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ORIGINAL ARTICLE

Cardioprotective effect of virgin coconut oil in heated palm oil diet-induced hypertensive rats

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Abstract

Context: Virgin coconut oil (VCO) contains high antioxidant activity which may have protective effects on the heart in hypertensive rats.
Objectives: The study investigated the effects of VCO on blood pressure and cardiac tissue by measuring angiotensin-converting enzyme (ACE) activity and its histomorphometry in rats fed with a heated palm oil (HPO) diet.
Materials and methods: Thirty-two male Sprague–Dawley rats were randomly divided into four groups: (i) control, (ii) orally given VCO (1.42 ml/kg), (iii) fed with a HPO (15%) diet, and (iv) fed with a HPO diet and supplemented with VCO (1.42 ml/kg, po) (HPO+VCO) for 16 weeks. Blood pressure was measured monthly. After 16 weeks, rat hearts were dissected for lipid peroxidation (TBARS) and ACE activity measurement and histomorphometric study.
Results: Systolic blood pressure was significantly increased in the HPO group compared with the control starting at week eight (112.91 ± 1.32 versus 98.08 ± 3.61 mmHg, $p < 0.05$) which was prevented by VCO supplementation (91.73 ± 3.42 mmHg). The consumption of HPO increased TBARS and ACE activity in heart, which were inhibited by VCO supplementation. The increases in the myofiber width and area as well as nuclear size reduction in the HPO group were significantly prevented by VCO supplementation.
Conclusion: These results suggested that VCO supplementation possesses a cardioprotective effect by preventing the increase in blood pressure via an antioxidant mechanism and remodeling in rats fed repeatedly with a HPO diet.

Keywords

Angiotensin-converting enzyme, heart, hypertension, myofiber area, myofiber width, oxidative stress

History

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Introduction

It is a common practice among Malaysians to use repeatedly heated palm oil (HPO) in their food preparation to save cost (Abdullah et al., 2010). The level of awareness among the Malaysian public regarding the negative effects of the use of repeatedly heated oil was moderate (Azman et al., 2012). Palm oil, when heated repeatedly at high temperature for a long period, will undergo a thermal oxidation process (Mozaffarian et al., 2006). A previous study showed that prolonged intake of repeatedly HPO had significantly increased blood pressure in rats, accompanied by an increase in plasma angiotensin-converting enzyme (ACE) (Leong et al., 2012), which was normally seen in hypertensive rats with cardiac remodeling (Varagic et al., 2012). ACE is required for the conversion of angiotensin I to angiotensin II, in which the latter is a potent vasoconstrictor. Increased activity of ACE leads to a rise in blood pressure and cardiac hypertrophy as well as vascular proliferation (Taylor & Pool, 2011).

Many studies nowadays have focused on the effects of plant-origin extracts on various pathological conditions. One of the plant-origin extracts of interest is virgin coconut oil (VCO), an extract from coconut milk. Due to its method of cold processing, VCO has greater retention of antioxidants such as total phenols than coconut oil which is prepared by subjecting its crude oil to high temperature up to 200 °C to deodorize the oil (Marina et al., 2009). It possesses beneficial effects of plasma lipid profile and antioxidant properties (Nevin & Rajamohan, 2004, 2008). VCO was recently reported to prevent blood pressure rise in rats fed repeatedly heated oil likely by improving endothelial functions (Nurul-Iman et al., 2013).

Due to its possible beneficial effects on the cardiovascular system, this study investigated the effects of VCO on blood pressure and cardiac ACE activity as well as histomorphometry in hypertensive rats which were induced by dietary repeatedly HPO.

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Materials and methods

Source and preparation of diet

Potatoes were bought from a local market. The skin was peeled and the potatoes were thinly sliced before being fried. Palm oil (Cap Buruh, Malaysia) (2.5 l) was used to fry potato chips (1 kg) in a stainless steel wok at 180 °C for 10 min. It was cooled at room temperature for at least 5 h before the next heating process. To produce five-times-HPO, the heating process was repeated for another four times (Leong et al., 2008). After that, 150 g oil was mixed with 850 g ground rat pellet and then remolded into pellets before left to air dry overnight.

