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Effect of Tocotrienol Rich Fraction Supplementation [TRF] on Lipid Profiles in Healthy Individuls

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Abstract

Tocotrienols are member of the vitamin E family, recommended as supplements for maintaining optimum health. Tocotrienols have multieffects as antiangiogenic, antiinflammatory, antidyslipidemic, pro-apoptotic, antiproliferative, free radical scavenger,radiation countermeasures. The main objective of this study was to determine the effects of TRF supplementation on lipid profile in healthy individuals at low dose of 160 mg/day for 6 months. *Method:* One hundred subjects were recruited from two age groups, 35-50 years (n=50) and above 50 years age (n=50) and randomly assigned to receive either TRF supplement or placebo capsules for six months. Blood samples investigated at 0, 3 and 6 months. *Result:* There were no significant alterations observed in total cholesterol, LDL, triglycerides while significant changes had been observed in HDL cholesterol, particularly 6 month TRF supplemented individuals ($P \leq 0.01$). *Conclusion:* Our result indicate that daily supplementation of TRF up to 6 months, raised plasma HDL cholesterol levels, reflects the antiatherogenic properties, predicts for the prevention of cardiovascular diseases risks. Further need of research study on lipid profile & other cardiac markers with different dose and duration of TRF is indispensable in cardiovascular and other diseases.

Keywords: TRF; Lipid Profiles; Free Redical; Cardiovascular Diseases.

Introduction and Conceptual Frame Work

Lifestyles of populations across the world have changed dramatically in the 20th century. These changes (collectively termed as epidemiological transition) have been brought about by a number of developments in science and technology that now affect every facet of human existence. Most human societies have moved from agrarian diets and active lives to fast foods and sedentary habits. Combined with increasing tobacco use, these changes have fuelled the epidemic of obesity, diabetes, hypertension, dyslipidaemia and cardiovascular diseases (CVD). In developed nations the rise in the burden of Cardiovascular diseases occurred over several decades due to a long period of epidemiological transition. In India, perhaps because of the rapid pace of economic development, epidemiological changes have spanned a much shorter time. As a consequence, cardiovascular disease (CVD) has emerged as the leading cause of death all over India, with coronary heart disease (CHD) affecting Indians at least 5-6 years earlier than their western counterparts [1,2]. Current estimates from disparate cross-sectional studies indicate the prevalence of CHD to be between 7-13 per cent in urban and 2-7 per cent in rural India [3]. Therefore recent interest has focused on finding compounds that could intervened the chemical process underlying age- related degenerative diseases in which has been demonstrated to be also responsible for the ageing phenomena. Evans and Bishop, in 1922, discovered that dietary supplements with alfalfa leaves (rich in vitamin E) prevent placental hemorrhage and reverse dietary sterility in rats [4].

Evans and his associates [4] isolated the compounds of vitamin E family and named them tocopherols . While alpha-tocopherol was the first vitamin E isomer to be recognized, eight chemically distinct isomers are now known, consisting of alpha (α), beta (β), gamma (γ) and delta (δ)-tocopherols and α, β, γ and δ-tocotrienols (T3), all of them are referred to as vitamin E. Tocotrienols are vitamins isomers which are "essential", meaning they cannot be manufactured by the human body. Hence, they have to be obtained from food and supplements. Tocotrienols variant of vitamin E typically only occurs at very low levels in nature [5].

