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Full Length Research Paper

Antihypercholesterolemia property and fatty acid composition of mardi-produced virgin coconut oils

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Virgin coconut oil (VCO), or in Malays known as '*minyak kelapa dara*', has gain a lot of attention recently due to various medicinal values. The aim of the present study was to evaluate the effect of feeding two types of VCOs, VCOA (produced via a standard drying method) or VCOB (prepared via fermentation process), that were produced by Malaysia Agriculture Research and Development Institute (MARDI) in lowering the plasma lipid parameter in rabbits. Nine groups of New Zealand White male rabbits (n = 6/group) were used in this study. Group 1 and 2 animals were treated with 0.9% normal saline, but fed either with a normal or cholesterol-added diet (negative control), respectively. Group 3 - 5 and 6 - 8 were given orally with the different volume (0.5, 1.0 and 2.5 ml/kg/day) of VCOA or VCOB followed by the cholesterol-added diet. Group 9 were treated with 5 mg/kg Atorvastatin and fed a cholesterol-added diet. All groups were treated for 8 weeks and blood samples were taken from the marginal ear vein prior to treatment (day 0), weeks 4 and 8 for the analysis of plasma. The rabbits fed with different volume of VCOs showed significant ($P < 0.05$) reduction in plasma cholesterol and LDL cholesterol levels compared to the control group in weeks 4 and 8. The triglycerides level increased significantly ($P < 0.05$) on week 4 before reduced on week 8, to a level that is still significant when compared to week 0. The HDL level also increased significantly ($P < 0.05$) on weeks 4 and 8 after treatment. Fatty acid analysis revealed the presence of all important fatty acids. Both VCOs showed insignificant effect on all parameters measured when compared together. In conclusion, the MARDI-produced VCOs appeared to possess great potentials as antihypercholesterolemic agent that required further in-depth study.

Key words: Virgin coconut oil, antihypercholesterolemia, atorvastatin.

INTRODUCTION

Hypercholesterolemia has been considered as a major risk factor for coronary heart disease and atherosclerosis (Romero-Corral et al., 2006). It is almost accepted that atherosclerosis is a disorder of lipid transport and metabolism. Cholesterol by- product would form thick, tough deposit called plague on the inner wall of the arteries, stiffening them and then starving the heart of blood, creating choke point where a clot could stop the flow entirely (Duff and Macmillian, 1951; Goldstein et al., 1979).

Although a range of synthetic drugs are available as antihypercholesterolemic drugs their numerous side effects and potential interference with drug metabolism are common (Moosmann and Behl, 2004). The search for compounds from nutraceutical sources for reduction of serum and plasma cholesterol and reduction of hypercholesterolemic atherosclerosis is still ongoing and might provide a useful source for therapy or alternatively as simple dietary adjuncts to existing therapies. (Sajjadi

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et al., 1998; Movahedianv et al., 2006; Prasad, 2005).

Coconut (*Cocos nucifera L*) provides food, drink, medicine, health, shelter, aesthetics and wealth to various types of people throughout the world. One of the primary natural products produced from coconut is coconut oil which has been used for a long time as food, food ingredient and functional foods. However, recent focus on the benefit of virgin coconut oil (VCO) has been on its uses in the pharmaceuticals, nutriceuticals, cosmetics and industrial fields (Che Man and Marina, 2006. VCO, which is extracted by a wet process directly from coconut milk under controlled temperature, may have more beneficial effects than copra oil since it retains most of its beneficial components (e.g. squalene, tocopherols and sterols) (Marina et al., 2009). VCO reduced total cholesterol, triglycerides, phospholipids, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol and increase high density lipoprotein in serum and tissues compared to copra oil. (Nevin and Rajamohan, 2004). Moreover, VCO is capable of increasing antioxidant enzymes and reduces lipid peroxi-dation content and also has more significant antithrom-botic effect over copra oil (Nevin and Rajamohan, 2005, 2007). In the present study, two types of VCOs were prepared using a standard drying method (VCOA) and a fermentation process (VCOB), respectively. In terms of their chemical properties, we have recently determined their iodine, saponification, peroxide and anisidine values as well as the percentage of free fatty acids. VCOA and VCOB were found to exhibit the respective iodine, saponification and peroxide values of 5.5 and 7.5 gI_2/100 g, 256.08 and 249.12 mg KOH/g oil, 0.21 and 0.62 m equiv oxygen/kg oil (Malarvili et al., 2010; personal comm.). In addition the percentages of free fatty acids and total phenolic contents of VCOA and VCOB were 0.094 and 0.981% and, 12.77 and 23.47 mg GAE/100 g oil, respectively. Interestingly, all of the values obtained were within the standard set by the Codex Alimentarius (Marina et al., 2009). The iodine value obtained for VCA and VCOB was considered low and indicates a high degree of saturation of both VCOs, which in turn, provides protection against oxidative rancidity. The sapo-nification values of VCOA and VCOB were very high indicating the presence of higher proportion of shorter chain fatty acids on the glycerol backbone. The peroxide values of VCOA and VCOB were relatively indicating that both VCOs are relatively fresh, stable and have a low susceptibility to oxidation, which could be linked to the presence of highly saturated and short chain fatty acids as described above. The difference in free fatty acid

values between the two VCO preparations could arise from the different methods of processing (Marina et al., 2009). The total phenolic contents of VCOA and VCOB are within the range of 11.82 and 23.14 mg GAE/100 g oil reported for various Malaysian-made VCOs (Marina et al., 2009). The different methods of production of VCO samples have been claimed as a major factor contributing to the variation in the phenolic content, wherein the use of heat in the production of VCO could also decrease the phenolic content.

