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SM Journal of Nutrition and Metabolism

Research Article

Antioxidant Enriched Nutri Bar Supplementation on the Serum Anti Oxidant Status and Performance of Track and Field Athletes

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Article Information

Received date: Jan 20, 2017
Accepted date: Feb 15, 2017
Published date: Mar 02, 2017

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Abstract

Background: Adequate nutrition knowledge among athletes becomes vital. Knowledge, Attitude and Practices (KAP) regarding food intake of athletes become essential more so to have antioxidant rich diets. Antioxidants supplementation may provide protection against negative health consequences of oxygen free radicals caused by aerobic and re-sustained exercise.

Aim: Is to find out the efficacy of anti- oxidant rich nutri bar supplementation on the anti-oxidant status and physical fitness components of the athlete's.

Methods: Single blind study was employed. Study group (n=20) subjects were supplemented with the formulated nutri bars daily for a period of 3 months and control group with a placebo. Bio-chemical parameters GSH, GSH-px, SOD, Vitamin C, lipid profile, serum LPO and physical fitness tests were assessed at baseline and after 90 days. Statistical analysis was performed using SPSS (version 15).

Results: The mean age was 18 ± 3.2 yrs. 60% of the selected athletes were males and 40% females. The main source of nutrition information was from coaches (56%) and magazines (30%). Supplemented group athletes showed a significant increase in serum levels of SOD (p=0.000), LPO (p=0.005), GSH (p=0.000) than control group. Significant decrease was observed in total cholesterol (p=0.000) and LDL Cholesterol (p=0.000) than placebo. Vitamin C levels and GSH-px, HDL decreased in supplemental group but there was a significant decrease in control group (p=0.000, p=0.02, p=0.000). Significant improvement in all six physical fitness tests was observed in supplemented group (p=0.000), whereas in control group pushups test, vertical jump and agility tests alone did not find statistical significance.

Conclusion: False beliefs, food fads, have brought in major drawback in performance; hence appropriate nutrition knowledge is necessary for winning. Antioxidant effects of the formulated food were useful in improving the endurance of athletes.

Introduction

Sports nutrition can be defined as the application of nutritional knowledge to a practical daily eating plan focused on providing the fuel for physical activity, facilitating the repair and rebuilding process following hard physical work and optimizing athletic performance in competitive events, while also promoting overall health and wellness.

The sport of athletics is defined by the many events which make up its competition programs. All events within the sport are forms of running, walking, jumping or throwing. These events are divided into the sub-sports of track and field, road running, race-walking and cross country running.

An adequate diet in terms of quality and quantity before, during and after training and competition will maximize performance.

Presently, the unorganized sports scenario in India is in the active transformation phase and steadily evolving to become more structured. Likewise, the field of sports medicine and sports science are gaining importance. A decade ago, this branch of medicine was non-existing. But, increased exposure of athletes, coaches, team officials to international sporting events has made them appreciate the importance of performance enhancement. Importance of a sports nutritionist has also increased demand. However, there is still a long way to go.

As far as the nutritious diet of sports person is concerned, there are lot many things that deserve proper attention like selection of foods, timing of food intake, selection of nutrition supplement and many more. It is well recognized that athletic performance is enhanced by optimal nutrition [1].

An active body consumes significantly more oxygen than a less active body, so it follows that

regular physical activity may result in a persistent state of oxidative stress and, therefore, greater antioxidant needs. Among the well known biological anti-oxidants- Glutathione (GSH), Glutathione Peroxidase (GSH-Px) and Superoxide Dismutase (SOD) have a significant role as a suppressor on scavenging free radicals [2]. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, researches have not been conclusive in antioxidant supplementation to be beneficial for athletic population. Studies that focused on the effect of antioxidant supplementation of athletes were scarce. Lack of awareness about different sports nutrient supplements among the athletes is the main concern of a sports nutritionist. The demand for sports nutrition products continued to be low, which poses a high demand on the nutritionist to concentrate on the various forms of nutrition supplements.

The novel way of incorporating the anti-oxidants into their diet is by involving indigenous, underutilized ingredients which are abundantly rich in anti- oxidants. The requirements vary at different sport level and they can provide wonders in athletes' performance.