Experimental design

Thirty-two male Sprague–Dawley rats weighing 200–250 g were randomly divided into four groups. The first group was given basal rat diet and served as the control. The second group was also fed with basal diet but given VCO (1.42 ml/kg) (Organic Gain Sdn. Bhd., Mentakab, Malaysia) via oral gavage. The last two groups were fed with the HPO diet (15%) (HPO), but the fourth group was also given oral VCO (1.42 ml/kg) (HPO + VCO). The treatment duration was 16 weeks. The dose of 1.42 ml/kg VCO used was based on the recommendation for the minimum daily intake of the VCO in human which was equal to one tablespoon (10 ml) (Organic Gain Sdn. Bhd., Mentakab, Malaysia). The fatty acid composition of VCO is shown in Table 1.

Systolic blood pressure of the rats was monitored monthly. At the end of 16 weeks, the rats were sacrificed and the hearts harvested. All animals were treated in accordance with the guideline for the care and use of laboratory animal and the animal handling procedures were approved by the Universiti Kebangsaan Malaysia Animal Ethical Committee.

Measurement of blood pressure

Before the systolic blood pressure measured by the tail-cuff method using PowerLab data acquisition systems (ADInstruments, Bella Vista, NSW, Australia), the rats were warmed for 10 min. Five readings were obtained from each rat and averaged.

Measurement of cardiac lipid peroxidation content

Lipid peroxidation content in the heart was assayed as thiobarbituric acid reactive substance (TBARS) using a commercial assay kit provided by Cayman Chemical Company (Ann Arbor, MI). The TBARS content was expressed as pmol malondialdehyde (MDA) per mg protein.

Table 1. Fatty acid composition in virgin coconut oil.

Fatty acid	Percentage[a] (%)
Caproic acid (C6:0)	0.5
Caprilic acid (C8:0)	8.4
Capric acid (C10:0)	6.5
Lauric acid (C12:0)	49.5
Myristic acid (C14:0)	17.9
Palmitic acid (C16:0)	8.0
Stearic acid (C18:0)	3.1
Oleic acid (C18:1)	5.1
Linoleic acid (C18:2)	1.0

[a]Obtained from the Organic Gain Sdn. Bhd. (Mentakab, Malaysia)

Measurement of cardiac ACE activity

ACE extract of heart was prepared by the method of Masuda et al. (1996) with some modifications. Briefly, cardiac was homogenized in five volumes of Tris-HCl (50 mM, pH 7.9) containing 0.3 M NaCl. The suspension was then centrifuged at 44 000*g* for 90 min at 4 °C. The pellets were suspended in the same buffer, homogenized, and recentrifuged (44 000*g*, 90 min, 4 °C). The pellets were resuspended in the above buffer containing 0.5% Triton X-100 and homogenized. After 1 h of incubation in ice, the homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatants were used as the enzyme source for ACE.

ACE activities were assayed colorimetrically according to the Cushman and Cheung (1971) method with some modifications. Hippuryl-histidyl-leucine (HHL) was dissolved in 0.1 M sodium borate buffer (pH 8.3) containing 0.3 M NaCl. An amount of 150 µl of 5 mM HHL was added to 100 µl of the enzyme extract sample and incubated for 30 min at 37 °C. The reaction was terminated by the addition of 250 µl of 1 M HCl. The HCl was added before the samples in zero-time control assays. The hippuric acid released by HHL was extracted from the acidified solution into 1.5 ml ethyl acetate by vortex mixing for 15 s. After brief centrifugation at 1000*g* for 10 min, 1 ml of upper layer was transferred to a clean tube and heated at 120 °C for 30 min. A volume of 1 ml of distilled water was added into the dried hippuric acid samples. The enzyme activity was determined by measuring its absorbance at 228 nm. The specific ACE activity was expressed as mU/g.