In terms of the pharmacological properties, we have recently reported on the antinociceptive and anti-inflammatory (Zakaria et al., 2010), antiulcer (Malarvili et al., 2010) and hepatoprotective (Zakaria et al., 2010) activities of both VCOS. The antinociceptive activity of both VCOs was observed at the peripheral and central levels while the anti-inflammatory activity was seen only against the acute, but not chronic, model of inflammation. Both VCOs were also effective in attenuating the paracetamol-induced hepatoxicity via modulation of the cytochrome P450 drug metabolism pathway and HCL/ethanol-induced gastric ulcer.

Despite those claims on traditionally produced VCO, no study has been made on the pharmacological properties of VCOs produced by the Malaysia Agriculture Research and Development Institute (MARDI) using animal models. It is the aim of the present study to investigate the potential role of MARDI-produced VCOs as antihypercholsterolemic agent by measuring its effect on the plasma lipid parameters (e.g., cholesterol (CHOL), triglycerides (TG), Low Density Lipoproteins (LDL) and High Density Lipoproteins (HDL)) that are responsible for hypercholesterolemia after 8 weeks of cholesterol-feeding.

MATERIALS AND METHODS

Samples

MARDI-produced VCOs, labeled as VCOA and VCOB, were donated by Dr. Kamariah Long from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia. Briefly, VCOA was prepared using a standard drying method while VCOB was prepared via fermentation process.

Experimental animals

All experiments were performed in male New Zealand White (NZW) rabbits weighing 1.9 - 2.5 kg placed in the animal house of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM). The animals (six per group) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 28 ± 2°C) with access to food and water *ad libitum.* All rabbits received approximately 100 g of standard rabbit pellets per day, with or without cholesterol added respectively. At all times, the rabbits were cared for in accordance with the current UPM principles and guidelines for the care of laboratory animals (reference code: UPM/FPSK/PADS/BRUUH/00221) and the UPM ethical guidelines for investigations of experimental pain in conscious animals as adopted from Zimmermann (1983).

Test solutions were administered orally via gastric gavage. Induction of hypercholesterolemia: The effects of VCO on normal and cholesterol-fed animals were studied for 8 weeks according to the method described by Zuraini et al. (2006) but with slight modifications. All rabbits were subjected to a trial period (week 0) prior to cholesterol feeding to estimate the amount of pellet taken daily and to let them adapt to confinement in cages. They were given a basal diet during this period. They were divided into 6 groups with 6 rabbits per group, the normal untreated control group (N), the cholesterol-control group (C), cholesterol-fed groups treated with 0.5, 1.0 and 2.5 ml/kg VCO or 5 mg/kg Atorvastatin.

Group N continued to be fed a basal diet (cholesterol free) for the next 8 weeks whereas Group C, VCO and Atorvastatin were given cholesterol -enriched diet (normal pellet + 0.5% cholesterol). The VCO were fed daily via oral gavage for 8 weeks (Zuraini et al., 2006).

Blood collection and storage

Approximately 5 ml of blood was collected from each animal at end of week 0 (day 0) and at week 4 and week 8 for the analysis of plasma lipids. Blood was taken from the marginal ear vein using 21G needles and collected in 6 ml EDTA tubes (B and D). The plasma was immediately separated by centrifugation at 3000 rpm for 10 min. They were then transferred into Hitachi cups and eppendorf tubes and stored under -80°C for further analysis (Zuraini et al., 2006).

Analysis of plasma lipids

The plasma lipids measured, namely Cholesterol (CHOL), Low Density Liporotein (LDL) cholesterol, High Density Lipoprotein (HDL) cholesterol and Triglycerides (TG), were determined enzymatically using Hitachi 902 automated analyzer. All reagents used for the biochemical analysis were purchased from Roche Diagnostics, USA (Zuraini et al., 2006).

Fatty acids analysis of VCOA and VCOB

The fatty acid composition of total lipids from VCOA and VCOB were analyzed using gas chromatography (GC). Fats were methylated with trimethylsulfonium hydroxide and then separated by GC using a system (HP 5890, Hewlett–Packard GmbH, Waldbronn, Germany) equipped with an automatic on-column injector, a polar capillary column (30 m FFAP, 0.53 mm ID, Macherey and Nagel, Du¨ren, Germany), a flame ionisation detector, and supplied with helium, used as carrier gas, at a flow rate of 5.4 mL/min (Butte., 1983; Eder and Brandsch, 2002). Fatty acid methyl esters were identified by comparing their retention times with those of individually purified standards.