Keeping the above mentioned importance of antioxidants, the purpose of the present study is to test an antioxidant rich nutri bar prepared from underutilized ingredients, which would be of great help in order to ensure the athletic performance. Nutri bars are mostly preferred by athletes as they are ready to eat foods and could be easily picked up by them before, during and after the event. Therefore, the study has been designed with the following objectives:

- To assess the nutritional status of selected athletic men and women (track and field events).
- To supplement an antioxidant rich nutri bar to the athletes and
- To find out the impact on the antioxidant status such as Superoxide Dismutase (SOD), Lipid Peroxides (LPO), Vitamin C, Gluathione (GSH) and Glutathione Peroxidase (GSH-px) of the athletes [3].

Materials and Methods

Study design and participants

This study is a single- blind randomized prospective intervention design with control group. Sample size calculation suggested statistical power of 0.80, with significance ($p<0.001$) which gave rise to 20 athletes and in order to take care of probable attrition, 40 subjects were recruited for the study.

The study has been done between March 2014 and May 2014. Inclusion criteria were athletic men and women aged between 18 and 22 years, who have a minimum of 3 years of experience with track and field events and who are willing to participate and who filled in the printed informed approval. These athletes were recruited from sport academies in Chennai, India. Exclusion criteria included having any chronic diseases, who were < 18 and > 22 years of age and those who are on other nutrient supplements were excluded from the study. Voluntary participation was emphasized and a printed informed approval was acquired from them to be integrated in the study. The study was officially declared by the institutional Ethical board of Human Research Ethics Committee of PSG College of Arts and Science Coimbatore, India for Nutrition departments. (REF: REC/NCND/K14 003).

3-Day record

Subjects belonging to the experimental and control group were requested to preserve a three-day food documentation so as to determine the modifications in the nutrient intake. It was collected at baseline, and at twelve weeks of the supplementation. Nutritional ingestion through 3 day record was evaluated via DIETCAL software.

Formulation

Initially a pilot study was conducted to arrive at the acceptable formulation. The most acceptable variation (variation B) was prepared thrice and evaluated to verify the consistency of results. The evaluation was carried out in a suitable ambience by the same set of semi- trained panel members. Thereafter, the nutri- bars were prepared for sensory evaluation. Acceptability test was conducted for the prepared bars in three variants by evaluating the different sensory attributes like appearance color, flavor, texture, taste and overall acceptability by ten semi- trained members. Five point hedonic scales were used to evaluate the nutri- bars.

The Nutri- bar was also subjected to organoleptic evaluation by the general public. The focus group considered were young adults with an age ranging from 18 to 25 yrs. Acceptability test was rated by 20 athletes of the Sports Academies in Chennai.

The Standardized recipe was followed for the bulk preparation of nutri- bars [4]. Exact quantity of the ingredients was measured each time and the detailed step by step procedures for preparation and cooking (including cooking temperature, time, type of size of utensil used) was followed at all times. The dry ingredients are mixed and baked into granola (nutri- bars). The nutri- bars were made up into 50 gm (each bar weight) and two bars were provided for the athletes as per the anti-oxidant content of the bars. 110 mg of anti-oxidants as per standardization is incorporated into 100 gm of the nutri- bars. This was divided into two bars weighing 50 gm each.

Estimation of nutrient analysis

The prepared bars using antioxidant rich ingredients were subjected to nutrient analysis [5]. The nutrients namely total carotene, vitamin A, C, E, energy, proteins, carbohydrates and fats were analysed by the following methods (Table 1).

1. Total Carbohydrate by Gravimetry
2. Protein estimation by Kjeldahl method
3. Fats by Soxhlet method

Table 1: Estimation of nutrient analysis.

Nutrients	Composition/100 gm
Energy	524.5 kcals
Protein	24.48 gms
Carbohydrates	39.14 gms
Fats	30.87 gms
Vitamin A	15.6 mcg
Vitamin E	20.2 mg
Vitamin C	50.6 mg
Total Antioxidants	115.8 mg

4. Vitamin E by HPLC liquid chromatography
5. Assay for ascorbic acid
6. Total anti- oxidant content was analyzed using DPPH method

Microbial analysis

Microbial analysis was carried out by Total Plate Count (TPC). Standard plating in nutrient agar was carried out, which is called as total plate count method. The Total Microbial Load (TPC) of nutri bars were determined in nutrient agar media according to the method given by Harrigan and McCance (1966), on 0th day and then again after 7 days and 14 days.

