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

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**Abstract:** Virgin coconut oil, rich in antioxidants was shown to attenuate hypertension. This study aimed to investigate the effects of virgin coconut oil on blood pressure and related parameters in kidneys in rats fed with five-time-heated palm oil. Thirty-two male Sprague-Dawley rats were divided into four groups. Two groups were fed with five-time-heated palm oil (5HPO) (15%) diet and the second group was also given virgin coconut oil (1.42 ml/kg, oral) daily for 16 weeks. The other two groups were given basal diet without (control) and with virgin coconut oil. Systolic blood pressure was measured pre- and post-treatment. After 16 weeks, the rats were sacrificed and kidneys were harvested. Dietary 5HPO increased blood pressure, renal thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO) contents, but decreased heme oxygenase (HO) activity. Virgin coconut oil prevented the increases in 5HPO-induced blood pressure and renal NO content as well as the decrease in renal HO activity. The virgin coconut oil also reduced the elevation of renal TBARS induced by the heated oil. However, neither dietary 5HPO nor virgin coconut oil affected renal histomorphometry. In conclusion, virgin coconut oil has a potential to reduce the development of hypertension and renal injury induced by dietary heated oil, possibly via its antioxidant protective effects on the kidneys.

Key words: virgin coconut oil; hypertension; kidney; heme oxygenase; nitric oxide.
Introduction

Cardiovascular disease has been implicated as the major cause of morbidity and mortality globally. This disease includes hypertension, ischemic heart disease, and heart failure. Hypertension may cause pathological changes in the blood vessels (Sollinger et al. 2014), heart (Kamisah et al. 2015) and renal system (Javkhedkar et al. 2015), which are believed via oxidative stress mechanisms. Kidney is one of the organs that are involved in blood pressure regulation through renin-angiotensin-aldosterone system (Schweda 2015). It was noted that reductions in renal nitric oxide bioavailability, a vasodilator (Chien et al. 2014) and heme oxygenase activity together with augmented oxidative stress (Lu et al. 2013) were associated with elevation of blood pressure in experimental animals.

One factor that contributes to the development of hypertension is consumption of repeatedly heated oil, which was demonstrated in experimental animals (Ng et al. 2012a). It has been used as an established animal model for hypertension (Leong et al. 2010; Ng et al. 2012b). The use of repeatedly heated vegetable oil has been a common practice in food preparation to save cost (Abdullah et al. 2010). Oils which are heated repeatedly undergo thermal oxidation and produce many oxidative byproducts (Mozaffarian et al. 2006).

Many plants with antioxidant property have been shown to exert protective effect against hypertension-induced changes in the kidneys (Sanae and Yasuo 2013; Moodley et al. 2014). Virgin coconut oil is one of the plant products that has been demonstrated to have beneficial effects in hypertensive animals (Nurul-Iman et al. 2013; Kamisah et al. 2015). It is produced using a cold process resulting an oil with a high retention in antioxidant content (Marina et al. 2009). Based on this information, the aim of the study was to investigate the effects of the oil on renal parameters in hypertensive rats induced by dietary repeatedly heated palm oil.
Materials and methods

Diet preparation

The diet was prepared according to the method described by Leong et al. (2012). Briefly, a kilogram of peeled and cut potatoes were fried in 2.5 L palm oil (Cap Buruh, Malaysia) in a stainless steel wok at 180°C for 10 min. To produce five-time-heated palm oil, the same frying process was repeated for another four times, with a cooling interval of at least five hours between frying. An amount of 150 g of five-time-heated palm oil was added into 850 g ground rat chow and then remolded into pellets before left to air dry overnight.

Experimental design

Thirty-two male Sprague-Dawley rats (Laboratory Animal of Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia) were randomly divided into four groups. Two groups were fed with five-time-heated palm oil for 16 weeks, while the other groups were only given rat chow. One group from each diet was administered 1.42 ml virgin coconut oil daily by oral gavage concurrently with or without five-time-heated palm oil diet. The dose of virgin coconut oil used was based on the recommended minimum daily intake of the oil in human which was equal to one tablespoon (10 ml) (Organic Gain Sdn. Bhd., Mentakab, Malaysia). Systolic blood pressure was monitored pre- and post-treatment via tail-cuff method using Powerlab data acquisition systems (ADInstruments, NSW, Australia) in pre-warmed rats. After 16 weeks, the rats were humanely sacrificed under ketamine (10 mg/kg)-xylazil (80 mg/kg) cocktail anesthesia (intraperitoneum) and kidneys were harvested. The experimental design and animal handling procedures were approved by the institutional Animal Ethics Committee (Qodriyah/14-July/309-August- 2010-August-2011).
Measurement of renal lipid peroxidation

