Original Research Article

In Vitro Inhibition of Dipetidylpeptidase-4 (DPP-4) and Arginase by a Dietary Supplement and Its Potential for Improving Quality of Life Factors in Adults

ABSTRACT

Aim: Somatopause is the progressive and age-related decline of growth hormone (GH) production due to changes in hypothalamic-pituitary axis signaling and results in typical physiological changes associated with the aging process. The purpose of our study was to investigate the anti-aging potential of several botanical ingredients, including noni fruit juice, as well as a dietary supplement formulation.

Methodology: Dipeptidylpeptidase-4 (DPP-4) influences hypothalamic-pituitary axis signaling and reduces GH production and secretion. Since DPP-4 concentrations increase with age, we evaluated the ability of several botanical ingredients to inhibit DPP-4 activity in vitro. We subsequently formulated an anti-aging dietary supplement with this blend of botanical ingredients by adding amino acids (L-arginine, L-Lysine, citrulline and beta-alanine), gamma-aminobutyric acid (GABA), flax seed oil and several nutrients. The supplement was evaluated for anti-arginase activity in vitro, as the expression of this enzyme also increases with age. The dietary supplement was also consumed by three men and one woman, ages 56 to 62, with relevant but disparate medical histories. Following short-term ingestion of the supplement, these volunteers were interviewed to ascertain any changes in health status.

Results: We discovered that a blend of the botanical ingredients synergistically inhibited DPP-4 in vitro by up to approximately 99%. A concentration-dependent reduction in arginase activity was also observed for the anti-aging dietary supplement. Further, all volunteers reported positive improvements in at least one quality of life factor, which included more restful sleep, reduced musculoskeletal discomfort, increased energy, improved physical stamina and better skin quality.

Conclusion: These initial findings suggest that the anti-aging dietary supplement can mitigate declines in quality of life associated with aging by reducing DPP-4 and arginase activities, thereby supporting healthy GH production and endothelial nitric oxide production.

Keywords: Growth hormone, anti-aging, somatopause, dipeptidylpeptidase-4 inhibition, arginase inhibition

1. INTRODUCTION

Growth hormone (GH) regulates childhood growth and helps maintain tissues and organs throughout life. It's produced by the anterior pituitary, a gland located at the base of the brain. In adults, GH helps maintain muscle and bone mass, promotes lipolysis, regulates carbohydrate metabolism, cardiovascular function, facilitates aerobic exercise capacity and cognitive function. But after their 30s, adults begin to experience a progressive decline of GH

secretion in which GH secretion tends to decrease by 15% for every decade of life [1]. Further, GH secretion rates may decline to less than one-quarter of the maximum achieved in mid- to late puberty [2]. Somatopause is term describing this progressive and age-related decline of GH production and the associated decline of insulin-like growth factor 1 (IGF-1), which are due to changes in hypothalamic-pituitary axis signaling [3]. This decline is associated with changes in body composition, physical and cognitive function, as well as declines in quality of life. Many aspects of aging resemble adult growth hormone deficiency (AGHD) syndrome, characterized by decreased muscle and bone mass, increased visceral fat, reduced exercise and cardiac capacity, changes in blood lipid profiles, thinning of skin, and psychological and cognitive complications [4]. Even though these symptoms are often milder in normal aged adults, they may still significantly impact quality of life.

The hypothalamus, a region of the forebrain, regulates the activity of the pituitary by sending hormonal signals to it. One of these signals is GH-releasing hormone (GHRH). The pituitary responds to GHRH by producing and releasing GH. Many age-related declines in GH levels are due, in part, to reduced hypothalamic secretion of GHRH [5]. Somatostatin, also known as growth hormone-inhibiting hormone (GHIH), is produced by the hypothalamus as well as some other tissues in the body. Somatostatin's effect is opposite that of GHRH. Pituitary production of GH is inhibited by somatostatin [6].

