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Effect of Mixed-Tocotrienols in Hypercholesterolemic Subjects

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Abstract

Background: Studies on the cholesterol lowering activity of tocotrienols have yielded mixed results, with some showing cholesterol lowering effect while some showing no activity.

Aim: A randomized, double–blind, parallel group study was conducted to investigate the cholesterol lowering activity of tocotrienols.

Methods: Thirty–two hypercholesterolemic subjects were randomly assigned to orally receive either 300 mg of mixed tocotrienols capsules daily or placebo capsules containing 300 mg of soya bean oil for a period of 6 months. The subjects were monitored before supplementation and monthly thereafter for their serum cholesterol as well as tocotrienol and tocopherol concentrations.

Results: The serum total cholesterol and low density lipoprotein (LDL) cholesterol of the subjects in the tocotrienol supplementation group were decreased significantly by –8.9 ± 0.9% and –12.8 ± 2.6% respectively after 4 months of supplementation and the reduction persisted till the end of the 6-month study, with a reduction of –10.8 ± 1.0% and –17.3 ± 1.8%, respectively from baseline. Moreover, there was a 22–fold increase in the total tocotrienol concentrations from baseline during supplementation compared to the placebo group, while the concentration of α–tocopherol recorded only a modest increase. On the other hand, the serum cholesterol, total tocotrienol and α–tocopherol concentrations of subjects in the placebo group remained essentially unchanged.

Conclusions: Supplementation with mixed tocotrienols at dose of 300 mg per day resulted in the lowering of the serum total and LDL cholesterol levels after 5 months of supplementation.

Keywords: tocotrienols, cholesterol-lowering, total cholesterol, LDL cholesterol, tocopherols

Background

Tocotrienols are compounds belonging to the vitamin E family and are found abundantly in palm oil and cereal grains. They are structurally quite similar to tocopherols and differ only in having an unsaturated isoprenoid side chain rather than a saturated phytyl tail [1,2]. Besides being a potent chain-breaking anti-oxidant [3], an increasing number of studies have shown that tocotrienols possess additional beneficial pharmacological actions such as inhibition of platelets aggregation, inhibition of monocytic adhesion [1], anti-tumor or chemopreventive property [4,5], neuroprotective activity [6], hypoapolipoprotein B property [7] as well as cholesterol lowering activity [8].

Of the above, the hypocholesterolemic activity of tocotrienols has received much attention due mainly to the well-established mechanism of cholesterol suppressive action. The cholesterol lowering activity of tocotrienols is ascribed to the ability of the side chain of tocotrienols to increase cellular farnesol, which in turn signals the proteolytic degradation of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, the enzyme responsible for the production of cholesterol [9].

Several studies have been conducted to investigate the cholesterol lowering activity of tocotrienols in hypercholesterolemic human subjects with mixed results. Two studies by Qureshi et al [10,11] showed that supplementation with 200 mg of mixed or pure tocotrienols in subjects restricted to a controlled (NCEP Step I) diet led to a reduction of 8-16% of total cholesterol and LDL cholesterol within 4 weeks. Moreover, studies conducted without dietary control also showed similar results with significant reduction in total cholesterol and LDL cholesterol [8,12]. However, there are other studies reported in the literature [13-15], which showed that supplementation with up to 240 mg of tocotrienols did not result in lowering of the serum cholesterol. The discrepancy might be due to differences in the composition of the supplements as well as differences in the bioavailability of dosage forms used. For example, a high α-tocopherol content (30% and above) in the tocotrienol supplement has been found to attenuate the cholesterol lowering activity of γ-tocotrienol [16] as well as reducing the oral bioavailability of the tocotrienols [17]. The product used by Wahlqvist et al [13] had a α–tocopherol content of 30% while that used by Mensink et al [14] had more than 35% α–tocopherol, and this might explain the absence of cholesterol lowering activity observed. On the other hand, the study by Mustard et al [15] was conducted over 28 days only and a longer period might be required to see the cholesterol lowering activity. Moreover, the bioavailability of lipid soluble drugs was reported to be greatly influenced by the type and volume of oil administered concomitantly [18] as well as the delivery systems used [19]. Thus, a formulation that gives both consistent and enhanced absorption of the tocotrienols will be advantageous.

