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EFFECT OF COMBINED VITAMIN E SUPPLEMENTATION AND TREADMILL EXERCISE ON SPECIFIC HAEMATOLOGICAL PARAMETERS OF THE SEDENTARY UNIVERSITY STUDENTS

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ABSTRACT: Background: Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues). There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism. Several research studies have pointed out the role of vitamin supplementation and amelioration from exercise induced oxidative stress. Vitamin E, potential lipid soluble antioxidant has profound roles in preventing muscle fatigue and enhances exercise performance. **Methods:** In this study we have taken 96 subjects of which half of them were given Vitamin E supplement. Various biochemical analyses were done according to the standard protocols. **Results:** The results of this present study showed that Vitamin E supplementation with treadmill exercise can alter haematological parameters of the sedentary university students. **Conclusion:** It can be concluded from the data of the present study that the combined Vitamin E and treadmill exercise supplementation significantly alter the haemoglobin content and total count which can improve the performance level of the sedentary university students.

INTRODUCTION: A Vitamin is a vital organic nutrient that an organism requires in limited amounts. Supplementation is important for the treatment of certain health problems. Earlier study represents that the *in vivo* effects of Vitamin E administration on local inflammatory responses in various muscles [1]. Experiments studied by Amelink et al., 1991 had shown that the susceptibility to exercise-induced muscle damage in male rats more than in female rats during Vitamin E deficiency [2]. By convention, the term vitamin includes various essential nutrients along with great number of other nutrients that promote health, and are required less often to maintain the health of the organism[3].

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Vitamin E refers to a group of compounds that include both tocopherols and tocotrienols. γ-tocopherol is the most common vitamin E form in the North American diet [4]. γ-Tocopherol can be found in various oil and margarine. α-tocopherol, the most biologically active form of Vitamin E, is the another most common form of Vitamin E which is present in the diet. Vitamin E has many biological functions among which antioxidant function is the most important and best one [5].

There is a considerable debate about the role and clinical meaning of exercise-induced reactive oxygen species (ROS) and antioxidant supplementation for exercise performance [6]. Several studies have presented evidence that exercise can increase ROS production and that exercise-induce oxidative stress can cause muscle fatigue [6, 7]. Earlier studies mainly showed the role of antioxidant supplements on potential harmful effects of exercise-induced ROS generation, and also in preventing muscle fatigue and enhance

exercise performance [8]. While several animal studies have found that antioxidant supplements delay muscle fatigue and improve exercise performance, the clinical efficacy of antioxidants remains uncertain [8, 9]. In spite the weak clinical evidence presented in these studies, anti-oxtidants are known to be very commonly used [9]. It was recently reported that polyphenolic flavonoids have strong antioxidant effects. This may stem not only from direct scavenging ROS but also from indirect modulation of multiple cell signaling pathway [10, 11]. The presence of free radicals and evidence of damage to muscle cells in normal rats suggest that lipid peroxidation might be prevented by increasing the Vitamin E content of the diet. Dillard and colleagues found that Vitamin E supplements for 2 weeks decreased pentane production during moderate exercise [12]. The concentration of vitamin E in the blood more than doubled following supplementation. Because exercising untrained muscles is accompanied by the release of muscle enzymes (e.g., creatine kinase) into the blood, increasing the vitamin E content of the tissues might reduce enzyme leakage through the muscle cell membrane.

However, no difference in the plasma concentrations of muscle enzymes was observed following exercise between Vitamin E supplemented and placebo groups [13]. There is very little evidence that vitamin E supplements actually improve performance. Most of the studies that have examined Vitamin E supplements and performance have used relatively large supplements, 400 mg alpha tocopherol or more per day, for several weeks. No significant improvements in maximal oxygen uptake, swimming performance, post-exercise plasma lactate or muscular strength and endurance were found in vitamin E supplemented subjects compared to subjects receiving placebos [14, 15]. Plasma vitamin E level will not be affected by Vitamin C and E supplementation in athletes and also exercise; instead there is an increase in vitamin E concentration in lymphocytes and PMNN[16].