Statistical analysis

Data are expressed in mean ± SEM where appropriate and analyzed statistically by using one-way ANOVA and those at $p<0.05$ are accepted as significant. Tukey's post hoc test is employed to further evaluate the differences between the groups.

RESULTS

Effects of VCOA and VCOB on lipid levels

Figures 1a and b show the effect of VCOA and VCOB treated groups on plasma cholesterol level of rats at week 0, 4 and 8 for as compared to normal and cholesterol-fed groups. Plasma cholesterol of cholesterol-treated group had increased significantly ($p < 0.05$) beginning from week 4 and week 8 compared to normal group and these changes were also significantly higher than other groups until the end of experiment. The feeding of VCOs, at all volumes, and atorvastatin significantly ($p < 0.05$) reduced the plasma cholesterol level at week 4 and 8. Overall, VCOA was more effective than VCOB in reducing the plasma cholesterol level.

The plasma LDL profile of VCOA- and VCOB-treated groups is shown in Figures 2a and b, respectively. The LDL cholesterol level of cholesterol-fed group increased significantly ($P < 0.05$) at week 4 and 8 (Figure 2). Pre-treatment with VCOA, at the concentrations of 1.0 and 2.5 ml/kg, significantly ($P < 0.05$) reduced the plasma LDL level until the end of the experiment. Interestingly, this effect is seen with all concentration of VCOB used.

The plasma TG profile of VCOA- and VCOB-treated group is shown in Figures 3a and b, respectively. The TG level of cholesterol-fed group increased significantly ($P < 0.05$) at week 4 and 8. The TG level was significantly ($P < 0.05$) reduced at 4^{th} week when treated with 5 mg/kg atorvastatin, 1.0 and 2.5 ml/kg VCOA, and 2.5 ml/kg VCOB, but were reduced on week 8 by 5 mg/kg atorvastatin, 1.0 and 2.5 ml/kg VCOA, and 0.5, 1.0 and 2.5 ml/kg VCOB.

The plasma HDL profile of VCOA- and VCOB-treated group is shown in Figures 4a and b, respectively. All concentrations of both VCOs significantly ($P < 0.05$) increased the plasma HDL level at all assessed weeks with their activity insignificant when compared to 5 mg/kg atorvastatin.

Fatty acids composition of VCOA and VCOB

The main fatty acids found in VCOA are lauric, myristic, caprylic, palmitic, capric and oleic acids while those of VCOB include lauric, myristic, palmitic, caprylic, oleic and capric acids (Table 1).

DISCUSSION

Hyperlipidemia, especially those associated with increased serum cholesterol and LDL levels, is a risk factor in the development of atherosclerotic heart disease (Romero-Corral et al., 2006). The ability of dietary cholesterol to induce hypercholesterolemia has been regularly verified, (Yokozawa et al., 2003) mostly by observing during their intake the rise in the levels of cholesterol or other lipids such as triglycerides in blood (Zuraini et al., 2006). In the present study, the rabbits fed with hypercholesterolemic diet showed a rise in plasma concentration of cholesterol as compared to rats given normal diet. A simultaneous increased was observed in plasma cholesterol, that is, the LDL cholesterol and also TG.

Long-term feeding of saturated fats has been associated with increased levels of plasma cholesterol in both animals and human (Kritchevsky et al., 1983; Kritchevsky, 1985). When VCO at volumes of 0.5, 1.0 and 2.5 ml/kg/body weight/day were administered to cholesterol-fed rabbits, there were significant decreased

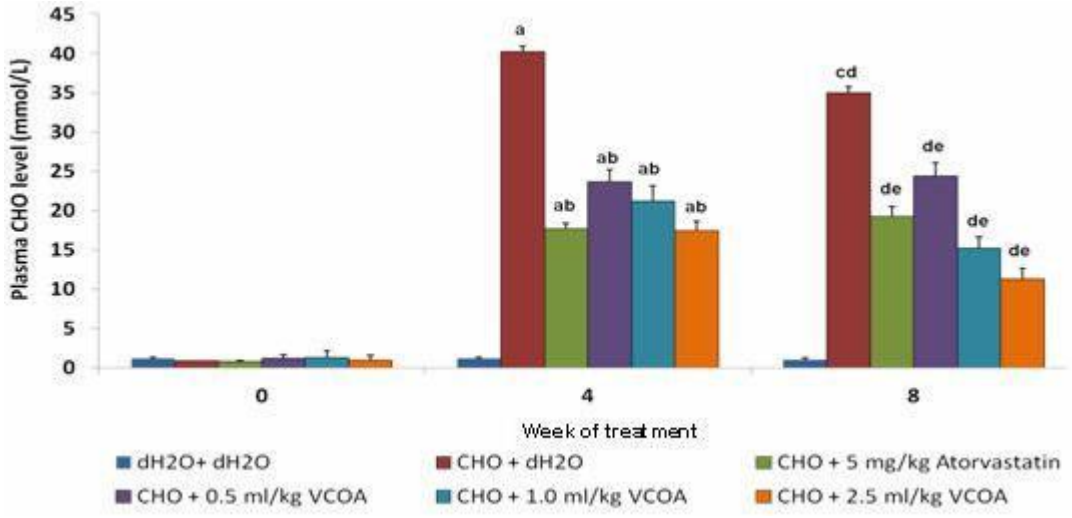

Figure 1a. Plasma cholesterol levels of VCOA-fed rabbits at week 0, 4 and 8 of treatment. [a] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 4.[b] Differ significantly when compared to the (CHO + dH_2O)-treated group at week 4.[c] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 8.[d]Differ significantly when compared to the (CHO + dH_2O)-treated group at week 8.