Decreased secretion of GHRH by the hypothalamus, a possible decline in pituitary responsiveness to GHRH and an increase in sensitivity of the pituitary to somatostatin might be partially responsible for the age-related declines in GH secretion [1]. But another likely contributor to age-related declines is increased dipeptidyl-peptidase 4 (DPP4) activity. DPP4 is a proteolytic enzyme that degrades GHRH, and its activity tends to increase with age [7, 8]. As such, DPP4 inhibitors may increase GH production in older adults. DPP4 also degrades glucagon-like peptide-1 (GLP-1), a peptide that helps decrease blood glucose levels by stimulating insulin secretion from pancreatic β cells [9]. Low glucose levels also stimulate GH release from the pituitary [10]. Therefore, DPP4 inhibition influences circulating GH levels through at least two biological pathways.

Nitric oxide (NO) is a reactive neurotransmitter that is produced from L-arginine by endothelial nitric oxide synthase (eNOS). NO regulates the release of several hypothalamic peptides and stimulates growth hormone secretion in the pituitary [11]. Dietary L-arginine promotes GH synthesis and secretion due, in part, to the ability of NO to reduce hypothalamic somatostatin release [12, 13]. Arginase competes with eNOS for L-arginine, it being a substrate of both enzymes [14]. Thus, inhibition of excessive arginase activity may increase NO synthesis and subsequent GH production and release.

To provide a safe therapeutic alternative to address the negative symptoms of the aging process, several healthy botanical ingredients were investigated for their potentials to influence DPP4 activity. Further, these ingredients were combined with several other dietary ingredients to produce an anti-aging supplement formulation. This dietary supplement was evaluated for anti-arginase potential and provided to human volunteers to determine its utility in improving the quality of life in adults undergoing somatopause.

2. MATERIAL AND METHODS

2.1 DPP4 Inhibition Assay

A DPP4 inhibition assay was performed according to a previously reported method, but with some modification [15]. Noni fruit juice from puree was filtered through a 0.45 µm filter and

then twice diluted to prepare a 0.25X concentration, as compared to single strength noni juice. Green tea extract (60%) was dissolved in ultrapure water, as was grape seed extract, blueberry fruit extract and curcumin (90%) to the desired concentration. These samples were also combined in equal volumes for tests that involved combinations, resulting in lower concentrations of each ingredient. The samples (10 µL) were mixed with 30 µL assay buffer (20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8). To this mixture was added 10 µL human recombinant DPP4 (Cayman Chemical, Ann Arbor, Michigan, USA). Afterwards, 50 µL of DPP4 substrate solution containing 100 µM H-Gly-Pro conjugated aminomethylcoumarin (Cayman Chemical) was added. Following incubation at 37 °C for 30 minutes, the fluorescence intensity of each sample reaction was measured with a microplate reader at 360 nm excitation and 460 nm emission. The fluorescence of the uninhibited (without sample) DPP4 enzyme activity was also measured in the same manner. The difference in fluorescence intensity of the samples versus that of uninhibited DPP4 was used to determine percent inhibition of the samples. Replicate samples were measured, and summary statistics (mean and standard deviation) were calculated.

2.2 Arginase Inhibition Assay

An anti-aging dietary supplement (Nutrifii™ Renew) containing the above herbal ingredients was formulated and contained additional ingredients which support growth hormone production and endothelial nitric oxide production. This dietary supplement formulation is described in Table 1 below. The additional ingredients were selected because they were known to be safe and had a previous history of human consumption. Further, each had been found in earlier studies to have the potential for influencing hypothalamus-pituitary signaling, the sleep cycle and/or cellular health.

