneficial in improving human exercise endurance and exerts various positive effects on physical performance. This herb has adaptogenic properties, meaning it can help the body adapt and cope better with stress, reducing fatigue and improving endurance during physical activity [5].

However, there is a research challenge due to the variability in commercial production of *Rhodiola rosea* products. Growing, harvesting, and extraction methods can significantly influence the chemical composition and quality of supplements available on the market, making standardization and comparison between studies difficult [6]. Despite these difficulties, evidence suggests that *Rhodiola rosea* supplementation may offer significant benefits for those who exercise. It has been observed that it can reduce pain and muscle injuries associated with exercise, in addition to reducing oxidative stress in muscle tissue. Anti-inflammatory effects have also been identified, which could contribute to the protection of skeletal muscle and improved recovery after physical exercise [7].

Additionally, this supplement has been shown to improve energy metabolism by increasing the synthesis of key molecules such as Adenosine Triphosphate (ATP) and creatine phosphate, which helps prolong endurance during exercise and promote faster recovery after exercise. Intense physical effort [8]. Although more research is needed to better understand its mechanisms of action and ensure consistency in product quality, available studies suggest that *Rhodiola rosea* may be a safe and effective option as a supplement to improve sports performance and reduce negative impact. of intense exercise on the body [9-11].

***Rhaponticum carthamoides* (Willd.) Iljin:** Among the plants rich in phytoecdysteroids is the endemic perennial *Rhaponticum carthamoides* (Willd.) Iljin from the *Asteraceae* family, commonly known as maral root or Russian leuzea [12]. In traditional medicine, it has been used to improve physical strength [13]. In 1969, Brekman and Dardimov systematized leuzea as one of the plant adaptogens and in recent decades, extracts from its rhizomes and roots have been used for physical weakness and to promote muscle growth. The main classes of chemicals isolated from the rhizomes and roots of *R.carthamoides* are not only phytoecdysteroids but also phenolic compounds (flavonoids and phenolic acids). Previous phytochemical reports on *R. carthamoides* ecdysteroids from the underground parts of the plants revealed the isolation of 20-hydroxyecdysone, also known as Ecdysterone (20E), Ponasterone A (PA), and Turkesterone (TU) [14]. Ecdysteroids are a group of polyhydroxylated sterols, structurally similar to androgens and possess important anabolic properties [15]. However, the similarity of the molecular structures of the ecdysteroids present in the extracts together with the high content of unrelated compounds of similar chromatographic characteristics makes the optimization of the separation, identification and isolation of ecdysteroids a difficult analytical task [16].

In 1976 it was shown that the introduction of phytiexdizone-exdysterone (0.5 mg/100 g) from *R. carthamoides* in rats for 7 days is accompanied by an accelerated increase in the tibialis anterior muscle with an increase in the total amount of protein. All the changes mentioned above are more marked when the substance is administered to growing rats (70-80 g). In experiments with castrated sexually immature rats, the androgenic action of exdysterone is not demonstrable [17].

It has been suggested that *R. carthamoides* inhibits disorders of energy metabolism (NAD+/NADH ratio, ATP and LD) and oxidative stress (SOD, ROS, etc.) in H9C2 cells and, in addition, increases the expression of *SIRT6* and *NRF2*. Next, mechanistic studies found that *SIRT6* knockdown reduced *NRF2* expression as well as the effects of the extract on ATP, LD, and ROS levels, while *NRF2* activation enhanced the effects of *R. carthamoides* on pod cells exert their effects through the *SIRT6*-mediated *NRF2* signaling pathway [18].

Fatty acids: Many athletes use a combination of fatty acids (Conjugated Linoleic Acid (CLA) and long and medium chain triacylglycerols) as it is claimed that these ergogenic aids are associated with a reduction in muscle glycogen breakdown, improved endurance capacity, reduction in body mass and a reduction in muscle damage and inflammatory responses [19]. Recent results suggest a new role for CLA supplementation in testosterone biosynthetic pathways, which improves maximal voluntary strength by increasing muscle mass and not by changing contractile properties, produces an increase in performance in sprinting by regulating neuromuscular transmission and affects endurance performance as it induces an increase in hemoglobin concentration and hematocrit [20]. Studies have been published that conclude that the effects of CLA in sport are produced, mechanistically, by the reduction of muscle glycogen degradation along with a decrease in body mass and muscle damage and inflammatory responses so that CLA would increase lean body mass and reduce body fat [21-23].