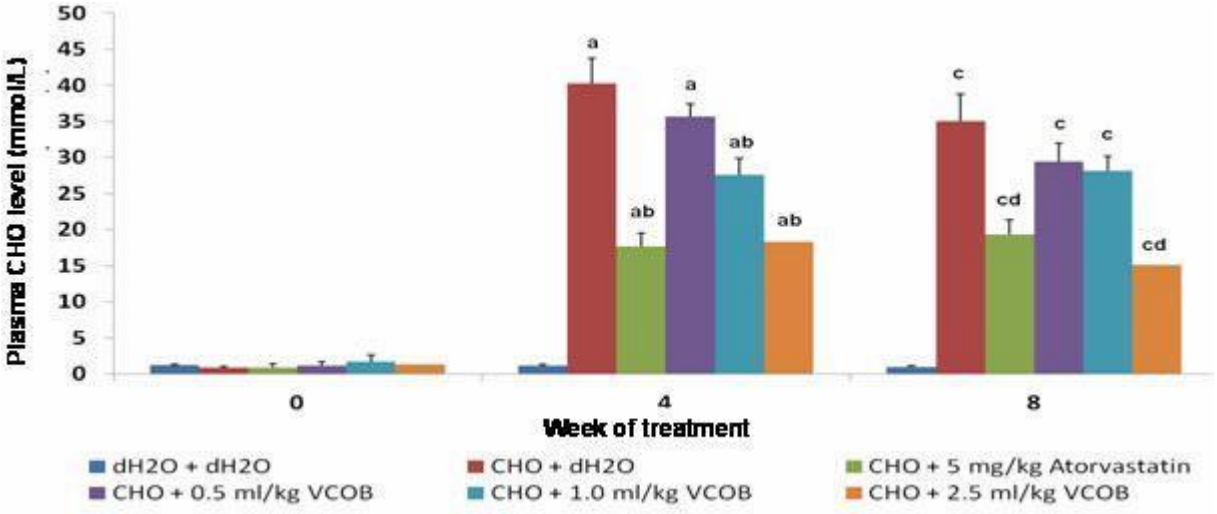

Figure 1b. Plasma cholesterol levels of VCOB- fed rabbits at week 0, 4 and 8 of treatment. [a]Differ significantly when compared to the (dH2O + dH2O)-treated group at week 4. [b]Differ significantly when compared to the (CHO + dH2O)-treated group at week 4. [c]Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 8. [d]Differ significantly when compared to the (CHO + dH_2O)-treated group at week 8.

in the values of cholesterol, LDL and TG compared to the cholesterol-fed group at both week 4 and 8. The VCO also exhibited significant increased in HDL level at both weeks measured, an indication of a beneficiary effect of the oil. The protective effect of HDL is most widely attributed to its key role in mediating the reverse cholesterol transport from the peripheral tissues to the liver for reutilization (Eckarstein et al., 2002). The results indicated that VCO had excellent lipid-lowering capabilities even during a continuous and prolonged feeding of dietary cholesterols. An explanation for the decreasing plasma cholesterol levels due to consumption of VCOs may be associated with its high polyphenol content (Nevin and Rajamohan, 2004). The high polyphenol content was capable of maintaining the normal levels of cholesterol and other lipid parameters in tissues and serum and also increased the concentration of HDL cholesterol (Nevin and Rajamohan, 2004). The suggested mechanisms of actions used by polyphenols include trapping of reactive oxygen species in aqueous