Table 1. Anti-aging dietary supplement ingredients

Component	Amount (label declaration)
Vitamin A (as beta-carotene)	200 ug
Vitamin B3 (as niacin and niacinamide)	25 mg
Vitamin B6 (as pyridoxine HCl)	5 mg
Vitamin B12 (as methylcobalamin)	10 ug
Magnesium (as magnesium amino acid chelate)	50 mg
Zinc (as zinc citrate)	15 mg
Selenium (as L-selenomethionine)	100 ug
Chromium (as chromium chloride)	50 ug
Flax (*Linum usitatissimum*) seed oil	15 mg
Amino acid and carotenoid blend: L-arginine, L-citrulline, L-lysine, beta-alanine, mixed natural carotenoids	3650 mg
Blend: Gamma aminobutyric acid, lutein, zeaxanthin isomers	515 mg
Herbal blend: *Morinda citrifolia* (noni) fruit, *Vitis vinifera* (grape) seed extract, *Camellia sinensis* (green tea) leaf extract, *Curcuma longa* (turmeric) root extract, *Vaccinium species* (blueberry) fruit extract	145 mg

This dietary supplement was evaluated for in vitro arginase inhibition potential using recombinant human arginase 1 (R&D Systems, Inc., Minneapolis, Minnesota, USA). The

assay employed has been described previously [16]. However, the protocol was slightly modified to better suit the evaluation of the anti-aging dietary supplement and did not involve solvent extraction. Briefly, 10 ug of arginase (>35,000 pmol/min/µg was) dissolved in 1500 µL deionized water. The dietary supplement was also dissolved in deionized water and filtered through 0.45 µm filter. An arginase inhibitor screening kit (Sigma-Aldrich, St. Louis, Missouri, USA) was used to evaluate the reduction in arginase activity when incubated with varying concentrations of the dietary supplement. The arginase solution (40 µL) was transferred to separate wells of a clear plastic 96 well plate. To control wells (no dietary supplement formulation) and blank wells (no enzyme substrate), 5 µL of deionized water was added. The same volume of varying concentrations of the dietary supplement and arginine solution (used as arginine alone controls) were added to the remaining wells. The plate was then incubated for 15 minutes at 25 °C. Afterward, 5 µL of substrate (arginine and manganese) buffer was added to each well except the blank wells, to which only 5 µL of water was added. The plate was then Incubated for 30 minutes at 25 °C. Next, 200 µL of urea reagent was added to all wells followed by 60 minutes incubated at room temperature. The absorbance of each well was at 430 nm. The control wells (no dietary supplement) represent 100% initial arginase activity. The absorbances of the dietary supplement wells were corrected by subtracting the absorbance of wells containing corresponding arginine concentrations (arginine alone controls). Arginase inhibition activity was calculated by the difference in the absorbance of the control well and the corrected dietary supplement wells, divided by the absorbance of the control well alone. Replicates of the controls, blanks and samples were measured.

2.3 Adult Volunteer Case Reports

Samples of the anti-aging dietary supplement formulation were provided to adult volunteers who had requested them, of their own volition, to consume the product and evaluate its effectiveness. The amounts and duration of consumption were chosen by the individual volunteers based, primarily, upon availability. Upon learning of this, the authors obtained consent to gather and report demographic data and medical histories from these people after they had used the supplement for some time. These volunteers were interviewed to gather information related to adverse events and changes in any preexisting health-related symptoms or quality of life.

3. RESULTS AND DISCUSSION

3.1 DPP4 Inhibition Assay

Table 2 reports the % DPP4 inhibition of each sample alone and in combination. The expected percentages of inhibition are the sum of each individual ingredient % DPP4 inhibition multiplied by the percentage of that ingredient in the blend. The combination of noni juice and green tea extract provides a slight (3.46%) increase in DPP4 inhibition above expected levels. However, the combination of grape seed extract and noni juice increases DPP4 inhibition by more than 16% above the expected amount. When these are all combined with curcumin , there is a 31.04% increase in enzyme inhibition above expected levels. The combination of noni juice, green tea extract, curcumin and blueberry fruit extract almost entirely inhibits DPP4 activity at 99.91%—revealing particularly strong synergism, as it is 48.44% greater than expected. The collective interaction of all the phytochemicals within each ingredient has a more powerful effect on DPP4 than those from any single ingredient. This is especially noteworthy when considering that the synergy occurs with significantly lower concentrations of each ingredient.

Table 2. Synergistic inhibition of DPP4 by herbal products and their blends.

