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Assessment of the efficacy and safety of protein Supplement with micronutrient fortification (NRL/2019/5PNV) to promote health and wellbeing in healthy adults: Randomized controlled clinical trial

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Abstract
Proteins are an essential component of the diet. As a source of good protein supplement for both male and female, Netsurf communication Pvt. Ltd. has developed NRL/2019/5PNV protein supplement with micronutrient fortification to help to manage health and quality of life. The current research depicts the efficacy and safety of the NRL/2019/5PNV. It is found to be safe and effective in promoting health and wellbeing in healthy adults. It improved skeletal muscle % and reduction in body fat %. There was increased physical endurance indicated as VO_2 max. NRL/2019/5PNV was found in improving energy levels in subjects along with improved digestive behavior. There was improved glycemic control and reduction in total cholesterol levels in NRL/2019/5PNV treated group. The blend of whey, soy, rice and peas protein with vitamin, micro minerals and rejuvenating herbal extracts like *Withania sominifera* and *Aloe vera* is definitely providing better assimilation and efficacy

Keywords: protein supplement, vo2 max, energy level, whey, soya, *Withania sominifera*, *Aloe vera*

1. Introduction

Proteins are an essential component of the diet needed for the survival of animals and humans. Nutritional quality of a food depends on protein content, digestion, absorption, and utilization of proteins. Amino acids availability depends on protein sources, processing treatments, and interaction with other components of the diet [1].

Source of proteins in human diet are mainly animal proteins (e.g. egg, milk, meat and fish) and plant proteins (e.g. pulses, cereals, nuts, beans and soy products). Animal proteins have high biological value than vegetable proteins as per amino acid composition and are more biologically complete. However two plant proteins such as legumes and grains or legumes and nuts/ seeds can be combined to formulate a complete protein from two incomplete proteins. There is gaining momentum to a fact that animal proteins are possessing concerns to many vegetarians and towards digestibility difficulties as well [2].

Legumes include peas, beans, lentils, peanuts etc. can be a good source of protein. Soybeans have captured more interest of researchers because of their concentrated source of isoflavones. It is being backed up by research that isoflavones reduce the risk of cancer, heart disease, and osteoporosis [3, 4]. Soy protein is thought by many researchers as almost equivalent to animal sources in protein quality [5]. According to a study, soy protein has beneficial effects in prevention of coronary heart disease, hypolipidemic effects [6]. Being rich source of isoflavone, soya protein help in improving bone health [7], menopausal symptoms [8] and preventing breast and prostate cancers [9]. Soy protein formulas are found suitable for feeding infants who exhibit lactose intolerance or have lactase deficiency [10].

According to U.S. Food and Drug Administration, the term "dietary supplement" defines any product that supplements the diet containing any one or more of a vitamin, mineral, herb, botanical, amino acid to increase the total dietary intake of added contents [11, 12]. Plant-based supplements are rich in fiber, beta carotene, vitamin K and C, folate, magnesium, and potassium which improve dietary health index [13, 14]. There is great need of supplementing on Vitamin B12. Its deficiency leads to neurological and cognitive health weakening [15]. Vitamin B12 is suggested to be neuroprotective, particularly for memory performance [16-18].

As per many studies, supplementation with vitamin C reduces the duration and severity of common cold symptoms in adults [21, 22]. When used in combination with zinc, vitamin C supplementation can relieve symptoms such as rhinorrhea in common cold [23, 24]. According to the study, vitamin D supplements can protect against respiratory tract infections and reduce the risk of acute respiratory illness and influenza [25]. Studies has shown an excellent rationale to combine vitamins C and D with zinc to support immune functions and help minimize the risk of respiratory infection [26]. Micronutrients have proved helpful in combating the detrimental health effects of prolonged contact with environmental factors that act as vehicles for pollutants [27]. It is a proven fact that plant-based proteins are presented as lower in calories and fat than animal proteins and higher in fiber and essential nutrients. Plant-based pea proteins can help gain muscle mass, reduce hunger cravings. Its high

micronutrient profile helps reduce postmenopausal symptoms. Pea's protein is easily digested and leads to less gastric distress than animal based source.

