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# Virgin Coconut Oil and Palm Tocotrienol Supplementation: Effects on Lipid Parameters of Experimental Rats

(Suplementasi Minyak Kelapa Dara dan Tokotrienol Sawit: Kesan ke atas Parameter Lipid pada Tikus Kajian)

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ABSTRACT

Virgin coconut oil (VCO) and palm tocotrienol (TT) possess cholesterol-regulating properties, but their combined effects remained unexplored. Thus, this study aims to determine their individual and combination effects on lipid parameters in ovariectomised (OVX) rats on hypercholesterolemic diet. Female Sprague-Dawley rats were ovariectomised except for the sham group, assigned into (i) sham-operated fed with basal rat diet, (ii) OVX control, (iii) V1 (OVX + VCO 1.43 mL/kg), (iv) V2 (OVX + VCO 4.29 mL/kg), (v) TT (OVX + TT 30 mg/kg), (vi) V1+TT (OVX + VCO 1.43 mL/kg + TT 30 mg/kg) and (vii) V2+TT (OVX + VCO 4.29 mL/kg + TT 30 mg/kg). Groups (ii) to (vii) were fed with 2% cholesterol mixed with five-time heated palm oil. VCO and TT alone or in combination reduced food intake, visceral fat weight, thiobarbituric acid reactive substances and HMG-CoA reductase activity significantly ($p<0.05$ vs the OVX control). HDL was reduced significantly in V1, V2, and V1+TT compared to the TT and V2+TT ($p<0.05$). The increase in LDL was the lowest in V1 compared to other groups ($p<0.05$). V2 and TT significantly reduced total cholesterol compared to other supplementations ($p<0.05$). All supplementations were found to reduce triglyceride compared to the OVX control group ($p<0.05$). The increase in apolipoprotein A was higher in V2+TT than other groups ($p<0.05$). The reduction in apolipoprotein B was higher in V1+TT and V2+TT than the V1, V2 and TT. VCO and TT exerted beneficial effects on lipid parameters, but the efficacy was not better than individual agents.

Keywords: Cholesterol diet; heated palm oil; lipid profile; tocotrienol; virgin coconut oil

ABSTRAK

Minyak kelapa dara (VCO) dan tokotrienol sawit (TT) mempunyai ciri kawal atur kolesterol tetapi kesan gabungannya belum pernah dikaji. Kajian ini adalah untuk menentukan kesan individu dan gabungannya ke atas parameter lipid pada tikus yang diovariektomi dan diberi diet tinggi kolesterol. Tikus Sprague-Dawley betina yang telah diovariektomi kecuali kumpulan sham, dibahagikan kepada (i) pembedahan sham yang diberi makan diet basal tikus, (ii) OVX kontrol, (iii) V1 (OVX + VCO 1.43 mL/kg), (iv) V2 (OVX + VCO 4.29 mL/kg), (v) TT (OVX + TT 30 mg/kg), (vi) V1+TT (OVX + VCO 1.43 mL/kg + TT 30 mg/kg), (vii) V2+TT (OVX + VCO 4.29 mL/kg + TT 30 mg/kg). Kumpulan (ii) hingga (vii) diberi makan kolesterol 2% dicampur dengan minyak sawit yang dipanaskan lima kali. VCO dan TT secara individu atau gabungan menurunkan pengambilan makanan, berat lemak viseral, bahan reaktif asid thiobarbiturik dan aktiviti HMG-CoA reduktase secara signifikan ($p<0.05$ banding OVX kontrol). HDL turun secara signifikan dalam V1, V2, dan V1+TT berbanding TT dan V2+TT ($p<0.05$). Peningkatan LDL adalah paling kurang dalam V1 berbanding kumpulan lain ($p<0.05$). V2 dan TT menurunkan kolesterol total secara signifikan berbanding suplementasi yang lain ($p<0.05$). Semua suplementasi didapati menurunkan trigliserida berbanding kumpulan kawalan OVX ($p<0.05$). Peningkatan pada apolipoprotein A adalah lebih tinggi dalam V2+TT berbanding kumpulan lain ($p<0.05$). Pengurangan pada apolipoprotein B adalah lebih tinggi dalam V1+TT dan V2+TT berbanding V1, V2 dan TT. VCO dan TT menunjukkan kesan baik ke atas parameter lipid tetapi efikasinya bukan lebih baik daripada kesan individu.

Kata kunci: Diet kolesterol; minyak kelapa dara; minyak sawit dipanaskan; profil lipid; tokotrienol

## INTRODUCTION

Food provides energy to the human body upon consumption, which may contain fat, cholesterol, protein, carbohydrates or even vitamins. It is then digested inside the stomach into various components, absorbed into the intestinal mucosa of the small intestine and then carried by lymph into the portal vein and transported directly to the liver (Tortora & Derrickson 2008). The liver is the largest organ in the human body and is the major site for the production and excretion of cholesterol. Furthermore, it plays a role in the detoxification and storage of glycogen, decomposition of red blood cells as well as in the synthesis of plasma protein (Opoku et al. 2007). However, the presence of reactive oxygen species (ROS) may trigger the release of cytokines such as tumour necrosis factor α (TNF-α), tumour necrosis factor β (TNF-β) and interleukin 6 (IL-6), thus causing hepatocyte injury (Robertson, Leclercq & Farrell 2001). Exposure to xenobiotics, environmental pollutants and chemotherapeutic agents can lead to hepatic injury (Zakaria et al. 2011). Since the liver is involved in various enzymatic metabolic activities, damage to this organ would lead to severe consequences for human health.

Frying is one of the oldest methods of cooking food (Choe & Min 2007). Although cooking oil is cheap and can be easily found in the market, a previous study has shown that Malaysians tend to repeatedly use the same oil for frying to reduce expenses, which has become a common practice in households and among street food vendors (Azman et al. 2012). However, cooking oil that is exposed to high temperatures for extended periods will be subjected to several chemical reactions, such as oxidation, hydrolysis and thermal polymerisation (Choe & Min 2007; Dobarganes, Márquez-Ruiz & Velasco 2000; Gertz 2000). The repeated heating process deteriorates the quality of oil as the oxygen solubility is increased during the time interval between repeated cooling and reheating (Clark & Serbia 1991). Repeatedly heated oil is detrimental to health as heating produces free radicals (hydroperoxides and aldehydes), thus producing thermally oxidised oil (Dobarganes & Márquez-Ruiz 2003; Owu, Osim & Ebong 1998). Free radicals are then absorbed into the fried food and later enter the systemic circulation (Kubow 1992). Intake of food containing oxidised oil has been shown to cause liver dysfunction (Izaki, Yoshikawa & Uchiyama 1984; Owu, Osim & Ebong 1998). Several studies had previously highlighted the harmful effects of oxidised dietary fats on experimental animals (Abbas & Elsamanoudy 2011; Falade et al. 2015; Imafidon & Okunrobo 2012; Jaarin et al. 2015; Leong et al. 2008; Nevin & Rajamohan 2006).

Conventional drugs used in the treatment of liver diseases are sometimes ineffective and related to serious adverse effects. Therefore, natural products, including vitamin E, emerged as a potential alternative treatment for liver disorders (Zakaria et al. 2011). Owing to its antioxidant (Nevin & Rajamohan 2006), antimicrobial, antiviral (Bergsson et al. 1998; German & Dillard 2004) and lipid peroxidation reduction (Nevin & Rajamohan 2004), a natural product called virgin coconut oil (VCO) has gained much attention worldwide. VCO has been shown to improve liver metabolism (Marina, Man & Amin 2009) and maintain blood coagulation in normal rats (Arunima & Rajamohan 2012).

