

Tocotrienol rich fraction supplement reduces oxidative stress in non familial hypercholesterolaemia: beyond the lipid lowering capability

^{1,2}Muid, S., ³Hamid, Z., and ^{1,2}*Nawawi, H,

¹Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA (UiTM), 68100 Batu Caves, Selangor, Malaysia

²Faculty of Medicine, Universiti Teknologi MARA (UiTM), 47000 Sungai Buloh, Selangor, Malaysia

³Sime Darby Plantations, Ara Damansara, Petaling Jaya, Selangor, Malaysia

Article history

Received: 7 April 2017

Received in revised form:
25 June 2017

Accepted: 27 June 2017

Abstract

The beneficial effects of Palm Tocotrienol Rich Fraction (TRF) in the reduction of cholesterol and oxidative stress in human especially in Non-Familial Hypercholesterolaemia (NFH) patients are still lacking and need to be further investigated. In this clinical trial, 37 NFH patients were recruited and randomized to either Palmvitee (60 mg/day TRF) [NFHe; n= 12] and atorvastatin 10 mg/day (NFHs; n=25). Fasting serum lipids, F2 -isoprostanes, oxidized LDL (ox-LDL) and malondialdehyde (MDA) were measured at baseline (BL), 2 weeks and 12 weeks. NFHe group showed significant reduction in Total cholesterol, TC, LDL, MDA, F2 – isoprostanes and ox-LDL at 12 weeks compared to BL. NFHs group showed a reduction in TC, LDL and TG, MDA and F2 – isoprostanes in 12 weeks compared to the BL. NFHs had greater % change reduction of TC, LDL, TG and MDA than NFHe in 12 weeks. Despite that, NFHe and NFHs had comparable % reduction of F2–isoprostanes in 12 weeks. NFHe had greater % change reduction of ox-LDL than NFHs. In conclusion, TRF reduces cholesterol level in NFH patients even though it is not as efficient as statins. The ability of TRF in the reduction of oxidative stress especially F2-isoprostanes is comparable with statins. TRF has a great potential in the prevention of atherosclerosis in part not only due to its cholesterol lowering activity, but perhaps more effective as a potent antioxidant.

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Keywords

Tocotrienols
Non familial
hypercholesterolaemia
Palmvitee cholesterol
Oxidative stress

Introduction

Hypercholesterolaemia is commonly associated with increased oxidative stress (Yang *et al.*, 2008). Oxidative stress plays a major role in the pathogenesis of atherosclerosis and coronary artery disease (CAD) (Nourooz-Zadeh *et al.*, 2001). Endothelial cells, vascular smooth muscle cells, neutrophils and monocytes can generate an oxidised LDL, a highly atherogenic property, and this process leads to the formation of lipid peroxidation products such as F2-isoprostanes, malondialdehyde (MDA) and reactive oxygen species (Ridker, 2003). Endothelium exposed to ox-LDL develops early signs of injury such as apoptosis and decreases the gene expression of the endothelial cell NO synthase (eNOS) leading to endothelial dysfunction (Ridker *et al.*, 2004). Ox-LDL induces the expression of adhesions molecules and attachment of monocytes and T lymphocytes to the endothelial cells. In the tunica intima, ox-LDL is taken up by macrophages to produce foam cells, a key event in the development of atherosclerosis (Mehta *et al.*, 2001).

Treatment with inhibitors of 3-hydroxyl-3-

methylglutaryl coenzyme-A (HMGCO-A) reductase (statin) reduces serum cholesterol levels and coronary events in patients with hyperlipidaemia (Gresser and Gathof, 2004). Beyond lipid lowering, statin also has beneficial effects on oxidative stress (Madamanchi *et al.*, 2005).

Vitamin E comprises of tocopherols and tocotrienols synthesized by plants and photosynthetic organisms. Palmvitee is a naturally acquired palm oil derived tocotrienol rich fraction (TRF). Vitamin E is an essential, fat-soluble nutrient that functions as an antioxidant in the human body (Galli *et al.*, 2017). Both tocopherols and tocotrienols have four different isomers (α , β , γ and δ) all of which are potent membrane soluble antioxidants (Aggarwal *et al.*, 2010). On the basis of the premise that vitamin E may reduce oxidative stress markers, many clinical trials tested vitamin E supplementation as a therapy to prevent various chronic diseases and the results is not promising (Niki, 2015).