Lipid peroxidation byproducts measured by thiobarbituric acid reactive substance (TBARS) was determined based on the method described by Ledwozyw et al. (1986) with some modification. Briefly, kidney homogenate (0.5 ml) was added to 2.5 ml trichloroacetic acid (1.22 M) in 37% HCl and incubated for 15 min at room temperature. The mixture was then mixed with 1.5 ml 67% thiobarbituric acid in 0.05 M NaOH before incubation at 100°C in a water bath for 30 min. After cooling, 4 ml n-butanol was added and the mixture was vigorously vortexed. It was then centrifuged at 3000 rpm for 10 min. The absorbance upper layer was measured using a UV spectrophotometer (Shidmadzu, UV-160A Japan) at 532 nm against 1,1,3,3-tetraethoxypropane standard curve. The results were expressed as nmol/g wet weight.

Measurement of renal nitric oxide content

Renal homogenate was prepared following a method by Mansour et al. (2011). Kidney was homogenized in three times volume of KCl (1.17% w/v, pH 7.4) before being centrifuged at 800 rpm at 4°C for 5 min. Supernatant produced was then recentrifuged at 10,000 rpm at 4°C for 20 min. The newly obtained supernatant was deproteinized by an addition of absolute ethanol (1:2). The nitric oxide content in the renal homogenate was indirectly estimated using Griess method by measuring its main metabolites, nitrite and nitrate. The mixture was then vortexed and centrifuged at 4,600 rpm at 4°C for 10 min. The absorbance of the supernatant was read at 540 nm (Miranda et al. 2001; Ghasemi et al. 2007).

Measurement of renal heme oxygenase activity

Heme oxygenase activity was determined based on the measurement of total bilirubin produced following a method described by Vera et al. (2006). Renal cortex was homogenized
in phosphate buffer (0.25 mM, pH 7.7) containing 0.25 M sucrose, 1 mm EDTA and 1 mM phenylmethylsulphonyl fluoride before being centrifuged at 13,000 rpm at 4°C for 15 min. Reaction was started by adding 5 mg protein of sample into a reaction mixture containing 2 mM glucose-6-phosphate, 0.2 unit glucose-6-phosphate dehydrogenase, 0.8 mM NADP and 20 µM hemin. The mixture was then incubated at 37°C in dark for one hour before addition of 1 ml of chloroform to extract the formed bilirubin. The samples were centrifuged at 7,000 rpm for 10 min and the change in optical density of the chloroform layer was read at 464-530 nm. The heme oxygenase activity was calculated using an extinction coefficient of 40 mM/cm for bilirubin.

**Measurement of renal histomorphometry**

Kidney samples were fixed in phosphate formalin buffer solution (10%) for a day, before being processed for histological sectioning using serial dehydration in alcohol. The kidneys were blocked in paraffin and sectioned at 5 μM before mounted on slides which later were stained with hematoxylin and eosin. The slides were read under 200X to measure luminal diameters of distal and proximal tubules using an image analyzer (ImagePro, USA) using a 3x3 grid method which was superimposed over images. Bowman space area, glomerular number and area were measured under 100X. Nine measurements from each kidney sample from three slides by two blinded assessors were averaged to obtain individual values.

**Statistical analysis**

All data were tested for normality test using Shapiro–Wilk test. As the data were all normally distributed, they were analyzed using a one way ANOVA followed by Tukey post-hoc test. The changes in systolic blood pressure pre- and post-treatment were analyzed using
paired Student’s t-test (SPSS version 19.0, Chicago, USA). Values of p less than 0.05 were considered significant. The data are presented as mean ± standard error mean.

RESULTS

Effect of virgin coconut oil on systolic blood pressure in heated oil fed rats

A significant increase in systolic blood pressure after 16 weeks was observed in rats fed with heated oil (5HPO) compared with the control (128.45 ± 1.57 vs 78.90 ± 1.78 mmHg, n=8, p < 0.05). While in rats given 5HPO and virgin coconut oil (81.69 ± 0.56 mmHg, n=8, p < 0.01), there was a significantly lower blood pressure compared with the 5HPO group, and was comparable with the control and virgin coconut oil groups (Fig. 1).

Effect of virgin coconut oil on renal TBARS content in heated oil fed rats

TBARS content was increased significantly in heated oil fed group compared with the control group (573.28 ± 37.03 vs 111.55 ± 16.34 nmol/g, n=8, p < 0.001). Virgin coconut oil supplementation decreased the content of renal TBARS in the group fed with 5HPO diet (416.02 ± 20.49 nmol/g, n=8). However, TBARS content in 5HPO+virgin coconut oil and virgin coconut oil only groups were significant higher than the control group (p < 0.01) (Fig. 2).