One of the families in vitamin E is tocotrienol (TT), known as the minor plant constituents that can be found abundantly in natural sources such as annatto fruit, palm oil, barley and rice bran (Nevin & Rajamohan 2008). TT has also been shown to function as an antioxidant that prevents the growth of cancer either *in vivo* or *in vitro* (Aggarwal et al. 2010; Nesaretnam & Meganathan 2011). Previous studies have shown that α-tocotrienol components can potentially improve cytochrome P450 protection better than α-tocopherol in fighting against oxidative damage (Packer, Weber & Rimbach 2001; Wada et al. 2005). In addition, previous studies showed that TT can improve lipid profile in renal patients (Serbinova et al. 1991), enhance lipid profile levels in hypercholesterolemic subjects (Mat Daud et al. 2013), prevent cardiovascular disease by suppressing HMG-CoA reductase activity (Qureshi et al. 2002) and delay the progression of Parkinson disease (Parker et al. 1993). Since VCO and tocotrienol exhibit many beneficial effects, this study was carried out to observe the effect of both natural products on lipid parameters.

## MATERIAL AND METHODS

### ANIMALS AND EXPERIMENTAL DESIGN

Forty-nine female Sprague-Dawley rats, weighing between 200 and 250 g, were obtained from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia and then randomly divided equally into seven groups: one sham group and six ovariectomised groups (control, V1, V2, TT, V1+TT and V2+TT) (n=7). All animals were kept in accordance with the recommended guidelines. The rats were kept in stainless-steel cages with wood shaving, fed with water *ad libitum* and maintained in a 12-hour light-dark cycle at 27 °C ± 2 °C. After 1 week of acclimatisation, the rats from the sham group underwent a sham-operated procedure while the other rats were ovariectomised. The (i) sham group was fed with basal rat diet only throughout the study period while ovariectomised groups, (ii) control ovariectomised (CONTROL) group, (iii) V1 (VCO 1.43 mL/kg), (iv) V2 (VCO 4.29 mL/kg), (v) TT (TT 30 mg/kg), (vi) V1+TT (VCO 1.43 mL/kg + TT 30 mg/kg) and (vii) V2+TT (VCO 4.29 mL/kg + TT 30 mg/kg), were fed with 2% cholesterol diet mixed with five-time heated palm oil (5HPO). The average food intake and body weight were

measured weekly during the study period. Withdrawal of blood was done at week 0 and week 24 for lipid profile analysis and determination of apolipoproteins A and B levels. After 24 weeks of study, the rats were sacrificed while visceral fats were harvested and weighed. In contrast, livers were harvested to determine HMG-CoA reductase activity and thiobarbituric acid reactive substance (TBARS) level. The ethical approval was obtained from Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (FR/FAR/2013/QODRIYAH/17-JULY/528-AUG-2013-JUNE-2015).

### OVARIECTOMY

Ovariectomy was carried out under Ketapex:Xylazil (1:1) anaesthesia with 0.1 mL injected intramuscularly for every 100 g body weight of the rats. Once anaesthetised, the fur on the abdomen area was shaved. An incision was made on the abdomen area, and both ovaries were identified. The fallopian tubes were tied before the ovaries were cut out. The abdomen was closed using a catgut suture for the inner layer of the skin, while mersilk thread was used for the outer layer. For sham-operated rats, the same procedure was applied, except for the ovaries, which were not removed. A combination of penicillin and streptomycin antibiotics (8:10 mg/kg) were given intraperitoneally for infection prophylaxis. Fucidic cream was available at hand to be applied when necessary, also for infection prevention. The rats were left to rest for 2 weeks before treatment commenced.

### SUPPLEMENTATIONS

The VCO was purchased from a local company (Bio Asli, Malaysia). The supplementations were given via oral gavage at doses of 1.43 mL/kg and 4.29 mL/kg. Both dosages were chosen based on the minimal and maximal recommended dose by Fife (2004). The palm tocotrienol used was Natural Full Spectrum Tocotrienol/Tocopherol Complex 80% Oil Suspension, purchased from Carotech, Malaysia. It consisted of a tocotrienol/tocopherol complex (89.8%), and the tocotrienol isomers were γ (44.8%), α (29.4%), δ (10.8%), and β (4.8%). The supplementation was given orally via oral gavage at the recommended dose of 30 mg/kg (Yu et al. (2006). The combination supplementations (V1+TT and V2+TT) were prepared by mixing the tocotrienol with a low and high dose of VCO.

### FIVE-TIME HEATED PALM OIL (5HPO) PREPARATION

Palm oil (Cap Buruh; Lam Soon Sdn. Bhd., Malaysia) and sweet potatoes were purchased at the local market. The heating was done following the process described by Owu, Osim and Ebong (1998). Two and a half litres of palm oil was exposed to the temperature of 180 °C for 10 min. Then, 1 kg of peeled sweet potatoes was fried inside the stainless-steel wok for 20 min. Next, the hot oil was cooled down at room temperature for 5 h. Then, another fresh batch of 1 kg sweet potatoes was fried without adding the fresh oil. This process was repeated until five times heated palm oil was obtained.

### DIET PREPARATION

About 850 g of 2% cholesterol pellet (MP Biomedical, Ohio) was grounded and mixed with 150 g of 15% (w/w) of 5HPO. Then, the mixture was reformed and dried in the oven at 70 °C overnight.

### LIVER THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) ANALYSIS

TBARS level in the liver was determined using a commercial kit from Cayman Chemical (USA) and read using an ELISA reader machine (Molecular Devices, USA) following the manufacturer's instructions.

### LIVER 3-HYDROXYL-3-METHILGLUTARYL CoA (HMG-CoA) REDUCTASE ANALYSIS

HMG-CoA reductase activity in the liver was recorded using commercial kits from Elabscience Biotechnology Co., Ltd. (China) and Bio-Rad (USA), which then was read using an ELISA reader machine (Molecular Devices, USA) following the manufacturer's instructions.

### SERUM LIPID PROFILE

Blood samples were taken from the tail veins of the rats. The blood collected was centrifuged by a centrifuge machine (Heraeus-Labofuge-400, Germany) at 3000 rpm for 10 min under room temperature to obtain the serum, which was then stored at -70 °C. Serum lipid profile level was determined using commercial kits from Abcam (England) and Bioassay (USA) and read using an enzyme-linked immunosorbent assay (ELISA) reader machine (Molecular Devices, USA) following the manufacturer's instructions.

### SERUM APOLIPOPROTEIN ANALYSIS

Serum Apolipoprotein A and B were evaluated using commercial kits from Cloud-Clone Corp. (USA) and then read using an ELISA reader machine (Molecular Devices, USA) following the manufacturer's instructions.

### STATISTICAL ANALYSIS

All analyses were performed using Statistical Product and Service Solutions (SPSS) software version 2.0 (SPSS Inc.,

Chicago, USA). The normality of all data was first determined by the Shapiro-Wilk test. Statistical differences were determined using one-way ANOVA followed by Tukey's HSD post hoc test. The intergroup difference for data not normally distributed was compared using non-parametric tests, namely Kruskal-Wallis, Mann-Whitney U and Wilcoxon-Signed Ranked Test. A value of $p<0.05$ is considered as significant. The data were presented as the mean ± standard error of the mean (SEM).