The effects of Medium Chain Triglycerides (MCTs) on energy expenditure, food intake, and fat deposition have been well researched and studies in humans and rodents have shown that MCTs also play a role in food intake and satiety [24]. Unlike Long-Chain Triglycerides (LCTs), MCTs will be broken down into glycerol and fatty acids, which will be directly absorbed into the bloodstream and thus transported to target organs [25]. It is this metabolic particularity that supports its role in muscle function and exercise performance [26]. Other studies on systemic hemodynamics, physical strength, and cardiac function during physical activity have demonstrated the role of certain fatty acids such as gamma-linolenic acid and oleic acid on hemorrheology, vascular function, inflammation, and the potential to improve cardiac function and physical performance [27].

Results and Discussion

Development of HR15

It is known that numerous factors affect the oral administration of different classes of isolated bioactive components (nutraceuticals), including phytochemicals, which is why different strategies are being addressed to improve their oral bioavailability including bioaccessibility, absorption and transformation [28]. Our main concern in formulating HR15 was to review the potential of the approaches used in oral administration trying to discover new strategies that could be

applied to bioactive compounds to overcome the obstacles in oral nutraceutical administration considering not only the physicochemical and physiological factors that affect the oral administration of nutraceuticals but others such as solubility, stability and intestinal permeability, which are the main factors that prevent the effective release of bioactive phytochemical compounds, so we proposed an administration system designed to overcome specific factors that can affect bioactive ingredients in particular [29].

"Phytochemicals" are a large group of compounds derived from plants, which have been studied for their potent nutraceutical activity although oral administration approaches must overcome solubility problems, provide a sufficient dose to overcome partial metabolism and increase the epithelial permeability [30].

Solubility is one of the first obstacles that must be overcome in the oral administration of bioactive molecules, so *in vitro* measurements of solubility and permeability (as suggested by the Food and Drug Administration (FDA) after adopting the Biopharmaceutical Classification System (BCS) in 1995) is the first strategy when formulating a nutraceutical [31]. In the development of HR15, we also took into consideration the proposal of McClements et al., who developed the Nutraceutical Bioavailability Classification System (NuBACS), taking into account the main problems that affect their oral bioavailability. NuBACS introduces the concept of "bioaccessibility", the ability of the bioactive compound to be accessible to the body for absorption from the delivery matrix [32].

Degradation and metabolism are obstacles that an oral delivery system must overcome once solubilization has been achieved. The Biopharmaceutical Drug Disposition Classification System (BDDCS) takes into account drug metabolism through phase I and II processes and is useful for predicting drug interactions that may occur in the intestine and liver [33].

Nutraceuticals may have a limited ability to penetrate the intestinal wall and before reaching the epithelia, the bioactives must pass through the intestinal mucus. Mucus is a complex hydrogel consisting of a mixture of glycoproteins, lipids, and desquamated epithelial cells. Interaction with mucus reduces the permeability of large molecules due to steric blocking [34]. Lipophilic bioactives can be transported through the mucus layer by mixed micelles formed from bile salts, phospholipids and free fatty acids [35].

Following diffusion through mucus, there are several routes by which a bioactive agent can penetrate the intestinal epithelium. Transport by the paracellular route requires movement through tight junctions and reduces the risk of intracellular metabolism, which is relevant for phytochemicals. Transcellular transport involves molecules crossing the apical membrane by passive diffusion, receptor mediation, or endocytosis. The epithelium of the small and large intestine has a multitude of transporters located in the apical membrane that play roles in nutrient uptake and absorption, so these membrane-bound proteins are relevant to the absorption of many nutraceuticals [36].

For the development of HR15 we assumed that a delivery vehicle can control the delivery and release of the bioactive components of the nutraceutical. Due to the obstacles that must be overcome to orally administer a biologically effecting dose of a nutraceutical, delivery vehicles are of increasing interest especially when based on the use of safe, food-grade ingredients [37]. In HR15 we have chosen a lipid-based system that has been shown to enhance intestinal permeation by improving mucodiffusion and paracellular and transcellular permeability of phytochemicals.