Protein and fiber rich diet as per study was demonstrated to support successful weight-loss [28]. High-protein diets improve satiety and appetite control thereby providing an excellent way to control and reduce body weight [29]. Hunger and satiation define appetite pattern [30]. From a credible study it was hypothesized that hormones improvise hunger and satiety response such as insulin, glucagon, pancreatic polypeptide (PP) and amylin from pancreas, leptin and adiponectin from adipose tissue and ghrelin, glucagon-like peptide 1 and 2 (GLP-1, GLP-2)from intestine; and dopamine, neuropeptide Y, growth hormone releasing peptide (GHRP) from hypothalamus [31-32]. Orexigenic hormones or peptides promote appetite and anorexigenic work antagonistically, by suppressing apetite. The sensory exposure to food (e.g., sight, smell, taste) has been shown to improvise appetite [33].

High protein diets have been shown to be an effective weight-loss strategy by improving the pancreatic response to reduce hunger [34]. Proteins produce greater satiety than carbohydrates and fats. It increases energy expenditure [35]. Many researches back up the fact that protein diet gives postprandial appetite suppression and subsequent reduction in energy intake [36].

Omega-3 fatty acid, docosahexaenoic acid (DHA), is essential for normal brain function. It is a key component to support brain and eye function throughout life [37, 38]. It is also an important component of tissues of cardiovascular system. The American Heart Association and USDA Dietary Guidelines recommend Omega-3 fats as good for cardiovascular health [39].

By taking in to consideration the pressing need of good protein supplement for both male and female, Netsurf Communication has designed and developed the NRL/2019/5PNV protein supplement with micronutrient fortification for both male and female to help to manage health and quality of life.

It contains essential vitamin, minerals, herbal extracts together with a blend of whey, soy, pea, rice and milk solids. The current research depicts the efficacy and safety of the NRL/2019/5PNV with micronutrient fortification to promote health and wellbeing in healthy adults.

2. Material and Method

2.1 Investigational product details

Product Code: NRL/2019/5PNV. Key ingredients are Milk Solids, Maltodextrin, Protein blend (Whey Protein Concentrate, Soy protein isolate, Rice protein isolate, Pea protein isolate), DHA (omega 3-Algal source), *Aloe vera* extract, *Withania sominifera* extract, Minerals (Calcium phosphate tribasic, potassium citrate, sodium citrate, magnesium sulphate, ferric diphosphate, zinc sulphate, manganese sulphate, copper sulphate, sodium selenite), Vitamins (ascorbic acid, vitamin E, nicotinamide, calcium-d-pantothenate, pyridoxine hydrochloride, thiamine mononitrate, riboflavin, retinyl acetate, folic acid, d-biotin, phylloquinone ,Vitamin D2 ergocalciferol, cyanocobalamin)

2.2 Study Design

A randomized, double Blind, controlled clinical study. The subjects were distributed randomly to test NRL/2019/5PNV treated and marketed product treated group. We had used leading market brand as a protein supplement in comparator arm.

2.3 Study Objectives & Purpose

The primary objectives were assessment of daily activity questionnaire, endurance levels (steppers test), perceived stress levels, immunity via number of events of recurrent UTI/RTI etc., anthropometric analysis, HbA1c and glycemic profile ie blood sugar levels, regulation of lipid levels The secondary objectives were assessment of quality of life, changes in digestive behavior assessment, changes in mood behavior, safety profiling by LFT, RFT and thyroid profile etc., assessment of adverse events, changes in sleep quality questionnaire, palatability with dosage compliance of test product

3. Selection and Withdrawal of Subjects

3.1 Inclusion exclusion criteria

Inclusion Criteria for the study were: age group: 18 years - 65 years, BMI in the range of 20-26, subjects in good physical condition and sound mental status, in the judgment of the Principal Investigator, able to comply with protocol requirements, willing to sign written informed consent, subjects with willingness to abstain from use of vitamin or mineral supplements, nutritional supplements and or medical foods if applicable.

Exclusion Criteria for the study were: allergies to ingredients in product/ milk, Subjects with uncontrolled and uncomplicated diabetes (HbA1C NMT 7.0 % and blood pressure NMT 140/90 mm hg) may or may not be on prescription, subjects with past history of addiction abuse and rehabilitation, subjects with current medical history of any major illness such as cancer, heart disease, COPD, Asthma etc. in the past, subjects with history of any acute or chronic illness that may affect the patient's participation in the study, use of prescription medications and/or nonprescription medications for weight loss, subjects with acute illness or history of major or minor surgery in the past one year, female subjects who are currently pregnant and/or breast feeding, subjects not willing to participate in study

3.2 Recruitment Plan

We intended to complete 100 subjects (50 in each group) at the end of the study. Additional subjects were recruited to complete the required number (100) of completed subjects for analysis. Precautions have been taken not to recruit the subjects belonging to possible vulnerable groups.