In consideration of the above factors that might affect the activity of the tocotrienols, the present study was thus conducted to investigate the effects of mixed tocotrienols on the serum cholesterol of hypercholesterolemic subjects using a preparation with a mixed tocotrienols content greater than 75% with respect to α–tocopherol and delivered as a self emulsifying formulation to facilitate the absorption of tocotrienols.

Methods

Study Population

Thirty-two hypercholesterolemic but otherwise healthy subjects (20 males and 12 females) aged between 31 and 53 were recruited into the study. They all had an initial baseline serum total cholesterol concentrations of ≥ 6.2 mmol/L, LDL cholesterol ≥ 4.0 mmol/L and triacylglycerol ≤ 3.8 mmol/L. Subjects were considered to be healthy based on an interview regarding their medical history. They were excluded from the study if their fasting serum total cholesterol concentrations were less than 6.2 mmol/L, body mass indexes of more than 30.0, known history of diabetes or having liver, renal and hypertensive diseases. Subjects who were taking (or within the previous 2 months) cholesterol lowering drugs or medications that have an effect on blood lipid profiles were also excluded. All subjects were briefed on the nature of the study and they were required to sign an informed consent statement prior to the commencement of the trial.

Experimental Design

The study was conducted according to a double–blind, placebo–controlled, parallel group design. Prior to the start of the study, the subjects were monitored for a period of two weeks (baseline period). Subjects were then randomly assigned to one of the two groups (16 per group) to receive either the mixed tocotrienols supplements or placebo for a period of 6 months (24 weeks supplementation period).

Subjects were advised to maintain their usual diet as well as physical activity and lifestyle throughout the study period. They were also not allowed to take drugs which were known to interfere with the blood lipid profiles and to refrain from additional vitamin E supplementation. Any signs of illnesses, medications taken, adverse reactions and deviations from protocol were recorded in diaries and reported to the investigators during their visits. A 24–hour recall of food intake was also conducted on each subject for at least thrice during the course of the study to make sure that they did not change their dietary pattern. The study procedures were approved by a Joint School of Pharmaceutical Sciences, University of Science Malaysia/Penang Hospital Institutional Review Board. The clinical trial was conducted according to Good Clinical Practice guidelines and the Declaration of Helsinki.

Dietary Supplements

During the supplementation period, subjects assigned to the placebo group were given soft gelatin capsules containing 200 mg of soya bean oil while those in the supplementation group received

mixed tocotrienols formulated in a self–emulsifying drug delivery system (Tocovid Suprabio®, Hovid Sdn Bhd, Malaysia). Each soft gelatin capsule contained 50 mg of mixed tocotrienols (30.8% α–tocotrienol, 56.4% γ–tocotrienols and 12.8% δ–tocotrienol). It also contained 22.9 IU of α–tocopherol.

The subjects were asked to take three capsules of the placebo or mixed tocotrienols after breakfast and another three at night after their dinner. Thus, the total daily intake of mixed tocotrienols for each subject was 300 mg. The capsules were provided on a monthly basis during each visit and compliance with the supplementation regimen was monitored by counting the capsules that were left over at the end of the month and through questionnaires given to the subjects.