In this regard, it should be noted that among the intestinal permeation enhancers that increase oral bioavailability are MCTs and particularly capric and caprylic as well as oleic acid that act by reorganizing proteins in the epithelial tight junction (for example, tricellulin and claudin-5) and through a mild detergent fluidizing effect on the plasma membrane allowing even poorly permeable molecules to transiently penetrate through tight junctions or possibly become trapped in mixed micelles with the ability to cross lipid bilayers [38].

MCT generally cause a reduction in Transepithelial Electrical Resistance (TEER) using *in vitro* and *ex vivo* intestinal epithelial models. This reduction suggests an opening of tight junctions or a disturbance of the epithelia. Furthermore, they have shown a significant increase in the apparent permeability of markers of paracellular transport across the intestinal mucosa. Progress in the use of MCT in nutraceutical formulations has also addressed safety issues that may be associated with increased oral bioavailability [39].

Furthermore, oleic acid can modulate tight junction integrity *in vitro* by improving permeability by opening tight junctions and increasing jejunal absorption [40]. Therefore, the use of MCT and oleic acid in a nutraceutical has the potential to improve the permeability of bioactive compounds and improve oral bioavailability. From the results of our studies we can conclude that the lipid carrier of HR15 is a clear advance in the adoption of nutraceutical strategies for oral administration to improve the solubility, stability and permeability of botanical bioactives. In particular, this solubilization technology overcomes the problems associated with the administration of hydrophobic compounds [41].

Improving absorption is an area that until now had not been used to improve the administration of oral nutraceuticals and in this sense HR15 constitutes a world novelty due to the use of a lipid vehicle that improves the absorption of lipophilic and hydrophilic compounds, through a combination of food grade ingredients that guarantees, in addition to its effectiveness, its safety. In addition to this lipid vehicle, two bioactive ingredients are used that have proven effective in human studies.

Rhodiola rosea is a plant whose bioactive components can function as adaptogens, thereby increasing resistance to stress and improving overall resilience. Some of these effects may influence exercise performance and adaptations by modulating energy substrate reserves and utilization, reducing muscle fatigue and damage, and altering antioxidant activity. However, the variability in the dose and duration of supplementation, the concentration of bioactive compounds, the characteristics of the participants, the exercise tests and the statistical considerations make the published studies disparate. It is for this reason and to explore the entire ergogenic effect of *Rhodiola* that we have

carried out specific clinical studies to establish the conditions in which supplementation facilitates performance and adaptations to exercise with a *Rhodiola rosea* extract standardized to 5% rosavin [42].

Another of the functional ingredients to formulate HR15 was an extract of *Rhaponticum carthamoides* titrated to 10% hydroxyecdysone for its adaptogenic and tonic properties that promote muscle growth and increase the body's resistance to stress, such as trauma and fatigue and with perspective. to improve muscle health and, in particular, obtain significant improvements in muscle strength and quality, as well as achieve a reduction in fat mass and an increase in muscle mass. In the last century, the qualities of Rhaponticum for muscle and strength development have been widely investigated in Russia, where various preparations to increase physical and psychological performance were commonly used by elite Soviet and Russian athletes who were exhausted by hard training. that ecdysteroids affect certain important metabolic pathways in mammals: Protein synthesis, lipid metabolism and carbohydrate metabolism. Several research studies, currently not available in English, suggest that phytoecdysteroids possess a broad spectrum of biological, pharmacological, and medicinal properties in mammals without androgenic effects [43,44].

The idea of combining both extracts arose after verifying the results of a study in rats, which was the first published work on the association of these two extracts with performance in resistance exercises [45]. Resistance exercise remains the best way to stimulate protein synthesis and induce chronic muscle adaptations. The aim of this study was to investigate the acute and chronic effects of resistance exercise together with R. rosea and *R. carthamoides* (Rho+Rha) supplementation on protein synthesis, muscle phenotype and physical performance. Findings from the acute study indicated that Rha and Rha+Rho supplementation after resistance exercise stimulated protein synthesis more than resistance exercise alone. After 4 weeks of training, average power performance increased in the Rha+Rho and Rha alone groups without any significant effect of supplementation on muscle weight or fiber cross-sectional area. A trend towards an increase in the proportion of type I/type II fibers was observed in the Rha/Rho treated groups compared to the trained control group. In conclusion, it was established that supplementation with *Rhodiola* and *Rhaponticum* after resistance exercise could synergistically improve protein synthesis, muscle phenotype and physical performance.