Supplementation

Those subjects in the study group who were assessed were supplemented with the formulated nutri bars daily for a period of 3 months (12 weeks). Two bars per day were given to the subjects during the day (morning and evening sessions) and it was monitored personally by the investigator whether athletes consumed the nutri-bar supplement or not. Those subjects in the control group were provided with appropriate nutritional counseling about the importance of anti- oxidant rich foods and a placebo was given. The nutritional intake and the blood reports were collected at 12 weeks of the supplementation from both the study and control groups to check for any improvement in the anti- oxidant status [6].

Blood analysis

The bio-chemical parameters, namely Glutathione peroxidase, Glutathione concentration, superoxide dismutase, Vitamin C concentrations and Lipid Peroxides (LPO) in plasma serum, were estimated before and after supplementation of anti- oxidant rich nutri- bars to find out the impact on these blood parameters.

A sterile disposable syringe was used to draw blood from the femoral vein in the morning. 10 ml of blood was drawn from each subject before and after the period of study. Part of blood was transferred into heparinized tubes and the rest used for serum separation. Resting blood samples were collected using two different vacutainers. The heparinized vacutainer was used to determine plasma TBARS, the erythrocyte glutathione concentration (GSH) level, and Glutathione Peroxidase (GSH–Px) activities. Five hundred micro liters of whole blood were stored at 4°C for one day before determining enzymes activity and 150 μL of whole blood were frozen at –20°C for GPx activity determination (with a maximum conservation of 20 days). For GSH analysis, 500 μL of whole blood were centrifuged at 2500 g for five min at 4°C. The plasma supernatant was discarded and the erythrocyte pellets were suspended in four volumes of MPA (6% 1:1 in water). After shaking vigorously, it was centrifuged at 3000 g (10 min, 4°C). The aciditic protein-free supernatants were stored at -80°C until analysis. The rest of the blood samples were centrifuged (1500 g, 10 min, 4°C) and the plasma used to determine LPO (500 μL) for experiment.

Physical fitness tests

The following physical fitness tests were also performed.

I. Cooper's 12 Minutes Test
II. Harvard Step test
III. Push- ups Test
IV. Vertical Jump test (Sargent Jump)
V. Sixty- metre dash
VI. Hexagon Agility test

Statistical analysis

Paired 't' tests were used to assess the difference between the groups (treatment and control groups). A significant P value ($P \leq 0.05$) for the interaction term was considered a demonstration of a treatment effect. Significance was found between the placebo and the treatment group at either time interval. Statistical analysis performed using SPSS (version 15).

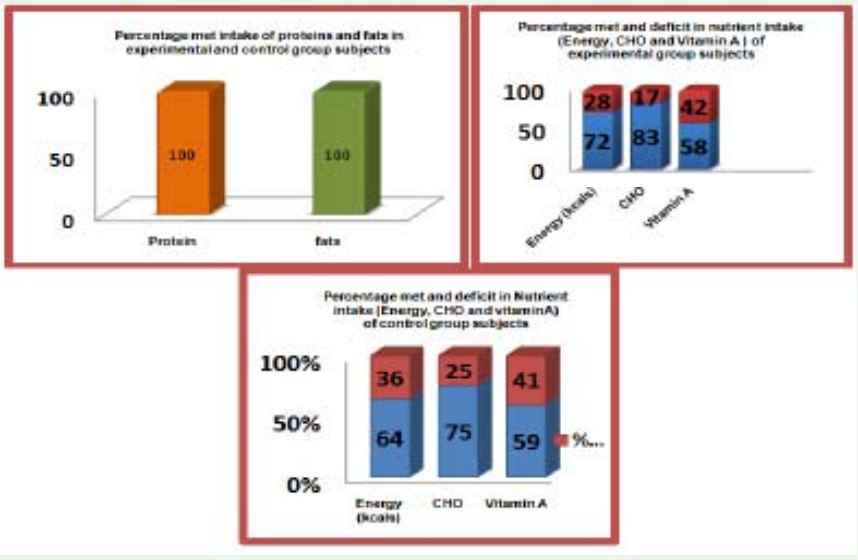

Figure 1: Dietary intake of athletes belonging to experimental and control groups.

