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ENHANCING ATHLETE PERFORMANCE: FUNCTIONAL FOOD MIX IMPACT

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Abstract
A prospective observational study was conducted to assess the impact of a formulated functional food mix on athletes'. According to the market survey, a sports food supplement prepared based on the functional ingredients that it was scientifically tested and proved can be stable for a storage period of 13 months. A total of thirty athletes (n = 30), 80% males with an average age of 24 years, participated for a period of 110 days (dietary intervention). The participants were grouped as experimental and naive ($n_1 = n_2 = 15$ athletes). All anthropometric parameters were taken and analyzed. Some of the variables showed slight increases, including the IQR of height (1.74–1.75 m), weight (64.09–65.08 kg), BMI (21.0–21.10 kg/m2), and marginal declines among waist circumference (28.83–28.63 cm), hip circumference (36.90–36.00 cm), and triceps skinfold thickness (8.13–8.09 mm). Biochemically, the functional food mix was found to have significant improvements ($p<0.01$) with anthropometric parameters. Total cholesterol decreased from 171 mg/dL to 162 mg/dL, triglycerides were reduced from 97.2 mg/dL to 91.08 mg/dL, HDL cholesterol increased from 45 mg/dL to 47.53 mg/dL, and LDL cholesterol dropped from 96.84 mg/dL to 94.08 mg/dL ($p<0.01$). The protein parameters showed positive changes; they were increased from 6.4 g/dL to 7.1 g/dL. Albumin and globulin concentrations significantly ($p<0.01$) increased with the A/G ratio (1.54 to 1.47). Both experimental and naive groups were demonstrated by logistic regression, and the results show a significant increase ($p<0.01$) in hemoglobin level (HB) and insignificant changes in uric acid. In terms of performance, the experimental group showed an 86.72% decrease in the too many episode of fatigue and maintained very good homeostasis. This food mix can serve as the best functional food for the development of muscles and bones in a younger population. It is more beneficial for regular athletes and other sports players because it offers diversified potential health benefits. It is useful for sports nutrition specialists to demonstrate new food mix formulations and can also support the development of food algorithms.

Keywords: *Sports nutrition, albumin, globulin, anthropometry, endurance, athletes*

Introduction

Agriculture and nutrition share a common entry point: food. Food is a key outcome of agricultural activities, and in turn, food is a key input to good nutrition. The research progress in the field of nutrition is most frequently dealt with in experimental nutrition, community nutrition, and clinical nutrition. But the rise in popularity of sports and exercise has led to a corresponding increase in the popularity of sports nutrition, which is a rarely focused area in nutrition. Sports nutrition is indeed a booming sector and a critically important branch of nutrition that focuses on the specific dietary needs and strategies of individuals engaged in physical activities, sports, and athletics. Sports nutrition plays a pivotal role in optimizing athletic endurance and ensuring athletes full potential (Kerksicket et *al.,* 2018). In recent years, there has been a growing recognition of the vital role nutrition plays in sports performance. According to a 2021 survey by the Indian Olympic Committee (IOC), 89 percent of elite athletes acknowledge the importance of nutrition in enhancing their performance and aiding in recovery. Additionally, a 2020 study found that 72 percent of elite athletes consult nutrition experts to optimize their performance (Tayechet et *al.,* 2020). The use of dietary supplements, particularly protein supplements, is prevalent among athletes, with 75 percent incorporating them into their regimens. Sports nutrition plays a significant role in injury prevention and recovery, as it helps reduce the risk of sports-related injuries (Turnagolet et *al.,* 2021). This growing awareness of the importance of nutrition in sports has led to a booming sports nutrition market, which is projected to reach $34.7 billion by 2028. Notably, youth sports also emphasize the significance of nutrition, with 80 percent of parents recognizing its importance (Arenas-Jal *et al.,* 2020). It's important to note that nutrition isn't limited to physical health alone; it also contributes to athletes mental well-being, stress management, and the management of issues like anxiety and mood disorders. Nutrition plays a vital role in supplying athletes with the necessary energy for their training, practice, and competitive endeavors. To maintain optimal energy levels, athletes must prioritize their carbohydrate intake. Additionally, essential amino acids obtained from protein sources are instrumental in supporting various physiological functions, most notably muscle protein synthesis. In endurance sports such as marathons and triathlons, strategies like carbohydrate loading and maintaining proper hydration are indispensable. Adequate hydration is particularly critical for performance, as a lack of hydration can result in reduced athletic performance, heat-related health issues, and the onset of muscle cramps, as emphasized by Nichols (2014). Athletes must also replenish electrolytes lost through sweat, especially during endurance events and in hot weather conditions. Pre-workout supplements containing the right balance of nutrients provide sustained energy and help prevent fatigue (Magro, 2019). Post-exercise nutrition is vital for recovery, as consuming carbohydrates and protein within the post-workout window helps replenish glycogen stores and supports muscle repair, facilitating fast recovery. Additionally, ensuring adequate intake of micronutrients can enhance athlete's immune systems. Each athlete has unique nutritional needs based on factors such as sport type, intensity, body composition, age, gender, and specific goals. Therefore, individualized nutrition plans are crucial for helping athletes achieve and maintain their ideal body composition, whether it involves building muscle, reducing body fat, or maintaining weight. Strategies like carbohydrate loading, using the functional ingredient grain amaranth, can enhance endurance by increasing glycogen stores in muscles and the liver (Pareek and Singh, 2021). Sports nutrition leads to short-term performance; it also supports long-term health by helping athletes strike a balance between health, nutrition, and performance. Properly balanced diets, hydration strategies, and nutrient timing are essential components of sports nutrition that help athletes excel in their chosen sports while maintaining overall well-being. Functional ingredients have gained attention in sports nutrition due to their potential to enhance athletic performance, aid in recovery, and support overall health (John and Singla, 2021). These ingredients, such as quinoa, chia, grain amaranth, pulses, and whole grains, offer targeted benefits when incorporated into a balanced diet. They help athletes optimize their physical abilities and achieve their performance goals. Continued research in sports nutrition is likely to further highlight the benefits of functional ingredients, driving their increased use among athletes and fitness enthusiasts (Arenas-Jal *et al.,* 2020). Teradalet et *al.* (2017) investigated the effect of wholesome grain-based functional food formulation on clinical and biochemical parameters in 24- to 30-month-old Wistar albino geriatric rats, corresponding to human age 60–75 years. Animals were randomly divided into five groups. Experimental diets were compared to the basal rat diet (Group I). Four food formulations were wheat-based (Group II), finger millet-based (Group III), wheat-based diet + fenugreek seed powder (Group IV), finger millet-based diet + fenugreek powder (Group V). These five types of diets were fed to the experimental rats for 6 weeks. This study clearly justifies the recommendation to use wholesome grain-based functional foods for the geriatric population. Properly balanced diets, hydration strategies, and nutrient timing are essential components for a sportsperson. Functional ingredients like quinoa, grain amaranth, and chia seeds offer targeted benefits and can help athletes optimize their physical abilities. Continued research in sports nutrition will likely uncover further benefits of these ingredients, contributing to the overall well-being and performance of athletes. Considering the foregoing, a product containing functional ingredients to aid athletes health and athletic performance was developed in the current study.

Methodology

Study design

A prospective-observational study was adopted for the study. Subjects for the study were chosen based on the following inclusion and exclusion criteria: The research was approved by the Institutional Ethical Committee for Human Research (IECHR).

Inclusion criteria

Healthy athletes aged 16–25 years old, involving themselves in sports regularly, and having the willingness to participate in the study were included.