To verify the effectiveness and safety of the HR15 product ingredients, we reviewed three preclinical studies and two clinical studies conducted by San Antonio technologies, a research center associated with the Catholic University of Murcia. These studies had investigated, among others, the specific ingredients contained in HR15. Our goal was to propose a final composition of bioactive ingredients and determine their optimal doses. By analyzing the results of these studies, we were able to integrate the individual findings and speculate on how they might work together. San Antonio Technologies conducted the reviewed studies. The involvement of a CRO (Contract Research Organization) ensures that studies meet high scientific and ethical standards.

Based on the data obtained from the studies, it can be determined which bioactive ingredients should be included in HR15 and at what dosage. This involves analyzing both the individual effects of each ingredient and their possible synergy when combined. By combining the results of preclinical and clinical studies, you get a more complete view of how the ingredients work together. This allows inferences to be made about their joint effectiveness and possible interactions, improving the final formulation of the product.

In summary, this detailed review of preclinical and clinical studies on the ingredients of HR15 made it possible to scientifically substantiate the final composition of the product, ensuring its safety and effectiveness before its launch on the market.

Preliminary preclinical and clinical results obtained with the ingredients of HR15

Preclinical studies: These studies are carried out in animal models or in the laboratory before testing the ingredients in humans. They help understand the mechanisms of action, effectiveness and safety of the ingredients.

In the first preclinical study, the activity of some components of HR15 on the lifespan of *C. elegans* was studied. The agar plates (Nematode Growth Medium (NGM)) used for the *C. elegans* feeding experiments were prepared with 2 mg/mL ingredients by adding the product directly to the agar surface (200 µL of the stock at 100 mg/mL). The *C. elegans* wild-type strain was age-synchronized by recovering adult eggs on the corresponding agar plates, already seeded with *Escherichia coli* OP50. NG medium was used as a control diet, the HR15 ingredients were mixed obtaining a 100 mg/mL solution and added to the agar surface at 2 mg/mL (200 µL). Worms were incubated at 20°C for 26 days in the different media, periodically moved to new plates, and scored as dead if they did not respond to a platinum wire. The experiments were performed in duplicate. Survival curves were compared using the log-rank survival significance test, provided by the GraphPad Prism 4 statistical software package. The conclusion of this study was that ingredients in HR15 produce an increase in the lifespan of *C. elegans* in the dose of 2 mg/mL.

The second preclinical study on other HR15 ingredients determined antioxidant activity in the *Caenorhabditis elegans* model. The *C. elegans* wild-type strain was age-synchronized by recovering adult eggs on the corresponding agar plates, already seeded with *Escherichia coli* OP50. NG medium was used as a control diet, NGM supplemented with vitamin C (10 µg/mL) and NG with 2 mg/mL of HR15 ingredients. The worms were incubated at 20ºC for 7 days in the different media, transferring them every two days to new fresh plates. Subsequently, the worms were treated with hydrogen peroxide (2 mM) and after 5 hrs, the survival of the worms in each feeding condition was analyzed. The experiments were performed in triplicate. HR15 ingredients showed a significant antioxidant effect (23.5% increase in worm survival *versus* control conditions), allowing us to conclude that these ingredients significantly protect acute oxidative stress in the *C. elegans* model.

In the third study, *C. elegans* was analyzed transcriptomically to

study the genes, processes and metabolic pathways affected by some HR15 ingredients.

Agar plates (NGM) used for *C. elegans* feeding experiments were prepared with 2 mg/mL of HR15 ingredients by adding the product directly to the agar surface (200 µL of the stock at 100 mg/mL). Transcriptomic profiling of *C. elegans* fed HR15 ingredients revealed changes related to protein metabolism, energy metabolism, glutathione metabolism, fatty acid metabolism, and xenobiotic metabolism. Some of these transcriptomic changes would explain the antioxidant activity of these ingredients and the ability to increase the lifespan of *C. elegans* (Table 1).

Clinical trials: These studies are performed in humans and are essential to confirm the safety and efficacy observed in preclinical studies. Clinical studies may have several phases to evaluate different aspects of the ingredients, such as dosing and side effects.

In a first clinical trial, the effects of some ingredients present in HR15 were investigated in healthy volunteers. This was a single-center, randomized, placebo-controlled, double-blind, month-long study with 3 groups: Placebo (group A), once-daily supplement (group B), and twice-daily supplement (group C).