Histomorphometry of cardiac tissue

The heart left ventricle was fixed in 10% formalin before being processed for histological sectioning using serial dehydration in alcohol. The heart was blocked in paraffin and cross-sectioned (5 µm thick) before mounting on slides. The slides were stained with hematoxylin and eosin before being examined under ×400 magnification using an image analyzer (Nikon Corporation, Tokyo, Japan). Three slides were obtained from each block of heart samples. Three fields of cross-sectional view from each slide were used for histomorphometric measurements by two blinded assessors.

Myofiber width and area were determined using a 3×3 grid method which was superimposed over images. Each myofiber that lay beneath each of the nine intersections of grid lines was taken for the measurement of myofiber area (Burkhardt et al., 1996). Nuclear count and size were counted in the surface area of each field ($100 \times 100\,\mu m^2$). Nine measurements from each heart sample were averaged to obtain individual values.

Statistical analyses

Data were expressed as the mean ± SEM. The normality of the data was determined by the Shapiro–Wilk test. The differences among groups were determined using one-way analysis of variance (ANOVA), followed by the *post hoc* test

Tukey HSD. The correlation between parameters was analyzed using Pearson's correlation test. A value of $p < 0.05$ was considered significant. All statistical analyses were performed using Statistical Package for Social Sciences version 16.0 software (SPSS, Inc., Chicago, IL).

Results

There was a significant increase in systolic blood pressure for five-times-HPO group compared with the control starting from week 8 towards week 16 ($p < 0.05$) (Figure 1). The rats that were fed with the HPO and given oral VCO had significantly lower blood pressure than the HPO group ($p < 0.001$). No significant difference in blood pressure among the control, VCO, and HPO+VCO was observed throughout the study.

The lipid peroxidation content in the heart measured as TBARS was significantly increased in the HPO group ($p < 0.05$). Supplementation with VCO had prevented the increase in this parameter induced by dietary HPO. Moreover, VCO supplementation also had further reduced the TBARS when compared with the control group ($p < 0.05$) (Figure 2).

Dietary repeatedly HPO for 16 weeks significantly augmented the activity of ACE in rat hearts (Figure 3) (14.70 ± 5.27 mU/g) more than four-fold compared with the control group (3.31 ± 1.22 mU/g). In the group which was also fed the same diet but supplemented with VCO, the enzyme activity (3.21 ± 1.02 mU/g) was significantly reduced compared with the unsupplemented group (HPO) ($p < 0.05$). The enzyme activities were similar in the control, VCO, and HPO+VCO groups.

The histological cross sections of cardiac left ventricle in rats fed with a repeatedly HPO and treated with VCO is shown in Figure 4. The sections from the control (Figure 4A) and VCO-supplemented (Figure 4B) groups showed normal arrangements of the cardiac myofibers. The nuclei appeared to be at normal size. Surrounding the cardiac myofiber was