Exclusion criteria

Subjects suffering from deficiencies or disorders, age groups below 16 and above 25 years, not involved in sports regularly, and unwilling to participate in the study were excluded. After scrutiny, a total of (n=30) athletes were purposively selected for the study.

Assessment of pre- and post-nutritional and athletic performance

Framed pretesting of a questionnaire - A structured questionnaire was used to formulated by reviewing past literature cited in scientific research papers and databases. Based on available attributes (which have been extracted from the literature), tested questionnaire was formed.

Sample size (n)

The target population of sports scholars who are engaged in different sports events, in particular cricket games. All participants were selected for the study intervention besides with inclusion and exclusion criteria

Selection of athletes

A total of 30 athletes (n = 30) were purposively recruited for the study with written consent. Importantly, the following criteria were used for the selection of individual respondents based on their health status, involvement in sports and duration of sports engaged by the respondents. For the purpose of uniformity of food habits while minimizing disparity in outcome, the sample size was grouped as a naive v/s experimental group for relative comparison and hypothesis testing. All the subjects were carefully examined with the consent of physicians and caretakers. We conducted a research study involving athletes or sportspeople, it's important to have a sample size that is statistically significant. While the minimum number of participants can vary depending on the specific research question, the nature of the sport, and the statistical power desired, a common guideline is to aim for a minimum of 30 participants. This number is often considered the threshold for achieving a reasonable level of statistical reliability and validity in many research designs. Certain limitations were found during the study period. The first is that the the sample size is very small because of the complexity and willingness of the participants to achieve specific objectives and goals. Certain attributes need a larger sample size to diagnose or test the variability among the participants.

Collection of Data

Before the data collection, an introductory awareness program on the current research work and health and hygiene aspects was conducted to educate about supplements, the need for a balanced diet, and the side effects of excess consumption of animal foods.

Food intervention study

The selected 30 healthy athletes were categorized into an experimental group (n = 15) and a naive group (n = 15). For both groups, blood samples were drawn and assessed for hemoglobin level, total protein content, lipid profile, uric acid, sodium and potassium levels. Once the blood test report was received, the food intervention study began. The naive group participants were on a regular diet with no food intervention. But for the experimental group, along with their regular diet, a protein-rich functional food mix in the form of a ready-to-drink beverage as a post-workout supplement was given. A total of 100 g of functional food mix reconstituted in 300 ml of water per day per athlete for a period of 110 days was consumed. Follow-up was done every day through a phone call and by meeting participants at least once a week. Issues regarding any kind of discomfort or dislike with the product were frequently recorded and rectified. In the food intervention period, about eight nutrition education classes covering various aspects of diet, health, and hygiene were covered. As a part of the research, an educational tool like a folder was developed and distributed to all 30 athletes.

Pre- and post-anthropometric assessment

Anthropometry is a widely used, inexpensive, simple, and easy-to-apply technique that comprises various body measurements. Anthropometric parameters included height, weight, waist circumference, hip circumference, waist-to-hip ratio, and skinfold thickness, which were considered the best tools to evaluate nutritional status.

Height (cm)

The height was measured by the method described by Jelliffe (1997) with the help of an anthropometric rod. The subjects were asked to stand upright against the wall on a flat surface, barefooted, with the heels touching each other and ensure that the buttocks, shoulders, and back of the head were held comfortably erect with arms hanging by the sides. Marking was done on the wall with the help of a scale, and then the height was measured in centimeters. Three readings were taken to avoid any errors, and then an average was taken. The mean of the recorded height was compared with ICMR (2010) standards.

Weight (kg)

The weight of the respondents was taken using a weighing balance calibrated in kilograms. The subjects were made to stand straight on the balance platform without touching anything nearby. The weight was recorded three times, and the mean was calculated. The mean weight was compared with ICMR (2010) standards.

Body Mass Index (kg/m2)

Body Mass Index (BMI) is a crude index for assessing nutritional parameters. It is a convenient, non-invasive screening tool for the assessment of weight status, estimating underweight, overweight, and obese. It was calculated (Kularathneet al., 2019) using the formula described by ICMR (2010). (Underweight<18.5, Normal range 18.5-22.9, Overweight 23.0-27.4, Obese >27.5)

BMI = body weight (kg)/height (m2)

Waist-to-hip ratio

Participants were asked to stand up straight and breathe out. A measuring tape was used to check the distance around the smallest part of the waist, just above the belly button. The measurement revealed the values for waist circumference. Similarly, the distance around the largest part of the hips, i.e., the widest part of the buttocks was measured to know the hip circumference. WHR was calculated by dividing waist circumference by hip circumference (Kularathne et al., 2019).

Skinfold thickness (mm)

We measured skinfold thickness in triceps, the Harpenden Skinfold Caliper was used to the nearest of 0.1mm by using the method of Jelliffe (1997).

Procedure for skinfold measurement

Firmly grasp a fold of the person's skin between the thumb and index finger and lift it up. The skinfold should include two layers of thickness: one of skin and one of subcutaneous fat, but not muscle. The calliper should be placed at a 90-degree angle to the skinfold, approximately 1 cm below the fingers. Slightly release the pressure between the fingers, but remain holding the skinfold so that a greater pressure is applied by the calipers. Release the handle of the calipers and read the needle to the nearest 0.1 mm, approximately 4 seconds after the pressure is released. To estimate the body fat, the triceps skinfold (backside middle upper arm) was measured between the acromion (bony point of the shoulder) and olecranon processes (bony point of the elbow). The arm was held freely to the side of the body.

Pre and post-assessment of biochemical parameters

Nutrients play an important role in many different metabolic processes, as the human body needs a constant supply of adequate nutrients to perform them. Biochemical assessment detects a number of nutrient-depletion stages, namely changes in nutrient stores, body fluids, functional levels of tissues, and enzyme activity. Biochemical assessment also has the ability to determine nutritional status at an earlier stage of deficiency, therefore enabling correction at an early stage of nutritional inadequacy. Athletes were assessed pre- and post-period for biochemical parameters such as hemoglobin level, protein profile, lipid profile, uric acid, sodium, and potassium levels, which were estimated in the laboratory.

Statistical analysis

All the analysis was performed in triplicate and presented as mean ± standard deviation. Data were analyzed by a student t-test using SPSS (Statistical Package for Social Sciences) software version 23. Differences were declared statistically significant at $P < 0.01$. The Wilcoxon sign test works with metric (interval or ratio) data that is not multivariate normal, or with ranked or ordinal data. Generally, it is the non-parametric alternative to the dependent sample t-test. The Wilcoxon sign test tests the null hypothesis that the average signed rank of two dependent samples is zero. The Wilcoxon test compares two paired groups and comes in two versions: the rank sum test and the signed rank test. The goal of the test is to determine if two or more sets of pairs are different from one another in a statistically significant manner. The clinical trial data was compiled using R-statistical software for Univariate logistic regression and Kaplan Mayer Survival Analysis model (equation 1 & 2) was used to check the significance and threshold of protein profile.

$$P(Y = y) = \frac{1}{expo^{\,a+\beta_0 X_0+\beta_1 X_1+\cdots\beta_k \cdot X_k}} \qquad (1.1)$$

$P(Y = y) = f(x) \sim$ N (μ, σ^2), again the model was standardized based on the simplification of each variables data traformed techniques. Finally sequences of odd ratio was simulated

$$odd\ ratio = Logi\ \left(\frac{P}{1-p}\right) \qquad (1.2)$$

Where, $P(Y = y) = dependent\ varaible,$ $\beta_0, \beta_1, .\beta_k$ is the regression co efficient, $X_0, X_1, X_1 \ldots. X_k$ is the independent variable . The dependent variable coded as '1' good performance '0' coded as not well performed after food mix intervention.