Additionally, the effects of subjects in arm B were evaluated after stopping intake (group B1) or 120 days of intake (group B2). Subjects were evaluated on days 1, 7, and 30 (37 and 44 for group B1; 60, 90, and 120 for group B2). The evaluation included antioxidant defenses, oxidative damage, inflammatory profile and change in emotional state. A total of 73 subjects were randomized, arm A=32, arm B=21 (group B1=11, group B2=10), arm C=20. The supplement significantly increased glutathione peroxidase levels (arms B and C), reduced glutathione (GSH) (arm C) and decreased oxidized glutathione (GSSG) (arms B and C). The GSH/GSSG ratio also showed a significant increase (group C).

The supplement also significantly increased the levels of vitamins C (group B and C; group B1) and E (group B2). Reduced levels of oxidative damage to proteins were observed (arms B and C). The supplement had a significant positive emotional effect on anxiety (group C), positivity (groups B and C), vitality (group C) and global score (group C). In conclusion, one month of supplementation increased antioxidant defense and reduced oxidative damage to proteins, with a good safety profile.

The second experimental nutritional study (randomized, crossover, with two study groups, double-blind and placebo-controlled) was carried out to evaluate the efficacy of other different components of the HR15 product in 35 male subjects who performed physical activity at least once a week. week, as an antioxidant, anti-inflammatory and anti-fatigue after performing physical activity of high intensity and long duration. In antioxidant results, significant differences were observed between placebo and ingredients of the HR15 product in the levels of 8 oxodG, carbonyl groups, oxidized glutathione and reduced/oxidized glutathione ratio. All the differences indicate that ingredients in the HR15 product reduce the oxidative damage generated by the stress test or improve the action of antioxidant defenses.

When the levels of total antioxidant capacity of the plasma were compared under basal conditions (before performing the tests) between the two study groups, it was seen that the consumption of ingredients from the HR15 product for 15 days increased the total antioxidant capacity of the plasma. In anti-inflammatory and muscle damage results, significant differences were observed between the placebo and ingredients of the HR15 product in the levels of creatine kinase and interleukin 6. The differences indicate that ingredients of the HR15 product reduce the muscle damage generated by the stress test.

Additionally, a reduction in muscle pain and damage was evident after exercise training, improving skeletal muscle damage, reducing oxidative stress, and improving athletic explosive power without reducing perceived exertion rating scores. In the fatigue study, significant differences were observed between placebo and ingredients of the HR15 product in the Borg scale score at minute 90 and in serum glucose results at the moments corresponding to minute 90 and minute 110. Using the Vitality subscale of the RAND-36, with cross-validation using the Visual Analogue Scale for Fatigue (VAS-F), a reduction in fatigue and an increase in resistance was observed, especially during periods of physical activity, suggesting a positive effect on athletic ability and sports performance. The differences between the groups indicate that the product contributes to reducing the fatigue generated by the stress test.

The conclusions of this study were that the consumption of HR15 ingredients for 15 days increases the total basal antioxidant capacity of the plasma, reduces the oxidative damage to DNA and proteins generated by a high intensity and prolonged stress test, reduces the damage muscle generated by said test and improve the athlete's fatigue during the performance of that same test.

In several studies carried out on a lipid mixture specifically designed as a carrier of bioactive ingredients for athletes, it has been shown how it affects hormonal signaling and the composition of cell membranes, in addition to influencing the molecular events that regulate genetic expression, thus impacting the distribution of metabolic fuels within and between organs. In addition, it has been shown to be able to regulate glucose, lean mass, total weight and adiposity, independently of factors such as physical activity and protein intake, which has a positive impact on body composition and long-term metabolic health. The lipid mixture appears to influence thermogenesis and energy distribution towards the accumulation of lean tissue, activating the sympathetic nervous system and increasing thermogenic activity in brown adipose tissue, contributing to the reduction of body fat accumulation.

It could also reduce the machinery for fat accumulation in white adipose tissue, improving the expression of peroxisome proliferator-activated receptors. It has also been shown how the lipid mixture reduces inflammatory patterns, particularly in muscles, by balancing the ratio of n6/n3 fatty acids and reducing protein oxidation, favoring protein retention and increasing lean mass. In addition, it could also influence hormonal secretion and sensitivity to anabolic hormones, as well as the regulation of protein synthesis and degradation in skeletal muscle (Table 2).