Tocotrienols are natural compounds found in selected vegetable oils, wheat germ, barley, saw palmetto and certain other types of seeds, nuts, and grains. Palm oil and rice bran oil represent two major nutritional sources of natural tocotrienol. Vitamin E isomers are tocochromanols having a polar chromanol head group with a long isoprenoid side chain. Depending on the nature of the isoprenoid side chain, tocopherols (containing a phytyl chain) or tocotrienols (geranyl chain) can be distinguished [6]. The name "tocotrienol" was first suggested by Dr. Banyan, for the isomers of vitamin E, with isoprenoid side chain present in nature, when isolated from the latex of the rubber plant, Havea brasiliensis [7]. Chemically, vitamin E in all its forms functions as an antioxidant. All the tocotrienols and tocopherol isomers have this antioxidant activity due to the ability to donate a hydrogen atom (a proton plus electron) from the hydroxyl group on the chromanol ring, to a free radical in the body. This process inactivates (quenches) the free radical by effectively donating a single unpaired electron (which comes with the hydrogen atom) to radical [8]. Tocotrienols have a very broad range of medicinal properties and are used as antioxidant, analgesic, antiinflammatory, antibacterial, antipyretic, antithrombotic, anticancer, cardioprotective, hepatoprotective, hypoglycemic, nephroprotective and neuroprotective etc [9,10]. In this research work, alterations in lipoprotein- lipid profile, total cholesterol, low density lipoproteins [LDL-C], high density lipoproteins[HDL-C] were measured in a randomized , placebo-controlled intervention study of low dose(160mg/ day) TRF supplementation.

Objective

The prime objective of this study was to analyzed the effects of low dose TRF supplementation on lipid profiles in healthy individuals.

Methodology

Study Design and Procedures

The study was conducted according to single-blind placebo controlled group design. The present study was approved by the Institutional Ethics Committee, Mayo Institute of Medical sciences, Barabanki, U.P. and IEC, MGM Institute of health sciences, Navi Mumbai, written informed consent was obtained from all the subjects. Healthy subjects were recruited through screening of a population study on oxidative stress and ageing. Subjects were selected from clinical division of mayo institute of Medical sciences with results of a pre-study consisting of a full examination , blood chemistry and hematology to conform study suitability . All the selected subjects were free from any other type of supplement or minerals except placebo controlled. Hundred subjects were recruited from two age group of 35- 50 years(n= 50)and above 50 years and they were assigned to receive either TRF single dose of 160mg/day(4EOL) or placebo capsules for six months. Blood samples were obtained at 0, 3rd and 6 months. healthy subjects were standardized as control to ensure the instrumentation and reference biochemical values for this study. All individuals were requested to consume the capsules after dinner to ensure proper absorption and encouraged to maintain their daily activities throughout the study period.

Sample Collection

Blood sampling will be perform at baseline (month 0), 3months and 6 months of TRF supplementation. Venous blood samples were drawn from fasting subjects into lithium heparin- coated and K2EDTA-containing tubes for plasma extraction . Plasma was immediately separated by centrifugation at 3000 rpm for 10 minutes. Plasma-EDTA aliquots were used for quantification of lipid profiles.

Lipid Profile Determination

A. Plasma cholesterol

B. Plasma Triglycerides

C. High density lipoprotein-cholesterol [HDL-C]

D. Low density lipoprotein- cholesterol [LDL-C]

Analysis of Cholesterol

Cholesterol was determined by CHOD- PAP method. It is based on modified Roeschlaus method [11].

Procedure

Pipette in to Tubes Marked	Blank	Standard	Test
Working reagent	1000µl	1000µl	1000µl
Distilled water	20µl	-	-
Standard	-	20µl	-
Test	-	-	20µl

Test tubes mixed well incubated at 37^0 C for 10 minutes. Aspirates blank followed standard and tests. Absorbance were read of standard and each test tubes against blank at 505 nm on semi auto analyzer.

Calculation $\text{Cholestrol } \left(\frac{\text{mg}}{\text{dl}}\right) = \frac{Absorbance\ of\ test}{Absorbonce\ of\ standard} X\ concentration\ of\ standard\ (\frac{mg}{dl})$

Normal Values

The normal range of cholesterol depends on age sex , diet race and geographical location. Normal range for reference values are 140-225 mg/ dl.

B. Analysis of HDL Cholesterol

HDL was determined by PEG/CHOD-PAP method [12].