A high dosage of tocopherol of vitamin E (400 IU/D/267 mg/D) has recently been reported to increase all cause mortality in human (Gee, 2011). However, this data was based on the results reported

*Corresponding author.

Email: hapizah.nawawi@gmail.com

on the various tocopherols but not naturally acquired tocotrienol-based vitamin E intervention clinical trials.

Several *in vitro* studies have reported the potent anti-oxidative effects of palm oil derived TRF (Kamat *et al.*, 1997; Mutalib *et al.*, 2003; Yam *et al.*, 2009). Tocotrienol has been shown to have more potent antioxidant activity compared to tocopherol yet most clinical trials use tocopherols instead of tocotrienols (Sen *et al.*, 2007). A high concentration of tocotrienol is present in crude palm oil which is extracted from the fruits of *Elaeis guineensis* (MacLellan, 1983). In various *in vitro* studies, palm oil derived TRF has been shown to have more potent antioxidant, anti-inflammatory, anti-cancer, anti-aging, anti-thrombotic and anti-angiogenic activities than tocopherols (Miyazawa *et al.*, 2008; Wu *et al.*, 2009; Aggarwal *et al.*, 2010). TRF but not tocopherol has been shown in *in vitro* studies to have hypocholesterolaemic activity by suppressing the activity of hydroxy-3-methylglutaryl co-enzyme A reductase (Pearce *et al.*, 1994). Several animal studies have shown its cholesterol lowering effects (Qureshi *et al.*, 2001). However, data on its effects on humans are still lacking and human studies reported controversial results in cholesterol reduction (Tomeo *et al.*, 1995; Mensink *et al.*, 1999; O'Byrne *et al.*, 2000; Wahlqvist *et al.*, 2016).

In addition, it is postulated that the optimal effects of palm oil derived TRF is not necessarily fall at high concentrations. Therefore, this study aimed to investigate the effects of TRF on serum lipids and oxidative stress biomarkers in comparison with statin treatment in patients with non-familial hypercholesterolaemia (NFH).

Materials and Methods

Subjects and study design

This was a randomized and prospective clinical trial. Ethics committee approval was obtained prior to the commencement of this study. A total of 37 non familial hypercholesterolaemia (NFH) patients (24 males, 13 females, age + SD age = 45.3 + 9.4 years) were recruited from the Lipid Specialist Clinics of a research institute in Malaysia. The diagnosis of NFH was based on the total cholesterol (TC) concentrations > 6.5 and/or low density lipoprotein (LDL) > 3.8 mmol/L and did not fulfill Simon Broome's Criteria. Patients with diabetes mellitus (type 1 and type 2), hypothyroidism, chronic alcoholism, liver and renal diseases were excluded from the study. Other exclusion criteria for the study were regular intake of antioxidants, acetosalicylate acid or other drugs

with anti-oxidative properties, contraindications or adverse reaction to statins, chronic inflammatory disorders or any condition limiting mobility and severe disease shortening life expectancy (e.g. malignancy). All patients gave written informed consent for participation before recruitment into this study.

Thirty-seven (37) NFH subjects were randomised into 2 treatment groups, which were palmvitee [NFHe; n= 12) or atorvastatin 10 mg/day (NFHs; n=25). Clinical data and blood collection were performed at baseline (BL) and at subsequent two weeks and 12 weeks followed-up visits. Palmvitee supplements contain palm oil derived TRF [67% tocotrienols (28.2% α -, 1.9% β -, 23.1% γ -, 13.5% δ - tocotrienols), 33% α -tocopherol and palm superolein] enclosed in soft gelatin capsules. Palm superolein is a mixture of triglycerides with oleic, palmitic and linoleic fatty acids. Palmvitee were provided by Sime Darby Biogenic, Sdn. Bhd, Malaysia. It has been reported that plasma concentrations of tocotrienol was significantly elevated after supplementation (Zahara *et al.*, 2010). Patient's compliance to medication was assessed by 'pill counting technique' during follow-ups visits.