Some studies have shown that the short-term effect of vitamin E supplementation against muscle damage induced by exercise is not physiologically significant [17]. The literature data present little evidence to support the beneficial effects of antioxidant vitamin C or E supplementation on exercise-induced muscle damages, although there is evidence that this can reduce some oxidative stress markers in muscles during exercise[18].

Physical training is one of the most important ingredients in training to achieve high level of performance. The objectives of physical training are to increase the athlete's physiological potential and to develop bio-motor abilities to the highest standards [19].

Therefore the present study has aimed to investigate the combined effect of vitamin E supplementation and treadmill exercise towards some specific hematological parameters.

METHODS AND SUBJECTS:

The subject:

Ninety six (n=96) university male students of twenty one to twenty five years of age group were selected randomly from University of Kalyani, Kalyani, Nadia, West Bengal for the said study. Those subjects were selected only on the basis of following criteria:

The selected subjects:

I. Were not of very high level elite athletes.

II. They have no sports achievement.

III. They have no regular habit of physical activity.

Experimental design:

The ninety six (n=96) subjects were distributed into four groups- (1) control group, (2) treadmill exercise group, (3) treadmill exercise and Vitamin E supplementary group, (4) only Vitamin E supplementary group [double positive].Treadmill exercise for ten weeks (4 day per weeks with two day rest between them) was applied as per the modified Bruce protocol [20] for the group no. 2, 3 and 4. Vitamin E were supplemented in the form of capsules (Evion ® 400 of Ranbaxy Pvt. Ltd which was selected by consultation with medical professional) at the dose one capsule daily after lunch for 4 days per week (as per Takanami et al., 2000) for 10 week duration for group no 3 and 4

only[21]. For group no 2 only performed treadmill running and for control group no exercise or supplementation was given. All the haematological parameters were taken twice i.e. prior to start of treadmill exercise before 10 weeks and after completion of tests i.e. after 10weeks for all of the four groups as mentioned above.

Instruments and facilities taken:
For collecting the data, the following instruments and facilities were taken from Human Performance Laboratory of Department of Physical Education of Kalyani University:

- Height was taken by anthropometric rod; weight was measured by weighing machine.
- Life Guard Laboratory from Kalyani Central Park area was used to measure hemoglobin.

Procedure of collecting data:
For the proposed study a pre test blood sample collection was conducted by supervision of pathological experts and researcher; again post test blood sample collection was done. The subjects were introduced to put on suitable minimal clothing. Then they were explained about the purpose of the study. All the subjects were very much interested and eager to appear for the test.

Height:
Participants are asked to remove their shoes heavy outer garments and hair ornaments. The participant is asked to stand with his back to the height rule, the back of the head and heels should be touching upright feet together. The top of ear canal should be level with inferior margin of the bony orbit (check bone). The participant is asked to straight [22].

Score: The score was measured to the nearest 0.1 cm

Weight:
The subject stood straight and barefooted on the weighing machine, looking forward. Researcher recorded student's weight by observing the reading of weighing machine [23].

Score: weight of the subject was recorded to 0.5 kg.

Blood sample collection and processing:
Blood samples were taken from the subjects. All of them were non smokers. Five ml of fasting venous blood sample (fasting time was > 12 hours) were taken from each donor before going to work. All samples were taken by venapuncture with anticoagulant (EDTA) evacuated tubes.

Hemoglobin (Hb) measurement:
Hemoglobin concentrations in blood were determined with the hemoglobin cyanide procedure[24]. In this method, cyanide and ferricyanide in an alkaline medium convert hemoglobin to the highly colored cyanme-themoglobin derivative measured spectrophotometrically at 540 nm. In brief, 0.02ml of whole blood and 5.0 ml of Drabkin solution were taken in a test tube, mixed well and allowed to stand for at least 15 minutes at room temperature (18-26°C). Then absorbance was read spectrophotometrically at 540 nm.

Differential and total count measurement:
The differential count and total count were measured according to the standard laboratory methods. The blood of the subject is collected with the help of expert pathologist in this concern.