Procedure

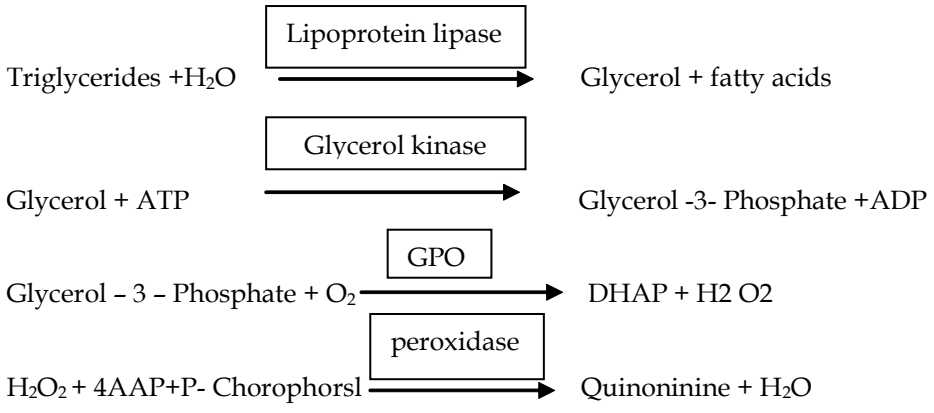
Pipette in to tubes marked	Blank	Standard	Test
Working reagent	450µl	450µl	450µl
Distilled water	5µl	-	-
Standard	-	5µl	-
Test	-	-	5µl

Test tubes mixed well and incubated for 5 main at 37^0C measured the absorbance of standard and test against blank at 578nm.

Calculation: $\text{HDL } \left(\frac{\text{mg}}{\text{dl}}\right) = \frac{Absorbance\ of\ test}{Absorbonce\ of\ standard} X\ Concentration\ of\ standard\ (\frac{mg}{dl})$

Normal Value: 40- 80 mg / dl

C.Analysis of Triglycerides: It was determined by enzymatic kit method [13]. Triglycerides hydrolyzed by lipoprotein lipase in to glycerol and free fatty acids. The glycerol phosphorylated to make glycerol-3 phosphate as product in presence of glycerol kinase enzyme . Now glycerol-3 phosphate oxidized in to dihydroxy acetone phosphate with the help of enzyme glycerol-3 phosphate oxidase (GPO) & H_2O_2 released as by product. Produced H_2O_2, reacts with p-chlorophenol and amino-4- antipyrine by the catalytic action of peroxidase to from a red colored quinine immune dye complex. Intensity of color formed is directly proportional to the amount of triglycerides present in sample.



GPO = Glycerol – 3- phosphate oxidize, 4AAP= 4 amino – antipyrine, DHAP = Dihydroxy acetone phosphate.

Procedure

Pipette in to tubes marked	Blank	Standard	Test
Working reagent	300µl	300µl	300µl
Distilled water	3µl	-	-
Standard	-	3µl	-
Test	-	-	3µl

Test tubes mixed will and incubated for 425 seconds at 37^0 C. measured the absorbance (A) of standard and tests against the blank at 500mg.

Calculation: $$\text{TG}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{Absorbance\ of\ test}{Absorbonce\ of\ standard} X\ standard\ concetrution$$

Reference Values: According to NCEP (American national cholesterol education programme) are:

Normal - < 150 mg/dl; Borderline high - 150-199 mg/ dl; High - 200-499 mg/dl; Very High - > 500 mg/dl

D. Determination of LDL – Cholesterol

In was determined by using fried Wald's formula[12].

$$\text{Concentration of VLDL} = \frac{\text{Concentration of Triglyceride}}{5}$$

Concentration of LDH = Total Cholesterol – (HDL + VLDL)

OR

LDH Cholesterol mg/ dl = Total cholesterol – (Tg/ 5) HDL Cholesterol

Normal Value : 62-185 mg / dl .

Results

Anthropometric Characteristics of Subject

The baseline characteristics of subject regarding to age, Blood pressure, BMI, gender, pulse in the TRF group were similar to those in the placebo groups. Systolic and diastolic blood pressure in the younger group were significantly lower than those of older group. None of these clinical parameters showed statistical differences upon supplementation.