3.3 Withdrawal Criteria

Subjects withdrawn from the study for the following reasons: At their own request i.e. withdrawal of consent at any time for personal reasons. If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being. Protocol deviations that could invalidate interpretation of the results (i.e. intake of not permitted concomitant treatments etc.)

4. Treatment of Subjects

4.1 Dosage and Treatment Duration

As per computer generated randomization list, subject was either be randomized to NRL/2019/5PNV (Test) or Marketed Product Group in 1:1 ratio. Subjects were advised to take given supplement in a dose of 10 gm in the morning with 150ml of milk for 90 days.

4.2 Visit Schedules
Subjects were asked to regularly visit at day 0, day 30, day 60 and day 90 of the treatment.

5. Measures taken to avoid bias
5.1 Randomization and study groups
All the subjects are randomly allocated (as per computer generated randomization list) to either one of the two treatment arms in 1:1 ratio. Subjects may be randomized to either NRL/2019/5PNV (Test) group or Marketed Product Group.

5.2 Blinding of the investigational products:
Identical comparator was provided as a marketed product. Primary packing of the test drug and standard was kept absolutely similar in order to keep both investigator as well as the subject blind.

6. Methodology
On screening visit, a written informed consent was obtained from the subjects for their participation in the study and their demographic details and vitals were recorded. The subject then proceeded to undergo clinical examination and their medical, surgical and treatment history was recorded. Current medication if any was noted in the case record from (CRF). The subject was considered for further evaluation as per the inclusion and exclusion criteria. Subject's blood sample were collected at the respective study centers for laboratory testing i.e. CBC, ESR, Hb%, Liver Function Tests, Lipid Profile, Renal Function test, HbA1c. During screening visit and the entire study duration subjects were advised to refrain from antioxidant agents, vitamins, anti-inflammatory drugs, hormones, Nutraceutical, Ayurvedic, Siddha, Unani, herbal /homeopathic medicines. On baseline visit, subject was recruited in the study if he or she m*et a*ll the inclusion criteria. Subjects were then being to be randomized to one of the two study groups as per the computer generated randomization list. Subjects were asked for occurrence of any adverse event during screening period. Subject undergone clinical examination. Subject's Daily activity, Endurance levels (steppers test), Perceived stress levels and number of events of recurrent UTI/RTI etc. were recorded. Subject's Anthropometric analysis was performed. Following questionnaires were assessed like Quality of life (Physical, mental and social) General Health Questionnaire-28 (GHQ-28), digestive behavior assessment, mood behavior, sleep quality questionnaire. On every follow up visit the above said parameters were assessed. At baseline visit and at every follow up visit (except last follow up visit), as per computer generated randomization list, subjects were provided with investigational products. Subject were advised to consume given supplement in a dose of 10gms in the morning with 150 ml milk. Subject were advised to continue his/ her concomitant medication other than antioxidant agents, weight loss management, vitamins, anti-inflammatory drugs, hormones, Nutraceutical, Ayurvedic, Siddha, Unani, herbal /homeopathic medicines etc. The record of concomitant medication was kept in the CRF. Drug compliance was assessed by the investigator on every follow up visit. Subjects who continuously miss dosing for >3 consecutive days or total missed dose > 9 days during the study period were to be treated as drop outs.

There were no drop outs as of all were compliant. Subjects were advised to continue the diet and exercise regimen (which they are already following) during the entire study period.

On last follow up visit (i.e. Day 90) apart from all baseline assessments, subject's safety biochemical parameters along with the clinical examination were repeated. Adverse events were critically evaluated.

After completion of 3 months of study treatment, all the subjects were being asked to stop trial medications and take advice of investigator for further treatment.

All the subjects were closely monitored for any adverse event starting from baseline visit till the end of the study visit.

7. Ethical consideration and registration of study
7.1 Ethical Consideration
The study was initiated only after a written approval was from Independent/ Institutional Ethics Committee (IEC) and subsequent registration of study on CTRI website. The study was conducted as per approved protocol and as per Good Clinical Practices guidelines.