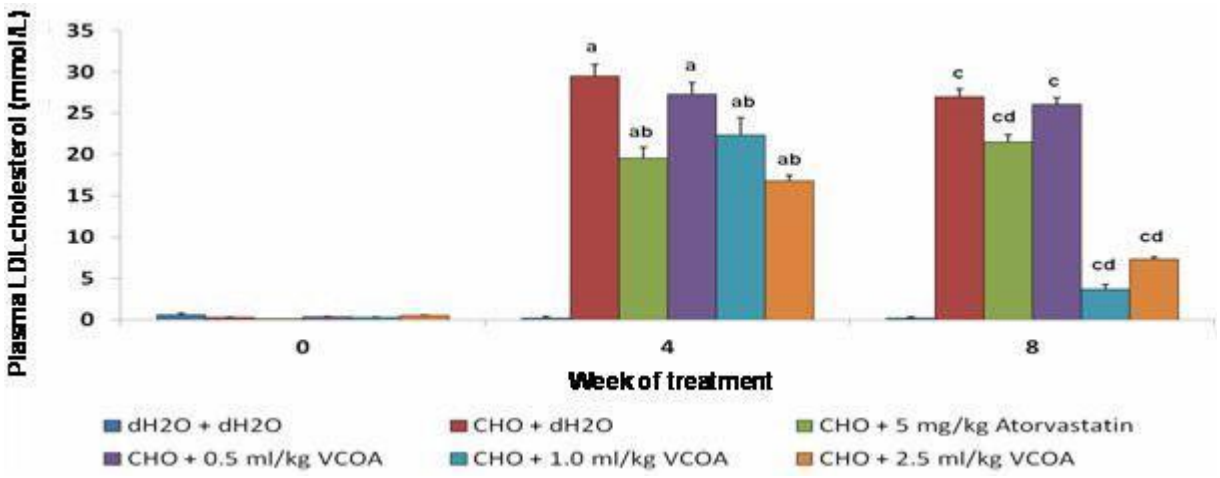

Figure 2a. Plasma low density lipoprotein (LDL) cholesterol of VCOA- fed rabbits at 0, 4 and 8 weeks of treatment.[a] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 4. [b]Differ significantly when compared to the (CHO + dH_2O)-treated group at week 4. [c] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 8. [d] Differ significantly when compared to the (CHO + dH_2O)-treated group at week 8.

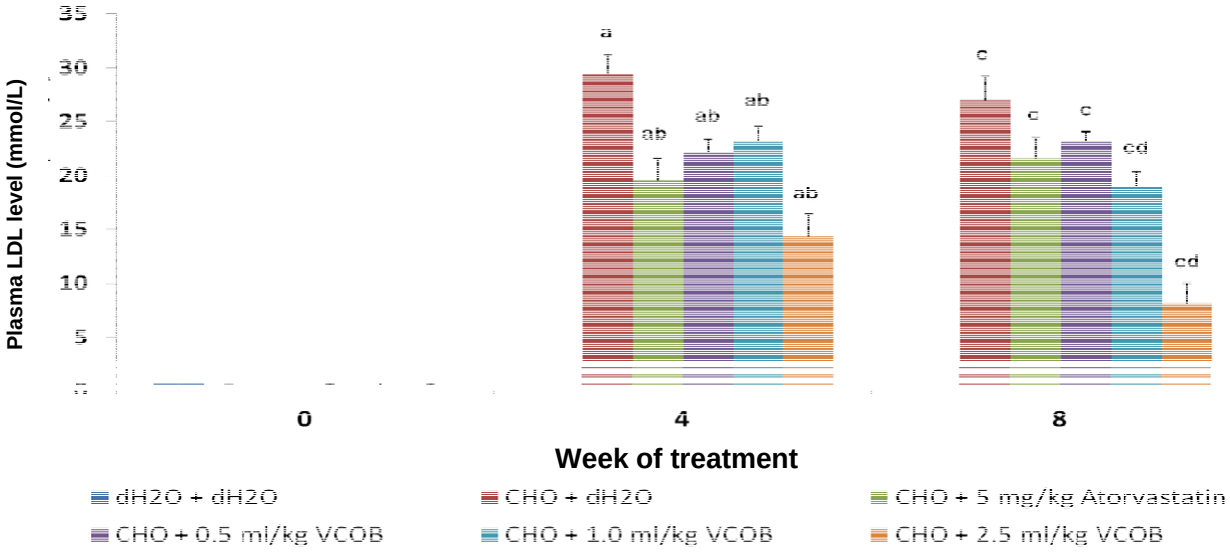

Figure 2b. Plasma low density lipoprotein (LDL) cholesterol of VCOB-fed rabbits at 0, 4 and 8 weeks of treatment.[a] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week. 4.[b] Differ significantly when compared to the (CHO + dH2O)-treated group at week 4. [c] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 8. [d] Differ significantly when compared to the (CHO + dH_2O)-treated group at week 8.

components such as plasma and interstitial fluid of the arterial wall thereby inhibiting LDL oxidation, reversal of cholesterol transport and reducing intestinal absorption of cholesterol (Eckarstein et al., 2002). We have recently demonstrated the antioxidant and antiulcerogenic activities of VCOA and VCOB (Malarvili et al., 2010) and have shown that these activities are due at least in part to their polyphenol content. The total antioxidant activity and total phenolic content measured for VCOA and VCOB were 75.7 and 82.4%, and 12.8 and 23.5 mg GAE/100 g oil, respectively. Based on these findings, it is postulated that the presence of polyphenolic compounds may contribute to the observed antihypercholesterolemia activity of both VCOs.