Table 2: Mean Organoleptic Scores of Nutri-Bars.

Attributes	Max score	A1	A2	A3
Appearance	30	19	20	21
Colour	30	19	19	22
Flavour	30	19	20	24
Texture	30	20	20	23
Taste	30	20	22	24
Overall acceptability	30	20	19	24
Total score	180	117	120	138

Results and Discussion

General profile

The mean age of participants were 18 ± 3.2 yrs with a range of 18 to 21 yrs. 60% of the selected athletes were males and 40% females. According to a report by a Statistical Review of Australia (2012) on sports and physical recreation, in organized sport, the participation rate was similar for men and women in organized sport.

Nutrient intake

Young athletes in their quest for victory by recognizing that children and adolescents generally need more calories and protein per kilogram of body weight than adults. Nutrient needs further elevate and reach their peak during adolescence. The results reveal that nutrition counseling is important for athletes to consume the nutrients as per the RDA as well as pertaining to their specific sport activity (Figure 1) [7,8].

Results of organoleptic evaluation

The three variants (A1, A2, and A3) were made different with varying proportions of rolled oats, dehydrated carrots, groundnuts and almonds to achieve a highly acceptable product. It was thus observed from Table 2 that variant A3 had scored highest in attributes of appearance, color, flavor, texture, taste and overall acceptability. The variant A3 was accepted well with a total score of 24. Overall, the A3 was acceptable and tasted good.

Table 3: Level of Significance of Glutathione (GSH) and GSH px (Glutathione peroxidase- GSH px) between experimental and control groups.

Parameters	Experimental MEAN ± SD	Control MEAN ± SD	't' test (P value)
GSH (µg/ml)Initial	209.76 ± 8.17	214.12 ± 7.4	1.76 (0.08^{NS})
Final	244.58 ± 33.36	188.88 ± 38.3	4.90 (0.00*)
Change	-34.82	25.24	
't' test (P value)	4.42(0.000*)	2.80(0.011**)	
GSH Px (µg/ml) Initial	17.15 ± 15.25	15.30 ± 12.13	0.42(0.67^{NS})
Final	18.65 ± 8.9	9.20 ± 6.32	3.84(0.000*)
Change	-1.5	6.1	
't' test (P value)	0.45(0.65^{NS})	2.48(0.02***)	

*- Significant at p<0.001, **- Significant at p<0.01, ***- Significant at p<0.05, NS- Not Significant.

Anti- oxidant Levels - SOD, LPO and Vitamin C of Experimental and Control Groups

The mean initial levels of SOD and LPO (Figure 2) between experimental and control groups showed no significant difference {SOD t value-0.12(0.90^{NS}) LPO t value-1.36(0.18^{NS})} indicating no difference in the levels at baseline, whereas mean initial levels of vitamin C {t value-2.38(0.02***)} alone showed a difference (p<0.05) between the groups. At the end of 90 days of supplementation, the mean final levels of SOD showed a significant difference (p<0.001) between the groups. However, LPO and vitamin C levels between experimental and control groups showed no significant difference {LPO t value-0.08(0.93^{NS}))} and vitamin C {t value-0.841(0.40^{NS}).

From the above table, it is evident that the biochemical parameters such as Superoxide Dismutase (SOD), LPO and vitamin C before supplementation were found to be statistically not significant between experimental and control groups. The mean standard deviation of Superoxide Dismutase (SOD) has been found to be 42.08 ± 13.16 in experimental group and 18.689 ± 21.158 in control group showing a vast difference between the two groups after supplementation (p<0.001), while other parameters such as LPO and Vitamin C showed not significant results.