Blood and data collection

Subjects were confirmed not to have any acute inflammatory disease at least two weeks prior to blood collection. Fasting venous blood samples were collected. Plasma and serum was collected prior to blood centrifugation at 3,000 rpm in 4°C and was stored frozen at -80°C until analysis. Blood pressure at sitting position, body mass index, waist hip ratio, smoking status, alcohol consumption status, personal and family history of CAD was documented. Systolic and diastolic hypertension classifications were based on the WHO criteria (Whitworth, 2003). BMI was calculated by this following formula: BMI = weight (kg)/height² (m²) (Nawawi *et al.*, 2003). Waist circumference was measured to the nearest 0.5 cm using a measuring tape at midway between the inferior margin of the last rib and the iliac crest in a horizontal plane. Hip circumference measurement was taken around the pelvis at the point of maximal protrusion of the buttocks. WHR was derived from the calculated waist/hip ratio.

Cholesterol and oxidative stress markers measurement

Fasting serum lipids (FSL), liver profile and fasting plasma glucose were analysed by an automated analyzer (Cobas Integra 700, Roche Diagnostics, Switzerland). MDA was determined by the method adapted from Ledwozyw *et al.* (1986)

based on the measurement of pink chromogen formed at 532 nm wavelength. F2-isoprostanes and ox-LDL were measured by ELISA, as instructed by the manufacturer (Merckodia, Uppsala, Sweden and Cayman Chemicals, USA respectively).

Statistical analysis

The sample size ($n > 10$) in this study was determined by the Power and Sample size program (PS) version 3.0 to provide a study power of 80% at 5% level of significance. Variables with normal distribution were expressed as mean \pm SEM. Comparison between two treatment groups was determined by independent t-test. Paired sample t-test was used to determine the effects of treatment in each NFH treatment groups. The criterion for statistical significance was at p value < 0.05 . The Chi-square was used to examine associations between categorical variables. Data was analysed by a statistical package program (SPSS) version 16.0

Results

BL characteristics

In this prospective study, 37 NFH patients were divided into 2 treatment groups (NFHe and NFHs). For NFHe group, 12 patients were recruited (mean \pm SEM age = 43.1 ± 10.5 , 7 males and 5 females). Twenty-five patients were randomised into NFHs group (mean \pm SEM age = 46.3 ± 9.0 , 17 males and 8 females). BL characteristics parameters among all groups were matched and did not differ significantly (Table 1). In terms of oxidative stress biomarkers, all groups were matched for their F2-isoprostanes and ox-LDL level. However, NFHs group had higher MDA levels compared to NFHe ($p < 0.0001$).

Lipid profile

Table 2 illustrated the lipid profile of NFHe and NFHs groups at BL (BL), 2 weeks and 12 weeks post-TRF supplementation. In NFHe groups, TC ($p < 0.05$) and LDL ($p < 0.05$) were significantly reduced after 12 weeks TRF supplementation compared to BL. However, there was a significant increase of TG levels ($p < 0.05$) at 12 weeks post-TRF supplementation compared to BL with no significant change in HDL levels. In the NFHs group, there was a significant reduction of TC ($p < 0.0001$) LDL ($p < 0.0001$) and TG ($p < 0.005$) at 2 weeks compared to BL ($p < 0.0001$) and the reduction was maintained at 12 weeks. However, there were no differences in HDL levels at 2 weeks and 12 weeks post-statin treatment compared to BL. In order to compare the effects of TRF versus statin, percentage (%) change of lipids in each NFHe and