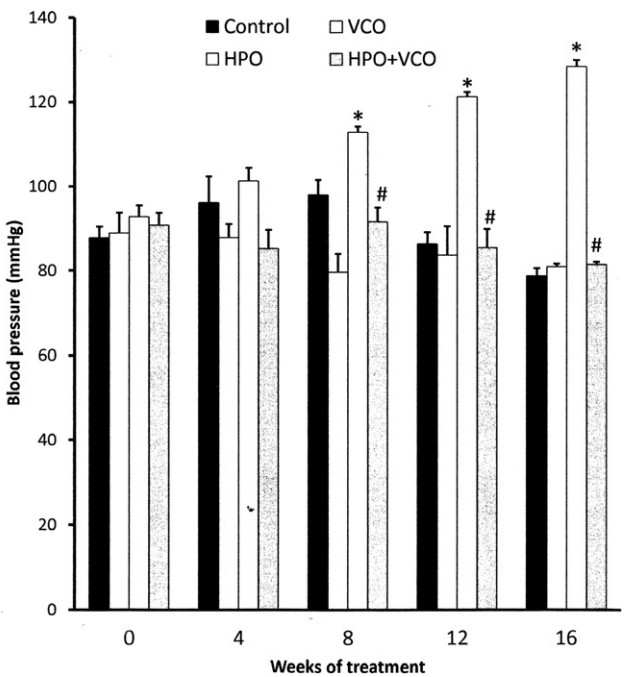

Figure 1. Effects of virgin coconut oil (VCO, 1.42 ml/kg, orally) supplementation on blood pressure in rats fed with a heated palm oil (HPO) diet for 16 weeks. Bars represent mean ± SEM ($n = 8$). *$p < 0.05$ compared with the control group and #$p < 0.005$ compared with the HPO group.

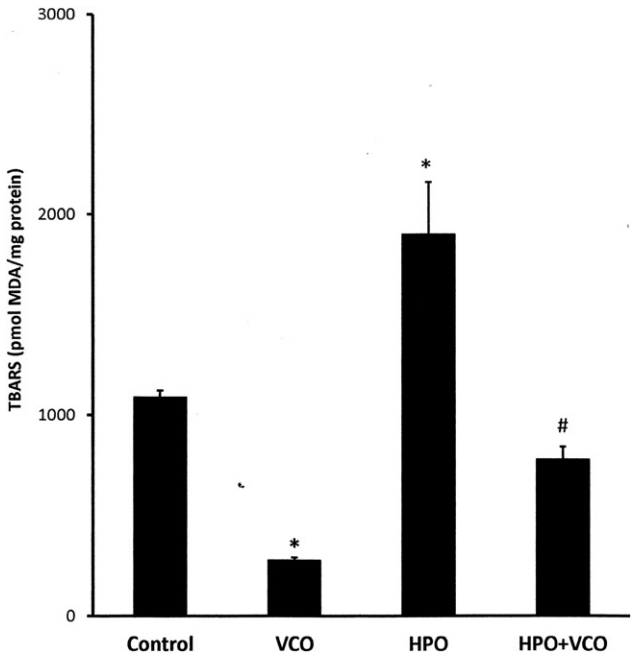

Figure 2. Cardiac lipid peroxidation content in rats that were fed with heated palm oil (HPO) diet and supplemented with virgin coconut oil (VCO, 1.42 ml/kg, orally) for 16 weeks. Bars represent mean ± SEM ($n = 8$). *$p < 0.05$ compared with the control group and #$p < 0.05$ compared with the HPO group.

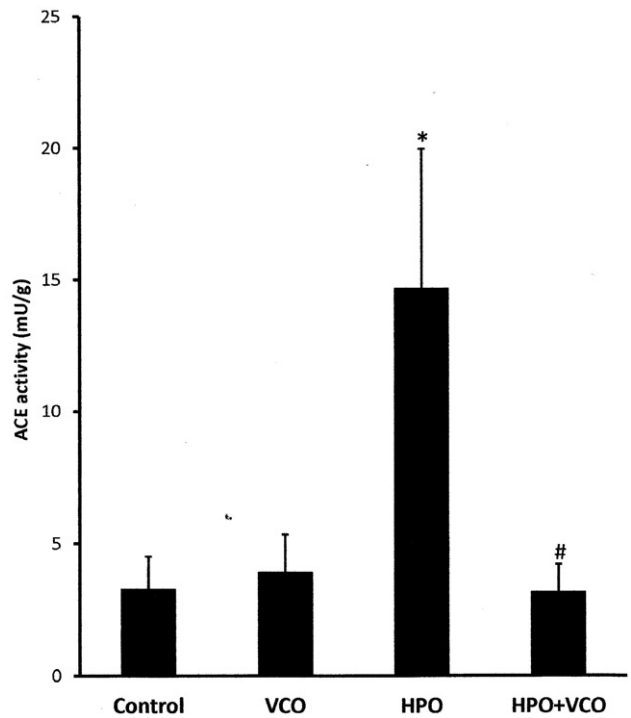

Figure 3. The angiotensin-converting enzyme (ACE) activity in the heart of rats that were fed with heated palm oil (HPO) diet and supplemented with virgin coconut oil (VCO, 1.42 ml/kg, orally) for 16 weeks. Bars represent mean ± SEM ($n = 8$). *$p < 0.05$ compared with the control group and #$p < 0.05$ compared with the HPO group.