Sample	% DPP4 Inhibition	Expected inhibition*
Noni juice 0.25X dilution	72.84	
Grape seed extract (2.5mg/mL)	82.27	
Green tea extract (2.5mg/mL)	83.45	
Noni 0.125X + Green tea extract (1.25 mg/mL)	81.61	78.15
Noni 0.125X + Grape seed extract (1.25mg/mL)	93.80	77.55
Curcumin (5mg/mL)	21.71	
Blueberry fruit extract (10 mg/mL)	33.98	
Noni 0.0625X + Green tea extract (0.625 mg/mL) + Grape seed extract (0.625 mg/mL) + Curcumin (1.25mg/mL)	96.11	65.07
Noni 0.0625X + Green tea extract (2.5 mg/mL) + Blueberry fruit extract (2.5 mg/mL) + Curcumin (2.5 mg/mL)	99.91	51.47

**Based on ingredient percentages in mixture*

3.2 Arginase Inhibition Assay

The anti-aging dietary supplement inhibited in vitro arginase activity in a concentration-dependent manner through the 25 – 250 µg/mL range (Figure 1). As the supplement contains several ingredients, the anti-arginase action (14% inhibition) at 25 µg/mL was notable, and effective reduction of arginase activity by individual supplement ingredients, or the combination of ingredients, appears to be evident. These results are not unexpected as some amino acids, including L-lysine and L-citrulline, have long been known to competitively inhibit arginase [17]. This observation is not only limited to in vitro studies but has been reported in human subjects. For example, plasma arginase activity in type 2 diabetic (T2DM) patients decreased by an average of 30%, with a concomitant modest improvement in H1Ac levels, after one month of L-citrulline supplementation [18].

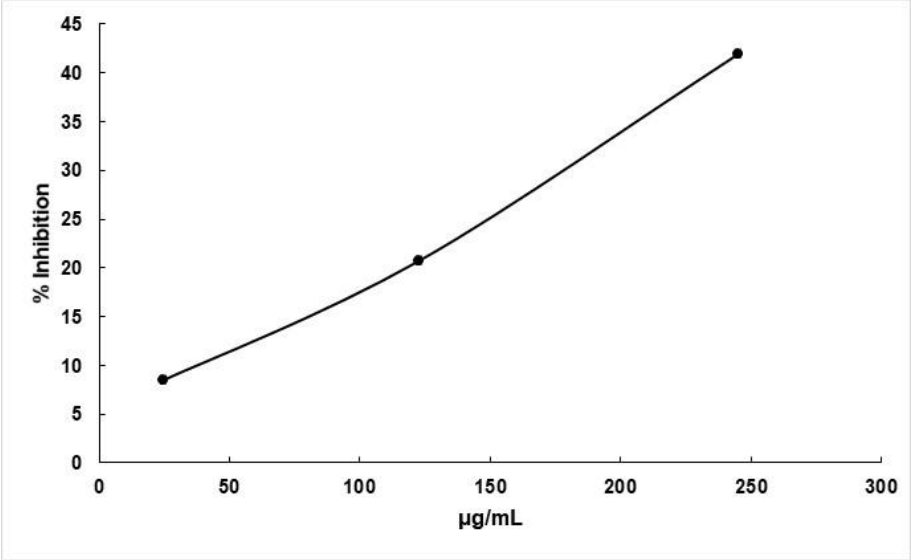

Fig. 1. Arginase inhibition assay of anti-aging dietary supplement formulation

3.3 Adult Volunteer Case Reports

3.3.1 Case 1

A middle-aged (58 yr.) Caucasian male presented with a medical history of musculoskeletal pain and cardiovascular disease. Over the course of the subject's lifetime, he sustained more than 30 bone fractures in multiple locations, as well as multiple other injuries due to active participation in athletics (high school and university) and professional motorsports (motocross and snowmobile racing). Most bone fractures occurred by age 32. Adolescent and early adulthood injuries include fracture of both ankles (requiring 4 surgeries), left anterior cruciate ligament (ACL) tear, at least 6 concussions, fractured ribs, fractured tibia (requiring plates and screws stabilization), fractured wrist, fractured clavicle, ruptured jugular vein, lacerated trachea and severed auricle. At age 55, another motorcycle accident resulted in tearing of the right ACL and an elbow injury that required surgical repair.