It is generally believed that the highly saturated nature of coconut fatty acid increases cholesterol synthesis in our body. VCO contains a high proportion of four saturated fatty acids (Table 1) and the high level of HDL cholesterol in VCO- treated animals compared to the other group could be attributed to the presence of these fatty acids (Cox et al., 1995). The main fatty acids found in VCOA included lauric (50%), myristic (18%), caprylsic

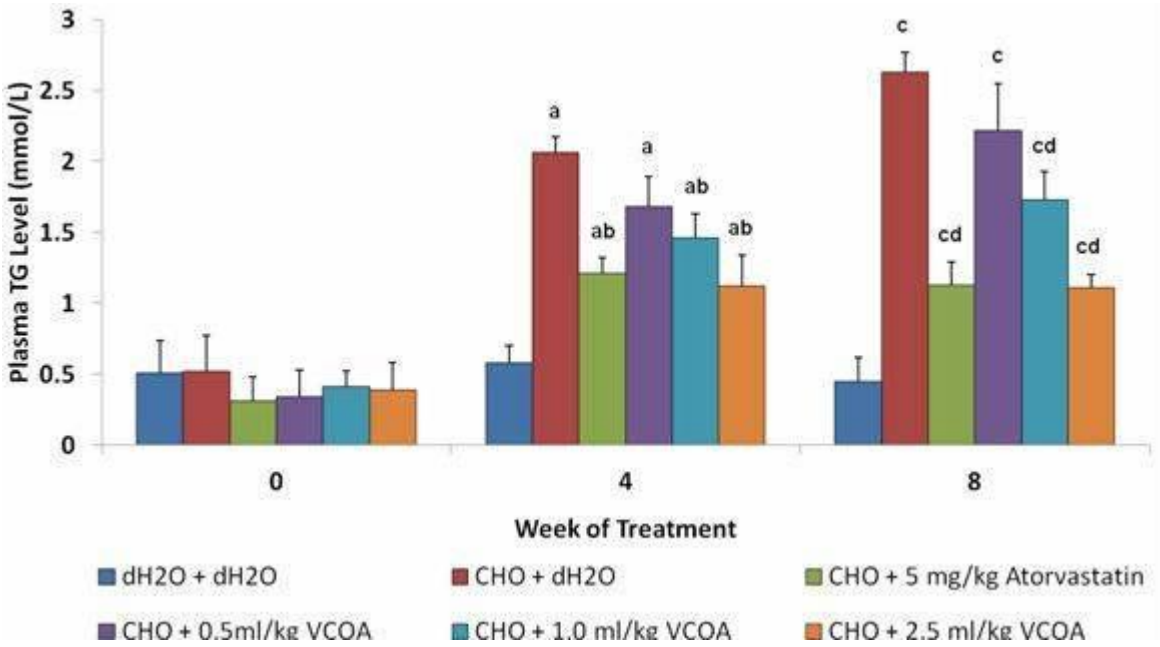

Figure 3a. Plasma triglycerides (TG) of VCOA-fed rabbits at 0, 4 and 8 weeks of treatment. [a] Differ significantly when compared to the (dH2O + dH2O) -treated group at week 4 [b] Differ significantly when compared to the (CHO + dH2O)-treated group at week 4 [c] Differ significantly when compared to the (dH2O + dH2O)-treated group at week 8 [d] Differ significantly when compared to the (CHO + dH2O)-treated group at week 8.

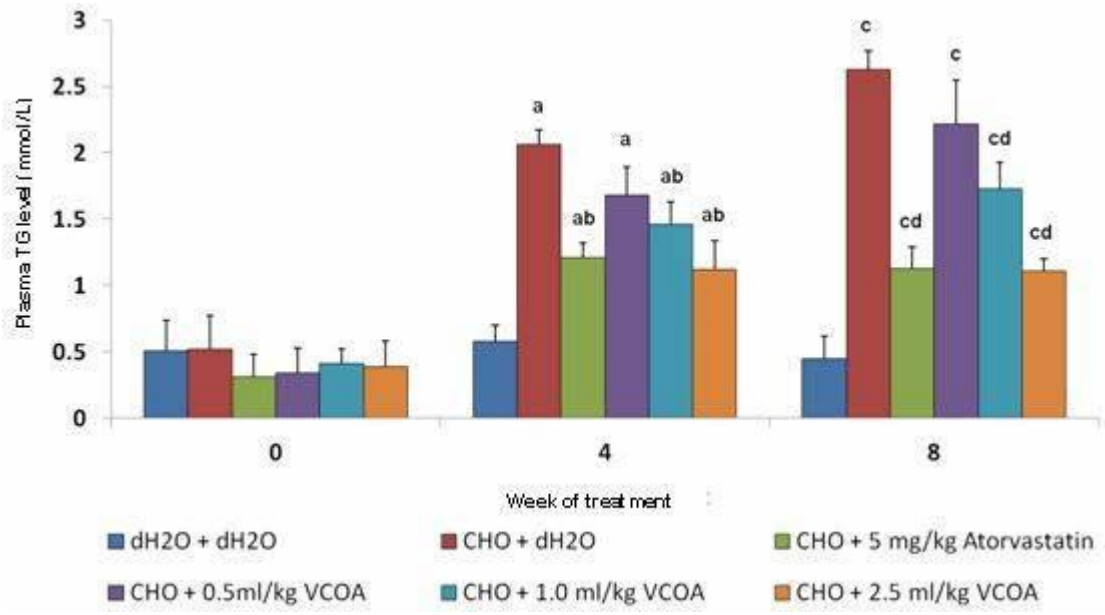

Figure 3b. Plasma triglycerides (TG) of VCOB-fed rabbits at 0, 4 and 8 weeks of treatment. [a] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 4. [b] Differ significantly when compared to the (CHO + dH_2 O)-treated group at week 4. [c]Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 8. [d] Differ significantly when compared to the (CHO + dH_2O)-treated group at week 8.