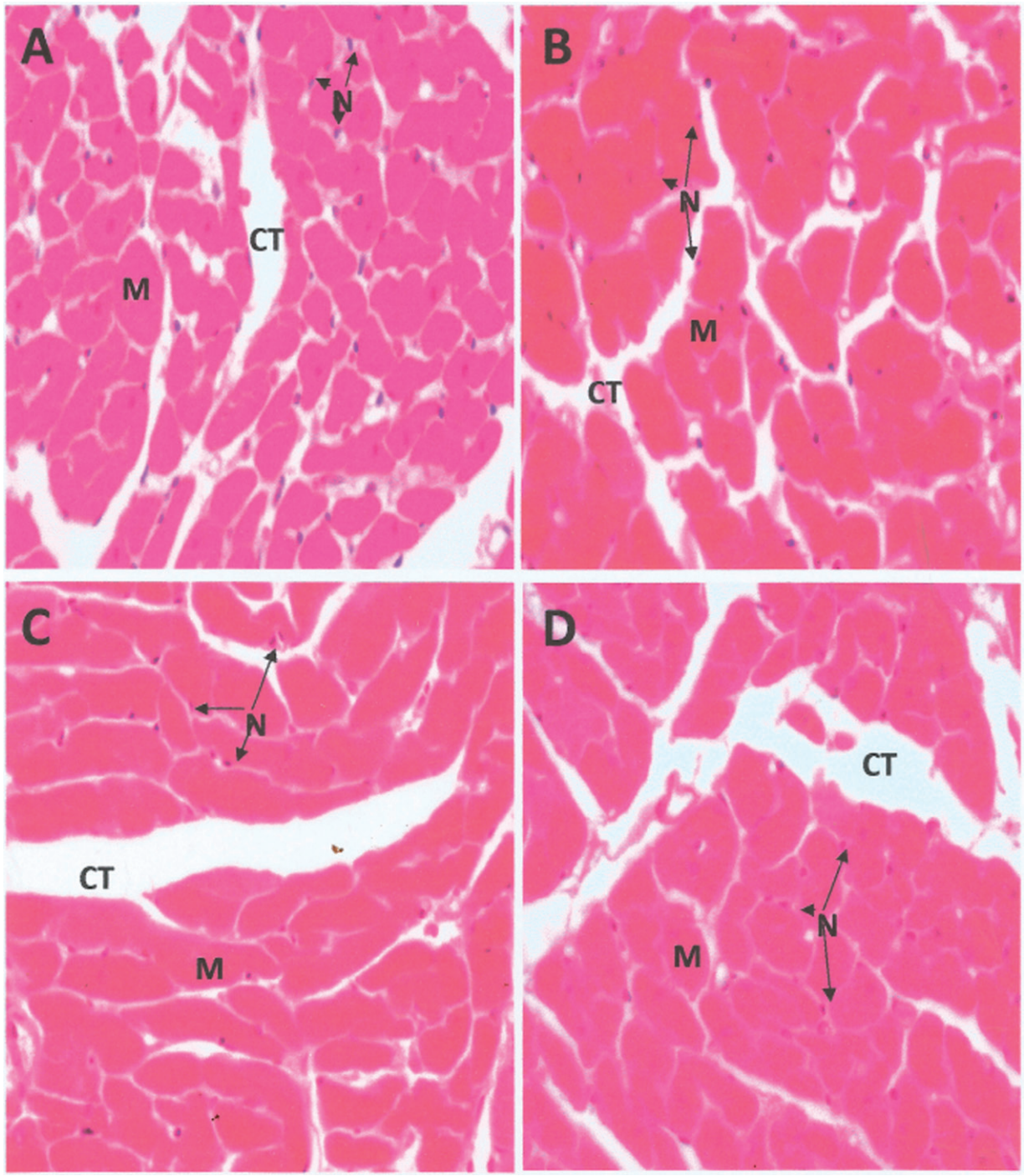

Figure 4. Representative cross sections of cardiac left ventricles stained with H&E (×400) of rats from the control (A), virgin coconut oil (VCO) supplemented (1.42 ml/kg, oral) (B), heated palm oil (HPO) (C), and heated palm oil plus virgin coconut oil (HPO+VCO) (D) groups. CT, connective tissue; M, myofiber; N, nucleus (shown by arrows).

the delicate sheath of connective tissues. In rats fed with HPO diet, there was an apparent increase in the size of myofibers in terms of width and area as compared with other groups. There was also evident reduction in the nuclear size in this group (Figure 4C). While in rats given HPO diet and treated with VCO, its histological findings were comparable with the control and supplemented group (Figure 4D).

Myofiber width and area in the HPO group were significantly larger ($p < 0.005$) than that of the control group (18.50 ± 0.40 versus $16.47 \pm 0.74\,\mu m$; 105.07 ± 2.46 versus $87.33 \pm 3.26\,\mu m^2$, $p < 0.005$), respectively (Table 2). The myofiber width and area in the HPO+VCO were significantly lower than that of the HPO group ($16.11 \pm 0.35\,\mu m$; $87.25 \pm 2.31\,\mu m^2$, $p < 0.005$, respectively). There was no significant difference in myofiber width and area among the control, VCO, and HPO+VCO groups. The nuclear count was similar in all groups, neither affected by the dietary repeatedly HPO nor affected by the VCO supplementation. The nuclear size was reduced in the HPO group, while the same reduction was prevented by the VCO supplementation ($p < 0.005$).

There was a positive correlation between blood pressure and the cardiac ACE activity ($r = 0.538$, $p < 0.001$), cardiac