7.2 CTRI Registration
After getting approval from the ethics committee, the study was registered on CTRI website. Patients were enrolled in the study only after registration of study on CTRI website. The Registration details of study are- CTRI/2019/10/021716 [Registered on: 18/10/2019]-Trial Registered Prospectively.

8. Statistics
8.1 Sample size consideration
Sample size calculation is derived taking considerations of primary and secondary outcomes by a qualified statistician. The software used for calculation of sample size is SPSS version 10.0. There were 50 subjects recruited per group.

9. Results and observations
9.1 Demographic details
In the present study, 107 subjects were screened. Out of 107 subjects, 6 lost to follow up in the study. 101 subjects were considered evaluable cases at the end of the study 50 in test and 51 in marketed product treated group.

Out of 101 completed subjects, the mean age of Test group subjects in were 33.2± 6.83 years and the mean age of Marketed product group subjects was 33.38 ± 8.55 years. If compared between the groups, the difference was statistically insignificant.

The ratio of approximately 75:25 for male and female was decided and maintained during recruitment of the study.

9.2. Efficacy Assessments
9.2.1. Assessment of Maximum Aerobic Capacity (VO_2 Max)
There was non-significant difference between VO_2 Max calculated by 6MWT in Test product and Marketed product treated group at baseline. After day 60, there was significant increase in VO^2 Max calculated by 6MWT with test product treated group as compared to Marketed product. After day 90, there was significant increase in VO_2 Max calculated by 6MWT i.e. 24.64± 2.77with test product treated group as compared to Marketed product.

Table 1: Assessment of Maximum Aerobic Capacity (VO_2 Max) between the groups

Duration (Days)	Mean VO_2 Max (Mean± SD)	
	Test (N = 50)	Marketed Product (N = 51)
Baseline	20.80± 3.14	20.52± 3.23
30	20.95± 3.27	20.53± 3.23
60	22.59± 2.93*	19.80± 3.27
90	24.64± 2.77**	19.87± 2.71

ANOVA multiple comparison Test: P > 0.05 Not Significant; significant p< 0.01*, p<0.001**

9.2.2. Assessment of Mood Disorder Questionnaire (MDQ) Score

After day 90, there was significant (p<0.001) increase (2.56±0.62 and 1.80±0.66 respectively) between MDQ score in Test product and Marketed product treated group. In case of intergroup analysis, test product treated group demonstrated significant increase in MDQ score at day 60 and 90 when compared to baseline value of the same group. In case of intergroup analysis, marketed product treated group demonstrated non- significant increase in MDQ score at day 30, 60 and 90 when compared to baseline value of the same group.

9.2.3. Assessment of Pittsburgh Sleep Quality Index (PSQI)

There was non-significant difference between PSQI in Test product and Marketed product treated group at baseline. After day 60, and 90 there was significant decrease (p<0.01) in PSQI with Test product treated group.

Table 2: Assessment of Pittsburgh Sleep Quality Index (PSQI) between the groups

Duration (Days)	Mean Pittsburgh Sleep Quality Index (Mean± SD)	
	Test (N = 50)	Marketed Product (N = 51)
Baseline	10.87±5.55	10.09±3.12
30	9.76±3.17	9.82±3.04
60	6.13±1.96*	9.64±2.19
90	3.22±1.43**	10.18±1.27

ANOVA multiple comparison Test: P > 0.05 Not Significant; significant p< 0.01*, p<0.001**

9.2.4. Assessment of Perceived Stress Score (PSS):

There was non-significant difference between PSS in Test product and Marketed product treated group at baseline. After 30, 60 and 90 days there was significant decrease (p<0.001) in PSS evident with Test product compared to Marketed product treated group.

Table 3: Assessment of Perceived Stress Score (PSS) between the groups

Duration (Days)	Mean Perceived stress questionnaire (Mean± SD)	
	Test (N = 50)	Marketed Product (N = 51)
Baseline	24.73± 8.70	26.09± 3.67
30	19.91± 3.55**	25.18± 3.18
60	12.71± 2.94**	22.42± 3.58
90	5.29± 1.70**	24.71± 3.27

ANOVA multiple comparison Test: P > 0.05 Not Significant; significant p< 0.01*, p<0.001**

9.2.5. Assessment of General Heath Questionnaire-28 (GHQ-28) Score

After day 60 and 90, there was significant increase (p<0.001) in GHQ-28 score of Test product treated group compared to Marketed product treated group. During intragroup analysis both groups showed significant (p<0.05) increase in GHQ-28 score from their respective baseline values.