Effect of virgin coconut oil on renal nitric oxide content in heated oil fed rats

Dietary 5HPO for 16 weeks increased renal nitric oxide significantly (641.11 ± 64.39 vs 407.32 ± 32.68 µmol/g, n=8, p < 0.05) compared to the control group. Supplementation with virgin coconut oil decreased the renal nitric oxide content significantly in rats that were given 5HPO diet (375.53 ± 22.90 µmol/g, n=8). The nitric oxide content in the virgin coconut oil-
supplemented groups with or without 5HPO were comparable with the control group ($p > 0.05$) (Fig. 3).

**Effect of virgin coconut oil on renal heme oxygenase activity in heated oil fed rats**

Consumption of 5HPO significantly reduced heme oxygenase activity compared with the control group, which was prevented with the virgin coconut oil supplementation ($11.08 \pm 3.13$ vs $37.09 \pm 4.65$, n=8 µmol/mg/h, n=8, $p < 0.05$). There was also no significant difference observed between the supplemented group fed heated oil and the control. However, there was a significant rise of the enzyme in the group given virgin coconut oil only compared with the control ($57.81 \pm 4.59$ vs $36.05 \pm 6.46$ µmol/mg/h, n=8, $p < 0.05$) (Fig. 4).

**Effect of virgin coconut oil on renal histomorphometry in heated oil fed rats**

Fig. 5 shows sections of renal cortex in all groups, comprising renal corpuscles and convoluted tubules. These sections showed normal histology of renal corpuscles which formed of glomerular tuft of capillaries surrounded by Bowman’s capsule with outer layer of simple squamous epithelium. Normal histological appearance of distal and proximal tubules was also observed. No prominent histological changes were seen between the groups. Table 1 shows histomorphometric parameters measured in the kidneys which were glomerular number and area, Bowman’s space area, luminal diameter of distal and proximal tubules. No significant difference in these parameters was seen among the groups.
DISCUSSION

Hypertension is one of major causes of premature death globally. It may cause end organ injury such as to the heart, brain and kidneys. One of the modifiable risk factors for hypertension is consumption of unhealthy diet like repeatedly heated vegetable oil. In this study, intake of 5HPO diet for 16 weeks elevated systolic blood pressure significantly, as similarly reported (Leong et al. 2010; Ng et al. 2012a). On repeated heating, the oil is thermally oxidized producing many oxidative products such as peroxides and aldehydes which can be absorbed into the fried foods (Choe and Min 2006). Consumption of heated palm and soy oils decreased vasorelaxation and increased vasoconstriction responses, leading to an increase in blood pressure in rats (Leong et al. 2010; Nurul-Iman et al. 2013). Intake of repeatedly heated canola oil also reduced vasorelaxation and caused early endothelial dysfunction in rats, possibly mediated by peroxynitrite formation in the aorta (Bautista et al. 2014). Collectively, intake of repeatedly heated oils causes vascular endothelial dysfunction, probably mediated by oxidative stress. The blood pressure-raising effect induced by the heated oil was associated with increased renal oxidative stress in the current study. Heating increased free radicals and reduced antioxidant contents in the oil, which contributed to the elevated oxidative stress (Jaarin and Kamisah 2012).

In the present study, virgin coconut oil prevented the increase in blood pressure induced by the heated oil diet after 16 weeks. It was shown to decrease aortic vasoconstrictory response in rats given heated oil (Nurul-Iman et al. 2013), therefore reducing mean arterial pressure without affecting baroreflex gain in spontaneous hypertensive rats (Alves et al. 2015). The beneficial effects of the virgin coconut oil might be attributable to its high antioxidant content in particular polyphenolic content (Marina et al. 2009) that combat the oxidative stress in the kidneys. The oil has been demonstrated to improve antioxidant status in kidneys (Arunima and Rajamohan 2013) and heart (Kamisah et al. 2015). However, the
renal TBARS in the group given virgin coconut oil only was significantly higher than the control group but comparable to the supplemented group fed heated oil. A similar trend of lipid peroxidation was not seen in the heart as recently reported (Kamisah et al. 2015). The increase in the group is not explainable since other parameters of the group showed positive effects of the oil.