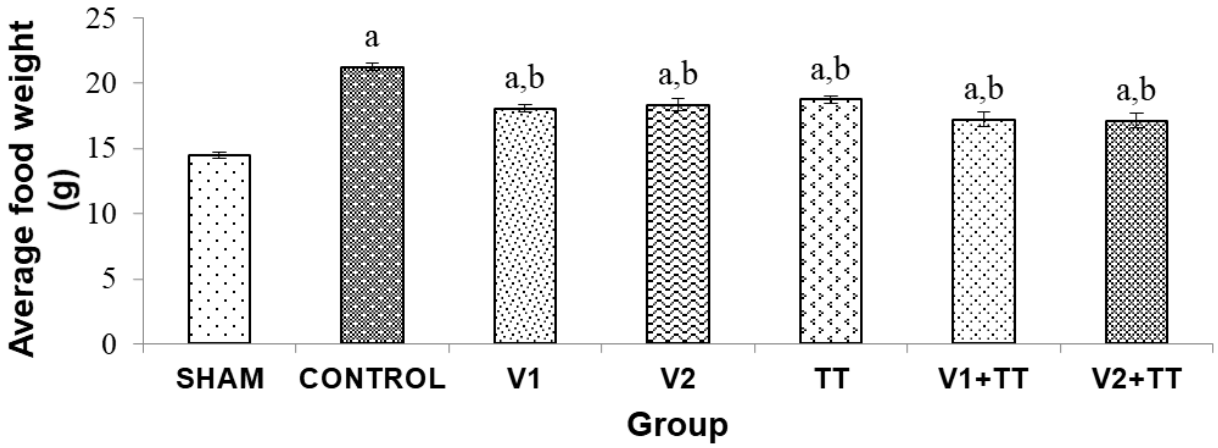
## RESULTS

### AVERAGE FOOD INTAKE

All ovariectomised rats displayed a significant increment of food intake compared to the sham (14.49 ± 0.27 g) rats fed with normal rat chow. However, all supplementations groups (V1 (18.03 ± 0.29 g), V2 (18.34 ± 0.44 g), TT (18.74 ± 0.29 g), V1+TT (17.22 ± 0.54 g), V2+TT (17.12 ± 0.59 g)) showed a significant reduction in food intake compared to control group (21.23 ± 0.28 g) although the value was not as low as the sham group. However, no significant difference was observed between supplementation groups (Figure 1).

### AVERAGE BODY WEIGHT

Figure 2 presents the effects of supplementation on body weight during the study period. The sham group showed no significant difference in body weight in week 0 (264.83 ± 5.88 g), week 12 (272 ± 3.82 g) and week 24 (282.5 ± 4.17 g). Meanwhile, control and all supplementation groups showed significant increment in body weight at week 12 (control (350.5 ± 12.31 g), V1 (360.67 ± 6.58 g), V2 (365.17 ± 19.53 g), TT (337.33 ± 18.81 g), V1+TT (371.17 ± 15.91 g), V2+TT (347.83 ± 18.05 g)) and week 24 (control (370 ± 11.99 g), V1 (370.17 ± 10.06 g), V2 (380.33 ± 17.08 g), TT (338.33 ± 25.58 g), V1+TT (394.5 ± 28.73 g), V2+TT (372.17 ± 22.29 g)) compared to the week 0 (control (268.67 ± 5.52 g), V1 (259 ± 8.93 g), V2 (272.83 ± 11.63 g), TT (262.5 ± 15.38 g), V1+TT (268.83 ± 13.77 g), V2+TT (269 ± 8.36 g)) within the same groups. However, no significant difference was observed between body weight at week 12 and week 24 within the same group. In comparison to the sham group in the same week, control and all supplementation groups showed a significant increase in body weight at weeks 12 and 24. However, no significant difference was seen in body weight between all supplementation groups at weeks 12 and 24. Overall, all ovariectomised rats experienced a body weight increment




[a] significantly different ($p<0.05$) compared to sham group; [b] significantly different ($p<0.05$) compared to control group. V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 1. Average food intake per day by rats in all groups at week-24. Value represents

compared to the sham group. At the same time, all supplementation was unable to reduce body weight compared to the sham group at the end of the study.

### AVERAGE VISCERAL FAT WEIGHT

Based on the results, feeding with 5HPO and 2% cholesterol diet have caused a visceral fat increment in all ovariectomised rats; control (10.73 ± 0.53 g), V1 (7.49 ± 0.56 g), V2 (7.29 ± 0.64 g), TT (8.03 ± 0.67 g), V1+TT (8.11 ± 0.67 g), V2+TT (8.04 ± 0.33 g) compared to the rat fed with normal pellet in the sham group (3.46 ± 0.22 g). All supplementations were able to reduce the visceral fat significantly compared to the control group, although the value was not as low as the sham group. However, no significant difference was observed between supplementation groups (Figure 3).

### LIVER TBARS LEVEL

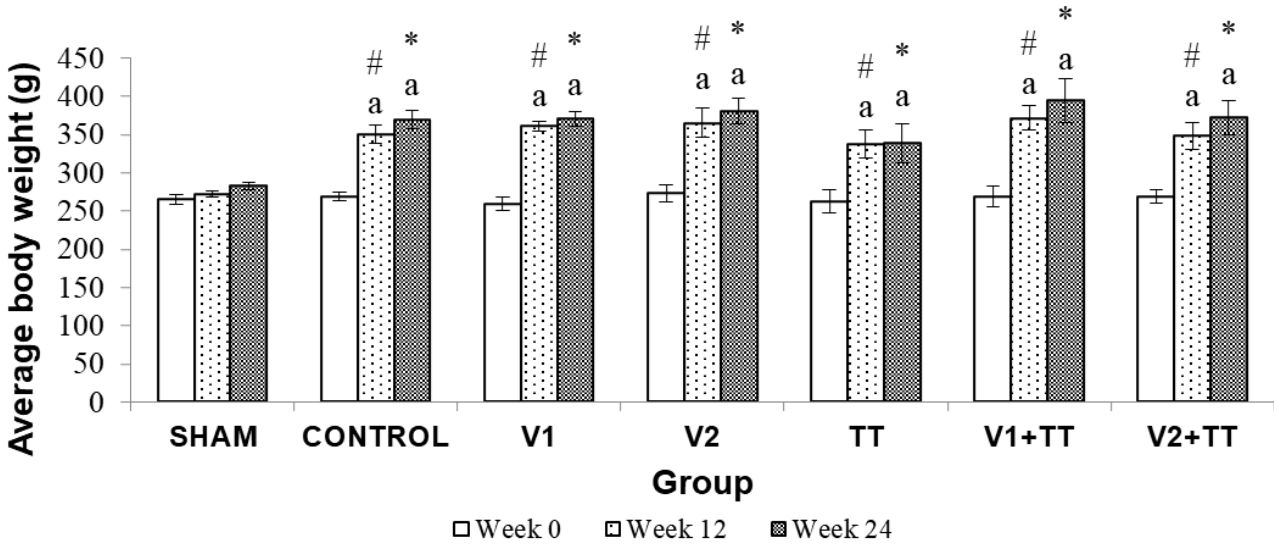
Feeding with 5HPO and 2% cholesterol diet caused a significant increase in TBARS level in the control group (54.17 ± 4.65 µM) compared to the sham group (16.67 ± 5.82 µM). At the end of the study, all supplementations: V1 (29.17 ± 7.55 µM), V2 (27.22 ± 6.98 µM), TT (19.44 ± 4.84 µM), V1+TT (24.44 ± 6.52 µM) and V2+TT (23.89 ± 2.84 µM) were able to reduce TBARS level significantly compared to the control group and reduced to the level near to the sham group. However, there was no significant difference between supplementation groups (Figure 4).