Table 2. Histomorphometric parameters of cardiac left ventricle in rats treated with virgin coconut oil and fed with heated palm oil for 16 weeks.

Group	Myofiber width (μm)	Myofiber area (μm^2)	Nucleus count (per $10^3 \mu m^2$)	Nucleus size (μm^2)
Control	16.47 ± 0.74	87.33 ± 3.26	29.50 ± 1.10	7.48 ± 0.63
VCO	15.78 ± 0.36	91.63 ± 2.79	27.50 ± 2.69	7.20 ± 0.67
HPO	18.50 ± 0.40*	105.07 ± 2.46*	31.88 ± 2.90	4.82 ± 1.00*
HPO+VCO	16.11 ± 0.35#	87.25 ± 2.31#	32.88 ± 2.02	7.92 ± 0.50#

Values represent mean ± SEM ($n = 8$).
*$p < 0.005$ compared with the control group.
#$p < 0.005$ compared with the HPO group.

Table 3. Correlation (r) between parameters.

	TBARS	Blood pressure	Nuclear count	Nuclear size	Myofiber width	Myofiber area
ACE	0.474**	0.538***	−0.064	0.158	0.415*	0.360*
Myofiber area	0.516**	0.685***	−0.489**	−0.107	0.402*	
Myofiber width	0.542**	0.568**	−0.184	0.082		
Nuclear size	−0.283	−0.494**	0.386*			
Nuclear count	0.079	0.168				
Blood pressure	0.711***					

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

myofiber area ($r = 0.685$, $p < 0.001$), myofiber width ($r = 0.568$, $p < 0.01$), and TBARS ($r = 0.711$, $p < 0.001$). However, blood pressure was negatively correlated to the cardiac nuclear size ($r = -0.494$, $p < 0.01$). The TBARS was also positively correlated to the myofiber area ($r = 0.516$, $p < 0.01$), width ($r = 0.542$, $p < 0.01$), and ACE activity ($r = 0.474$, $p < 0.01$). The nuclear count was negatively correlated to the myofiber area, but positively correlated to the nuclear size. There was also a positive correlation between the myofiber width and the ACE activity as well as the myofiber area (Table 3).