Table 1. BL characteristics of NFHe and NFHs patients

Parameters	NFHe (n=12)	NFHs (n=25)	p value
^a Age (years)	43.1 \pm 10.5	46.3 \pm 9.0	NS
^b Gender (M/F) (%)	7/5 (58/42)	17/8 (68/32)	NS
^b Race (M/C/I) (%)	13/2/0 (92/8/0)	20/5/0 (80/20/0)	NS
^b Current smoker (%)	2/10 (17)	4/25 (16)	NS
^a BMI (kg m ⁻²)	25.3 \pm 4.4	26.0 \pm 4.3	NS
^a Waist (cm)	89.6 \pm 12.0	89.6 \pm 10.6	NS
^a WHR	0.87 \pm 0.02	0.87 \pm 0.06	NS
^a SBP (mm Hg)	128.1 \pm 14.9	131.8 \pm 17.9	NS
^a DBP (mm Hg)	75.8 \pm 10.1	78.2 \pm 11.0	NS
^b TC (mmol L ⁻¹)	7.4 \pm 0.7	7.0 \pm 0.9	NS
^c TG (mmol L ⁻¹)	1.4 \pm 0.8	2.0 \pm 1.4	NS
^c LDL-c (mmol L ⁻¹)	5.5 \pm 0.8	4.8 \pm 0.7	NS
^c HDL-c (mmol L ⁻¹)	1.3 \pm 0.4	1.3 \pm 0.3	NS
^c MDA (nmol g ⁻¹)	183.2 \pm 7.2	282.2 \pm 60.3 ****	< 0.0001
^c F2-isoprostanes (pg ml ⁻¹)	1700.3 \pm 241.1	1331.8 \pm 229.5	NS
^c ox-LDL (U L ⁻¹)	79.7 \pm 9.4	64.9 \pm 8.2	NS

Note: ^aData are expressed as mean + SD (age)/SEM and ^bData are expressed as proportion (%). M/F indicates Male/Female; M/C/I: Malay, Chinese, Indian; BMI: Body Mass Index; WHR: Waist to Hip Ratio; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; TC: Total Cholesterol; TG: Triglycerides; LDL-c: Low Density Lipoprotein Cholesterol; HDL: High Density Lipoprotein Cholesterol; MDA: Malondialdehyde; ox-LDL: oxidized LDL; NS: Not significant.

Table 2. Fasting serum lipid (FSL) levels at BL and post-treatment periods in NFHe and NFHs groups

Groups	TOTAL CHOLESTEROL (mmol L ⁻¹)			p value
	BL	2 weeks	12 weeks	
NFHe (n=12)	7.4 \pm 0.2	7.1 \pm 0.2	7.0 \pm 0.2*	< 0.05
NFHs (n=25)	6.9 \pm 0.2	5.1 \pm 0.2****	5.0 \pm 0.2****	< 0.0001
Groups	LOW DENSITY LIPOPROTEIN (mmol L ⁻¹)			p value
	BL	2 weeks	12 weeks	
NFHe (n=12)	5.3 \pm 0.2	5.1 \pm 0.6	4.9 \pm 0.2*	< 0.05
NFHs (n=25)	4.6 \pm 0.2	3.0 \pm 0.2***	3.0 \pm 0.2****	< 0.0001
Groups	TRIGLYCERIDES (mmol L ⁻¹)			p value
	BL	2 weeks	12 weeks	
NFHe (n=12)	1.4 \pm 0.2	1.5 \pm 0.3	1.8 \pm 0.3*	NS
NFHs (n=25)	2.0 \pm 0.4	1.5 \pm 0.3***	1.6 \pm 0.3***	< 0.005
Groups	HIGH DENSITY LIPOPROTEIN (mmol L ⁻¹)			p value
	BL	2 weeks	12 weeks	
NFHe (n=12)	1.3 \pm 0.1	1.3 \pm 0.1	1.2 \pm 0.1	NS
NFHs (n=25)	1.3 \pm 0.1	1.4 \pm 0.1	1.3 \pm 0.1	NS

Note: Data are expressed mean + SEM. **** $p < 0.0001$, *** $p < 0.005$, ** $p < 0.01$, * $p < 0.05$ compared to BL. NFH indicates Non-Familial Hypercholesterolaemia; NS: Not Significant.

NFHs group was calculated at 2 weeks and 12 weeks treatment period and illustrated in Table 3. NFHe had lower % change reduction of TC compared to NFHs at 2 weeks ($-2.4 \pm 3.3\%$ vs. $-24.9 \pm 3.3\%$, $p < 0.0001$) and 12 weeks ($-4.6 \pm 2.3\%$ vs. $-27.9 \pm 2.6\%$, $p < 0.0001$). Similarly, NFHe had lower % change

Table 3. Comparison of % Delta change of Fasting serum lipid (FSL) among NFH groups