### HMG-CoA REDUCTASE ENZYME ACTIVITY

Figure 5 illustrates the average HMG-CoA reductase enzyme activity at the end of the study. The result showed that feeding with 5HPO and 2% cholesterol increased the HMG-CoA reductase enzyme activity significantly in the control group (3.03 ± 0.16 ng/mg) compared to the sham group (1.83 ± 0.05 ng/mg). Other than that, all supplementation groups: V1 (2.32 ± 0.16 ng/mg), V2 (2.21 ± 0.12 ng/mg), TT (2.09 ± 0.16 ng/mg), V1+TT (2.26 ± 0.13 ng/mg) and V2+TT (2.28 ± 0.15 ng/mg) successfully reduced the HMG-CoA reductase enzyme activity significantly compared to the control group and as low as the sham group. However, no significant difference was observed between all supplementation groups.

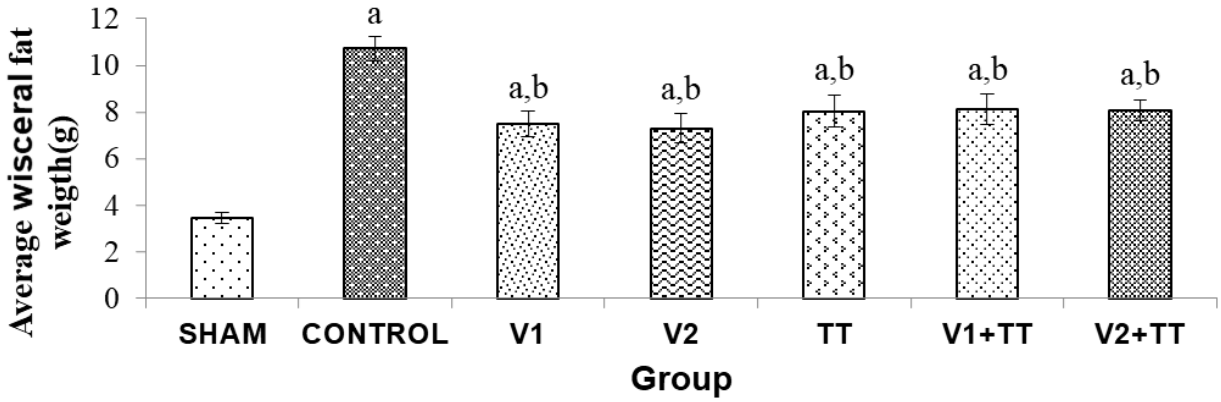
### SERUM LIPID PROFILE

When compared to the control group, all supplementation groups reduced HMG-CoA reductase activity, HDL



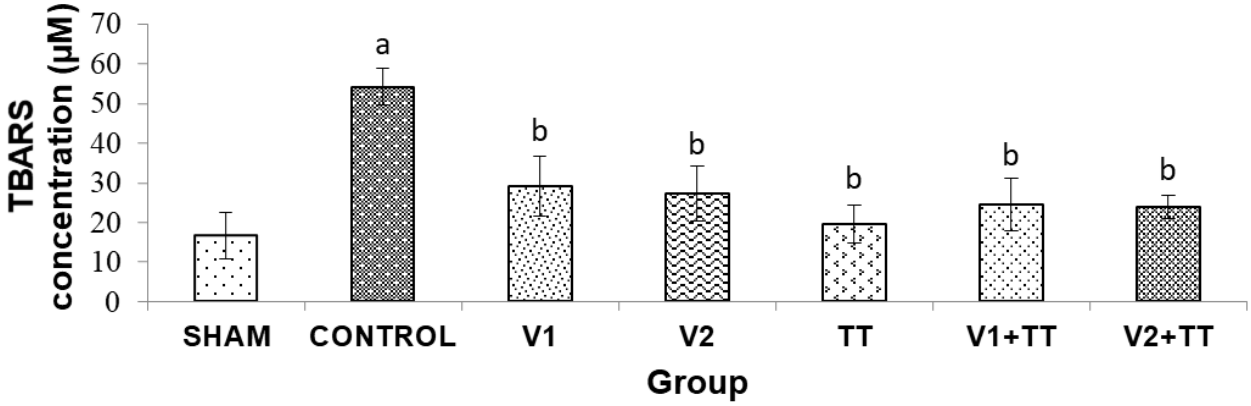

[a]significantly different ($p<0.05$) compared to week-0 within the same group; [#]significantly different ($p<0.05$) compared to sham group at week-12; [*]significantly different ($p<0.05$) compared to sham group at week-24. V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 2. Average body weight of rat in all groups at week-0, 12 and 24. Value represents the mean ± SEM




[a]significantly different ($p<0.05$) compared to sham group; [b]significantly different ($p<0.05$) compared to control group
V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 3. Average visceral fat weight of rat in all groups at week-24. Value represents the

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V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

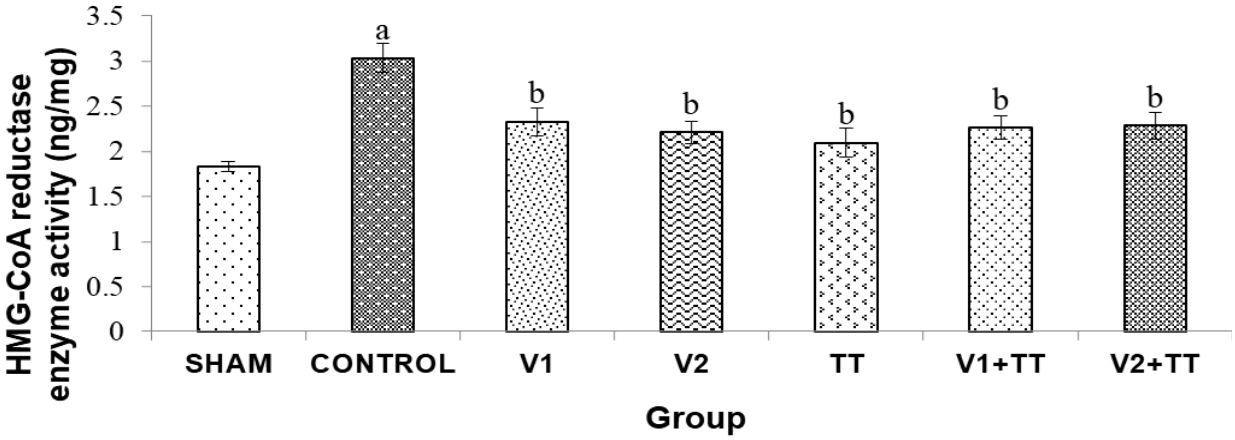
FIGURE 4. Concentration of TBARS (µM) in all groups at week-24. Value represents the mean ± SEM

reduction, LDL increment, total cholesterol, triglyceride, and apolipoprotein B but increased apolipoprotein A. Nevertheless, there were differences among the supplementation groups. V1, V2, and V1+TT supplementations showed less reduction in HDL level compared to the TT and V2+TT supplementations (Figure 6), while V1 supplementation increased LDL level less compared to other supplementation groups (Figure 7). V2 and TT supplementations significantly reduced total cholesterol (TC) levels compared to other supplementations (Figure 8). All supplementations were found to significantly reduce triglyceride levels compared to the control group (Figure 9). Supplementation with V2+TT significantly increased the apolipoprotein A (ApoA) level more than other supplementation groups (Figure 10), while supplementation with V1+TT and V2+TT significantly reduced apolipoprotein B (ApoB) level more than V1, V2 and TT supplementations (Figure 11).