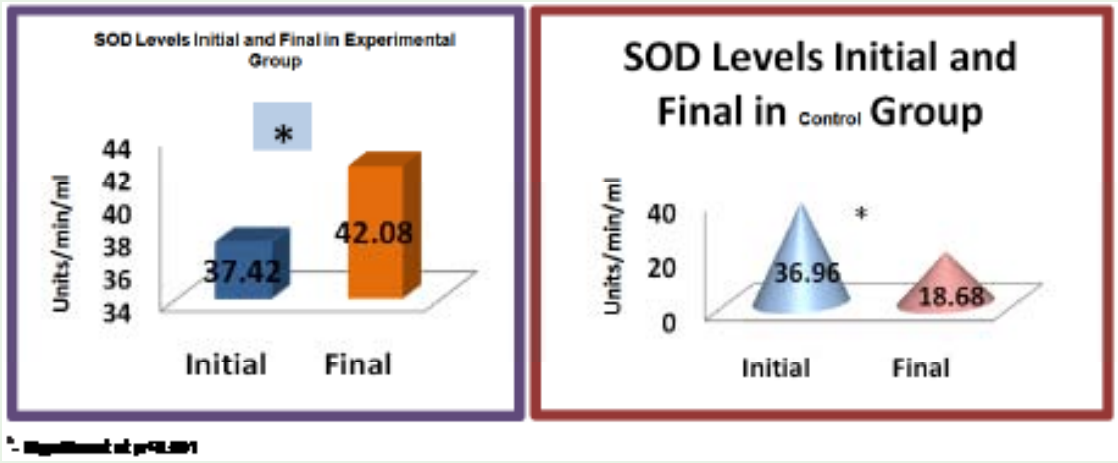

Figure 2: Mean SOD levels (Initial and Final) of experimental and control groups.

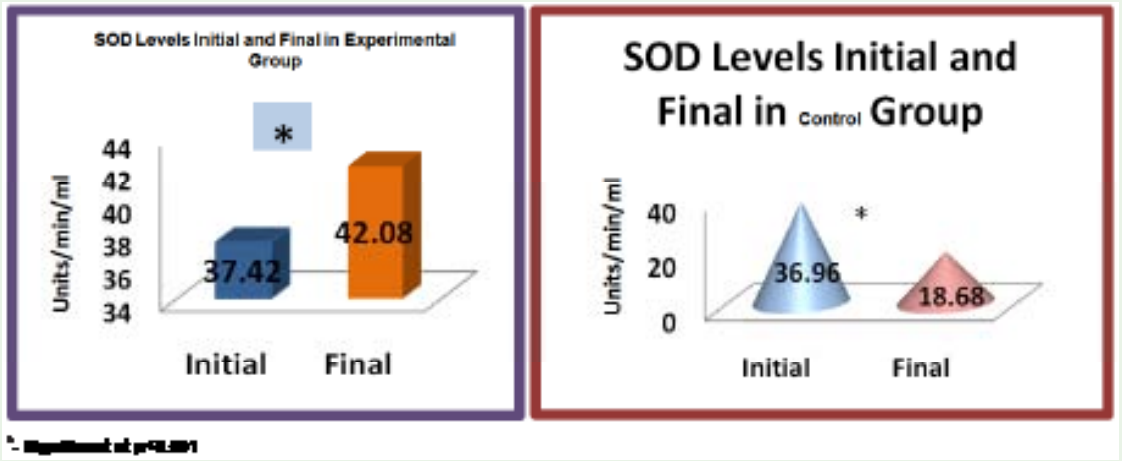

Figure 3: LPO levels (Initial and Final) of experimental and control groups.

- The significant improvement in the SOD levels($p<0.001$) of the experimental group (initial 37.42 ± 12.01 Units/min/ml, final-42.08 ± 13.16 Units/min/ml) is due to the daily consumption of anti-oxidant rich nutri bars formulated by the investigator and effective nutritional counselling provided. The mean initial level of SOD was 36.96 ± 10.79 Units/min/ml which decreased to18.68 ± 21.15 Units/min/ml at the end of 90 days ($p<0.001$) in the control group. Since the athletes of this group were not supplemented with anti-oxidants, the SOD values were decreased.
- In experimental group, the mean initial levels of LPO was 2.89 ± 0.82 μg/ml which increased to 3.80 ± 1.37 μg/ml post intervention ($p<0.01$) in view of daily consumption of anti- oxidant rich nutri bars. In the control group, there was a significant increase from 3.44 ± 1.59 μg/ml to 3.85 ± 1.87 μg/ml ($p<0.05$).This significant increase could be due to the fact that the athletes of the control group were also well trained (Figure 3).
- Vitamin C levels decreased after 90 days of supplementation from 4.14 mg/dl to 3.62 mg/dl in the experimental group with statistically not- significant results. However, in the control group there was a significant decrease from 5.14 mg/dl to 3.32 mg/dl ($p<0.001$) (Figure 4).