Results

Baseline characteristics of athletes

The baseline characteristics of the experimental and naive groups are depicted by the descriptive statistics. The majority of the participants in the study were male (80%). The maximum, average, and IQR age of athletes was 24- 19.5 years and 16 years, respectively. The educational qualifications of subjects were PUC (80%) and UG (20%). Around 37% of the subjects had participated in state-level cricket competitions.

Table 1 Pre and post anthropometric measurement of experimental group (n=15)

Anthropometric measurements	Intervention period	Mean ± SD	t-value
Height (cm)	Pre	1.74 ± 0.05	4.02**, ≤ 0.01
	Post	1.75 ± 0.05	
Weight (Kg)	Pre	64.09 ± 6.45	3.07**, ≤0.01
	Post	65.08 ± 6.46	
BMI (Kg/m^2)	Pre	21.00 ± 2.09	0.94**, ≤0.01
	Post	21.10 ± 1.89	
WC (cm)	Pre	28.83 ± 2.46	1.10**, ≤0.01
	Post	28.63 ± 1.93	
HC (cm)	Pre	36.90 ± 1.64	4.32**, ≤0.01
	Post	36.00 ± 1.59	
Triceps (mm)	Pre	1.13 ± 0.41	3.34**, ≤0.01
	Post	1.08 ± 0.38	

** Significant @ 1% level (p<0.01)

Table 2 Pre and post lipid profile of naive group (n=15)

Lipid Profile	Intervention period	Mean ± SD	t-value
Cholesterol (mg/ dL)	Pre	187.93 ± 44.81	3.24**, ≤0.01
	Post	189.93 ± 44.52	
Triglycerides (mg/ dL)	Pre	123.93 ± 70.90	5.77**, ≤0.01
	Post	125.66 ± 71.33	
HDL Cholesterol (mg/ dL)	Pre	45.33 ± 9.39	2.10^{ns}
	Post	45.73 ± 9.75	
LDL Cholesterol (mg/ dL)	Pre	118.93 ± 32.38	1.25^{ns}
	Post	119.04 ± 32.48	
VLDL Cholesterol (mg/ dL)	Pre	24.90 ± 14.29	2.14^{ns}
	Post	25.13 ± 14.27	

** Significant @ 1% level (p<0.01), ns- non-significant

Table 3 Pre and post protein profile of experimental group (n=15)

Protein Profile	Intervention period	Mean ± SD	t-value
Total protein (g/ dL)	Pre	6.40 ± 0.71	5.29**, ≤0.01
	Post	7.10 ± 0.55	
Albumin (g/ dL)	Pre	3.89 ± 0.38	6.32**, ≤0.01
	Post	4.23 ± 0.32	
Globulin (g/ dL)	Pre	2.51 ± 0.53	1.52**, ≤0.01
	Post	2.87 ± 0.26	
A/G ratio	Pre	1.54 ± 0.29	0.32*, ≤0.05
	Post	1.47 ± 0.13	

** Significant @ 1% level (p<0.01), * Significant @ 5% level (p<0.05)

Table 4 Pre and post protein profile of naive group (n=15)

Protein Profile	Intervention period	Mean ± SD	t-value
Total protein (g/ dL)	Pre	7.74 ± 0.35	4.90**, ≤0.01
	Post	7.86 ± 0.33	
Albumin (g/ dL)	Pre	4.42 ± 0.24	6.85**, ≤0.01
	Post	4.53 ± 0.22	
Globulin (g/ dL)	Pre	3.32 ± 0.17	0.43^{ns}
	Post	3.33 ± 0.16	

A/G ratio	Pre	1.32 ± 0.08	4.25**, ≤0.01
	Post	1.36 ± 0.06	

** Significant @ 1% level (p<0.01), ns- non-significant

Table 5 Pre and post biochemical parameters of experimental group (n=15)

Biochemical parameters	Intervention period	Mean ± SD	t-value
Hemoglobin (g/ dL)	Pre	14.94 ± 1.70	6.16**, ≤0.01
	Post	15.32 ± 1.52	
Uric Acid (mg/ dL)	Pre	5.32 ± 1.31	0.45^{ns}
	Post	5.34 ± 1.26	
Sodium (mmol/ L)	Pre	141.86 ± 3.13	3.77**, ≤0.01
	Post	139.80 ± 1.85	
Potassium (mmol/ L)	Pre	4.16 ± 0.21	0.144^{ns}
	Post	4.15 ± 0.17	

** Significant @ 1% level (p<0.01), ns- non-significant

Table 6 Pre and post biochemical parameters of naive group (n=15)

Biochemical parameters	Intervention period	Mean ± SD	t value
Hemoglobin (g/ dL)	Pre	15.13 ± 2.00	7.04** , ≤0.01
	Post	15.38 ± 1.99	
Uric Acid (mg/ dL)	Pre	5.25 ± 1.52	20.91**, ≤0.01
	Post	5.58 ± 1.50	
Sodium (mmol/ L)	Pre	138.2 ± 1.93	5.28**, ≤0.01
	Post	140 ± 1.85	
Potassium (mmol/ L)	Pre	3.88 ± 0.21	6.24**, ≤0.01
	Post	4.12 ± 0.25	

** Significant @ 1% level (p<0.01)

Table 7 Total time spent on workout per day in both groups (n=30)

Groups	Intervention period	Total time spent on workout per day (hours)	t-test
Naive group (n=15)	Pre	3.40 ± 0.82	ns
	Post	3.40 ± 0.82	
Experimental group (n=15)	Pre	3.08 ± 1.43	1.89**≤0.01)
	Post	3.53 ± 1.26	

** Significant @ 1% level (p<0.01), ns- non-significant

Table 8 Pre and post assessment of fatigue in both groups(n=30)

Groups	Perception on feeling of fatigue	Pre-intervention period (%)	Post-intervention period (%)	Wilcoxon test
Naive group (n=15)	Rarely	26.7	26.7	ns
	Frequently	73.3	73.3	
Experimental group (n=15)	Rarely	13.3	86.7	** ≤0.01)
	Frequently	93.3	6.67	

** Significant @ 1% level (p<0.01), ns- non-significant

Anthropometric measurement of athletes

The anthropometric measurements for two groups, the experimental and naive groups, before (pre) and after (post) the food intervention are presented in Tables 1 and 2. In the experimental group, average height increased slightly from 1.74 m to 1.75 m, weight increased from 64.09 kg to 65.08 kg, and BMI (body mass index) rose from 21.0 to 21.10. Waist circumference (WC) decreased slightly from 28.83 cm to 28.63 cm, hip circumference (HC) decreased from 36.90 cm to 36.00 cm, and triceps skinfold thickness decreased from 8.13 mm to 8.09 mm. The naive group's measurements followed a similar pattern, but without intervention. These changes in anthropometric measurements provide insights into how the intervention impacted the physical characteristics of the experimental group compared to the naive group.