	NFHe (n=12) (% Δ)	NFHs (n=25) (% Δ)	NFHe vs. NFHs
TC			
2 weeks	-2.4 ± 3.3	-24.9 ± 3.3	< 0.0001
12 weeks	-4.6 ± 2.3	-27.9 ± 2.6	< 0.0001
LDL			
2 weeks	-2.7 ± 4.8	-33.2 ± 5.7	< 0.0001
12 weeks	-8.9 ± 3.8	-36.9 ± 3.5	< 0.0001
TG			
2 weeks	16.4 ± 11.0	-7.9 ± 23.5	NS
12 weeks	24.0 ± 9.4	-14.0 ± 6.0	< 0.01
HDL			
2 weeks	-4.0 ± 3.7	3.0 ± 2.7	NS
12 weeks	-5.7 ± 4.2	-2.2 ± 4.3	NS

Note: Data are expressed mean ± SEM.

NS indicates not significant. % Δ indicates percentage delta change

reduction of LDL compared to NFHs at 2 weeks (-2.7 ± 4.8% vs. -33.2 ± 5.7%, p<0.0001) and 12 weeks (-8.9 ± 3.8% vs. -36.9% ± 3.5%, p<0.0001). For TG, there was no significant difference in percentage change reduction at 2 weeks between NFHe and NFHs groups. However, at 12 weeks, NFHe showed higher percentage change increment of TG compared to NFHs.

Oxidative stress markers

Table 4 shows MDA, F2-Isoprostane and ox-LDL levels at BL, 2 weeks and 12 weeks post-treatment periods in NFHe and NFHs. In order to compare the effects of TRF with the statin, percentage change of MDA, F2-Isoprostane and ox-LDL in NFH groups was calculated at 2 weeks and 12 weeks treatment period and was illustrated in Table 5.

In NFHe group, there was a significant reduction of MDA levels at 2 weeks (p<0.05) and 12 weeks (p<0.05) post-treatment compared to BL. NFHs treatment group expressed significant reduction of MDA levels after 2 weeks (p<0.05) post-treatment compared to the BL and declined further at 12 weeks (p<0.0001) post-treatment. NFHe and NFHs had similar percentage change reduction of MDA at 2 weeks post-supplementation. However, NFHe had a significantly lower percentage change reduction of MDA at 12 weeks compared to NFHs (-8.7 ± 2.7% vs. -41.4 ± 3.3%, p<0.0001).

In NFHe group, there was a significant reduction of F2- isoprostanes levels at 12 weeks (p<0.05) post-treatment compared to BL. NFHs group showed a reduction of F2- isoprostanes at 2 weeks (p<0.05) and 12 weeks (p<0.05) compared to BL. NFHe and NFHs had similar % change of F2- isoprostanes at 2 weeks and 12 weeks post-supplementation.

There was a significance reduction of ox-LDL levels at 12 weeks compared to BL in NFHe (p<0.05)

Table 4. Oxidative stress markers at BL and post-treatment periods in NFHe and NFHs groups

Groups	BL	MDA (nmol g ⁻¹ protein)		p value
		2 weeks	12 weeks	
NFHe (n=12)	183.2 ± 7.2	164.7 ± 7.8*	166.6 ± 6.3*	< 0.005
NFHs (n=25)	289.4 ± 16.0	242.1 ± 16.0*	159.8 ± 7.3****@@@	< 0.0001
Groups	BL	8-epi-PGF _{2α} (pg ml ⁻¹)		p value
		2 weeks	12 weeks	
NFHe (n=12)	1701.6 ± 251.1	1642.5 ± 256.1	1038.3 ± 124.9*®	NS
NFHs (n=25)	1415.6 ± 239.4	924.3 ± 190.4*	837.9 ± 171.5*	< 0.0001
Groups	BL	Ox-LDL (UL ⁻¹)		p value
		2 weeks	12 weeks	
NFHe (n=12)	88.0 ± 12.4	89.3 ± 10.1	61.0 ± 6.7* @@@	<0.005
NFHs (n=25)	60.0 ± 6.9	54.2 ± 6.1	66.6 ± 6.1®	NS

Note: Data are expressed mean ± SEM.