## DISCUSSION

In Malaysia, affordable prices and availability in the market have made palm oil a widely used cooking oil among Malaysians. Unfortunately, some people tend to use cooking oil repeatedly to save costs for food preparation, especially food outlet operators (Azman et al. 2012). The present study illustrates the effects of VCO and palm TT supplementation on factors related to cardiovascular diseases, particularly lipid profile and lipid peroxidation in the rats fed with 5HPO and 2% cholesterol diet compared with rats without supplementation (control group) as well as rats fed with the normal diet (sham group). A high cholesterol diet (2%) and estrogen deficiency condition were used to enhance the oxidative stress in the rats (López-Varela, Sánchez-Muniz & Cuesta 1995). In previous studies, a 2% cholesterol diet was found to be atherogenic in rabbits (Adam et al. 2008), while estrogen deficiency indicated low antioxidants to protect against lipid peroxidation (Jaarin, Nafeeza & Ngang 1994).

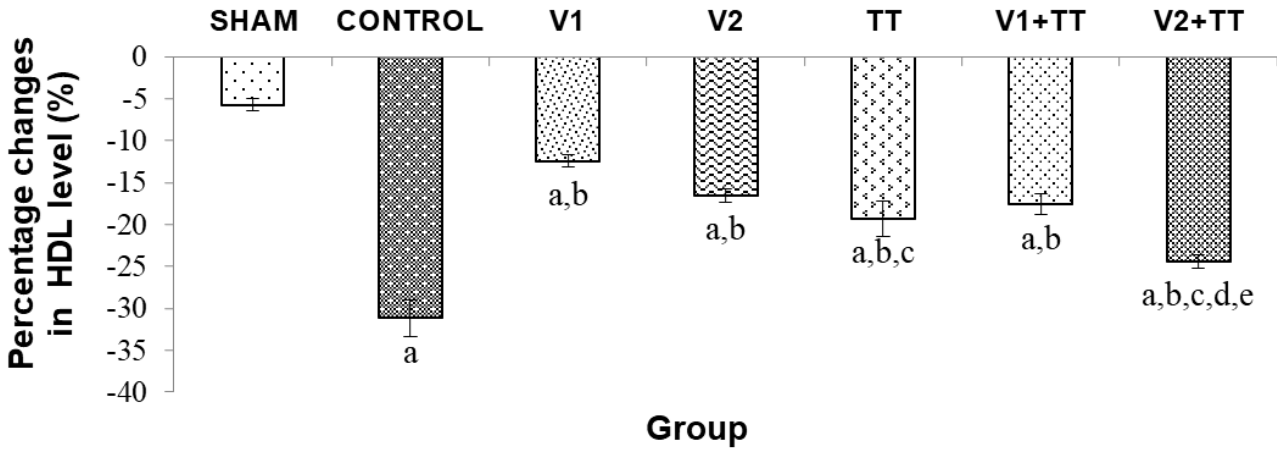
The result showed that feeding with a 5HPO and 2% cholesterol diet increased food intake and body weight in the rats compared to the normal diet. Previous studies have found that repeatedly heating oil reduces food intake since the heated oil changes taste and texture and gives a foul odour (Baker et al. 2003; Liu, Yamada & Osawa 2010). Other previous studies also reported no difference in food intake, either rats fed with fresh oil or heated oil (Falade et al. 2015; Izaki, Yoshikawa & Uchiyama 1984). This difference was probably due to the addition of a 2% cholesterol diet that was not included in those previous studies. The addition of fat to the food was reported to increase appetite (Narasimhamurthy & Raina 1999).




[a]significantly different ($p<0.05$) compared to sham group; [b]significantly different ($p<0.05$) compared to control group

V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

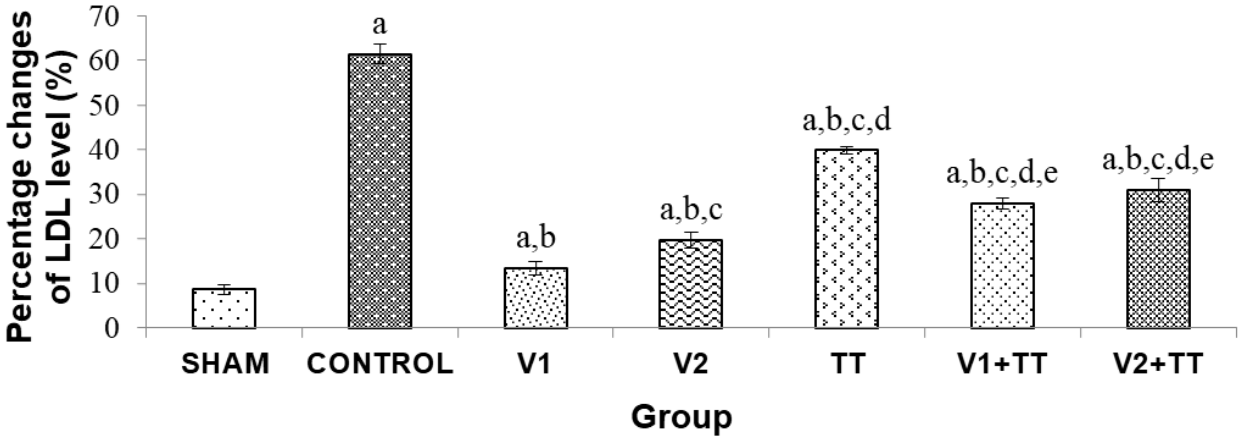
FIGURE 5. Activity of HMG-CoA reductase enzyme in all groups at week-24. Value represents the mean ± SEM




[a]significantly different (*p<0.05)* compared to sham group; [b]significantly different (*p<0.05*) compared to control group; [c]significantly different (*p<0.05*) compared to V1 group; [d]significantly different (*p<0.05*) compared to V2 group; [e]significantly different (*p<0.05*) compared to V1+TT group

HDL, high density lipoprotein; V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

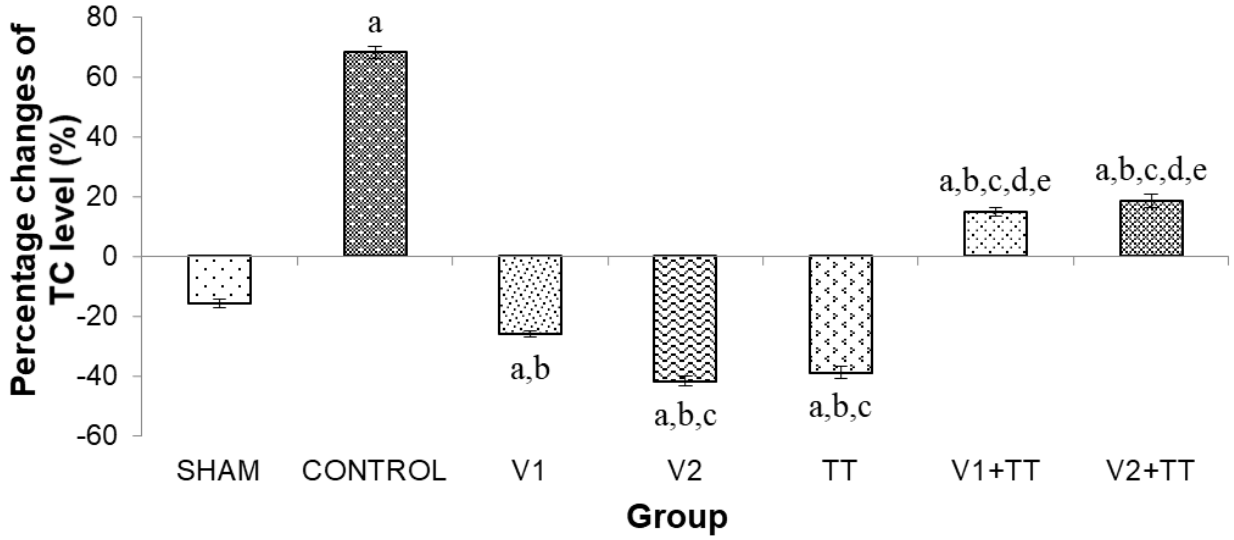
FIGURE 6. Percentage changes of HDL level in all groups at week-24. Value represents the mean ± SEM




[a]significantly different (*p<0.05*) compared to sham group; [b]significantly different (*p<0.05*) compared to control group; [c]significantly different (*p<0.05*) compared to V1 group; [d]significantly different (*p<0.05*) compared to V2 group; [e]significantly different (*p<0.05*) compared to TT group.