Table 2Pre and post anthropometric measurement of naive group (n=15)

Anthropometric measurements	Intervention period	Mean ± SD	t-value
Height (cm)	Pre	1.74 ± 0.06	2.25*, p<0.05
	Post	1.74 ± 0.06	
Weight (Kg)	Pre	65.62 ± 9.17	2.62*,p<0.05
	Post	66.61 ± 8.98	
BMI (Kg/m^2)	Pre	21.65 ± 2.69	1.5^{ns}
	Post	21.85 ± 2.68	
WC (cm)	Pre	29.00 ± 2.59	0.80^{ns}
	Post	29.06 ± 2.71	
HC (cm)	Pre	36.73 ± 2.54	2.44*, p<0.05
	Post	36.93 ± 2.51	
Triceps (mm)	Pre	1.17 ± 0.44	3.05**, p<0.01
	Post	1.21 ± 0.43	

** Significant @ 1% level (p<0.01), * Significant @ 5% level (p<0.05)

Assessment of lipid profile, protein profile, hemoglobin, uric acid, and electrolyte levels among athletes. The pre- and post-intervention lipid profiles for both groups are exhibited in Tables 3, 4, and Fig. 1.3. The experimental group saw a significant (p<0.01) reduction in mean total cholesterol levels, from 171 mg/dL to 162 mg/dL, and triglyceride levels decreased significantly from 97.2 mg/dL to 91.08 mg/dL. The average HDL-cholesterol levels increased significantly from 45 mg/dL to 47.53 mg/dL, and LDL-cholesterol levels decreased significantly from 96.84 mg/dL to 94.08 mg/dL. In contrast, the naive group showed a significant increase in mean total cholesterol (from 187.93 mg/dL to 189.93 mg/dL) and triglyceride levels (from 123.93 mg/dL to 125.66 mg/dL). While both groups exhibited an increase in mean HDL-cholesterol levels, the naive group's change was non-significant (from 45.33 mg/dL to 45.73 mg/dL), and the mean LDL-cholesterol levels in the naive group showed a non-significant increase (from 118.93 mg/dL to 119.04 mg/dL). The VLDL-cholesterol level increased significantly in the naive group (from 24.90 to 25.13 mg/dL) but non-significantly in the experimental group. These results indicate that lipid profile parameters changed before and after the intervention, with the experimental group generally showing improvements while the naive group exhibited a slight increase.

Table 3Pre and post lipid profile of experimental group (n=15)

Lipid Profile	Intervention period	Mean ± SD	t-value
Cholesterol (mg/ dL)	Pre	171 ± 18.6	5.15**, p<0.01
	Post	162 ± 17.0	
Triglycerides (mg/ dL)	Pre	97.20 ± 31.88	4.28**, p<0.01
	Post	91.08 ± 29.08	
HDL Cholesterol (mg/ dL)	Pre	45.00 ± 5.66	5.31**, p<0.01
	Post	47.53 ± 6.40	
LDL Cholesterol (mg/ dL)	Pre	96.84 ± 18.56	4.83**, p<0.01

	Post	94.08 ± 17.99	
VLDL Cholesterol (mg/ dL)	Pre	19.80 ± 6.92	4.50**, p<0.01
	Post	18.96 ± 6.88	

** Significant @ 1% level (p<0.01)

Pre and post protein profile of both groups

The pre- and post-protein profiles of both groups are shown in Tables 5, 6, and Fig. 1.4. The average concentration of total protein in the experimental group was 6.4 g/dL before the intervention (pre) and significantly (p<0.01) increased to 7.1 g/dL after the intervention (post). The average concentration of albumin and globulin in the experimental group significantly (p<0.01) increased from 3.89 g/dL to 4.23 g/dL and 2.51 g/dL to 2.87 g/dL, respectively. The average A/G ratio in the experimental group was 1.42 before the intervention and decreased significantly (p<0.01) to 1.39 after the intervention. The average concentration of total protein and albumin in the naive group was 7.74 g/dL, 4.42 g/dL, and showed a significant increase to 7.86 g/dL, 4.53 g/dL, respectively. The average concentration of globulin in the naive group remained relatively stable (3.32 g/dL (pre) and 3.33 g/dL (post). The average A/G ratio significantly (p<0.01) increased from 1.32 to 1.36 (Table 5, 6, and Fig. 1.4).

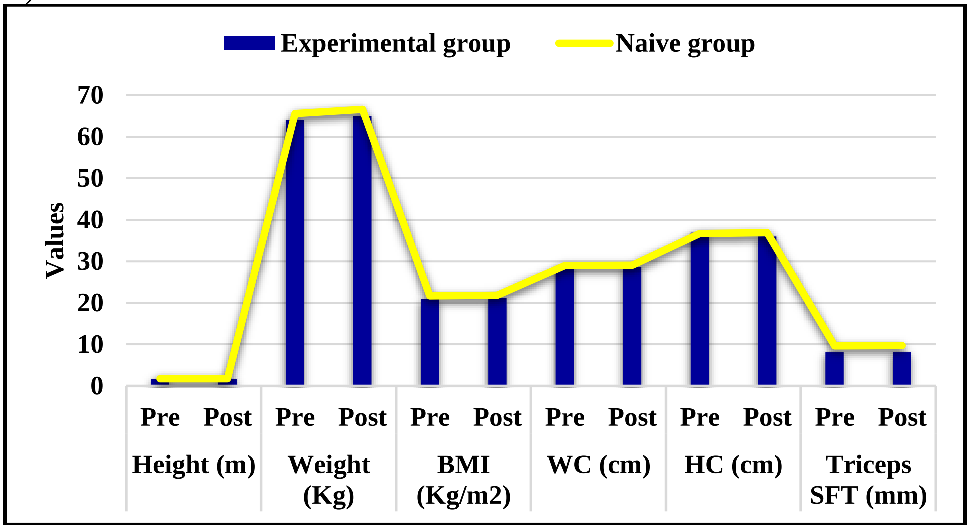


Fig. 1.2 Pre and post anthropometric measurements of experimental and naive groups

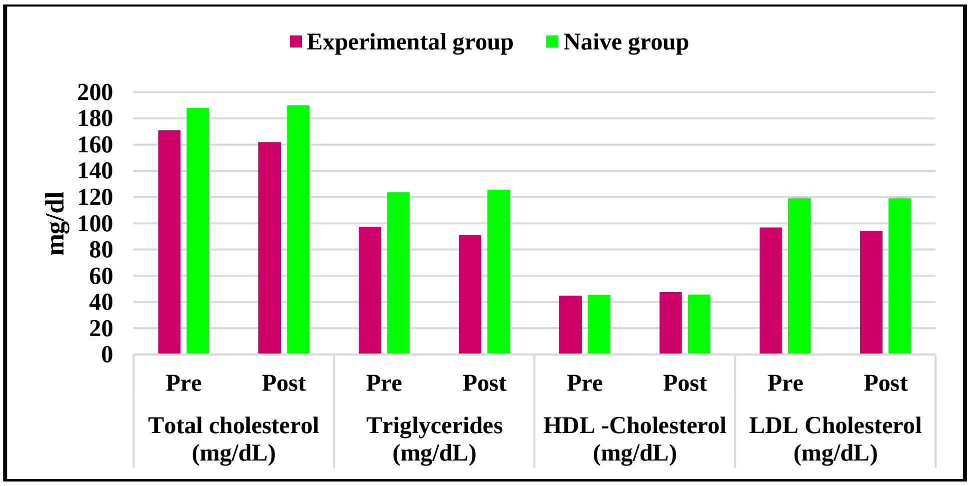


Fig. 1.3 Pre and post lipid profile of experimental and naive groups (n =30)