Measures

Initial measures that were obtained included subjects' height, weight, blood pressure, and history of significant diseases, medications and alcohol use. Prior to the supplementation (baseline period), venous blood samples of 10 ml volume each were drawn twice at two weeks apart after an overnight fast for determination of their cholesterol profiles and the mean were taken as the baseline data. The blood samples were left to clot for approximately 30 minutes before centrifuged. The serum was promptly separated and kept frozen at –20°C until analysis. The venous blood samples were analysed for total cholesterol, high density lipoprotein (HDL) cholesterol, LDL cholesterol, triacylglycerol, α–tocotrienol, γ–tocotrienol, δ–tocotrienol and α–tocopherol. During the study period of 24 weeks, the subjects were also monitored for the above parameters after every 4 weeks of supplementation. All the blood samples were collected in the morning after an overnight fast. The body weight and blood pressure of the subjects were also recorded during each visit.

Analytical Method

The serum was analysed enzymatically for total cholesterol, HDL cholesterol and triacylglycerol within 1 week after collection using an autoanalyzer (550 Express Plus®, Ciba-Corning, USA). The concentrations of LDL cholesterol were then calculated according to the methods of Friedewald et al [20]. The serum was analysed again in a batch for each subject at the end of the trial. The results obtained from both measurements were closely similar and the mean values of the two measurements were subsequently used for the statistically analysis.

The concentrations of α–tocotrienol, γ–tocotrienol and δ–tocotrienol in the serum obtained from the subjects were determined as a block at the end of the trial using a high performance liquid chromatography (HPLC) method with fluorescence detection [21]. On the other hand, the levels of α–tocopherol in the serum was determined using the HPLC method with ultraviolet detection reported by Julianto et al [22].

Statistical Analysis

The data collected at baseline and during the supplementation period for both groups were compared using an analysis of variance procedure (ANOVA) for a two factor repeated measures split-plot experimental design [23]. If a statistically significant difference was observed, a post-hoc simple main effects test was employed to locate the pair that gave rise to the difference observed.

The homogeneity of the baseline characteristics between the two groups, namely age, weight, body mass index and the cholesterol levels was assessed using an independent Student's t–test. A statistically significant difference was considered at $p < 0.05$.

Results

It was estimated that a sample size of 16 subjects per group was required to achieve a statistical power of 80% for detecting a 10% change in the serum total cholesterol, being similar to that reported by Mustard et al [15] in their study which also investigated the effect of tocotrienols on the serum cholesterol.

Table 1. Baseline characteristics of study subjects

	Tocotrienol Supplementation (Mean ± SD)	Placebo (Mean ± SD)
Age (years)	43.2 ± 5.6	44.8 ± 8.9
Weight (kg)	76.1 ± 10.8	66.3 ± 7.1
BMI (kg/m^2)	26.7 ± 3.1	24.5 ± 3.4
Total Cholesterol (mmol/L)	6.9 ± 0.5	6.9 ± 0.6
LDL Cholesteroln (mmol/L)	4.8 ± 0.7	4.6 ± 0.6
HDL Cholesterol (mmol/L)	1.3 ± 0.3	1.4 ± 0.3
Triacylglycerol (mmol/L)	1.9 ± 0.7	1.8 ± 0.9

All 32 subjects enrolled into the study completed the entire 6–month course (72 weeks). There was no dropout, exclusion or withdrawal of consent from the subjects during the study period. Both treatment groups had equal numbers of male and female patients, each consisting of three females and thirteen males with age ranging from 31 to 62 years old. There was no statistically significant difference in any demographic characteristics between the two groups, namely, age, weight, body mass index and ethnicity (table 1). Moreover, the initial cholesterol levels in both treatment groups were comparable and no statistically significant difference was detected.

Compliance with the study protocol and supplementation regimen was satisfactory, as evidenced by the maintenance of body weight throughout the study period and capsule counts during their monthly visit. No severe adverse drug reaction related to the administration of the drug product and placebo was recorded, thus indicating that long term administration of 300 mg of mixed tocotrienols for up to 6 months was tolerable among the subjects.