Lipid Profiles

It was observed that the total plasma cholesterol was within the normal range in all subjects before TRF supplementation. Therefore, there was no significant difference in lipid level between the younger and older groups at baseline. A statistically significant effect for duration of treatment was

Table 1: Lipid Profile study of subjects

	35-50 years		>50 years	
	PLACEBO	TRF	PLACEBO	TRF
Triglycerides (mg/ dl)				
Baseline (0 month)	100 ± 14	123.11 ± 17.80	110.71 ± 14.17	131.08 ± 22.14
3 Months	108.94 ± 10.62	124.89 ± 17.71	139 ± 19.48	128.82 ± 22.14
6 Month	90.34 ± 8.90	129.31 ± 23.01	127.54 ± 22.14	139.06 ± 26.57
Total Cholesterol (mg/ dl)				
Baseline (0 month)	197.99 ± 7.35	206.88 ± 6.97	204 ± 7.73	209.97 ± 7.35
3 Months	189.86 ± 6..58	208.43 ± 6.57	208.43 ± 6.97	209.97 ± 7.38
6 Month	194.89 ± 6.58	211.91 ± 7.00	206.49 ± 8.12	210.36 ± 7.38
High Densely lipoprotein (HDL) (mg / dl)				
Baseline (0 month)	53.00 ± 2.70	17.00 ± 2.33	52.20 ± 3.48	52.59 ± 3.86
3 Months	57.23 ± 3..86	51.04 ± 2.32	56.8. ± 2.70	51.04 ± 3.86
6 Month	54.53 ± 3.86	57.23 ± 2.70	49.99 ± 2.70	56.84 ± 2.70
Low Density lipoproteins (LDL) (mg/dl)				
Baseline (0 month)	126.83 ± 6.96	131.47 ± 7.73	126.83 ± 7.83	132.25 ± 5.80
3 Months	115.62 ± 6.57	134.00 ± 5..80	122.97 ± 6.57	133.41 ± 6.96
6 Month	121.81 ± 6.57	131.47 ± 5.80	136.50 ± 6..18	124.90 ± 6.90