Table 4: Assessment of General Heath Questionnaire-28 (GHQ-28) Score between the groups

Duration (Days)	Mean General Heath Questionnaire-28 (Mean± SD)	
	Test (N = 50)	Marketed Product (N = 51)
Baseline	25.60±8.35	27.60±5.32
30	44.51±5.92	43.49±5.92
60	64.42±5.78**	58.02±9.17
90	80.04±2.26***	67.64±9.42

ANOVA multiple comparison Test: P > 0.05 Not Significant; significant p<0.001**, p<0.0001***

9.2.6. Assessment of Energy Audit Questionnaire

The frequency distribution of energy events depicted difference in "Very High" score is increased significantly in test treated group than marketed product treated group. The frequency distribution of energy events depicted difference in "High" score is increased significantly in test treated group than marketed product treated group. There was shift of events from low, neutral and moderate category of energy events to High and very high score.

The frequency distribution of energy events depicted that after treatment the number of subjects representing "High" and "Very High" energy level scores were increased and "Moderate" to "Very Low" energy levels were decreased. The similar results are evident from Mean Score of Energy Audit and frequency of energy events.

9.2.7. Assessment of Digestive Behavior Score

At day 30, 60 and 90 there was significant decrease in the mean score of bloating, constipation, loss of appetite and post prandial fullness in test treated group than marketed product treated group.

9.2.8. Assessment of Anthropometric parameters

According to the results obtained regarding anthropometric parameters of Test and Marketed product treated groups, it was observed that there is no significant difference in both the groups in parameters like body weight, circumferences, BMI and arm fat index etc. There was small increase in body weight (of average 1 to 1.5 kg in 3 months) in both the groups after treatment for 90 days but the different was not significant.

There was significant reduction in fat % at day 90, in test group when compared to marketed product treated group. There was significant increase in the skeletal muscle % at day 90 in test group when compared to marketed product treated group.

9.2.9. Global assessment for overall improvement by investigator

In test group, 43 (86 %) subjects reported very much overall improvement and 07 (14 %) subjects reported much overall improvement at the end of the study.

In marketed product group, 04 (7.84%) subjects reported minimum overall improvement, 45 (88.23%) subjects reported no change and 01 (1.97 %) subject reported minimal worsening in the condition at the end of the study.

If compared between the groups, test treated group, performed significantly better than marketed product treated group.

9.2.10. Global assessment for overall improvement by subject

In test group, 45 (90 %) subjects reported very much overall improvement and 05 (10 %) subjects reported much overall improvement at the end of the study.

In marketed product group, 04 (7.84%) subjects reported minimum overall improvement, 45 (88.23%) subjects reported no change and 01 (1.97 %) subject reported minimal worsening in the condition at the end of the study. If compared between the groups, test treated group, performed significantly better than marketed product treated group.

9.2.11. Immunity events by recurrent RTI/ UTI

In test group, at baseline, 12 subjects of test group reported average of at least 12 events of RTI/ UTI in last month to commencement of project.

In test group, 10 subjects (20%) reported recurrent episodes of either RTI/UTI during three months' period. The number frequency of events were 13 events with two to three days' duration. Resolved completely before completion of the study duration.

In marketed product group at baseline, 10 subjects of test group reported average of at least 11 events of RTI/ UTI in last month to commencement of project. In marketed product group, 9 (17.64%) subjects reported 11 events of recurrent RTI/ UTI during three months' period and found to be completely resolved in two to three days. If compared between the groups, there was no significant change in both the groups.

9.3 Safety assessment

9.3.1. Changes in Mean parameters of Complete Hemogram, liver, renal, thyroid function test and random blood sugar levels

All the parameters in the complete Hemogram, liver, renal, thyroid function test and random blood sugar levels were within normal range and comparable between groups at baseline.

After treatment at day 90, mean value of parameters of said parameters did not show any significant change from baseline in test and marketed product treated groups. The difference was statistically insignificant.

9.3.2. Changes in Mean parameter of Lipid Profile

All the parameters of lipid profile were within normal limits at baseline visit in both the groups. After completion of the treatment, no significant change in all other parameters of lipid profile was observed in both the groups i.e. test and marketed product treated group apart from total cholesterol. There was significant $p<0.05$ reduction in test product treated group at day 90.