Table 2. Mean (± SEM) fasting serum lipids at baseline and after supplementation (mmol/l)

	Tocotrienol Supplementation (month)							Placebo (month)						
	Baseline	1st	2nd	3rd	4th	5th	6th	Baseline	1st	2nd	3rd	4th	5th	6th
Total Cholesterol	6.91 (0.11)	6.93 (0.15)	6.85 (0.16)	6.90 (0.14)	6.74 (0.14)	6.29 (0.13)	6.17 (0.13)	6.89 (0.14)	6.76 (0.21)	6.93 (0.18)	6.74 (0.27)	6.88 (0.29)	6.73 (0.20)	6.78 (0.23)
LDL cholesterol	4.82 (0.17)	4.83 (0.17)	4.77 (0.19)	4.70 (0.15)	4.52 (0.15)	4.20 (0.17)	3.99 (0.16)	4.59 (0.16)	4.60 (0.14)	4.59 (0.25)	4.64 (0.25)	4.68 (0.23)	4.62 (0.20)	4.61 (0.20)
HDL cholesterol	1.34 (0.07)	1.30 (0.08)	1.32 (0.09)	1.30 (0.07)	1.39 (0.10)	1.43 (0.11)	1.33 (0.06)	1.39 (0.07)	1.37 (0.10)	1.37 (0.11)	1.31 (0.09)	1.33 (0.08)	1.38 (0.09)	1.33 (0.09)
LDL: HDL Ratio	3.59	3.72	3.62	3.62	3.25	2.94	3.00	3.30	3.36	3.35	3.54	3.52	3.35	3.47
TC: HDL Ratio	5.16	5.33	5.19	5.31	4.85	4.40	4.64	4.96	4.93	5.06	5.15	5.17	4.87	5.09
Triacylglycerids	1.88 (0.17)	1.90 (0.12)	1.92 (0.14)	1.98 (0.17)	1.91 (0.13)	1.82 (0.13)	1.84 (0.16)	1.76 (0.23)	1.89 (0.21)	1.90 (0.28)	1.89 (0.26)	1.81 (0.23)	1.84 (0.19)	1.83 (0.18)

Mean concentrations of the fasting serum cholesterol during the baseline and supplementation period are shown in table 2. It is apparent that significant lowering of the total and LDL cholesterol was only observed after more than 4 months (16 weeks) of supplementation with the mixed tocotrienols. The total and LDL cholesterol reduction from the baseline values were more substantial at the 5^{th} month, being 8.9 ± 0.9% ($p < 0.05$) and 12.8 ± 2.6%, respectively. At the 6^{th} month, the two parameters showed a further reduction to 10.8 ± 1.3% ($p < 0.05$) and 17.3 ± 1.8% ($p < 0.05$), respectively. In contrast, subjects in the placebo group recorded negligible changes in their total and LDL cholesterol concentrations from the baseline throughout the 6–months (24 weeks) of supplementation. Moreover, significant differences between the two treatment groups for the total and LDL cholesterol values were observed at the 5^{th} and 6^{th} month ($p < 0.05$) but not during the first four months. It should be emphasised that the total and LDL cholesterol values of all subjects in the mixed tocotrienols supplementation group showed a decrease after 4 months of supplementation

and no poor respondent (serum cholesterol fell by less than 3%) was identified. On the other hand, the triacylglycerol and HDL cholesterol levels of the subjects in both treatment groups did not change significantly during the supplementation period.

Nevertheless, as shown in Table 2, there was a significant difference between the two supplemented groups in terms of reduction in the TC:HDL ratio from baseline, being 14.7% and 10.1% at 5th & 6th month, respectively observed with tocotrienol supplementation while the placebo group showed a minimal reduction of 1.8% from baseline at 5th month and a slight increase of 2.6% from baseline at 6th months. A similar trend was also observed with LDL:HDL ratio. There was a significant difference between the two supplemented groups in terms of reduction in the LDL:HDL ratio from baseline, being 18.1% and 16.4% at 5th & 6th month, respectively observed with tocotrienol supplementation while the placebo control group showed a slight increase of 1.5% from baseline at 5th month and a slight increase of 5.2% from baseline at 6th months.