Discussion

Repeatedly HPO is an established model for hypertension (Leong et al., 2008; Ng et al., 2012). In the present study, dietary five-times-HPO increased the systolic blood pressure as early as 8 weeks, consistent with other findings (Leong et al., 2008, 2012; Ng et al., 2012). Chronic intake of HPO was shown to attenuate vasorelaxation and augmented vasoconstrictory responses in rats (Leong et al., 2009), which may explain the increase in blood pressure. Repeated heating at high temperature causes thermal oxidation in the oil which can be detected as peroxide values and changes in its fatty acid composition (Kamisah et al., 2012). Heating also reduces the antioxidant content such as vitamin E in the oil and this reduction renders the oil to lose its beneficial property, thus increases the oxidative instability in the oil (Adam et al., 2007). Rats being fed heated oil showed increased oxidative stress in plasma and liver (Leong et al., 2012; Srivastava et al., 2010). Their findings were in agreement with our finding in which an elevation of TBARS in rats fed heated oil was observed in this study. Oxidative stress was reportedly to be one of the mechanisms involved in the development of hypertension (Kawarazaki et al., 2012), confirmed by its positive correlation to hypertension in our study. It had been proven that reactive oxygen species (ROS) plays a fundamental role in the development of cardiovascular disease like hypertension, atherosclerosis, and heart failure (Touyz & Briones, 2011; Xu et al., 2006). ROS causes vascular injury by promoting vascular cell growth, inflammation, endothelial dysfunction, and increased vascular tone. As a result, it will lead to hypertension (Xu et al., 2006).

Supplementation of VCO in rats that were fed repeatedly HPO prevented the increase in blood pressure. It was demonstrated that VCO significantly attenuated vasoconstrictory response of the aortic rings in rats fed HPO without affecting its vasorelaxation. It also prevented the loss of plasma nitric oxide, a potent vasodilator (Nurul-Iman et al., 2013). The protective effect of the VCO is most probably due to its high content of antioxidant, as evident by a lower cardiac lipid peroxidation in the group fed heated oil and supplemented with VCO. Ferulic acid and *p*-coumaric acid are two major phenols that present in the oil in addition to α-tocopherol (Marina et al., 2009). These phenols possess a good antioxidant properties (Pragasam et al., 2012; Wang et al., 2012). The antioxidant components of the oil might counteract the harmful effects of repeatedly HPO consumption. This may explain the protective effect of the VCO on the development of hypertension. Moreover, in normal rats, VCO had further reduced the lipid peroxidation content in the heart when compared with the control group, suggesting the beneficial effect of its antioxidant content even without the presence of a stressor.

In our study, the repeatedly HPO increased ACE activity in the heart. The increase in plasma ACE was also reported in rats that were fed repeatedly heated palm and soy oils (Leong et al., 2010, 2012). The enzyme activity was measured because its increased activity is very important for the development of hypertension (Ceroni et al., 2010). Its elevated activity was seen in hypertensive animals and associated with increased oxidative stress (Ceron et al., 2012), in agreement with our finding which showed a positive correlation between the enzyme activity and TBARS. A significant correlation between the enzyme activity in the

cardiac and the blood pressure was also observed in the present study. ACE plays an important role in the regulation of blood pressure. It converts angiotensin I to angiotensin II, a potent vasoconstrictor and possesses proinflammatory property (Nguyen Dinh Cat et al., 2013). Increased production of the angiotensin II could mediate hypertrophic growth by stimulating transforming growth factor-β and endothelin signaling pathways (Gray et al., 1998; Sano et al., 2000). Increased activity of this enzyme was found in hypertrophied heart of experimental animals (Gómez-Roso et al., 2009; Murça et al., 2012). Cardiac hypertrophy is one of the adverse changes induced by hypertension.