**** p<0.0001, *** p<0.005, * p<0.05 compared to BL. @@@@ p<0.0001, @@ p<0.005 and @ p<0.05 compared to 2 weeks. ssss p< 0.0001 and sss p< 0.005 and sp<0.05 compared to 12 weeks. NS indicates not significant.

and not in NFHs. In NFHe, ox-LDL levels at 12 weeks had been decreased gradually from 2 weeks and it was statistically significant (p<0.005). However, in NFHs group, ox-LDL levels at 12 weeks was gradually increased from 2 weeks post-treatment (p<0.05). NFHe and NFHs had similar % change of ox-LDL at 2 weeks post-treatment. At 12 weeks, NFHe showed significantly higher % change reduction of ox-LDL compared to NFHs (-18.6 ± 8.0% vs. 37.1 ± 19.5%, p<0.05).

Discussion

The main finding of this study was the reduction of cholesterol (TC and LDL) and oxidative stress (MDA, F2-isoprostanes and ox-LDL) after 12 weeks of TRF supplementation in NFH patients. Epidemiological evidence consistently documents that an increased intake of dietary antioxidants reduces the risk for CAD (Rimm *et al.*, 1993; Kushi *et al.*, 1996). Antioxidant vitamins, for example vitamin C and E may provide vascular defense against oxidative stress by scavenging free radicals and thereby protecting nitric oxide (NO) from inactivation (Ting *et al.*, 1997). The anti-atherogenic actions of antioxidants are commonly linked to the inhibition of lipoprotein oxidation (Alwan *et al.*, 2011; Heinecke 1998). Although the results from the in vitro studies have clearly showed vitamin E as an effective lipid peroxidation chain breaking antioxidant and potentially anti-atherosclerotic properties, results

Table 5. Comparison of % Delta change of oxidative stress markers among NFH groups

MDA	NFHe (n=12) (% Δ)	NFHs (n=25) (% Δ)	NFHe vs. NFHs
2 weeks	-10.0 ± 2.7	-13.4 ± 6.9	NS
12 weeks	-8.7 ± 2.7	-41.4 ± 3.3	p<0.0001
F ₂ -isoprostanes	NFHe (n=12) (% Δ)	NFHs (n=25) (% Δ)	NFHe vs. NFHs
2 weeks	-2.1 ± 11.7	2.8 ± 29.1	NS
12 weeks	-26.9 ± 15.3	-15.8 ± 24.4	NS
ox-LDL	NFHe (n=12) (% Δ)	NFHs (n=25) (% Δ)	NFHe vs. NFHs
2 weeks	-8.7 ± 4.5	26.1 ± 20.0	NS
12 weeks	-18.6 ± 8.0	37.1 ± 19.5	p < 0.05

Note: NS indicates not significant. Data are expressed mean ± SEM. % Δ indicates percentage delta change

from the vitamin E clinical trials are scarce and contradictory (Niki, 2015). However, most of the clinical trials used α -tocopherol as the main source of vitamin E. When used as secondary prevention, α -tocopherol did not reduce the cardiovascular endpoints and was not suggested for inclusion of future primary and secondary prevention trials in patients at high risk of CAD (Vivekananthan *et al.*, 2003). In the same study, it was reported that vitamin E effectively inhibits the progression of atherosclerosis at the early stage. Therefore, in this study, patients with non-familial hypercholesterolaemia (NFH) who had no previous CAD were recruited. Furthermore, TRF has been used as a source of vitamin E in this study due to its potential as a more potent antioxidant compared to tocopherol. In addition, it has been proposed that α -Tocopherol at high concentrations might have pro-oxidant effects (Mutalib *et al.*, 2003). Supplementation with high doses of α -Tocopherol (2000 mg/day) in a randomised placebo-controlled study which involved healthy adults as the subjects had no effect on the reduction of lipid peroxidation products (Meagher and FitzGerald, 2000). It has suggested that α -Tocopherol supplementation in human subjects is undesirable as it does more harm than good (Gee, 2011). Experimental data have clearly demonstrated that tocotrienols have therapeutic potential against various degenerative diseases, including atherosclerosis and its complications such as CAD, whereas tocopherol is ineffective. It is hence timely for pure tocotrienol isomers and TRF to be evaluated clinically to fully explore their chemopreventive and therapeutic potential in view of the numerous positive *in vitro* and *in vivo* studies, and to a very limited extent, human intervention trials (Gee, 2011).