9.3.3. Changes in Mean HbA1C

Change in Blood HbA1C levels were non-significant at baseline visit in both the groups. After completion of the treatment, there was significant decrease in HbA1C level in test treated group compared to marketed product treated groups.

Table 5: Changes in Mean HbA1C% between the groups

Duration (Days)	Mean HbA1c % (Mean± SD)	
	Test (N = 50)	Marketed Product (N = 51)
Baseline	5.34 ± 0.97	5.30±0.68
90	4.80 ± 0.46*	5.22±0.08

Student's t test P > 0.05 Not Significant, P<0.05 Significant

9.3.4. Changes in Vital parameters

Pulse, respiratory rate, blood pressure of subjects of both the groups was within normal limits at baseline visit. After completion of the treatment, no significant change in said parameters. If compared within the groups, the difference was statistically insignificant.

9.3.5. Tolerability of study drugs assessed by physician and subjects

As per physician and subjects, all the subjects (100%) from both the groups reported excellent tolerability to given intervention ie test product and marketed product.

9.3.6. Palatability and compliance

All the subjects ie 100% from both the groups reported excellent palatability and mix ability of the product. Both the groups also presented excellent compliance to both of the investigational products.

9.3.7. Profile of adverse events

In test group, 15 (30%) subjects reported a total of 17 adverse events during the study period. These adverse events included fever, menstrual pain, hyperacidity, injury, body ache, dry & irritable eyes, cold, heartburn and headache. All these adverse events were mild in severity except headache which was moderate in nature. These adverse events were resolved completely after rescue medication was given. Study treatment was not stopped during these adverse events. All these adverse events were not related to the study drug. In marketed product group, 11 (32%) subjects reported a total of 16 adverse events during the study period. These adverse events included fever, menstrual pain, headache, vomiting, lumbar pain, body ache, mouth ulcer and hyperacidity. All these adverse events were mild in severity and resolved completely after rescue medication was given. Study treatment was not stopped during these adverse events. All these adverse events were not related to the study drug. If compared between the groups, the difference was statistically insignificant.

10. Discussion

In the present study NRL/2019/5PNV was compared for its safety and clinical efficacy against Established Marketed Product. The interpretation of the study parameters obtained are expressed as follows.

VO_2 max, also known as maximal oxygen uptake which measures the maximum amount of oxygen a person can utilize during intense exercise. It is a common measurement used to establish the physical endurance of an individual before and after an oxygen requirement. Factors Affecting VO_2 max are genetics, age, lifestyle & fitness status and boy composition. Shifting body composition to lean mass improvement can improve VO_2 max to considerable level. This in turn upgrades motivation to exercise/ activeness of lifestyle and thereby improving health status. VO_2 max

Directly measures body oxygen consumption, also get direct measurement of maximum heart rate by recording heart rate during the test. It is a measure of how well the heart and lungs work to deliver oxygen and energy to working muscles [40].

After day 60, there was significant increase in VO_2 Max calculated by 6MWT with test product treated group as compared to Marketed product. After day 90, there was significant increase in VO_2 Max calculated by 6MWT i.e. 24.64± 2.77 with test product treated group as compared to Marketed product.

Assessment of anthropometric parameters revealed following facts. There was slight increase (non-significant $p>0.05$) in body weight in both NRL/2019/5PNV and marketed product treated groups. It was evident from results that there was significant decrease in body fat % i.e. 25.13± 6.84 in NRL/2019/5PNV vs. 27.95± 7.08 % at day 90 in Marketed product treated group. Though the body weight is slightly increasing in both groups, the fat content in NRL/2019/5PNV treated group decreases that contributes to its probable anabolic effect in improving skeletal muscle mass and %. It was clearly evident in comparison of skeletal muscle % at day 90 in both groups. Skeletal muscle % was 32.30± 8.97 in NRL/2019/5PNV and 29.02± 6.05 in marketed product treated group (Significant change $p<0.05$), which indicates clinical superiority of NRL/2019/5PNV in positively modifying body composition by reducing body fat and improving skeletal muscle%. The same effect may be involved in improving VO_2 max by NRL/2019/5PNV.