Overall, the concentrations of total tocotrienols (α, γ and δ–tocotrienol) were markedly higher in the serum of subjects who consumed mixed tocotrienols supplementation compared to those in the placebo group (Figure 1). While a 22–fold increase in the total serum tocotrienol level (from the baseline concentration) was recorded for the treatment group after 6 months of supplementation, there was no appreciable increase in the placebo group. As for the individual isomers, the highest increment was observed with α–tocotrienol, followed by γ–tocotrienol and δ–tocotrienol (data not shown). Ikeda et al (24) reported that α–tocotrienol was preferentially absorbed compared to γ– and δ–tocotrienols. Similarly, Yap [17] also reported that the oral bioavailability of the α–tocotrienol was higher than that of the other two isomers.

Fig. 1. Mean (±SEM) plasma total tocotrienols concentration after supplementation with placebo and mixed tocotrienols

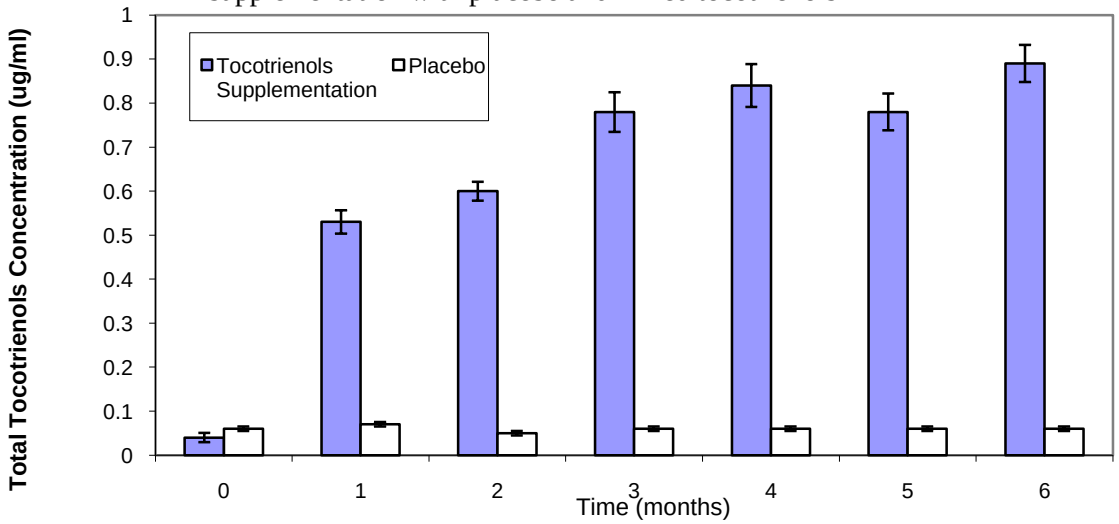

Fig. 1. Mean (±SEM) plasma total tocotrienols concentration after supplementation with placebo and mixed tocotrienols. ■ = tocotrienol supplementation, □ = placebo

Fig. 2. Mean (±SEM) plasma tocopherol concentrations after supplementation with placebo and mixed tocotrienols

