

Effect of Tocotrienols on Lipid Profile in Rabbits

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Abstract

Background: To evaluate the effect of tocotrienols on serum lipids in cholesterol-fed rabbits.

Methods: In this experimental study, thirty six, male New Zealand white rabbits were randomly allocated to three equal groups. They were given either normal rabbit diet (Group-I); or 2% high cholesterol diet (Group-II); or 2% high cholesterol diet with tocotrienols 6mg/kg body weight/day (Group-III) orally for six weeks. Serum lipid profile was analyzed at the start and end of study.

Results: Baseline levels of serum lipids were not statistically different among the three groups ($p > 0.05$). By the end of experiment, Group-I showed insignificant changes ($p > 0.05$) while Groups-II&III exhibited significant increase ($p < 0.001$) in serum levels of total cholesterol, LDL-C, HDL-C and triglyceride in contrast to their baseline levels. However, serum total cholesterol, LDL-C and triglyceride levels were 27% ($p < 0.05$), 29% ($p < 0.05$), 13% ($p > 0.05$) lower respectively and HDL-C concentrations were 4% higher ($P > 0.05$) in tocotrienols-supplemented group compared with rabbits using only high cholesterol control diet.

Conclusion: Tocotrienols may be therapeutically helpful as cholesterol lowering agents.

Key Words: Tocotrienols; Lipid profile;

Introduction

Hypercholesterolemia is well known to be the unique and sufficient cause for the development of coronary artery disease, even if the other known hazards are absent. The pooled results of many trials using diverse cholesterol-lowering regimens pointed out that for each 1 mmol/L decrease in total cholesterol level, there was a decline in mortality associated with coronary artery disease, by at least 24.5%.¹

High blood cholesterol levels and especially the retention of LDL fraction in the vessel wall lead to oxidative stress. Recent suggestions for lowering the blood cholesterol levels are aimed at increasing dietary antioxidants.² Vitamin E comprises four tocopherols and four tocotrienols [α (α), β (β), γ (γ) and δ (δ)]. However, only 1% of the entire pubmed literature on vitamin E deals with tocotrienols and rest

of all studies were intended for α -tocopherol. Interestingly, tocotrienols emerged more potent antioxidants than tocopherols. Dissatisfaction with outcomes of the trials testing the hypocholesterolemic properties of α -tocopherol can be handled with tocotrienols.³

Materials and Methods

The study was carried out in the centre for research in experimental and applied medicine, Army Medical College, Rawalpindi, National University of Sciences & Technology Pakistan. Thirty six adult male New Zealand white rabbits, weighing 1.5 to 2.5 kg were obtained from National Institute of Health, Islamabad, Pakistan (NIH). Rabbits were kept individually in wire-bottomed cages, at a standard temperature of $21 \pm 2^\circ\text{C}$ with 12 hour light/dark cycle. Each animal was given 100 g/day powdered normal rabbit diet (NIH). Water was available *ad libitum*. Rabbits were adapted to experimental conditions for seven days and then randomly segregated into three equal study groups. Group-I was maintained on the normal rabbit diet for six weeks. For the same period, Group-II&III were given 2% high cholesterol diet [2g cholesterol powder (Applichem, Germany) mixed with 100g normal rabbit diet /head/day], however, animals in group-III were also supplemented with annatto tocotrienols [mixture of 90% δ - and 10% γ -tocotrienol (Kabco, USA) in a dose of 6 mg/kg body weight/day orally]. Body weight and blood samples were taken as baseline (week 0) and at the end of experiment (week 6).

Serum cholesterol was measured by cholesterol oxidase method, triglyceride was measured by GPO-POD method, High density (HDL-C) and low density lipoprotein cholesterol (LDL-C) were analyzed by homogenous enzymatic assays. Coefficient of variation of the lipids profile assays was $<$ than 4.7%. For comparison between means of initial and final lipid profile within each group, paired sample 't' test was applied. Final 6 week data among all the three groups were analyzed by one way ANOVA followed by Tukey's post-hoc test. All the results were considered

significant at a P-value less than 0.05.

Results

Baseline levels of all the variables were not statistically different among the three groups ($p > 0.05$). By the end of experiment, Group-I showed insignificant changes ($p > 0.05$) while Groups-II&III exhibited significant increase ($p < 0.001$) in serum levels of total cholesterol, LDL-C, HDL-C and triglyceride in contrast to their baseline levels. However, serum total cholesterol, LDL-C and triglyceride levels were 27% ($p < 0.05$), 29% ($p < 0.05$), 13% ($p > 0.05$) lower respectively and HDL-C concentrations were 4% higher ($p > 0.05$) in tocotrienols-supplemented group compared with rabbits using high cholesterol control diet (Table I).