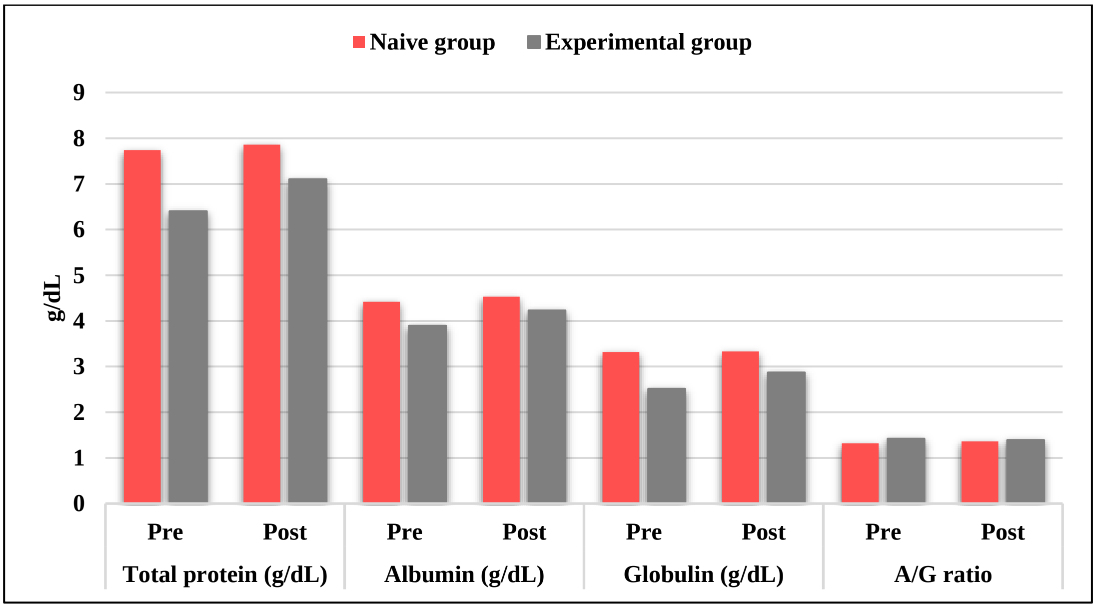
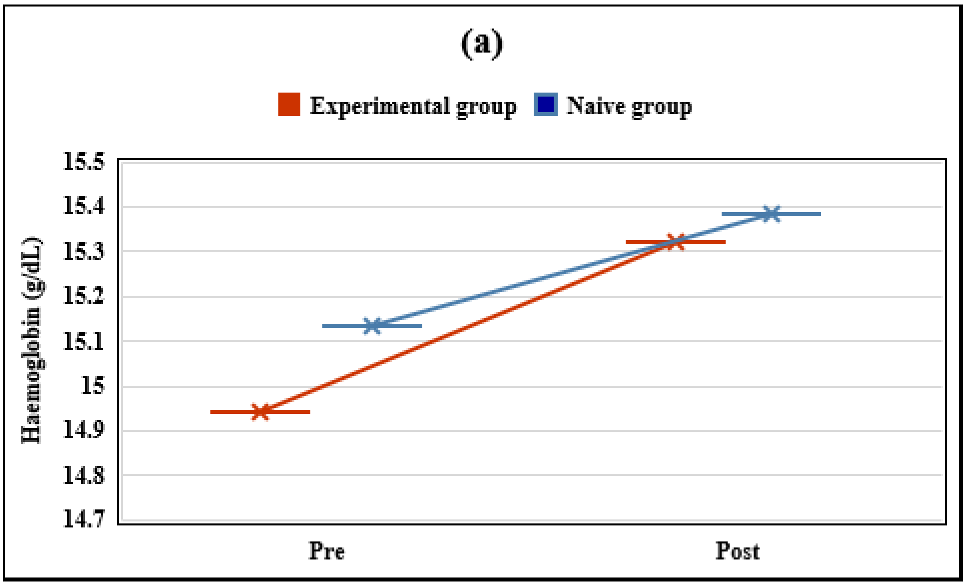


Fig. 1.4 Pre and post protein profile of experimental and naive groups (n =30)

Pre and post biochemical parameters of both groups

Pre- and post-biochemical parameters of the experimental and naive groups are presented in Tables 7, 8, and Fig. 1.5. A highly statistically significant difference ($p < 0.01$) between the pre- and post-intervention hemoglobin levels (14.94 to 15.32 g/dL) was noticed. The difference in uric acid levels between the pre- and post-intervention stages is not statistically significant (5.32 to 5.34 mg/dL). There was a statistically significant decrease ($p<0.01$) in sodium levels before and after food intervention from 141.86 to 139.80 mmol/L. Potassium levels did not differ significantly in the pre- and post-intervention analyses. Table 17 demonstrates significant changes in hemoglobin, uric acid, sodium, and potassium levels in the naive group following the intervention. The highly significant ($P<0.01$) t-values for these parameters suggest strong statistical evidence of change. Specifically, there was an increase in hemoglobin (15.38 g/dL), uric acid (5.58 mg/dl), sodium (140 mmol/L), and potassium levels (4.12 mmol/L) after the intervention period of 110 days.



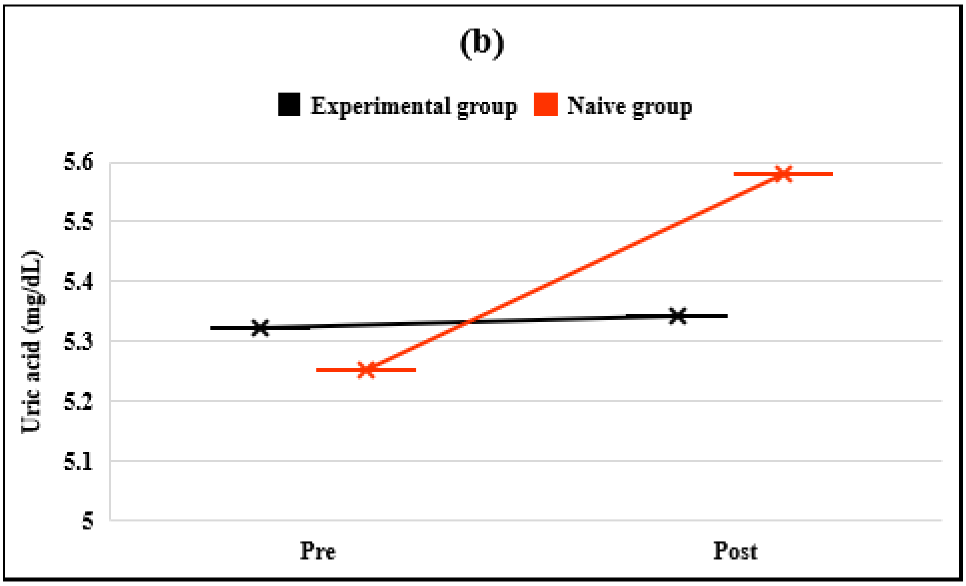


Fig. 1.5 Pre and post (a) Haemoglobin (g/dl) (b) Uric acid (mg/dl)

The physical performance of athletes

Comprehensive data regarding the daily workout duration in both the naive and experimental groups before and after an intervention is furnished in Table 9. In the naive group, the workout time remained relatively consistent at 3.40 hours per day, with no noteworthy change, as denoted by NS. (not significant). Conversely, the experimental group exhibited a statistically significant ($p<0.01$) upsurge in workout time following the intervention. This indicates that the intervention had a substantial impact on the exercise routines of the experimental group, resulting in a notable increase in their daily workout duration.