In terms of lipid lowering effects of TRF, the reports from various investigators have been

controversial and need to be further investigated (Tomeo *et al.*, 1995; Mensink *et al.*, 1999; O'Byrne *et al.*, 2000). In the literature review, the evidence of TRF in the reduction of cholesterol and oxidative stress especially in NFH patients are scarce and controversial. In this present study, we have found significant reduction of TC, LDL, MDA, F₂-isoprostanes and ox-LDL levels by 12 weeks post-treatment with TRF in NFH patients.

Supplementation of TRF containing 40 mg/day tocotrienols has shown significant reduction in TC and LDL in NFH patients at 12 weeks with % reduction of 5 and 9% respectively. Although a placebo group was not included in this study, both treatments arms were not on lifestyle modification including no dietary counseling was given throughout 12 weeks study period. Subjects were advised to continue with their usual lifestyle. Qureshi *et al.* (2002) reported the similar finding with the higher percentage reduction of TC and LDL by 14 and 18% reduction respectively. This may be due to the restricted dietary regimen using AHA Step 1 diet experienced by the HC subjects before being enrolled in that study and slightly higher dosage of tocotrienol (50 mg/day).

It has been suggested that, the lipid lowering activity of tocotrienol was due to the presence of three double bonds in the isoprenoid chain that appear to be essential for the inhibition of cholesterologenesis by higher cell penetration (Qureshi *et al.*, 2002). Tocotrienols influence the mevalonate pathway in mammalian cells by post-transcriptional suppression of HMG-CoA reductase (HMGR). This lipid lowering activity of tocotrienol was also due to the side chain unique ability to increase cellular farnesol, a mevalonate derived product, which signals the proteolytic degradation of HMG-CoA reductase (Vasanthi *et al.*, 2012). It has been suggested that gamma and delta tocotrienol (lack of 5 methyl substitution) are significantly more potent than alpha tocotrienol in suppressing HMGR (Vasanthi *et al.*, 2012).

Oxidative stress status *in vivo* can also be measured by secondary lipid peroxidation products such as thiobarbituric acid reactive material or malondialdehyde (MDA) and isoprostanes (Gniwotta *et al.*, 1997). In this study, we found the reduction of MDA levels at 2 weeks and 12 weeks post-TRF supplementation (40 mg/day) in NFH patients. It is reported that TRF effects are beyond the lipid lowering activity (Sen *et al.*, 2007). Similarly, (Tomeo *et al.*, 1995) reported the significant decrease of TBARS after 12 months supplementation with TRF in patients with chronic atherosclerosis.

Another study done by Kawakami *et al.* (2007) reported that in rats given 0.8 mg/day (48 mg/day if converted in human), TRF have no effects on MDA level after 3 weeks post-treatment. This result is contradicting with the results obtained from this present study where 40 mg/day of TRF was sufficient to significantly reduce the MDA level by 2 weeks post-treatment. The difference in the results can be explained by the different composition of isomer contained in TRF in these two studies and different models of the study (human vs. rats). Kawakami *et al.* (2007) have used TRF containing only γ - isomer whereas, TRF (Palmvitee) contains a mixture of α , β , γ and δ isomers. The composition of the tocotrienol isomers in each TRF (Palmvitee) capsule is in this order; $\alpha > \gamma > \delta > \beta$ (42.3% > 34.6% > 20.2% > 2.9%). This suggests that the TRF mixture may have a stronger and more potent effect in reducing of lipid peroxidation compared to γ - tocotrienol alone.

Although α -tocopherol have been suggested to reduce the F2-isoprostanes formation in hypercholesterolaemia, reports on the effects of TRF on plasma F2-isoprostanes levels are scarce and controversial (Davi *et al.*, 1997). In this study, we found that the ability of TRF (40 mg/day) in reducing F2-isoprostanes is comparable to statin at 12 weeks post-supplementation. However, it is not in the agreement with the findings by (Mustad *et al.*, 2002) who reported that, there was no reduction of F2- isoprostanes with TRF supplementation.