Table 1: Summary of preclinical studies

Study	Purpose	Methodology	Results	Conclusion
Study 1	To study the activity of HR15 components on the lifespan of *C. elegans*	Agar plates with 2 mg/mL HR15 ingredients; *C. elegans* incubated at 20°C for 26 days; survival curves compared using log-rank survival significance test	Increased lifespan of *C. elegans* at 2 mg/mL dose	HR15 ingredients increase the lifespan of *C. elegans*
Study 2	To determine antioxidant activity of HR15 ingredients in *C. elegans*	*C. elegans* fed with HR15 ingredients, Vitamin C (10 µg/mL), and control; incubated at 20°C for 7 days; treated with hydrogen peroxide (2 mM); survival analyzed	Significant antioxidant effect; 23.5% increase in worm survival	HR15 ingredients significantly protect against acute oxidative stress in *C. elegans*
Study 3	To analyze transcriptomic changes in *C. elegans* fed with HR15 ingredients	Agar plates with 2 mg/mL HR15 ingredients; transcriptomic profiling performed	Changes in protein metabolism, energy metabolism, glutathione metabolism, fatty acid metabolism, xenobiotic metabolism	Transcriptomic changes explain the antioxidant activity and lifespan increase in *C. elegans* fed HR15 ingredients

Table 2: Summary of clinical studies

Study	Purpose	Methodology	Results	Conclusion
Study 1	Investigate effects of HR15 ingredients in healthy volunteers	Single-center, randomized, placebo-controlled, double-blind, month-long; 3 groups (placebo, once-daily, twice-daily); 73 subjects; evaluated on days 1, 7, 30 (and additional days for certain groups)	Increased glutathione peroxidase levels, reduced GSH and GSSG, increased GSH/GSSG ratio, increased vitamin C and E levels, reduced oxidative damage to proteins, positive emotional effects on anxiety, positivity, vitality, and global score	One month of supplementation increased antioxidant defense, reduced oxidative damage to proteins, and had a good safety profile
Study 2	Evaluate efficacy of HR15 components in physically active males	Randomized, crossover, double-blind, placebo-controlled; 35 male subjects performing high-intensity, long-duration physical activity	Significant differences in oxidative damage markers, total antioxidant capacity, creatine kinase, interleukin 6, muscle damage, perceived exertion, fatigue, and athletic performance	HR15 ingredients reduce oxidative damage, muscle damage, and fatigue; improve antioxidant capacity, athletic performance, and reduce oxidative stress
Study 3	Investigate effects of lipid mixture designed as a carrier of bioactive ingredients for athletes	Various studies on lipid mixture affecting hormonal signaling, cell membrane composition, genetic expression, and metabolic fuel distribution	Regulation of glucose, lean mass, total weight, and adiposity; improved body composition and metabolic health; influenced thermogenesis, energy distribution, and reduced fat accumulation; reduced inflammation and protein oxidation; increased lean mass	Lipid mixture positively impacts body composition, reduces inflammation, enhances metabolic health, and supports athletic performance

Conclusion

Preclinical studies to evaluate the effects of HR15 components in *Caenorhabditis elegans* demonstrated that they increased the lifespan of *C. elegans* in a dose-dependent manner, showed a significant antioxidant effect protecting against oxidative stress in *C. elegans* and identified changes in metabolic pathways related to antioxidant activity through transcriptomic analysis. Clinical results on HR15 ingredients in healthy volunteers for one month improve antioxidant defenses, reduce oxidative damage and have a positive impact on emotional well-being. Furthermore, in active individuals, components of HR15 showed promising effects in combating oxidative stress, inflammation, muscle damage and fatigue induced by high-intensity physical activity after a 15-day intake.

Clinical results of HR15 ingredients in subjects who perform physical activity demonstrate that it reduces fatigue and increases endurance especially during periods of physical activity, suggesting a positive effect on athletic ability and

sports performance. In addition, a reduction in muscle pain and damage is evident after physical training, improving skeletal muscle damage, reducing oxidative stress and improving athletic explosive power. The lipid mixture of HR15 affects energy intake and energy expenditure, reducing fat accumulation and increasing lean mass through various molecular and metabolic mechanisms, which completes and complements the action of other bioactive ingredients. The consumption of the ingredients of HR15 daily was safe.

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