Pre and post assessment of fatigue in both groups

A detailed assessment of fatigue perception in both the naive and experimental groups before and after an intervention tested by the Wilcoxon test, specifically the Wilcoxon signed-rank test for paired data or the Mann-Whitney U test for independent samples, is typically used for analyzing quantitative data. It's a non-parametric test designed for ordinal or non-normally distributed continuous variables. In the naive group, there was no notable alteration in fatigue perception. In contrast, the experimental group exhibited a highly significant ($p<0.01$) decrease in the frequency (86.7%) of reporting fatigue after the intervention.The significance of protein profile and electrolytes for the experimental and naïve had a significant $p<0.01$, and all the selected parameters with different follow-up periods (pre and post-period) were simulated by the Kaplan-Mayer survival analysis model. The cut-off value and descriptive statistics were simulated with greater accuracy (95%) (Methodology, equations 1.1 and 1.2). The uric acid and potassium were found to be significant at 1 percent level ($p<0.01$) as compared with the naïve group with a mean value of uric acid (5.33+0.34 mg/dl) and a cut-off value of 5.00 mg/dl with a confidence interval (CI 95% 2.75 to 7.90) and good specificity and sensitivity of 86 percent and 64 percent, respectively (Area Under Curve = 0.76).The same simulation results were found in the protein profile. All the parameters of the protein were tested at the desired level of significance. The results were found to be statistically significant with naive ($p<0.01$). An absolute mean total protein (7.21+0.18 g/dl) with a cut-off value (7.03 g/dl) and confidence interval (CI 95% 5.81 to 8.61) ambient level of Area Under Curve (AUC=0.82).Further, the rest of the parameter associations were tested by the same model, and the results found that the mean albumin was 4.18+0.10 g/dl, with a cut-off value of 4.08 g/dl and a confidence interval of 95% (3.43 to 4.93), and the absolute mean globulin was 3.03+0.14 g/dl, with a with a cut-off value of 2.89 g/dl and a confidence interval of 95% (1.98 to 4.08). Finally, albumin globulin ratio was tested by the KPM model, with an average of 1.42+0.08 and a cut-off value of 1.35 with a confidence interval of 95% (0.84 to 2.00). From the above model, all selected parameter potency levels before and after intervention statistically differ in experimental and naive groups

($p<0.01$). Relatively, the protein profile shows a positive association with athletes. As per the confirmative parameters, this product can be used as an indicator or boosted transcriptase enzymatic food for the sports-dependent population and as a good nutritional supplement for the younger population. After the post-follow-up period, the parameters of the protein profile significantly differed from those of the naive group with good specificity and sensitivity. All the respondents showed marginal differences in protein level ($p<0.05$).

Discussion

Anthropometric measurements of athletes Quinoa, grain amaranth, and chia seeds are rich in essential nutrients, including protein and certain vitamins and minerals, which can support overall growth and health. Adequate nutrition from these foods may have contributed to maintaining proper bone health and growth, potentially resulting in a slight increase in height. The weight increase (Tables 1, 2, and Fig. 1.2) could also be influenced by overall calorie intake, physical activity, and metabolism. Functional ingredients used are high in fiber, which can aid in digestion and potentially support weight loss. However, other factors like exercise and overall calorie intake also influence waist circumference (Tables 1, 2, and Fig. 1.2). Changes in hip circumference (Tables 1, 2, and Fig. 1.2) can be influenced by overall weight changes, including fat loss or muscle gain. Triceps skinfold thickness can be an indicator of body fat levels. A decrease may suggest a slight reduction in body fat. High-fiber foods like quinoa, grain amaranth, and chia seeds can help control appetite and support weight management. A similar study reported that the mean height (183.34 cm), weight (70.21 kg), waist circumference (79.60 cm), and hip circumference (90.65) of athletes as described by Lamboonavar (2008) were high compared to the present study. Whereas BMI (20.82 kg/m^2) was low (Table 1, 2 & Fig. 1.2) compared to the current study indicating the normal and ideal body composition of athletes consuming a developed functional food mix.Assessment of lipid profile, protein profile, hemoglobin, uric acid, and electrolyte levels among athletes. The functional food mix consumed by the experimental group boasts a significant dietary fiber content, particularly soluble fiber. This soluble fiber plays a crucial role in lowering total cholesterol levels by binding to cholesterol in the digestive tract, preventing its absorption into the bloodstream. Moreover, ingredients such as quinoa, grain amaranth, and chia seeds are rich in healthy fats, including omega-3 fatty acids. These fats have a positive impact on triglyceride levels, contributing to their reduction (Table 3, 4, and Fig. 1.3). The consumption of this developed functional food mix also led to an increase in HDL (high-density lipoprotein) cholesterol, often referred to as "good" cholesterol. This elevation can be attributed to the functional food mix's healthy fat content and potential anti-inflammatory effects. Furthermore, the combination of fiber, healthy fats, and antioxidants present in quinoa, grain amaranth, and chia seeds may work synergistically to lower LDL (low-density lipoprotein) cholesterol, commonly known as "bad" cholesterol. This reduction can help mitigate the risk of plaque buildup in the arteries, promoting cardiovascular health. These results could also be influenced by factors such as overall diet, physical activity, genetics, and the duration of the dietary intervention. Similarly,an athlete administering a calorie-restricted diet for a period of 6 weeks reported a reduction in total cholesterol (197.2 to 171.4 mg/dL), LDL (107.6 to 102.5 mg/dL), and triglycerides (102.3 to 89.7 mg/dL). The reduction was higher compared to the current investigation. The HDL cholesterol, also known as good cholesterol, exhibited a reduction (51.6 to 47.0 mg/dL) after the food intervention conducted by Holowko*et al. (2019)*, but in the present study, the value of HDL significantly increased, proving the positive impact of a functional food mix on athletes. The pre- and post-protein profiles of both groups, the total protein level, and the ratio of albumin to globulin can help detect several kinds of health problems, including liver and kidney disease as well as nutritional deficiencies. A high A/G ratio is associated with dehydration, malnutrition, and other gastrointestinal conditions. The presence of plant-based proteins in the functional food mix likely contributed to the observed increase in protein concentration among participants in the experimental group during the intervention period. Notably, the protein content in the experimental group matched the results seen in the naive group, who were consuming a commercial protein formulation. Quinoa, grain amaranth, and chia seeds, being rich sources of essential amino acids and proteins, may have played a role in raising albumin and globulin levels. These proteins are vital for the proper functioning of various bodily processes, including immune system maintenance. The decrease in the albumin/globulin (A/G) ratio could be attributed to the relative increase in globulin levels compared to albumin. This ratio can serve as an indicator of changes in the body's immune response and overall protein balance. The observed increase in protein-related measurements and the slight shift in the A/G ratio suggest that incorporating quinoa, grain amaranth, and chia seeds into the diet during the intervention could have had a positive impact on protein levels and potentially on immune function within the experimental group. However, it's important to recognize that individual responses to dietary changes can vary, and other factors may have contributed to these findings. Quinoa, grain amaranth, and chia seeds are nutrient-rich foods that provide a wide array of essential nutrients, including iron, various vitamins, and minerals. Iron plays a pivotal role as it's a vital component of hemoglobin, the protein found in red blood cells responsible for transporting oxygen throughout the body. The functional food mix developed is notably rich in protein, which is crucial for overall cell health, including the synthesis of hemoglobin. Furthermore, it supplies essential amino acids like lysine, which is necessary for hemoglobin production. Uric acid levels primarily depend on factors related to purine metabolism and can be influenced by dietary sources of purines, such as specific meats and seafood. It's important to note that while quinoa, grain amaranth, and chia seeds are highly nutritious and offer various health benefits, they are not recognized for being high in purines, the primary precursors of uric acid. Additionally, these foods naturally have a low sodium content, which could potentially account for the observed decrease in sodium levels. The reduced sodium intake from the functional food mix contributed to maintaining a healthier sodium balance in the body. Quinoa, grain amaranth, and chia seeds are notable sources of potassium, a mineral critical for regulating blood pressure and electrolyte equilibrium. The absence of a significant change in potassium levels (Tables 7, 8, and Fig. 1.5) suggests that these foods may have helped sustain stable potassium concentrations throughout the intervention. A similar finding was suggested that , the hemoglobin of athletes playing basketball increased from 13.0 to 14.59 g/dL, as reported by Lamboonavar (2008). The value is low compared to the present result (Tables 7, 8, and Fig. 1.5). A high red blood cell volume facilitates high oxygen transport to the active skeletal muscles by facilitating a high cardiac output. A higher volume of red blood cells equals higher athletic performance. Consequently, improvement of hemoglobin mass has such a prominent role in the training of high-performance athletes (Reljicet et *al*., 2013).