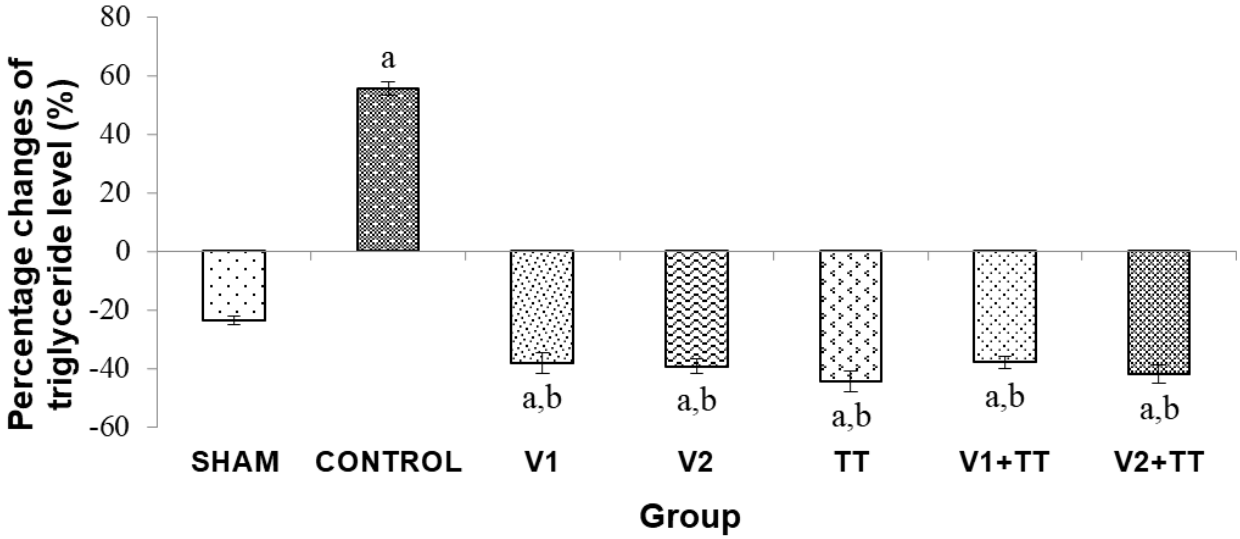
LDL, low density lipoprotein; V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 7. Percentage changes of LDL level in all groups at week-24. Value represents the mean ± SEM



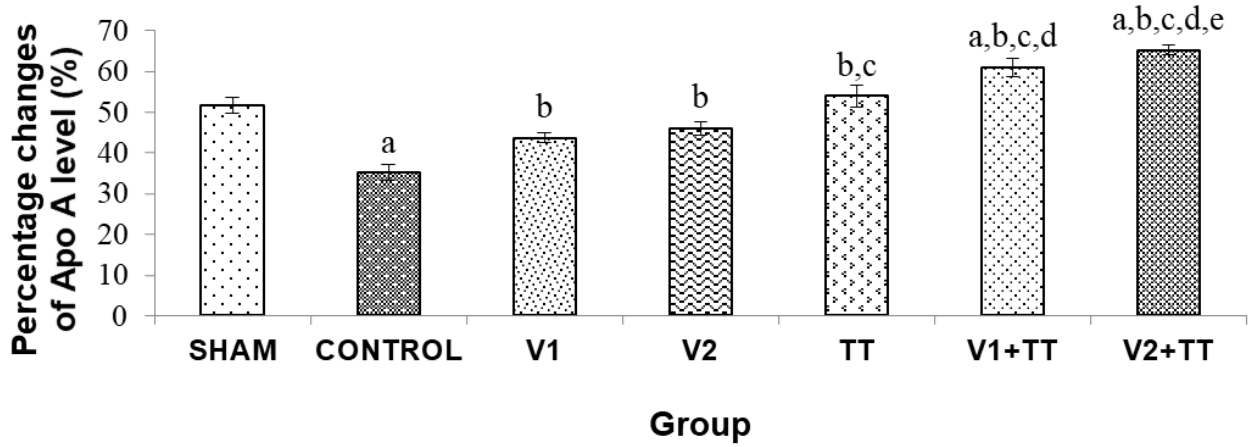

[a]significantly different ($p<0.05$) compared to sham group; [b]significantly different ($p<0.05$) compared to control group; [c]significantly different ($p<0.05$) compared to V1 group; [d]significantly different ($p<0.05$) compared to V2 group; esignificantly different ($p<0.05$) compared to TT group.
TC, total cholesterol; V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 8. Percentage changes of total cholesterol level in all groups at week-24. Value



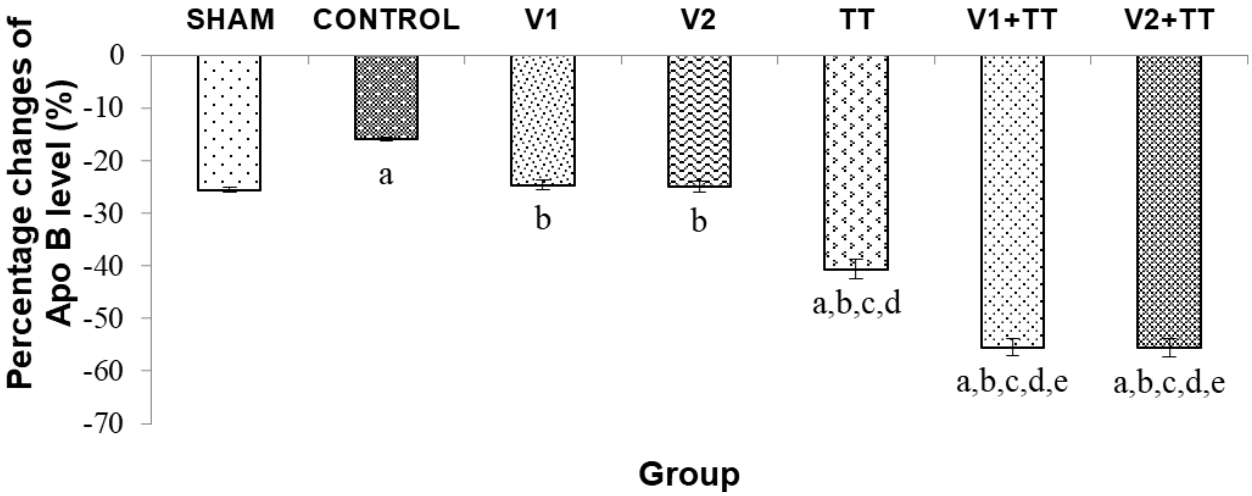

[a]significantly different ($p<0.05$) compared to sham group; [b]significantly different ($p<0.05$) compared to control group
V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 9. Percentage changes of triglyceride level in all groups at week-24. Value represents the mean ± SEM




[a]significantly different ($p<0.05$) compared to sham group; [b]significantly different ($p<0.05$) compared to control group; [c]significantly different (p<0.05) compared to V1 group; [d]significantly different ($p<0.05$) compared to V2 group; [e]significantly different ($p<0.05$) compared to TT group.
Apo A, apolipoprotein A; V1, VCO 1.43 mL/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 10. Percentage changes of Apo A level in all groups at week-24. Value represents the mean ± SEM