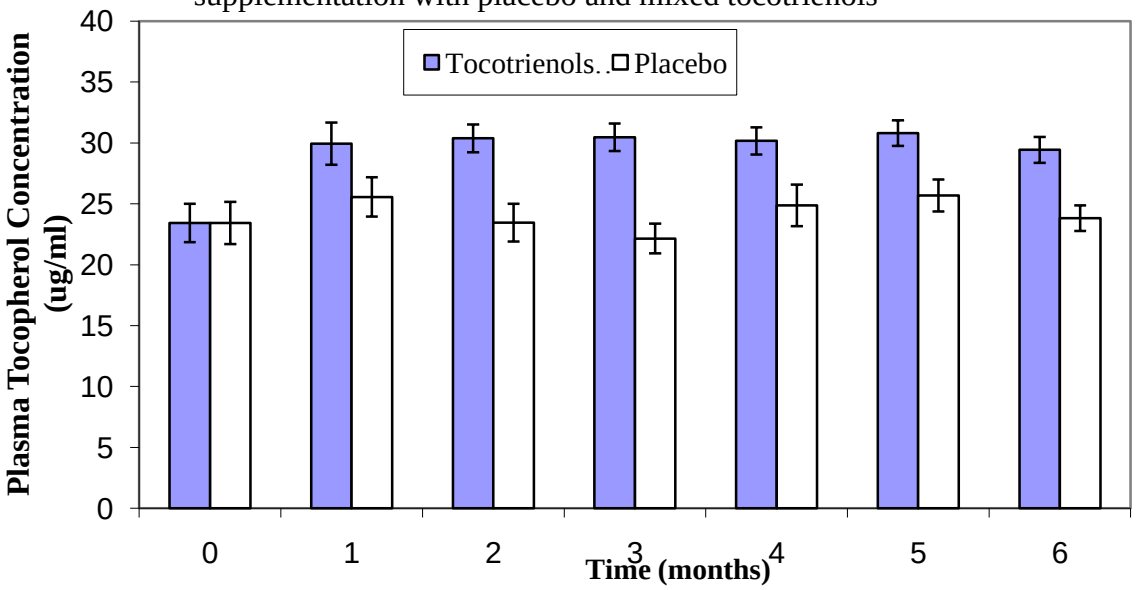

Fig. 2. Mean (±SEM) plasma tocopherol concentrations after supplementation with placebo and mixed tocotrienols. ■ = tocotrienol supplementation, □ = placebo

Referring to figure 1, it can also be seen that a marked increase in the mean total tocotrienol level was evident after the first month of supplementation, reaching a plateau after the 3rd month while that of the placebo group remained essentially unchanged throughout the study period.

Figure 2 shows the mean α–tocopherol levels of the two groups over the study period. It can be seen that the α–tocopherol levels in the mixed tocotrienols supplementation group was slightly increased after the first month of supplementation and remained essentially unchanged thereafter. The increase was approximately 20% from the baseline, being markedly less than that observed with the total tocotrienols. On the other hand, the subjects in the placebo group did not record any appreciable increase in the α–tocopherol concentration.

Discussion

The results of this study show that a reduction in the total and LDL cholesterol was observed after 4 months (16 weeks) of daily supplementation with 300 mg of mixed tocotrienols, while the HDL cholesterol and triacylglycerol levels remained essentially unchanged. The results obtained are consistent with those reported by Qureshi et al [8,10,11] and Tan et al [12], although the dosage and duration of tocotrienol supplementation, diet control as well as magnitude of cholesterol reduction varied from the present study.

In addition, it is also evident that there was a significant reduction in the ratio of TC:HDL after 4 months of tocotrienol supplementation as compared to the placebo group. TC:HDL ratio which contains both an atherogenic and antiatherogenic lipid component, has been accepted as a predictor of cardiovascular disease. An increased value of TC:HDL ratio has been associated with

an increased risk of ischemic stroke [25]. Furthermore, a significant reduction in LDL:HDL ratio can be also be observed with the tocotrienol supplemented group. In comparison, the placebo group did not show a similar reduction in LDL:HDL ratio. Instead, a slight increase in the ratio was observed. Low level of LDL-cholesterol has traditionally been associated with reduced risk of cardiovascular disease while HDL cholesterol is protective against cardiovascular diseases and stroke, particularly non-fatal stroke and ischemic stroke in the elderly [25]. Hence, a lower LDL:HDL ratio infers a reduced risk of cardiovascular diseases.