Physical performance of athletes

The functional food mix, rich in complex carbohydrates, provides a steady release of energy. This sustained energy can support longer and more effective workouts by preventing energy crashes during exercise. Quinoa and grain amaranth are good sources of plant-based protein, which is essential for muscle recovery and growth. Adequate protein intake can improve endurance and performance during workouts. Chia seeds contain omega-3 fatty acids, which have anti-inflammatory properties and can aid in reducing exercise-induced muscle soreness. This may encourage individuals to engage in longer and more frequent exercise sessions. Similarly, 25 percent of the players expressed an interest in football, with approximately 16 percent engaging in football once a week, while a smaller percentage (9%) played the sport twice a week. In contrast, about 16 percent of the players showed an interest in handball, and roughly 9 percent of them participated in this game once a month, with approximately 6 percent playing it twice a week (Lamboonavar, 2008). There is a strong relationship between the feeling of fatigue (Table 10), thirst, urine color, and urine specific gravity. Dehydration, indicated by increased thirst, darker urine color, and higher urine specific gravity, can contribute to fatigue by reducing blood volume, compromising circulation, and affecting various physiological processes. Staying adequately hydrated is essential for maintaining energy levels and overall well-being. This underscores that the intervention had a substantial and beneficial impact on reducing the perception of fatigue within the experimental group. Functional foods are an excellent choice for vegetarians. Quinoa and grain amaranth are complex carbohydrates, which means they provide a steady release of energy over a longer period of time. This can help athletes maintain their energy levels during workouts and competitions. These nutrients can aid in reducing the risk of fatigue (Table 10) and muscle cramps (Navruz-Varli and Sanlier, 2016). The sports nutrition used in this study contains a balanced combination of carbohydrates and protein. Recent research from a prominent exercise science laboratory has revealed that sports supplements with the right balance of protein and carbohydrates can extend fatigue resistance even more effectively than supplements solely consisting of carbohydrates. This finding aligns with the results reported by Koopman *et al.* (2004) and Saunders (2004). Their studies indicated that supplementing with carbohydrate and protein during exercise reduces endogenous protein oxidation, enhances the protein balance rate, and increases the availability of amino acids. This combination also aids in limiting the breakdown of muscle protein during prolonged exercise, thus mitigating muscle damage.

Conclusion

The market survey revealed a gap in sports supplements meeting the specific needs of athletes. The development of a functional food mix with quinoa, amaranth, and chia seeds garnered high acceptability scores. The functional food mix demonstrated nutritional richness, including antioxidants and amino acids, as well as moderate glycemic impact. Long-term storage stability was confirmed, and the cost was reasonable. Notably, the food intake study shows positive effects on anthropometric parameters, lipid profile, protein profile, and workout performance. Dietary patterns shifted towards healthier choices, whereas participants' knowledge, attitude and practice well improved. This comprehensive study underscores the potential of the developed functional food mix to benefit athletes and promote better dietary choices and nutrition awareness. Hence, the developed functional food mix is the best alternative to be chosen by the athletes.

Limitations of the study

As per the study research findings, the present study was conducted on a sample level, which will support only a few objectives of interest. However, in the interest of the food industry and stakeholders, a population-based study is necessary to fill the research gap to a greater extent (formulations of different kinds of food mixtures). The present trial has focused on a two-stage process (food formulation and human intervention). As per ethical considerations, we have recruited only a small number of respondents for the research. In this paradigm, little information has been correlated with the present objective of interest. With the aim of magnifying the research hypothesis for the development of newer algorithms and the formulation of food mixtures, the present research finding has ample scope to escalate different hypothetical assumptions about the rationality of the research.

Conflict of interest – Nil

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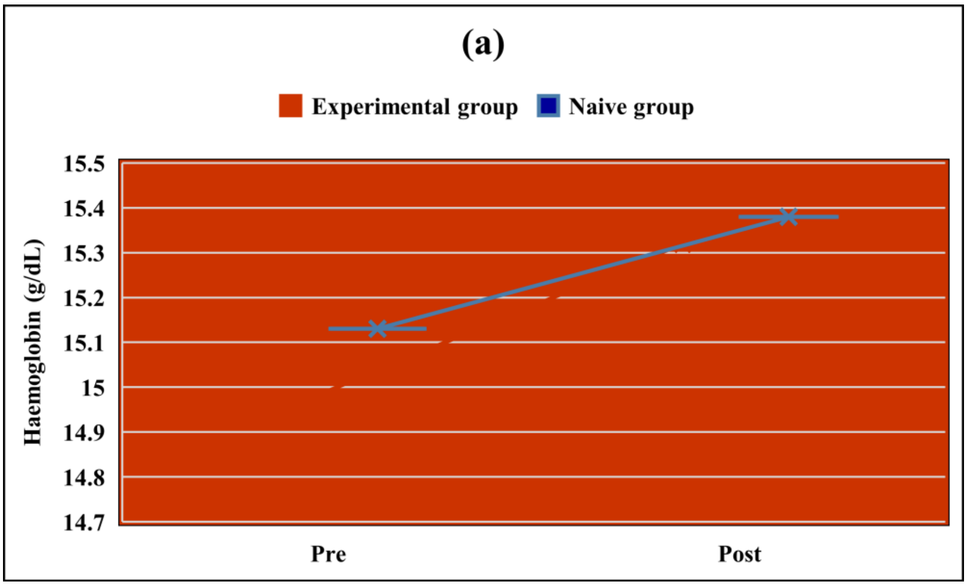
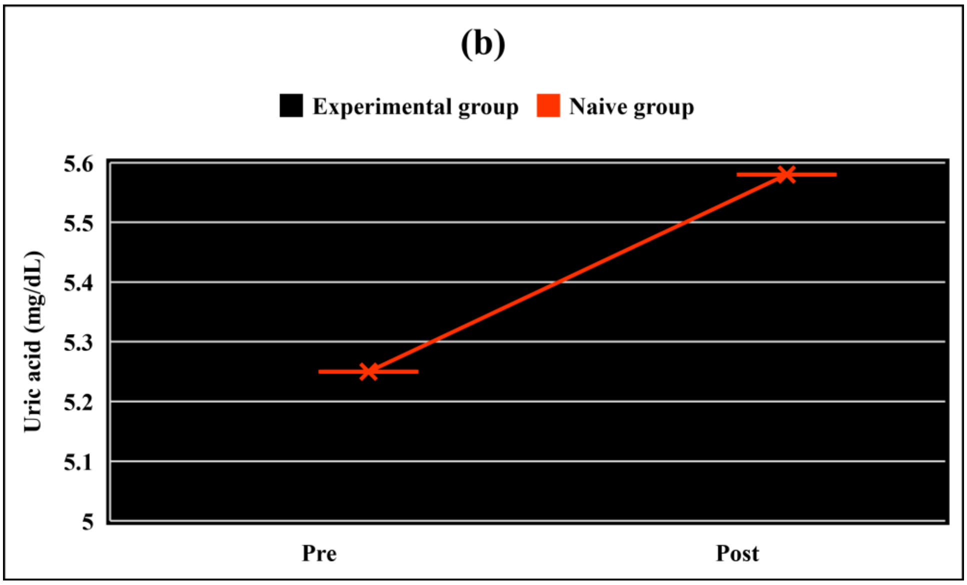
Annexure -1