(9%), palmitic (7%), capric (7%), oleic (5%) and stearic (3%) acids while those of VCOB include lauric (47%), myristic (19%), palmitic (9%), caprylic (8%), oleic (6%), capric (6%) and stearic (3%) acids. Fatty acids such as oleic and stearic acids have been shown to attenuate inflammation by suppressing (Zakaria et al., 2007). The

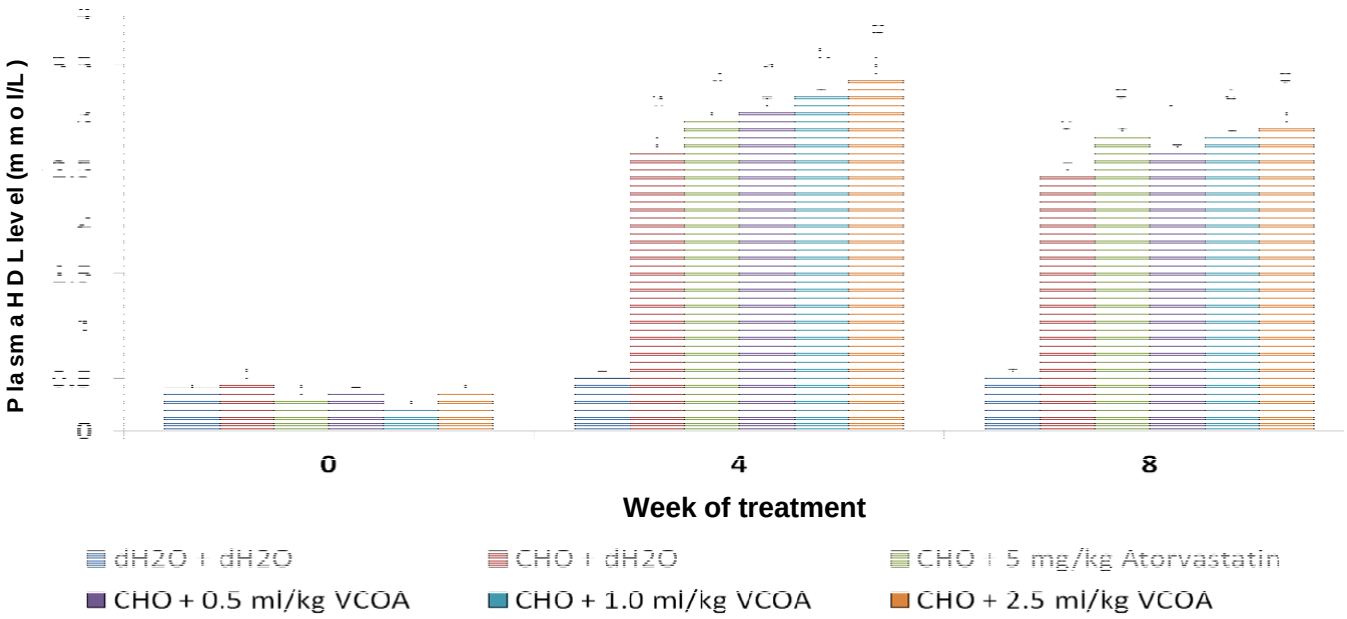

Figure 4a. Plasma high density lipoprotein (HDL) cholesterol of VCOA- fed rabbits at 0, 4 and 8 weeks of treatment. [a] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 4. [b] Differ significantly when compared to the (CHO + dH_2O)-treated group at week 4. [c]Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 8. [d] Differ significantly when compared to the (CHO + dH_2O)-treated group at week 8.