[a]significantly different ($p<0.05$) compared to sham group; [b]significantly different ($p<0.05$) compared to control group; [c]significantly different ($p<0.05$) compared to V1 group; [d]significantly different (p<0.05) compared to V2 group; [e]significantly different ($p<0.05$) compared to TT group.
Apo B, apolipoprotein B; V1, VCO 1.43 ml/kg supplementation group; V2, VCO 4.29 mL/kg supplementation group; TT, tocotrienol 30 mg/kg supplementation group; V1+TT, VCO 1.43 mL/kg + tocotrienol 30 mg/kg supplementation group; V2+TT, VCO 4.29 mL/kg + tocotrienol 30 mg/kg supplementation group

FIGURE 11. Percentage changes of Apo B level in all groups at week-24. Value represents the mean ± SEM

Ovariectomy also contributes to the factor that increases food intake (National Research Council 1972). Estrogen plays a role in regulating hypothalamus activity by controlling the central nucleus of appetite and satiety (Muhammad et al. 2012). However, estrogen deficiency due to ovariectomy has caused dysregulation of the hypothalamus to satiety, thus, increasing the appetite. In normal conditions, leptin acts in controlling food intake and energy expenditure at the hypothalamus (Cnop et al. 2002; Lizcano & Guzman 2014). Yet, estrogen deficiency can still lead the adipose tissues to reduce leptin production, thus causing an increment in food intake.

Based on this present study, all supplementations (V1, V2, TT, V1+TT, and V2+TT) managed to reduce food intake compared to the control group. However, there was no significant difference recorded between supplementation groups. Medium-chain fatty acids (MCFA) were reported to increase satiety in the human study (Mayes & Watson 2004). Therefore, MCFA content in the VCO could be the reason for the reduction of food intake by slowing down the emptying process inside the stomach, thus promoting fullness (St-Onge 2005). An animal study by Wong et al. (2012) suggested that food intake increased after the administration of TT along with a high-fat diet (Nurul-Iman et al. 2013). This result showed that TT intake cannot reduce food intake. On the other hand, this present study demonstrated a contradictory result due to the long duration of the study, which was 24 weeks, while the study by Wong et al. (2012) was done within only 16 weeks. Although mono supplementation showed more reduction effect in food intake, the combination supplementation did not display more reduction.

The present result showed that body weight increment was correlated to the food intake increment. The body weight of the ovariectomised rat was significantly increased at week 12 and week 24 compared to week 0 after being fed with 5HPO and a 2% cholesterol diet. Nevertheless, no significant increment was recorded between week 24 compared to week 12 within the same supplementation group. According to Leong et al. (2008), the repeatedly heated process caused the saturated fatty acid content inside the oil to become higher compared to the unsaturated fatty acid. Intake of saturated fatty acid was believed to increase adipose tissue (Wong et al. 2012). Saturated fatty acid triggered the peroxisome proliferator-activated receptor (PPAR) to proliferate and undergo adiposity differentiation and apoptosis, which produces more fat tissue, thus increasing body weight (Leong et al. 2010). A similar finding was also observed by Shastry et al. (2011), where feeding with repeatedly heated oil increased the body weight of the rats compared to that with fresh oil (Wajchenberg 2000). Similarly, Leong et al. (2008) and Hamsi et al. (2015) demonstrated that the intake of repeatedly heated oil increased animal body weight at the end of their studies. Moreover, ovariectomy also influenced the body weight increment. Estrogen deficiency was claimed to influence the satiety signal at the hypothalamus, thus increasing food intake while decreasing energy consumption (Hamsi et al. 2015; Babaeiet al. 2010). Likewise, a study by Nguyen et al. (2004) suggested that feeding using a high-fat diet with rich energy and low-energy consumption led to a significant increase in a cat's body weight compared to the low-fat diet (Camara et al. 2014).

From this result, supplementations of either mono or a combination of antioxidants failed to decrease body weight, but many previous studies reported different findings on VCO. These included a clinical study using VCO 30 mL per day that showed no body weight changes after four weeks of study (Nguyen et al. 2004), while another study showed that VCO 1.42 mL/kg successfully reduced rat's body weight after 16 weeks (St-Onge 2005). In a similar fashion, a study using a medium-chain triglyceride diet has been proven to reduce animal body weight compared to a high-fat diet (Liau et al. 2011). However, it should be noted that the design of this study and the previous ones are very different in terms of ovariectomy, administration of cholesterol diet, 5HPO, different dosage, and study duration. These could be the reason why this study showed different results compared to them. Meanwhile, supplementation of 120 mg/kg of TT was demonstrated to reduce body weight gain in animal studies (Han et al. 2003), and supplementation with 60 mg/kg of TT was also reported to reduce body weight in rats with bone loss due to estrogen deficiency (Nakamura et al. 2001). Unfortunately, a different result was seen in this study, which could be due to the high dosage of TT used by previous researchers and the absence of a cholesterol diet in their research. Although many studies showed that VCO and TT supplementations successfully reduced body weight, it was suggested in this study that mono and combination supplementation could not reduce body weight.

Administration of 5HPO on rats with estrogen deficiency produced more visceral fat than the sham rat. The increment in fat quantity was parallel with studies by Babaei et al. (2010) and Camara et al. (2014), who claimed that ovariectomy caused an increment in body weight and accumulation of visceral adipose tissue in rats with estrogen deficiency. Meanwhile, estrogen deficiency due to ovariectomy also caused estrogen receptor-α (Erα) to be less effective in reducing lipogenesis, thereby inhibiting triglyceride accumulation in the adipose tissue (Kozakowski et al. 2017; Muhammad et al. 2013). In the same vein, Bonora (2000) and Howard et al. (2004) pointed out that menopause causes central adiposity among postmenopausal women. In addition, saturated fatty acid intake was claimed to cause lipase enzyme reduction and increase adipose

tissue production, which was related to body weight gain (Leong et al. 2010).

All supplementations of mono and a combination of VCO and TT were found to reduce visceral fat weight, although not as low as the sham group. However, there was no significant difference between supplementation groups observed. Polyphenol from VCO was observed to reduce adipose cell and fat droplet size via reduction of triacylglyceride synthesis and increasing lipolysis; whereby indirectly reduced fat percentage in the body (Bonora 2000). Previous researchers also proved that VCO reduced visceral adipose by reducing waist circumference in obese people (Nguyen et al. 2004), while MCFAs content in the VCO reduced visceral fat in BMI < 23 kg/m$^2$ subjects (Wang et al. 2014). However, a study with a tocotrienol-rich fraction (TRF) of 120 mg/kg/day showcased a different result compared to the present study by failing to reduce the visceral adipose index in animals induced with high-fat diet (Nurul-Iman et al. 2013). The reason for this was not clear. However, it could be due to the different dosages used. Overall, although mono supplementation of VCO demonstrated a little higher visceral fat reduction, combination supplementation did not give more reduction in visceral fat parameters.

Feeding with a 5HPO and a 2% cholesterol diet has increased the TBARS level. This result was similar to the finding by local researchers who discovered that the administration of repeatedly heated oil, especially 5HPO, increased the TBARS level compared to the administration of 2% cholesterol only and fresh palm oil (Leong et al. 2012; Liu, Yamada & Osawa 2010). Previous studies suggested administration of cholesterol has increased rabbit's TBARS level (Abdelhalim 2010; Esa et al. 2013; Tsuji et al. 2001), although with 1% cholesterol intake (Arunima & Rajamohan 2012). Other than that, ovariectomy was also confirmed to involve the increment of malondialdehyde (MDA) level in another study (Hsu, Lee & Chen 2001).