Table I. Comparison of serum lipid profile (mmol/l) among three groups

Parameters	Group-I	Group-II	Group-III
Total Cholesterol			
0 Weeks	2.045 ± 0.102	1.914 ± 0.084	2.015 ± 0.086
6 Weeks	1.964 ± 0.100	31.478 ± 2.222* #	22.850 ± 1.898#
Triglyceride			
0 Weeks	0.874 ± 0.037	0.820 ± 0.047	0.900 ± 0.047
6 Weeks	0.882 ± 0.023*	1.613 ± 0.209#	1.407 ± 0.153*
LDL-C			
0 Weeks	1.172 ± 0.114	1.085 ± 0.070	1.144 ± 0.073
6 Weeks	1.087 ± 0.097	27.661 ± 2.276* #	19.536 ± 1.760#
HDL-C			
0 Weeks	0.523 ± 0.049	0.454 ± 0.015	0.459 ± 0.021
6 Weeks	0.472 ± 0.013*	2.579 ± 0.145#	2.678 ± 0.178*

Data are mean ± SE. n = 12 per group. * denotes significance ($P < 0.05$) when compared to other groups. # denotes significance ($P < 0.05$) when compared between 0 & 6 weeks of each group.

Discussion

The present study uncovered the effect of tocotrienols on serum lipids in New Zealand white rabbits which were chosen as a model on account of being most susceptible and sensitive to present cardiovascular diseases, quite similar to and with the involvement of same molecular mechanisms as in humans, through high cholesterol diet.⁴ Tocotrienols have achieved a substantial momentum during the last few years owing to their powerful antioxidant and anti-inflammatory properties and δ -tocotrienol was found to be the most potent isoform. Cholesterol lowering properties of tocotrienols have been reported by some researchers but with conflicting results.^{5,6} But,

tocotrienol rich fraction of either rice bran oil or palm oil was used. Rice bran oil is a natural source of tocotrienols but it contains 50% tocopherols and 50% tocotrienols.⁷ Crude palm oil contains 70% tocotrienols and 25% tocopherols.⁴ Annatto (*Bixa orellana* L.) seeds extract is the only source containing 100% tocotrienols.⁸ Current state of knowledge warrants that tocopherols interfere with the absorption of tocotrienols.⁹ Results from supplements containing pure tocotrienols may have more promising results. Substantial beneficial response to tocotrienols also depends on fed or fasted conditions. The fed state increased the onset as well as extent of absorption of tocotrienols by greater than two folds.¹⁰ Tocotrienols alter the metabolism of steroids and promote their clearance from intestine and are speculated to stimulate cholesterol catabolism.^{11,12}

Conclusion

Human trials should be considered for final elucidation of tocotrienols as cholesterol lowering agents.

References

- Gould AL, Davies GM, Alemao E. Cholesterol reduction yields clinical benefits. *Clinical Therapeutics*, 2007; 29(5): 778-94.
- Minhajuddin M, Beg ZH, Iqbal J. Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. *Food and Chemical Toxicology*, 2005; 43:747-53.
- Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: the other half of the natural vitamin E family. *Mol Aspects Med*, 2007; 28:692-728.
- Shahid U, Butt SA, Mubarak A. Site specific response to high cholesterol diet in aorta of rabbit. *Pak Armed Forces Med J*, 2010; 60:505-10.
- Khan DA, Khan FA, Ijaz A, Qureshi AA, Dilawar M. Cholesterol lowering effects of lovastatin and tocotrienols. *Pakistan Journal of Pathology*, 2004; 15:93-99.
- Budin SB, Othman F, Louis S J, Bakar MA. The effects of palm oil tocotrienol-rich fraction supplementation on oxidative stress and the vascular wall. *Clinics*, 2009; 64(3): 235-44.
- Qureshi AA, Mo H, Packer L. Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic properties. *Journal of Agricultural and Food Chemistry*, 2000; 48(8): 3130-40.
- Frega N, Mozzon M, Bocci F. Identification and estimation of tocotrienols in the annatto lipid fraction by gas chromatography. *JAOCs*, 1998; 75: 1723-27.
- Hasselwander O, Kramer K, Hoppe P. Effects of feeding various sources on plasma lipids and aortic atherosclerotic lesions in cholesterol-fed rabbits. *Food research international*, 2002; 35(2-3): 245-51.
- Yap SP, Yuen KH, Wong J W. Pharmacokinetics and bioavailability of alpha-, gamma- and delta-tocotrienols. *J Pharm Pharmacol*, 2001; 53(1): 67-71.
- Zhou C, Tabb MM, Sadatrafiee A, Grun F. Tocotrienols activate the steroid and xenobiotic receptors, SXR, and selectively regulate expression of its target genes. *Drug Metabolism & Disposition*, 2004; 32:1075-82.
- Chen CW and Cheng HH. A rice bran oil diet increases LDL-receptor and HMG-CoA reductase mRNA expressions and insulin sensitivity in rats. *J Nutr*, 2006; 136(6): 1472-76.