The very practical and strong index of improving physical health and well-being is feeling of energy and stamina throughout the day. There was assessment of energy audit questionnaire to assess subjective changes of the energy levels of the subject divided in 4 parts of day i.e. early morning till breakfast. Breakfast to lunch, lunch to evening snack time and late evening to bedtime. There was comparable baseline energy levels in both the treatment groups before starting of treatment. The energy levels were divided into Very high, High, Moderate, Neutral, Low and Very low depending on the expression of the energy at particular time [41]. The subject reported their reading in terms of score for the day. The results are expressed as mean score and frequency of events in each score title. There was significant increase in shift of individuals experiencing Very High and High energy evidences than Low, Moderate and Neutral energy level in NRL/2019/5PNV treated group than that of marketed product. Energy levels strongly depend on the digestion behavior and muscular strength and vitality together with mental state to perceive stress. The NRL/2019/5PNV contains plant based protein and herbal extracts of *Withania somnifera* and *Aloe vera*. The plant based proteins are easy to digest and could improve digestion cycle. *Withania somnifera* by virtue of its active constituents is known for its rejuvenating, anti-stress activity [42]. *Aloe vera* is known to potentially improve digestion by reducing gastric inflammation and mild laxative action [43]. The composition of NRL/2019/5PNV must have contributed to improve wellbeing of the subjects by improving their energy levels throughout the day.

After 30 and 60 days there was again non-significant difference between MDQ score in Test product and Marketed product treated group. After day 90, there was significant ($p<0.001$) increase (ie2.56±0.62 and 1.80±0.66 respectively) between MDQ score in NRL/2019/5PNV and Marketed product treated group. The NRL/2019/5PNV proved to be more efficacious in improving mood status in subjects than the marketed product. The effect could be attributed to the improved physical stamina, energy levels and rejuvenating effects of *Withania sominifera* as a combination [42, 44].

Quality of sleep determines physical, mental well-being and work productivity. It was demonstrable from the study that NRL/2019/5PNV is more efficacious in improving sleep quality in subjects. The assessment was performed by Pittsburgh Sleep Quality Index. The NRL/2019/5PNV showed significant $p<0.001$ improvement in healthy uninterrupted sleep pattern in subjects. There is much stronger linkage in improvement of quality of sleep and energy levels. The relationship is witnessed in this research. The available scientific data support the conclusion that *Withania sominifera* is a real potent regenerative tonic (Rasayana of Ayurveda), due to its multiple pharmacological actions like anti-stress, neuroprotective, analgesic and anti-inflammatory etc. It is useful for prevention stress related sleep deprivation. The effect could be attributed to rejuvenating action of *Withania sominifera* [45].

Stress is psychological phenomenon and is greatly influenced by individual's ability to perceive the state of stress. The perception to the stress is largely dependent of lack of physical stamina, energy levels, digestion and healthy sleep pattern. NRL/2019/5PNV has demonstrated significant ($p<0.05$) reduction in perceived stress score from 30 days of treatment up to 90 days when compared to marketed product. Protection to the human physiological system against diverse stressor, recent studies shows that the *Withania sominifera* having adaptogens which could induce a state of non-specific increase of resistance to affect internal homeostasis. The adaptogens improve the response to stress and help the body to adapt by normal physiological processes in times of increased stress. The anti-stress activity of *Withania sominifera* must have contributed through its adaptogenic action to combat stress situation. Antistress and adaptogenic properties of *Withania sominifera* have been investigated in all these studies using adult rats were carried out by swimming endurance stress test [42].

The right combination of essential vitamins and minerals are very important to keep the health and wellbeing to its maximum. NRL/2019/5PNV has blend of multivitamin and multimineral to improve and restore the normal physiology of body and exert immunomodulatory and antioxidant potential. Omega 3 fatty acid present in NRL/2019/5PNV is known to restore cellular functioning, integrity and energy balance. Omega 3 fatty acids are cardio protective, neuroprotective nature.

It is well researched fact that protein's nutritional value also depends on its rate of digestion in the gastrointestinal tract. Whey proteins were digested more rapidly than casein. Whey proteins, which are highly soluble in acidic conditions, pass through the stomach and are rapidly hydrolyzed in the duodenum, causing rapid absorption. Soy proteins are digested faster than casein and are slower than whey proteins. Thus, the postprandial muscle protein synthesis rate is more in whey and soy proteins than milk proteins. Plant proteins like rice and pea proteins are not as good as improving anabolic effect but are easy to digest and

improve short term goals in body composition and help in skin, hair and nails health. It can adjust the overall digestibility of the protein blend [46].