This subject also experienced a heart attack at age 46, requiring four stints. Subsequent treatment has included statin (currently rosuvastatin), ACE inhibitor (lisinopril), beta-blocker (metoprolol) and low-dose aspirin pharmacotherapy. Since the heart attack, he has reported muscle pain and joint stiffness. Statin medications have been changed four times in attempt to ameliorate the muscle pain, without complete success. This subject described experiencing flu-like aches every morning, as well as frequent evening muscle cramping. He scored this pain as moderate to severe (5 - 6) on the visual analogue scale (VAS). Shoulder pain during sleep was also present, preventing side-sleeping. He had begun taking an omega-fatty acid dietary supplement in the evening which seemed to reduce the severity of the morning pain. Typically, the pain dissipated by mid-morning. He had also been consuming multivitamin and mineral supplements which did not influence pain severity.

This subject also experienced significant limitations in mobility within the past five years. Still a frequent operator of motorcycles, he had found it difficult to lift and swing his leg over the seat while mounting and dismounting. In the "sit-rise" functionality test [19], the subject had

required the use of a tucked knee and grabbing a chair or table to aid rising from the floor, resulting in a score of 3 out of 5 for the rising portion of the test.

After two days of consuming 3.4 grams (1/2 serving) of the anti-aging dietary supplement, this subject experienced a noticeable reduction in morning pain and stiffness with his reported pain level decreasing to minimal to mild (VAS score of 1 - 2). One example provided by the subject was an absence of ankle stiffness and pain when descending stairs shortly after waking, stiffness which had been present for 10 years previously. He discontinued use of the supplement and pain symptoms returned after two days. He abstained from ingesting the supplement for 3-weeks due to it being unavailable to him during that time. Afterwards, he began again ingestion of just one-half serving of supplement daily, which reduced pain. Within 5 days, he reported an almost complete resolution of pain. Further, evening muscle cramping did not recur. He reported having more energy in the afternoon than before ingesting the supplement. Concomitantly, he reported sleeping better. Flexibility also improved, with no limitations in motorcycle mounting and dismounting. Improved performance in the rising portion of the "sit-rise" functionality test was also observed, achieving a maximum score of 5 due to not requiring any aid in standing up from a seated position on the floor. This latter observation is entirely consistent with the results of a 12-week, placebo-controlled clinical trial of L-arginine and L-lysine supplement use by elderly women [20].

3.3.2 Case 2

The medical history of a healthy middle-aged (58 yr.) Caucasian male included only a few sports related injuries, such as sprains and arthroscopy of the knee. Results of clinical laboratory tests, performed as part of a routine annual physical, were all within normal ranges. This subject did not smoke or consume alcohol, nor did he have a history of doing so. He exercised regularly, 3 to 5 times per week, and consumed daily multivitamin and mineral, coenzyme Q10, omega fatty acid and plant-based antioxidant products.

This subject consumed 3.4 grams (1/2 serving) of the anti-aging dietary supplement daily for three days. He reported that he experienced improved sleep (more rested and invigorated in the morning) and "felt younger"—less age-typical morning stiffness and discomfort—after the first day of consuming the supplement. To discern if what he was experiencing was truly associated with the supplement, the subject deliberately avoided its consumption to see if morning discomfort would return to previous levels. Within two days, the discomfort had increased to previous levels. After three days of not ingesting the supplement, he consumed 3.4 grams daily again for three more days. The next morning following resumption of anti-aging supplement use, he felt the morning stiffness and discomfort decline significantly, as well as a return of feeling invigorated due to improved sleep. During a one-month period of not consuming the supplement, due to unavailability of samples, the previous symptoms returned. When he was again able to take the supplement, he experienced the same effects—reduced discomfort and improved energy levels only when consuming the supplement. He also reported feeling that his muscle tone was improved during and following resistance training exercises.