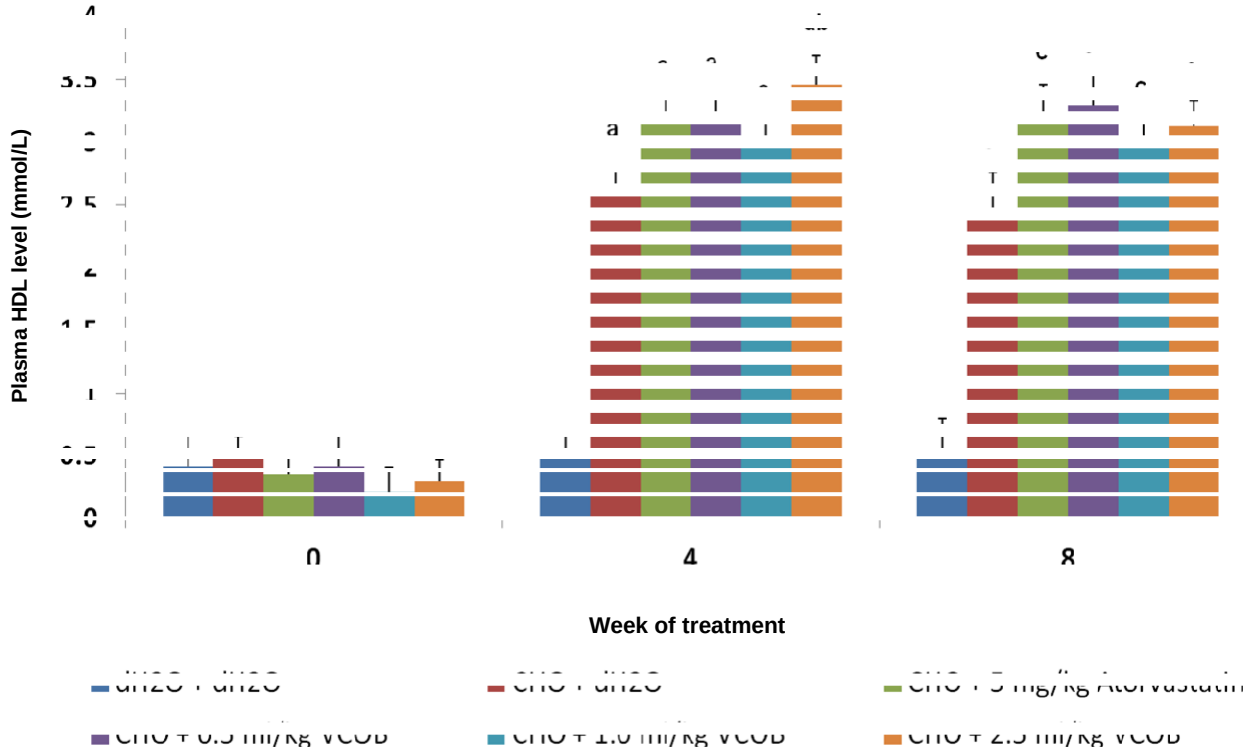

Figure 4b. Plasma high density lipoprotein (HDL) cholesterol of VCOB-fed rabbits at 0, 4 and 8 weeks of treatment. [a] Differ significantly when compared to the (dH_2 O + dH_2O)- treated group at week 4. [b] Differ significantly when compared to the (CHO + dH_2O)-treated group at week 4.[c] Differ significantly when compared to the (dH_2O + dH_2O)-treated group at week 8.

Table 1. Fatty acids composition of VCOA and VCOB.

Types of fatty acids		Range of fatty acids composition (%)	
		VCO A	VCO B
Caproic acid	$C_{6:0}$	0.9292 ± 0.1649	0.8961 ± 0.0153
Caprylic acid	$C_{8:0}$	8.9807 ± 0.4142	7.6717 ± 0.2279
Capric acid	$C_{10:0}$	6.5883 ± 0.1956	5.9170 ± 0.1413
Lauric acid	$C_{12:0}$	50.0686 ± 0.0387	46.8120 ± 0.5860
Myristic acid	$C_{14:0}$	18.1748 ± 0.2947	19.003 ± 0.1076
Palmitic acid	$C_{16:0}$	7.2997 ± 0.1678	9.4196 ± 0.2899
Stearic acid	$C_{18:0}$	2.7150 ± 0.0642	3.1119 ± 0.2030
Oleic acid	$C_{18:1}$	4.5586 ± 0.1130	6.1478 ± 0.2989
Linoleic acid	$C_{18:2}$	0.6850 ± 0.0190	1.0209 ± 0.0608
Linolenic acid	$C_{18:3}$	ND	ND

proportion of fatty acids integrated into membrane phospholipids have been suggested to affect membrane fluidity, thus affecting cell function. This is due to the fact that membrane fluidity is affected by the chain length and saturation of phospholipid fatty acids. It is plausible to suggest that these fatty acids also contribute to the recently reported antinociceptive and anti-inflammatory activity of VCOA and VCOB (Zakaria et al., 2010).

Despite the different in the VCOs' methods of preparation and the slightly high polyphenolic content of VCOB compared to VCOA, both VCOs contained almost similar content of fatty acids and exerted almost the same degree of antihypercholesteorlemic effect. Thus, it is not appropriate to recommend one of the VCOs is better than the other one. It is suggested that consuming VCO will give the consumers its beneficial effects regardless of their methods of preparation.

To conclude, our preliminary data strongly suggest that MARDI-produced VCOs, regardless of their methods of preparation, are capable of lowering the levels of cholesterol and other lipid parameters in plasma and also increased the concentration of HDL cholesterol in rabbits. These effects of VCO may be due to the differences in the absorption, transport and catabolism of the consistent fatty acids and polyphenol content. Further experiments are needed to test the mechanism of antihyper-cholesterolemic activity of MARDI-produced VCO.

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