Mustad *et al.*, (Mustad *et al.*, 2002) have reported the non-beneficial effects of tocotrienol (200 mg/day) towards the reduction of F2-isoprostanes among hypercholesterolaemic patients after 28 days post-treatment. It is possible that the differential in TRF dosage may have contributed to the contradictory finding by Mustad *et al.*, (2002) compared to our present study (200 mg/day vs. 40 mg/day). TCT at lower concentration (40 mg/day) is more potent in the reduction of F2-isoprostanes compared to TCT at highest concentration (200 mg/day). So far, clinical trials investigating the effect of TRF on isoprostanes levels among HC patients are lacking. However, the beneficial effects of palm oil derived TRF in the reduction of isoprostanes level in animal study with other CAD risks (hypertension and diabetes) were positive and promising. Yoshida *et al.* (2007) for example have reported decreased levels of F2-isoprostanes in a group of rats supplemented with α -tocotrienol for 1 month compared to control mice which were given a vitamin E-free diet. Bayorh *et al.* (2005) have studied the effect of crude palm oil on F2-isoprostanes level in hypertensive rats and found that crude palm oil potentially suppressed the F2-

isoprostanes elevation in hypertensive salt-induced rats. In that study it was suggested that the protective effect of palm oil may be related to the decrease in oxidative stress and preservation of endothelial function. In diabetic induced rats, it was found that crude rice bran derived TRF had reduced urinary F2-isoprostanes and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels compared to placebo (Kanaya *et al.*, 2004). In this study, NFHe and NFHs are reported to exhibit similar reduction of F2-isoprostanes at 12 weeks and are not reflected by the greater cholesterol lowering activity of statin.

Treatment to oxidatively modify LDL-c should be considered in addition to lowering its plasma LDL-c levels to prevent CAD. To date, no study has reported the effect of TRF on ox-LDL levels in NFH patients. To the best of our knowledge, this present study is among the earliest to report it. Supplementation of tocopherol alone has shown no beneficial effects in the reduction of ox-LDL, and furthermore it increased the plasma ox-LDL concentrations after 12 weeks post-treatment in dialysis patients (Diepeveen *et al.*, 2005). In this study, ox-LDL levels was found to be significantly decreased in serum of NFH patients after 12 weeks supplementation with TRF compared to BL. It is postulated that when ox-LDL levels is reduced, the formation of foam cells may be attenuated thus leading to the regression of the atherosclerotic plaque (Tedgui and Mallat, 2006). In addition, ox-LDL reduction may lead to decrease monocyte adhesion to endothelial cells and foam cells formation (Itabe *et al.*, 2011). Therefore, tocotrienol at 40 mg/day in NFH patients may provide beneficial effects in the prevention of atherosclerosis. In this study, it has been shown that the NFHe group had greater percentage reduction of ox-LDL at 12 weeks compared to NFHs and again it is not reflected by the greater cholesterol lowering activity of statin.

Oxidative stress is pivotal in atherogenesis and plaque instability leading to atherosclerosis related complications such as acute myocardial infarction (AMI) (Falk, 2006). Despite of being less potent in LDL lowering effects compared to atorvastatin, TRF is comparable to atorvastatin in terms of attenuating oxidative stress. This is particularly so in a marked reduction of ox-LDL with TRF on effects which were absent with statin. Given that oxidative stress and ox-LDL play an important role in atherogenesis, there is a great potential for TRF as an anti-atherosclerotic agent perhaps in part as a cholesterol lowering agent but more so as an antioxidant.

Conclusion

TRF reduces serum LDL-c level in NFH patients but to a lesser degree compared to statin. However, despite a less potent cholesterol lowering effects, TRF reduces oxidative stress which is comparable to statin but it is more potent in attenuating ox-LDL. TRF has a great potential in the prevention of atherosclerosis in part not only due to its cholesterol lowering activity, but perhaps more effective as a potent antioxidant. Further studies are needed to address clinical endpoints and other surrogate markers of regressions of atherosclerosis.

Acknowledgements

We would like to express our gratitude to Andrew Sinit for technical support in the malondialdehyde (MDA) assay. We would to acknowledge Dr Annuar Rapae for his contribution in patient management in the clinic. This study was supported by a research grant awarded to the corresponding author, Prof Dr. Hapizah Nawawi from the Malaysian Ministry of Science, Technology and Innovation (grant no: IRPA 06-02-02-0132). Palmvitee capsules were a gift from Sime Darby Sdn. Bhd., Malaysia. Each author has no conflict of interest involved in this study.

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