VCO supplementation was able to prevent the increase in the activity of ACE induced by dietary HPO. Bamboo shoot which was also high in phenolic compounds, namely ferulic and *p*-coumaric acids, similar to VCO, was demonstrated to significantly reduce blood pressure and lung ACE in spontaneous hypertensive rats. When combined with an ACE inhibitor, it exerted a synergistic effect (Liu et al., 2012). Another study had also reported ACE inhibitory activity of ferulic acid extracted from the *Ficus racemosa* stem bark (Ahmed et al., 2010). This suggests that the phenolic compounds might have a direct inhibitory effect on the enzyme. It might also neutralize the negative effects of ROS from the dietary, for its high antioxidant content. Therefore, it blocked the possible consequent changes which include the increase in the cardiac ACE activity. VCO supplementation has been shown to increase the antioxidant status in rats (Nevin & Rajamohan, 2006). In our study, we did not compare or combine the VCO supplementation with an ACE inhibitor because this study was more of preventive rather than treatment. Vaněčková et al. (2012) had shown that combined administration of an ACE inhibitor (trandolapril) and angiotensin receptor blocker (losartan) prevented the development of cardiac hypertrophy in hypertensive rats.

Increased cardiac myofiber area and width were associated with an increase in cardiac angiotensin II and ACE expression (Huang et al., 2012). Similar findings were also seen in the present study where significant positive correlations between these two parameters and ACE activity were observed. The increase in cardiac myofiber area and width suggestive of heart hypertrophy was prominently seen in the group fed repeatedly HPO only. The impaired vasorelaxation and increased vasoconstriction in rats induced by the chronic intake of the diet increased the blood pressure (Leong et al., 2009), which later could insult the heart. Prolonged increase in blood pressure will cause cardiac hypertrophy and remodeling process due to hemodynamic overload in the heart. In our study, the myofiber area and myofiber width were positively correlated to the blood pressure. There was also a positive correlation between the myofiber area and the myofiber width. The initial structural changes in the heart as a compensatory mechanism to the high pressure load and stress might later trigger maladaptive hypertrophy of cardiomyocytes by increasing in size (Leonard et al., 2012). Dilation, myocyte loss, either due to necrosis or due to apoptosis, and myocyte hyperplasia are involved in the process (Mandarim-de-Lacerda & Pereira, 2000; Sonnenblick & Anversa, 1999). Positive correlations observed between TBARS and myofiber area as well as myofiber width, strongly suggest that oxidative stress is one of the culprits involved in the development of cardiac hypertrophy. The reduction in the nuclear size observed in the present study with no significant change in the cardiac nuclear count was suggestive of nuclear condensation which might indicate an early sign of pyknosis without any cell proliferation. It could be concluded that HPO diet had induced cardiomyopathy which might lead to cardiovascular dysfunction. Nonetheless, the nuclear count was unaffected, it was positively correlated to the nuclear size, while the nuclear size was negatively correlated to the blood pressure and the myofiber area.

Supplementation of VCO was able to prevent the histopathological changes in the cardiomyocytes. The cardioprotective effect of the supplement was most likely at the earlier stage that was by preventing the development of hypertension, attributable to its high antioxidant content. In addition to the antioxidant, VCO also contains almost 50% of lauric acid, a saturated fatty acid. The consumption of the fatty acid was shown to decrease the ratio of total cholesterol to high-density lipoprotein cholesterol (TC:HDL) in a study (Micha & Mozaffarian, 2010). While another study demonstrated that patients with heart failure together with metabolic syndrome had a lower proportion of lauric acid in addition to a higher proportion of dihomo-γ-linolenic acid when compared with heart failure patients without metabolic syndrome (Lee et al., 2012). These reports might portray the beneficial effects of the lauric acid, hence the VCO on cardiovascular disease as well as metabolic syndrome.

In conclusion, our study has demonstrated the cardioprotective effect of VCO supplementation against the development of cardiac remodeling, possibly by counteracting the blood pressure-raising effect induced by dietary repeatedly HPO, and thus preventing the increase in cardiac oxidative stress, ACE activity, and related cardiac histomorphometric changes.

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Declaration of interest

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