In the present study, it can be observed that the reduction in serum cholesterol in the supplemented group was accompanied by a significant concomitant elevation in the serum tocotrienol levels, being similar to that observed in the study by Qureshi et al [8]. Moreover, the elevation in tocotrienol levels from the baseline values was accompanied by a relatively negligible increase in the tocopherol level. This, in turn, would translate into a significant increase of approximately 18 times in the ratio of tocotrienol to tocopherol levels (T3:T1) after supplementation or when compared to the placebo group. A similar observation was also reported by Qureshi et al [8].

As mentioned earlier, inconsistent cholesterol lowering effect of tocotrienols have been reported in the literature. Three studies have shown that supplementation with up to 240 mg per day of mixed tocotrienols did not cause a decrease in the serum cholesterol levels [13-15]. In all these 3 studies, the serum tocotrienol levels were only modestly increased when compared to the baseline concentrations or the placebo group. This could be attributed to the poor and erratic bioavailability of tocotrienols when administered as a normal lipid formulation. The self–emulsifying formulation used in the present study was reported to produce a more consistent as well as enhanced absorption of the tocotrienols. In the study by Mustard et al [15] in which the mixed tocotrienol preparations were taken after meal, the increase in the tocotrienol levels was still relatively small when compared to the placebo group although the authors claimed otherwise. The small increment observed could also be ascribed to poor subject compliance towards the supplementation regimen. Moreover, the duration for tocotrienol supplementation of 4 weeks might not be sufficient to achieve significant cholesterol reduction in view of the comparatively small increase in the tocotrienol levels. In the present study, the serum tocotrienol levels were observed to increase even after 4 weeks of supplementation, reaching a plateau only after 12 weeks.

Interestingly, the studies by Wahlqvist et al [13], Mensink et al [14] and Mustard et al [15] (which showed no cholesterol lowering activity) did not display a significant increase in the ratio of serum T3:T1 as compared to the study by Qureshi et al [8] that showed hypocholesterolemic effect of tocotrienols. This might be due either to the modest increment in the serum tocotrienol levels or there was a concomitant increase in the tocopherol levels. Qureshi et al [16] has demonstrated that α–tocopherol could attenuate the cholesterol lowering ability of γ–tocotrienols, and this might help to explain the absence of hypocholesterolemic activity of tocotrienols in the three studies. Thus, it could be inferred that the hypocholesterolemic activity upon ingestion of mixed tocotrienol preparations could only be attained if there was a significant increase in the serum tocotrienol

concentrations relative to the tocopherol concentrations. This may account for the discrepancy observed in the cholesterol lowering activity of tocotrienols among the studies. In view of the erratic oral bioavailability of the tocotrienols, they might best be given via a suitable delivery system that could ensure more consistent and higher oral bioavailability such that the beneficial effects of the tocotrienols could be attained.

Conclusion

Lowering of the total and LDL cholesterol levels in hypercholesterolemic subjects could be achieved through supplementation with mixed tocotrienols, and was accompanied by a significantly higher serum tocotrienol concentration relative to the tocopherol level. The cholesterol lowering activity can be attained after 4 months of supplementation.

Abbreviations: Low density lipoprotein (LDL), High density lipoprotein (HDL), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), High performance liquid chromatography (HPLC), an analysis of variance procedure (ANOVA), Tocotrienol to tocopherol levels (T3:T1)

Competing interest

The authors declare that they have no competing interest.

Authors' Contribution

Kah Hay Yuen is the principal investigator. Involves in the conceptualization of the study amd writing study proposal and manuscript as well as the overall conduct of study.

Jia Woei Wong coordinated the research, conducted statistical analysis and assisted in writing the manuscript.

Ai Beoy Lim coordinated the research and performed the lab work for the study. *Bee Hong Ng* coordinated the research and performed the lab work for the study

Wai Peng Choy coordinated the research and performed the lab work for the study.

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