3.3.3 Case 3

A 62-year-old Caucasian male with excellent health regularly attended a certified CrossFit® fitness class (five days per week). Despite being the oldest in his class (cohort), he placed consistently in the top 10% of fitness measurements during the previous 12 months. He began ingesting 6.8 grams (one serving) of the anti-aging dietary supplement within an hour of retiring to bed. For the first six days, this individual did not experience any improvements

in health. On the 7th day, he noticed a significant improvement in physical stamina during five rounds of the following weight training exercises: 12 repetitions of dumbbell deadlift, 9 repetitions of hang power cleans and 6 push jerks (each performed with 16 kg dumbbells in each hand). He completed the found rounds in 4 minutes 53 seconds, whereas the next best time in all the classes following the same workout that day was 8 minutes. The following day, this subject participated in another workout consisting of a 50-Calorie row followed by completing as many rounds as possible within 20 minutes of ten box-over jumps (24 inches) and twelve repetitions of 6.4 kg wall ball squats. The goal was to complete at least 9 rounds, with the class record being 11 rounds. This subject was able to complete 14 rounds. His performance on these two days was well above the previous and expected level. He reported that he had experience significant improvements in cardiovascular fitness and stamina over the previous several years. However, further incremental improvements had been more difficult to achieve in recent months due to having already achieved a high level of fitness. The only change to his regiment prior to experiencing this significant increase in performance was the ingestion of the anti-aging dietary supplement

3.3.4 Case 4

A 54-year-old Caucasian female in excellent health had, in recent months, began experience declines in sleep quality, skin aging and some menopausal symptoms. She began consuming one serving of the anti-aging dietary supplement every evening. Within a few days, sleep quality improved with deeper and longer sleep, resulting in feeling more refreshed in the mornings. After daily use of the supplement for one month, she felt that her skin on her face, neck, arms, and thighs appeared smoother and more youthful than the previous months. She also reported that wrinkles in the forehead appeared less prominent. Afterwards, she abstained from ingesting the supplement for two weeks, due to unavailability. During this time, sleep quality and daily energy levels began to decline. Upon resumption of daily ingestion of the anti-aging dietary supplement, her sleep quality improved.

A novel feature of the anti-aging formula is the blend of herbal extracts, featuring noni juice. DPP4 inhibition had been previously reported for some of these ingredients [21]. But this is the first time that synergistic effects have been demonstrated for the combination of these ingredients. The formula also features amino acids that are well-known to impact GH production. L-arginine, especially in combination with L-lysing, suppress the production and release of somatostatin by the hypothalamus [22]. This causes an increase in GH secretion by the pituitary, and this appears to be dose dependent. Therefore, improved absorption and transport of these amino acids across the blood brain barrier will impact GH production. L-arginine and L-lysine are actively transported across the blood brain barrier by System y+ (gamma-plus) transporters [23]. The combination of L-arginine and L-citrulline ingestion has been found to increase blood levels of L-arginine more than just L-arginine alone [24]. This is because, unlike dietary L-arginine, L-citrulline avoids first pass metabolism by the liver and that our bodies can convert L-citrulline into L-arginine [25]. The subsequent increase in circulating levels will result in greater available of L-arginine to the hypothalamus, following active transport across the blood brain barrier. This will result in greater suppression of somatostatin release by the hypothalamus, which then allows greater GH release from the pituitary. Such an effect was evident in previous studies. In a human clinical trial [26], co-ingestion of 1200 mg L-lysine and 1200 mg L-arginine provoked a release of GH. This phenomenon was reproducible, and the GH secreted in response to this stimulation was biologically active, as demonstrated by a radioreceptor assay and somatomedin induction.

In a randomized crossover clinical trial, seven days of oral supplementation with L-citrulline and L-arginine (1200 mg of each per day) improved 10-min full-power cycling test

performance in male collegiate soccer players in a randomized crossover clinical trial. Muscle power output was significantly greater with the L-citrulline and L-arginine combination than in the placebo group. Ingestion of this amino acid combination also significantly improved post-exercise subjective perception of “leg muscle soreness” and “ease of pedaling” [27].