Statistical analysis:
Analysis of covariance (ANCOVA) was used to determine the differences, among the adjusted post test means on selected criterion variables separately. The level of significance was fixed at .05 level of confidence. Percentage changes in mean value of each of the three parameters before and after test were measured. All the statistical analysis was done by using MICROCAL ORIGIN PRO 7 version.

RESULTS:
Personal data of subjects were measured and these have been shown in **Table 1**.

TABLE 1: PERSONAL DATA OF THE SUBJECTS

Group	Height(cm)	Weight(kg)
Control (n=24)	168.33±3.44	59±3.22
Treadmill (n=24)	166.83±2.04	54.5±3.61
Treadmill+Vit-E (n=24)	165±3.57	64.16±3.57
Vitamin-E (n=24)	167.16±3.18	60.66±1.50

Values are expressed as Mean± SD for all of the four groups.

Percentage analysis **(Table 2**) has shown that neutrophils were increased in all the three groups (treadmill group, treadmill+Vitamin-E group and vitamin-E group) when compare to the control group. Highest increment was observed in combined treadmill and vitamin E supplementation group. Lymphocytes were increased (**Table 2)** that in treadmill group, treadmill+ vitamin-E group and Vitamin–E group with respect to control group. It was interesting to note that highest increment of lymphocytes has been observed in case of the subjects of combined Vitamin E and treadmill exercise supplementation. Monocytes were decreased (**Table 2**) in the two groups (treadmill+ vitamin-E group and vitamin–E group) when compared to percentage changes of the control group after experimental time period. Eosinophils were decreased (**Table 2**) in the two groups (treadmill+ vitamin-E group and vitamin–E group) when compared to percentage changes of the control group after experimental time period. It was interesting to note that highest decrement of eosinophils has been observed in case of the subjects of combined Vitamin E and treadmill exercise supplementation.

TABLE 2: PERCENTAGE CHANGES OF THE DIFFERENT WHITE BLOOD CORPUSCLES (WBC) PARAMETERS IN ALL THE FOUR GROUPS.

Neutrophils (%)	Control (n=24)	Treadmill (n=24)	Treadmill+Vitamin-E (n=24)	Vitamin-E (n=24)
pre	53.33±5.92	57.00±1.68	52.00±5.13	50.83±5.07
post	50.83±8.75	58.33±4.85	68.50±7.28	55.00±4.91
% changes	4.68 ↓	2.33 ↑	31.73 ↑	8.20 ↑
Lymphocytes (%)	Control	Treadmill	Treadmill+ Vitamin-E	Vitamin-E
pre	39.00±3.52	34.50±9.16	34.50±2.20	39.16±2.56
post	33.33±6.19	36.83±10.20	45.00±4.18	41.17±1.66
% changes	14.54 ↓	6.45 ↑	30.43 ↑	5.13 ↑
Monocytes (%)	Control	Treadmill	Treadmill+Vitamin-E	Vitamin-E
pre	1.83±0.20	1.63±0.27	1.76±0.32	1.74±0.15
post	1.53±0.18	2.02±0.41	1.02±0.09	1.32±0.02
% changes	16.39 ↓	23.93 ↑	42.05 ↓	24.13 ↓
Eosinophiles (%)	Control	Treadmill	Treadmill+Vitamin-E	Vitamin-E
pre	5.83±0.32	4.16±0.97	5.50±1.08	4.89±0.45
post	4.50±1.09	5.01±0.85	3.50±0.73	3.95±0.65
% changes	22.81 ↓	20.43 ↑	36.36 ↓	19.22 ↓

Values are expressed as percentage for all the four parameters, it is to be noted arrow head sign gives the indication of increment as well as decrement of the value after experimental time period i.e. post test.

Table 3 shows that there was a significant difference in haemoglobin content and total count between the control group, treadmill exercise group, treadmill exercise and vitamin E supplementation group, only Vitamin E supplementation group. One Way ANCOVA analysis was performed to study the effect of exercise and Vitamin E supplementation on the total count and haemoglobin content. Adjusted post test mean was done for quantification of covariate in the analysis.