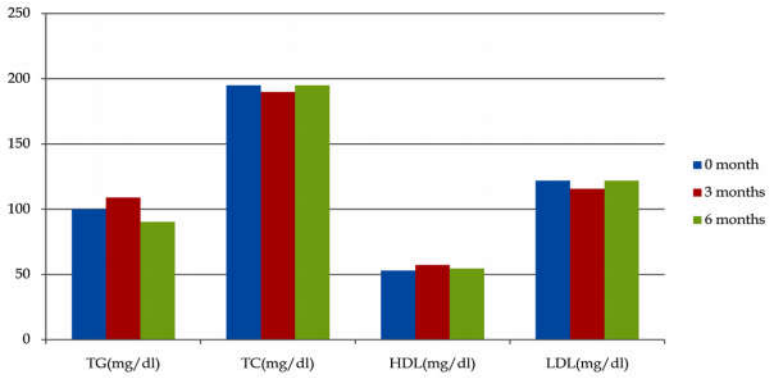


Fig. 1: Lipid profile in Placebo Group aged between 35 – 50 years

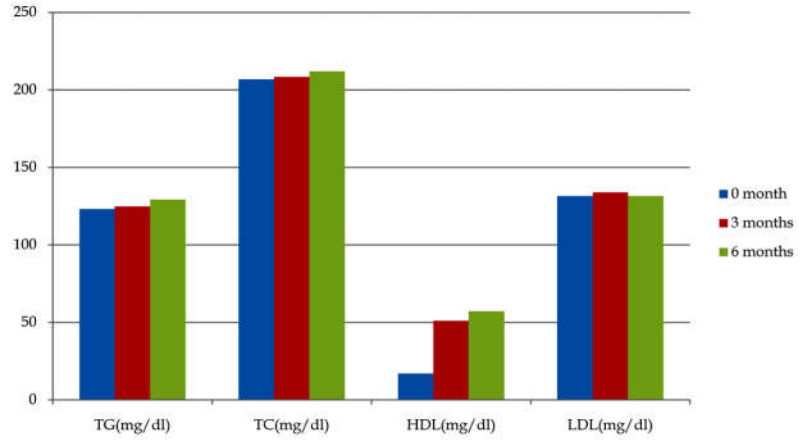


Fig. 2: Lipid profile in TRF Group aged between 35 – 50 years

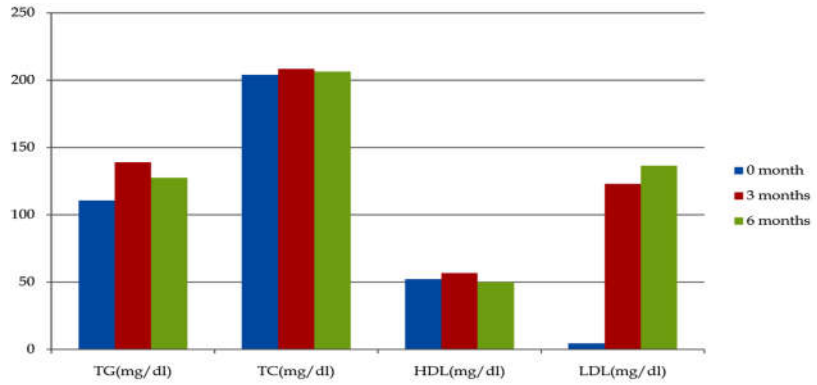


Fig. 3: Lipid profile in Placebo Group aged > 50 years

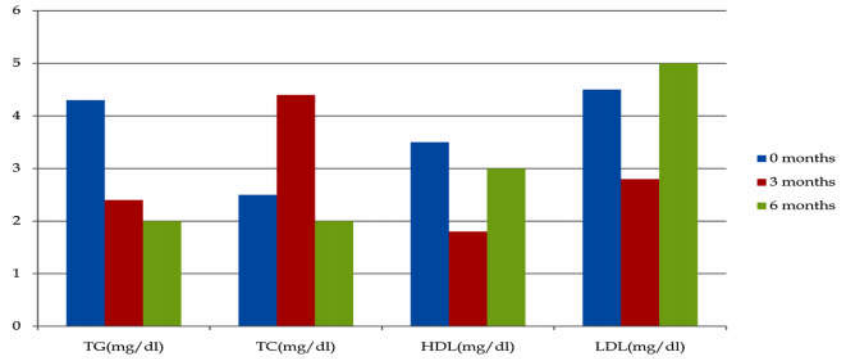


Fig. 4: Lipid profile in TRF Group aged > 50 years

observed in HDL-C of TRF group. The HDL cholesterol level in the younger group enhanced significantly($p=0.014$) after six months of tocotrienol supplementation as compared to baseline(0 month) . While in the ≥50 years old a non significant elevation of 8% was observed at the dose of 160mg/day[Table1].

Discussion

There are various factors affecting the ageing process, though genetic and lifestyle-related factors such as diet, exercise, pollution etc are having prominent impact. Dietary interventions are feasible, as nutrients can affect the rate of ageing by changing the type and quantity of proteins synthesized (ref) by modulating gene expression(ref),thereby altering the redox status of individuals [14,15].

Our result indicate that daily supplementation of TRF up to 6 months, raised plasma HDL cholesterol levels as early as 3 months, thereby increasing the HDL-cholesterol/Total cholesterol ratio. This ratio reflects the proportion of antiatherogenic to atherogenic lipids and has been suggested as better predictor of cardiovascular disease risk than the individual lipoprotein values (ref). In this way TRF might be helpful to reduce the risk of cardiovascular diseases in healthy older adults. The higher dose and durations of TRF supplementation might be beneficial for monitoring and prevention of other factors involved in cardiovascular disorders and also may confers its protective benefits via protection against oxidative

stress, involvement in oxidized protein repair, and redox homeostasis through signaling.

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