All supplementations were shown to have successfully reduced TBARS level compared to the control group. This finding indicates that all supplementations could reduce oxidative stress to the same level as the sham group. The reduction of TBARS level could be attributed to the capability of antioxidants in VCO to stop lipid peroxidation (Arunima & Rajamohan 2012). In 2006, Nevin and Rajamohan discovered that polyphenols in VCO inhibited lipid peroxidation via *in vitro,* therefore causing a reduction in TBARS level in the animal study. The same researchers also claimed that polyphenol fraction in VCO could prevent LDL-induced copper oxidation by reducing the LDL oxidation as well as reducing the formation of TBARS and carbonyl (Nevin & Rajamohan 2004). Similarly, the MDA level in the liver of rats fed with a cholesterol diet was reduced due to the increased antioxidant enzyme activity in VCO (Malone et al. 2014). Higher antioxidant capacity in the TT helped in fighting against oxidative change by heated oil (Nevin & Rajamohan 2009). In another study, the use of vitamin E reduced TBARS content in the hypercholesterolemic rat by entrapping the peroxyl radical-chain (Kamisah et al. 2012). Although mono supplementation of VCO and TT was able to reduce TBARS level, when two antioxidants were combined, they could not reduce more oxidative stress.

Generally, HMG-CoA reductase is involved in cholesterol homeostasis by detecting the changes in cholesterol levels of the membrane cell and modulating the transcription of proteins involved during cholesterol biosynthesis and cholesterol intake from plasma lipoprotein (Gökkusu, Özden & Mostafa 2004). However, cholesterol from diet reduces the effectiveness of protein translation, thus producing less cholesterol. Estrogen deficiency state due to ovariectomy can cause an increment in serum cholesterol level, as reported by Trapani and Pallottini (2010). HMG-CoA reductase enzyme activity was significantly decreased in all supplementation groups, which was similar to the study conducted by Arunima and Rajamohan (2012). The researchers discovered that the higher total polyphenol content in VCO affected the liver lipogenesis process by reducing the HMG-CoA reductase activity. Meanwhile, there were studies reporting that TT inhibited the HMG-CoA reductase enzyme activity via a post-transcriptional mechanism in HepG2 cells (Minhajuddin, Beg & Iqbal 2005; Ness & Chambers 2000; Qureshi et al. 2002) and that the administration of TT in many dosages could reduce HMG-CoA reductase enzyme activity significantly (Ness & Chambers 2000). Therefore, all mono supplementations were able to reduce HMG-CoA reductase enzyme activity. Unfortunately, when two antioxidants were combined, they were unable to reduce more HMG-CoA reductase enzyme activity due to unidentified reasons.

After 24 weeks of study, feeding with 5HPO and 2% cholesterol diet reduced the high-density lipoprotein (HDL) level while increasing low-density lipoprotein (LDL) level, total cholesterol (TC) and triglyceride significantly in the control group. This finding was similar to the previous study in which the intake of repeatedly heated oil at an extended duration disturbed lipid profile metabolism by reducing HDL cholesterol levels and increasing LDL, TC and triglyceride levels (Falade et al. 2015; Jaarin, Nafeeza & Ngang 1994; Minhajuddin, Beg & Iqbal 2005). Administration of a 1% cholesterol diet in animal subjects was demonstrated to increase the triglyceride level significantly (Arunima & Rajamohan 2012), while a 2% cholesterol diet increased the serum TC level significantly (López-Varela, Sánchez-Muniz & Cuesta 1995). Basically, estrogen controls the accumulation of fat in the liver by increasing the exportation of triglyceride from the liver for excretion. Therefore, estrogen deficiency can be said to cause triglyceride level

increment and HDL level reduction, as claimed by several studies (Babaei et al. 2010; Camara et al. 2014; López-Varela, Sánchez-Muniz & Cuesta 1995). Discovery by Zhu et al. (2013) has supported that ovariectomy and a high-fat diet caused triglyceride level increment in the animal study.

All supplementations, either mono or a combination of VCO and TT, prevented the HDL reduction while lowering the LDL, TC and triglyceride levels. Nevertheless, mono and combination supplementations illustrated different results on different parameters in the lipid profile. Only V1, V2, and V1+TT supplementations showed less reduction in HDL level compared to other supplementation groups. Meanwhile, only both VCO mono supplementations showed less increment in LDL level compared to other supplementation groups. Other than that, V2 and TT supplementations showed a higher reduction in TC level compared to V1 and both combination (V1+TT and V2+TT) supplementations. Nevertheless, all supplementations were able to reduce triglyceride levels without statistical significance. Moreover, both combination supplementations increased the ApoA level while reducing the ApoB level compared to mono supplementations of VCO and TT.

The findings of this study were similar to those obtained in previous studies. It has been reported that polyphenolic compounds in the VCO improved lipid metabolism and increased the plasma antioxidant, especially in rats fed with cholesterol (Chacko & Rajamohan 2011). Unsafonification components such as polyphenol, tocotrienol, tocopherol, β-carotene and phytosterol played an important role in VCO hypolipidemic effects (Leontowicz et al. 2002). In 2004, a study by Nevin and Rajamohan stated that VCO polyphenol was able to inhibit LDL oxidation via *in-vitro*. Polyphenols act by entrapping the reactive oxygen species (ROS) from plasma and interstitial fluid of the artery wall, inhibiting the oxidation of LDL. In addition, polyphenols can increase the reverse transportation of cholesterol and reduce cholesterol absorption by the intestine (Seneviratne & Dissanayake 2008). Furthermore, a study reported that VCO polyphenol was able to increase the serum HDL level (Malone et al. 2014). The same researchers added that the increment of ApoA1 level was in accordance with the increment of HDL level in the rat fed with 8% of VCO. Apart from that, they reported that feeding a combination of VCO with 1% cholesterol was able to reduce LDL, TC and triglyceride levels significantly compared to the combination of coconut oil and sunflower oil with 1% cholesterol (Arunima & Rajamohan 2012). On the other hand, a study by Liau et al. (2011) pointed out that VCO 30 mL/day for four weeks did not cause any changes in lipid profile level. The short duration of their study could be the reason why the VCO was unable to improve the lipid profile level. Tocotrienol induced the down-regulation of HMG-CoA reductase activity, therefore inhibiting the synthesis of cholesterol and indirectly reducing the TC and LDL levels (Ness & Chambers 2000; Zakaria et al. 2010). The result from this study was also supported by previous studies, which reported that TT was able to improve lipid profile by reducing TC, LDL and ApoB levels (Chin et al. 2011; Qureshi et al. 1991a; Yu et al. 2006).

The ability of VCO and TT to improve the lipid profile, increasing ApoA levels and reducing ApoB levels in humans and animals is supported by many previous studies. As the apolipoprotein bound together with its lipoprotein, the increment of ApoA level was in accordance with the increment of HDL level, while the reduction of ApoB level was in accordance with the reduction of LDL (Chin et al. 2011; Marina, Man & Amin 2009; Mat Daud et al. 2013; Ness & Chambers, 2000; Qureshi et al. 2001; Qureshi et al. 1991b; Tan et al. 1991). Although all supplementations of VCO and TT were able to improve the lipid profile in this study, the combination of both antioxidants at a lower dose was more capable of increasing HDL levels compared to the combination of a higher dose. This observation was postulated due to the role of polyphenol changes from antioxidant to prooxidant, especially at the higher dose (Pearson et al. 2006; Yordi et al. 2012).

## CONCLUSION

In conclusion, the finding of this study indicated that supplementation of VCO and TT, either in monotherapy or combination, gave better effects but different on each lipid parameter. The beneficial effects of both natural products may be caused by their role as antioxidants, as well as the high polyphenol contents of VCO and TT.

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