The sleep cycle plays a very important role in GH production [28]. The purpose of gamma-aminobutyric acid (GABA) in the anti-aging dietary supplement formulation is augmentation of sleep. GABA (100 mg) reduced sleep latency (time required to fall asleep) by several minutes in human volunteers [29]. A 100 mg oral dose of GABA significantly shortened sleep latency and improved the early stages of sleep, while increasing the total non-rapid eye movement (non-REM) sleep time [30]. In another placebo-controlled clinical trial, sleep latency decreased while sleep quality increased in insomnia patients who were given 300 mg GABA for 4 weeks [31].

The potential for GABA to increase GH production is not just limited to indirect improved-sleep evidence. Ingestion of 100 mg GABA along with 10 gm of whey protein for 12 weeks significantly increased growth hormone levels and increased lean mass in young men, especially when compared to ingesting whey protein alone [32]. In an 8-week clinical trial, ingestion of 40 to 69 mg of GABA significant improved in triglycerides, fat/lean mass, growth hormone levels, insulin-like growth factor levels, muscle strength and joint flexibility in middle-aged Japanese women [33]. Further, a placebo controlled, double-blind 8-week study involving 36 women with dry skin, fatigue and sleep disorder revealed that ingesting 100 mg GABA/day increased skin elasticity of the cheek [34]. Such skin improvements are associated with increased GH levels.

There are likely multiple mechanisms of action by which the ingredients improve growth hormone levels. These mechanisms are summarized in Table 3 and help further explain the improvement in symptoms seen in the case reports.

Table 3. Growth hormone promoting mechanisms of action of ingredients in anti-aging dietary supplement

Ingredient Group	Mechanisms of Action
Amino acids (L-arginine, L-lysine and L-citrulline)	Increases GH via suppression of somatostatin secretion from the hypothalamus. The blend also increases nitric oxide production for improved vascular health. L-lysine is an arginase inhibitor, which further shunts L-arginine metabolism towards nitric oxide production by eNOS [35].
GABA (gamma-aminobutyric acid) and Beta-alanine	Improves sleep, causing increased GH release with better sleep cycle. Beta-alanine is a structural intermediate of GABA as well as possesses neurotransmitter properties. It is also a precursor to carnosine, a potent geroprotective antioxidant peptide [36, 37].
Vitamins, minerals, and carotenoids	Nutritional support for L-arginine absorption across blood brain barrier, GH production, cellular health and viability. For example, nicotinamide prevents stress-induced apoptosis [38].
Botanicals (i.e. noni, grape seed extract, etc.)	Synergistically inhibits DDP4, thereby increasing GHRH signal to the pituitary and causing GH release.

4. CONCLUSION

The current study has revealed, for the first time, the synergistic action of the combination of noni, green tea extract, blueberry fruit extract, grape seed extract and turmeric in inhibiting DPP4 activity. Just as we observed synergy amongst the botanical ingredients, it is reasonable to assume that the combination of several different mechanisms of action, from the different ingredients in the anti-aging dietary supplement, work towards the improved health outcomes reported in each case report. Such a multi-faceted approach that involves well-known and safe ingredients may be very useful in alleviating some of the symptoms associated with the aging process.

CONSENT

All authors declare that consent was obtained to publish the information described in the case reports.

ETHICAL APPROVAL

All authors hereby declare that all human volunteers initiated their involvement after the dietary supplement had been developed and samples produced with ingredients known to be safe for human consumption. Use of the supplement and reporting of data relative to their experience was entirely voluntary and under their control. Authors collected information from the volunteers afterward. As such, their involvement in this study was in accordance with the ethical standards laid down by the International Committee of Medical Journal Ethics Protection regarding patients' rights to privacy and the 1964 Declaration of Helsinki.

COMPETING INTERESTS DISCLAIMER

Authors declare that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. The research was also supported by the personal efforts of the authors.

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