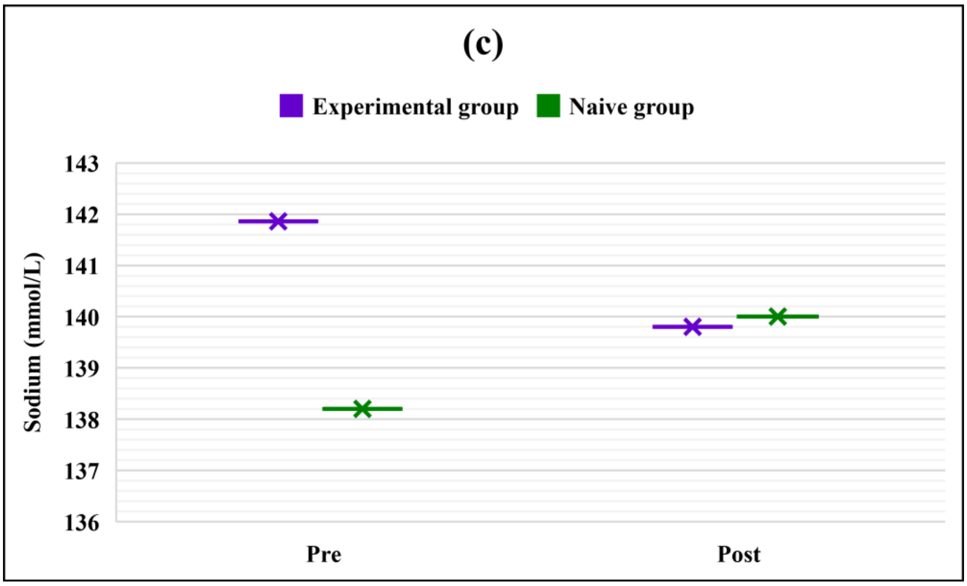
Table 11: Significance protein profile and electrolytes for the experimental and naive group (n=30)

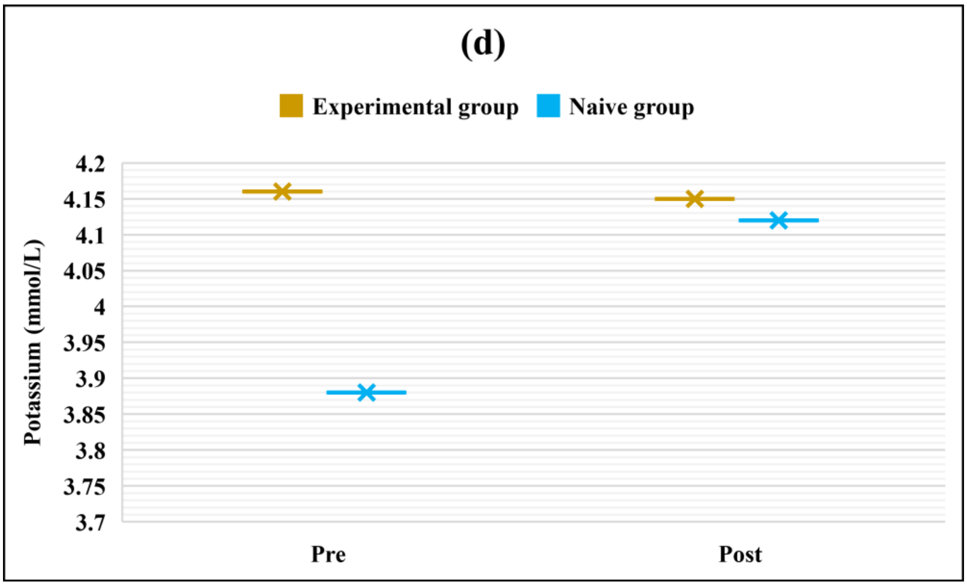
Statistics	Experimental group (n=15)																	
	Electrolytes,										Protein profile							
	Uric Acid (mg/dl)		Sodium (mmol/l)		Potassium (mmol/l)		Chloride (mmol/l)		Haemoglobin (g/dl)		Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		A/G ratio	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Mean	5.33	5.34	141.87	139.80	4.16	4.15	105.47	104.80	14.95	15.32	7.21	7.47	4.18	4.31	3.03	3.19	1.42	1.40
SD	1.31	1.27	3.14	1.86	0.22	0.17	2.56	2.43	1.71	1.53	0.71	0.56	0.38	0.33	0.54	0.26	0.30	0.14
SQRT	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87
SE	0.34	0.33	0.81	0.48	0.06	0.04	0.66	0.63	0.44	0.39	0.18	0.14	0.10	0.08	0.14	0.07	0.08	0.04
Cut off value	5.00	5.02	141.08	139.34	4.11	4.11	104.83	104.19	14.52	14.94	7.03	7.33	4.08	4.23	2.89	3.13	1.35	1.37
CI 95% Lower	2.75	2.86	135.72	136.16	3.73	3.81	100.45	100.04	11.60	12.33	5.81	6.38	3.43	3.67	1.98	2.68	0.84	1.13
CI 95% Upper	7.90	7.82	148.01	143.44	4.59	4.49	110.48	109.56	18.30	18.31	8.61	8.57	4.93	4.95	4.08	3.70	2.00	1.67
Specificity (%)	86		83		78		73		63		69		79		83		88	
Sensitivity (%)	65		70*		56		63		71		80		55		92		63	
AUC	0.76*		0.69 ^{ns}		0.82*		0.66 ^{ns}		0.63 ^{ns}		0.82*		0.83*		0.86*		0.88*	
	Naive group (n=15)																	
Mean	5.25	5.59	138.20	140.00	3.89	4.12	101.53	104.20	15.13	15.38	7.75	7.87	4.42	4.53	3.33	3.33	1.33	1.36
SD	1.52	1.51	1.93	1.85	0.22	0.25	2.56	2.37	2.01	1.99	0.35	0.34	0.25	0.23	0.17	0.16	0.08	0.07
SQRT	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87
SE	0.39	0.39	0.50	0.48	0.06	0.06	0.66	0.61	0.52	0.51	0.09	0.09	0.06	0.06	0.04	0.04	0.02	0.02
Cut off value	4.87	5.21	137.72	139.54	3.83	4.06	100.89	103.61	14.63	14.88	7.66	7.78	4.36	4.48	3.28	3.29	1.31	1.34
CI 95% Lower	2.27	2.63	134.41	136.37	3.46	3.63	96.52	99.56	11.20	11.48	7.06	7.21	3.93	4.09	3.00	3.01	1.18	1.23
CI 95% Upper	8.24	8.54	141.99	143.63	4.31	4.61	106.55	108.84	19.07	19.28	8.44	8.52	4.91	4.98	3.65	3.65	1.48	1.49
Specificity (%)	66		74		69		63		70		59		70		79		73	
Sensitivity (%)	45		62		47		55		53		67		63		55		58	
AUC	0.55 ^{ns}		0.52 ^{ns}		0.45 ^{ns}		0.36 ^{ns}		0.63 ^{ns}		0.48 ^{ns}		0.59 ^{ns}		0.68 ^{ns}		0.63 ^{ns}	

** Significant @ 1% level ($p<0.01$), * Significant @ 5% level ($p<0.05$), ns – non-significant


(a)
Experimental group
Naive group
15.5
15.4
15.3
15.2
15.1
15
14.9
14.8
14.7
Haemoglobin (g/dL)
Pre
Post


(b)
Experimental group
Naive group
5.6
5.5
5.4
5.3
5.2
5.1
5
Uric acid (mg/dL)
Pre
Post


(c)
Experimental group
Naive group
143
142
141
140
139
138
137
136
Sodium (mmol/L)
Pre
Post


(d)
Experimental group
Naive group
Potassium (mmol/L)
4.2
4.15
4.1
4.05
4
3.95
3.9
3.85
3.8
3.75
3.7
Pre
Post