After assessment of Digestive Behavior Score following were the observations. At day 30, 60 and 90 of treatment, there was significant decrease in the mean score of bloating, post prandial fullness and constipation ($p<0.001$ respectively for 30, 60 and 90 days) in NRL/2019/5PNV treated group than marketed product treated group. At day 30, 60 and 90 there was significant decrease in the mean score of loss of appetite ($p<0.001$ respectively for 30, 60 and 90 days) in test treated group than marketed product treated group. There is significant decrease in occasional abdominal pain and heartburn in NRL/2019/5PNV treated group as compared to marketed product, at day 90 ($p<0.001$). The results suggest not only the ease in digestion of the NRL/2019/5PNV but also improvement in overall digestive behaviour. The attribution might go to the balanced blend of Whey, soya and plant based proteins like rice and peas together with goodness of *Aloe vera* as a digestive promoter.

Earlier epidemiological studies demonstrate that poor glycaemic control is an independent risk factor for CVD. Whey protein administration has been demonstrated to reduce postprandial glycaemia, mediated through various mechanisms including an enhancement of insulin secretion. Whey protein ingestion has also been shown to elicit an incretin effect, enhancing the secretion of glucose-dependent insulinotropic peptide and glucagon-like peptide-1, which may also influence appetite regulation and glycemic control [47]. Soy protein is reported to possess low glycemic index, suggesting that soy protein tend to improve control of blood glucose and insulin levels. Plant proteins like rice and pea proteins are reported to alter the glycemic and appetitive responses as per a clinical trial conducted which help promoting glycemic control [48]. The research evidence suggests a possible effect of *Aloe vera* on glycemic control in prediabetes and type 2 diabetes.

In the present study, though random blood sugar is not significantly reduced, but the HbA1C levels are significantly reduced in NRL/2019/5PNV treated group than that of the marketed produce treated group. This can suggest possible potential of NRL/2019/5PNV in improving glycemic control by virtue of the protein blend and herbal blend present in product.

Pre-diabetics are at risk of developing type-two diabetes. Many of the Indians are at risk of development of diabetes in near future as a result of borderline or higher levels of blood sugar and cholesterol levels. A randomized controlled trial with pre-diabetes reported reduction in the cholesterol levels after treatment of *Aloe vera* extracts [50].

The lipid-lowering effect of a soy-based protein supplement was evaluated by researchers in patients with hypercholesterolemia. It was evident that soy protein supplementation may effectively reduce serum cholesterol levels and therefore is likely to diminish the risk for cardiovascular disease. Previous studies have suggested that whey supplementation may have beneficial effects on lipid profile. Whey protein proved to be modest in lowering total cholesterol and triglycerides.

In the present study there was significant ($p<0.05$) reduction in total cholesterol but no change in TG, HDL, LDL and VLDL in NRL/2019/5PNV treated group at day 90 of treatment.

GHQ-9 is a standard questionnaire to assess Quality of Life. The assessment of the GHQ-9 score depicted that there was improved score at day 60 and 90 in NRL/2019/5PNV treated group than marketed product treated group.

11. Conclusion

NRL/2019/5PNV is safe and effective in promoting health and wellbeing in healthy adults. There was increased physical endurance by NRL/2019/5PNV treatment indicated as VO2 max compared to marketed product. This can be linked to exercise motivation, work efficiency and improved quality of life. The NRL/2019/5PNV was equally tolerated and compliant as that of marketed product. NRL/2019/5PNV was better in improving energy levels of the subjects throughout the day, elevating mood, improving quality of sleep. Together these parameters are true measures of wellbeing. NRL/2019/5PNV is promoting wellbeing and thus its consumption can elevate health status. NRL/2019/5PNV was found better in reducing the perception to the stress and thus improving quality of life. NRL/2019/5PNV improved digestive behavior of the subjects like reducing constipation, bloating, and loss of appetite and post prandial fullness. These are essentially complaints with sedentary lifestyle. NRL/2019/5PNV consumption is suitable for people with sedentary lifestyle experiencing digestive disturbances. There was improved glycemic control indicated as significant reduction in HbA1C in NRL/2019/5PNV. A balanced blend of proteins like whey, soy, rice and peas along with proportionate vitamin and micro mineral fortification and addition of rejuvenating herbal extracts like *Withania sominifera* and *Aloe vera* is definitely providing better assimilation of the product and efficacy.

12. References

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