Comparison of GSH and GSH px between experimental and control groups

The Table 3 highlights the effect of GSH and GSH-px between experimental and control groups before and after supplementation. The mean glutathione levels were found to be 209.76 ± 8.17 in the experimental group and 214.120 ± 7.42 in the control group respectively. There seems to be not much of a difference before supplementation between the two groups. But after supplementation the mean glutathione levels were 244.58 ± 33.36 in the experimental group and 188.88 ± 38.3 in the control group respectively, which was found to be significant ($p<0.01$). Whereas, athletes of control group who were not supplemented found to show a decreased GSH level (214.120 ± 7.42 to 188.88 ± 38.3) and is not significant ($t=2.803$). This could be due to depletion of GSH level during the aerobic exercise/ training program.

GSH-Px level found to be 17.15 ± 15.25 in experimental group in the initial stage and mildly elevated to 18.65 ± 8.9 during study course, showing statistically not significant results. This may due to indication of long exercise during marathon induce caused modification of GSH-Px activity. GSH-Px level was found to be decreased from15.30

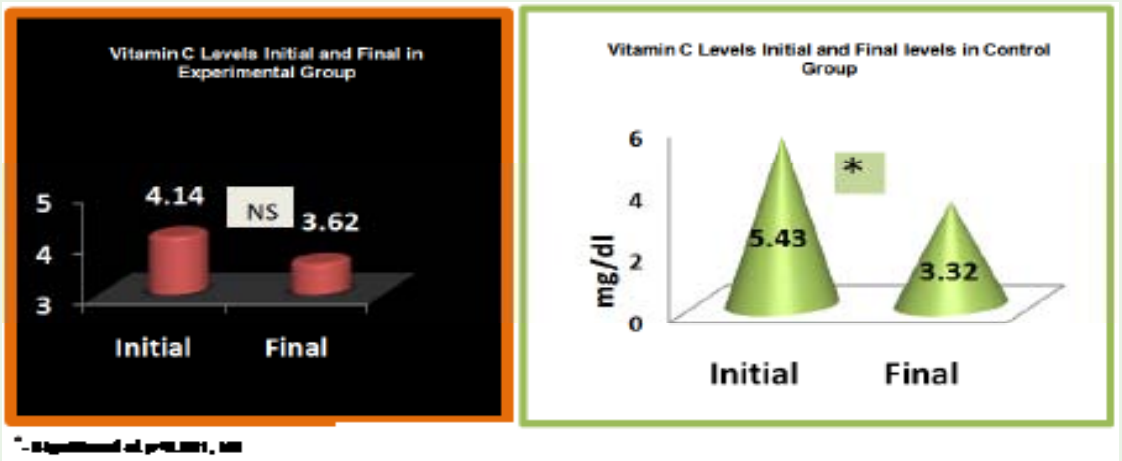

Figure 4: Vitamin C Levels (Initial and Final) of experimental and control groups.

± 12.13 to 9.20 ± 6.32 in control group showing not significant results. Studies have shown that glutathione and reduced glutathione levels are enhanced with anti-oxidant supplementation. The diet supplementation with vitamin E, vitamin C and β-carotene cocktail enhancing the basal neutrophil antioxidant enzymes in athletes for 90 days' supplementation with placebo or an antioxidant cocktail of vitamin E (500 mg/day) and β-carotene (30 mg/day) and the last 15 days also with vitamin C (1 g/day) on sportsmen's basal neutrophil antioxidant defenses and analyzed the activities of catalase, glutathione peroxidase, glutathione reductase. The antioxidant-supplemented group presented a significantly higher glutathione versus glutathione disulfide ratio in neutrophils (about 20%) than the placebo one.

Physical fitness tests (Table 4)

Table 4: Results of Physical Fitness Components.