TABLE 3: ANALYSIS OF COVARIANCE AND 'F' RATIO FOR LEG STRENGTH, EXERCISE OXYGEN UPTAKE AND EXPLOSIVE STRENGTH OF CONTROL GROUP, TREADMILL EXERCISE GROUP, TREADMILL + VITAMIN- E SUPPLEMENTATION GROUP, AND VITAMIN- E GROUP

$F_{.05\,(3,\,24)} = 3.01$ $F_{.01\,(3,\,24)} = 4.72$

Variable Name	Group Name	Control Group (n=24)	Treadmill Exercise Group (n=24)	Vit E supplementation (n=24)	Tredmill exercise+Vit E (n=24)	F ratio
Haemoglobin level(gm/dL)	Pre-test Mean ±S.D	13.83±0.78	13.69±0.69	13.95±1.91	13.31±1.60	1.935
	Post-test Mean ±SD	12.65±0.61	13.55±1.24	13.88±1.37	13.89±0.81	7.246*
	Adj Post test Mean ±S.D	11.33±0.21	10.36±1.10	9.32±1.11	10.12±0.35	16.754*
Total count (cu/mm)	Pre-test Mean ±S.D	6350±499.16	6741±1112.39	6908±1089.6	6754±455.48	0.697
	Post-test Mean ±SD	6375±657.83	6766±655.48	5975±1071.31	6952±355.18	12.855*
	Adj Post -test Mean ±S.D	2113.155±230.14	2151.002±655.48	4114.970±6255.48	4115.763±615.48	6.058*

* Significant at .05 level of confidence. (The table value required for significance at .05 level with df =3and 24 and 3.01 and 4.72 respectively).

Values are expressed as percentage for all the four parameters, it is to be noted arrow head sign gives the indication of increment as well as decrement of the value after experimental time period i.e. post test.

DISCUSSION: In the field of sports medicine, many studies of vitamin E have been conducted originally from the point of view of its effects on physical performance. Some of the earlier studies indicated that vitamin E supplementation could improve physical performances. Recent studies suggest that exercise may produce free radical in the body, and vitamin E may play an important role in prevention of the damages due to free radical generation in association with exercise. Although there is evidence of free radical involvement in exercise-induced muscle injury, vitamin E supplementation might not be expected to prevent muscle damage caused by exercise in humans without a vitamin E deficiency. Supplementation with 100 to 200mg of vitamin E daily can be recommended for all endurance athletes to prevent exercise-induced oxidative damage and to reap the full health benefits of exercise.

Our earlier studies showed that the combined Vitamin E and treadmill exercise supplementation improved significantly the leg strength, maximum exercise oxygen uptake and power of leg (explosive strength) on the other hand vitamin E supplementation improved the performance level of hill land football players but it could not possible to draw a conclusion with studying footprint parameters [19, 25].

In our studies we have observed that there is significant difference of all the parameters amongst the subjects of control, only treadmill exercises group, vitamin E and treadmill exercise and only vitamin E group. Moreover whenever we have compared the percentage increment values of total count, haemoglobin and each of the components of the differential count (neutrophil, eosinophil, lymphocyte and monocyte) we have found that the post test value has been increased in both the treadmill exercise group and treadmill exercise with vitamin E supplementation group in respect to control and double positive group. The possible explanation might be is that Vitamin E has profound effect in increasing muscle strength and free radical scavenge while treadmill exercise increases muscle strength also; whenever these two combined together we have found significant increment in all the three parameters.

As we know treadmill running is the exercise which can increase the cardiovascular endurance, we have observed the increment of haemoglobin level in both the two groups as more increment of haemoglobin level will cause more combination with oxygen and thereby increase the ventilation and perfusion ratio.

However due to time bound and limitation we have not performed Schaffer S test to analyze quantitative parameter increment. Moreover to analyze and study elaborately we have to work on large samples. Therefore for common people as well as sports persons Vitamin E supplementation as well as designed treadmill exercise could be a device by which greater muscular strength and endurance can be achieved.

CONCLUSION: It can be concluded from the data of the present study that the combined Vitamin E and treadmill exercise supplementation significantly alter the haemoglobin content and total count which can improve the performance level of the sedentary university students.

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