Parameters	Experimental MEAN ± SD	Control MEAN ± SD	't' test (P value)
12 mins (m) Initial	1.44 ± 0.142	1.43 ± 0.132	0.16(0.87^{NS})
Final	1.56 ± 0.134	1.47 ± 0.13	2.24(0.03***)
Change	-0.12	-0.04	
't' test (P value)	20.99(0.000*)	7.02(0.000*)	
Step test (nos) Initial	68.65 ± 5.51	64.65 ± 6.38	2.12(0.04***)
Final	78.35 ± 4.004	69.90 ± 6.82	4.77(0.000*)
Change	-9.7	-5.25	
't' test (P value)	16.83(0.000*)	12.81(0.000*)	
Push-ups (nos)Initial	22.25 ±3.97	25.65 ± 4.55	2.51(0.02***)
Final	29.70±3.40	26.20 ± 3.86	3.04(0.004**)
Change	-7.45	-0.55	
't' test (P value)	20.34(0.000*)	0.73(0.46 ^{NS})	
vertical jump(cm)Initial	60.00 ± 5.620	53.50 ± 7.96	2.98(0.005**)
Final	63.75 ± 6.043	53.25 ± 9.49	4.17(0.000*)
Change	-3.75	0.25	
't' test (P value)	7.55(0.000*)	0.25(0.80^{NS})	
Speed(sec) Initial	6.5940 ± 0.258	6.6010 ± 0.312	0.07 (0.93^{NS})
Final	6.7435 ± 0.25	6.5415 ± 0.30	2.26(0.02***)
Change	-0.14	0.06	
't' test (P value)	6.67(0.000*)	2.55(0.01**)	
Agility (Sec) Initial	12.255 ± 0.42	12.205±0.39	0.38(0.70^{NS})
Final	12.400 ± 0.51	12.110±0.52	1.76(0.08^{NS})
Change	-0.15	0.095	
't' test (P value)	2.48(0.02***)	1.36(0.18^{NS})	

*- Significant at p<0.001, ****- Significant at p<0.01,**
***- Significant at p<0.05, **NS- Not Significant**

Table IV revealed:

- The initial values of 12min run tests in experimental group was 1.44 ± 0.142m and the final values did show a significant rise (P<0.001). However the distance covered by the control group in 12 minutes was 1.43 ± 0.132m at baseline and 1.472 m after 90 days, though the increase was minimal, yet it was significant at p<0.001.
- The number of steps taken by athletes of experimental group had significantly increased from 68.65 ± 5.518 to 78.35 ± 4 (P<0.001) while there was only a marginal increase from 64.65 ± 6.385 to 69.90 ± 6.820 in the control group (P<0.001).
- In vertical jump test the athletes of the experimental group subjects showed a significant increase from 60.00 ± 5.620cm to 63.75 ± 6.043cm (P<0.001) and control group showed a marginal decrease from 53.50 ± 7.964cm to 53.25 ± 9.497cm which is statistically not significant.
- Push- ups tests which demonstrates the improvement in the endurance of athletes showed a significant increase from 22.25 ± 3.972 to 29.70 ± 3.404 (p<0.001) in the experimental group. Though there was an increase from 25.65 ± 4.55 to 26.20 ± 3.861 in control group, it was not statistically significant.
- The athletes belonging to experimental group had a significant increase from 6.5940 ± 0.2588 seconds to 6.7435 ± 0.2576 seconds (P<0.001) in the overall speed, while in the control group it decreased from 6.6010 ± 0.3126 sec to 6.5415 ± 0.303 sec (P<0.01).
- Agility tests showed the improvement in the endurance of athletes with significant increase from 12.255 ± 0.42 sec to12.400 ± 0.51 sec (p<0.05) in the experimental group. In control group a decrease from12.205 ± 0.39 sec to12.110 ± 0.52 sec was observed statistically not significant.

Limitations of the Study

1. Anthropometrical measurements such as MAC, TSF and MAMC have not been performed.
2. Dietary information was not obtained after the supplementation.

Conclusion

Knowledge about free radicals and anti-oxidants is quite poor. It is apparent that further research examining the relationship between nutrition knowledge and dietary behaviors is warranted.

Antioxidant effects of the formulated food improved the bio-markers and physical fitness components. Supplementation of antioxidant rich nutri bars hence had beneficial effects on the oxidative stress of the athletes. Future research may explore the effect of other nutritional antioxidants that can benefit the athletes. The anti-oxidant requirements for athletes have to be computed for recommendations.

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