Increased oxidative stress induced by heated oil diet augmented renal nitric oxide content which was in agreement with other models such as in renal ischemia-reperfusion injury (Sezgin et al. 2013) and cisplatin-induced renal injury (Yousef and Hussien 2015). The heated oil diet increased the nitric oxide content possibly by downregulating endothelial nitric oxide synthase (eNOS) that physiologically synthesizes nitric oxide, and upregulating inducible nitric oxide synthase (iNOS) which activity is induced by oxidative stress (Sarath et al. 2014). Increased activity of iNOS also augments free radical production, which couples with nitric oxide forming peroxynitrite (Cook 2006). Therefore, excess synthesis of nitric oxide is not favorable despite of its physiological property in vasodilation. However, a few other studies showed reduction in nitric oxide bioavailability in presence of oxidative stress (Nurul-Iman et al. 2013; Senbel et al. 2014). It could be that the amplitude of oxidative stress in the rat kidneys in the current study was more, which then triggered the stimulation of iNOS.

Virgin coconut oil prevented the heated oil-induced renal nitric oxide increase in this study. It could be that the virgin coconut oil exerted its protective effect by reducing the activity of iNOS via its antioxidant property (Vysakh et al. 2014) afforded by major polyphenols (ferulic and p-coumaric acids) present, therefore reducing the nitric oxide content and associated production of free radicals. Both ferulic and p-coumaric acids inhibited the expression of iNOS at the transcriptional level (Lampiasi and Montana 2015; Yoon et al. 2015).
Heated oil-induced oxidative stress decreased renal heme oxygenase activity. A similar decrease was also reported in plasma in rats fed heated oils (Leong et al. 2010). Increased activity of the enzyme increases formation of bilirubin (Kamisah et al. 2014), an antioxidant and carbon monoxide, a potent vasodilator and anti-inflammatory agent. These byproducts are believed to play a role in attenuating hypertension (Cao et al. 2009). Therefore, reduced activity of heme oxygenase in heated oil-fed rats indicates increased oxidative stress as observed in the elevated renal TBARS, and reduced vasodilatory effects of its byproducts. This might explain the increase in blood pressure in the rats. Virgin coconut oil supplementation on the other hand, was able to restore the enzyme activity to the level comparable to that of the control. However, so far studies regarding the effects of virgin coconut oil on this enzyme are still lacking. In rats given virgin coconut oil without heated oil, the renal heme oxygenase activity was significantly higher than the control. The positive effect of the virgin coconut oil on the heme oxygenase activity could be attributable to its high content of polyphenols which have good antioxidant properties (Pragasam et al. 2012; Wang et al. 2012). Ferulic acid and p-coumarin were able to upregulate the expression of heme oxygenase-1 and it is believed that the antioxidant property of these components is mediated by this mechanism (Yeh et al. 2009; Fetoni et al. 2010).

Neither dietary heated oil nor virgin coconut oil supplementation affected the renal histomorphometry. All structures in renal cortex appeared normal. No shrunken or congested glomeruli, widened Bowman’s space or dilated lumens of distal and proximal tubules were noted. Both treatments had no effect on kidney structures despite of their observed effects on biochemical parameters. This could be that the biochemical changes were more sensitive and had taken place earlier than the histological changes. As previously reported, virgin coconut oil did not interfere with food intake in rats, despite of a reduction in body weight compared
with the control (Nurul-Iman et al. 2013). Therefore, it was not suggestive that the observed beneficial effects of virgin coconut oil were due to decreased caloric intake.

**Conclusion**

Based on the obtained findings, virgin coconut oil supplementation for 16 weeks was able to reduce the adverse changes in rat kidneys induced by five-time-heated palm oil. It is suggestive that the virgin coconut oil has a potential renoprotective effects in hypertensive rats possibly by reducing oxidative stress via its high antioxidant contents.

**Conflict of Interest**

The authors declare no conflict of interest.

**Acknowledgment**

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Table 1. Renal histomorphometry in rats given dietary heated palm oil (5HPO) and treated with oral virgin coconut oil (VCO) for 16 weeks

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<th>Control</th>
<th>VCO</th>
<th>5HPO</th>
<th>5HPO+VCO</th>
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<tr>
<td>Glomerular area (µm²)</td>
<td>8133.42 ± 694.01</td>
<td>8180.74 ± 316.66</td>
<td>8394.19 ± 646.66</td>
<td>8300.23 ± 195.29</td>
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<tr>
<td>Glomerular number (per µm²)</td>
<td>16.88 ± 0.89</td>
<td>17.25 ± 0.56</td>
<td>17.13 ± 0.97</td>
<td>17.29 ± 0.42</td>
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<tr>
<td>Bowman space area (µm²)</td>
<td>2311.12 ± 269.28</td>
<td>2577.68 ± 136.31</td>
<td>2487.04 ± 249.13</td>
<td>2525.67 ± 84.93</td>
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<tr>
<td>Luminal diameter of proximal tubule (µm)</td>
<td>21.95 ± 1.50</td>
<td>21.35 ± 0.69</td>
<td>21.11 ± 0.62</td>
<td>20.29 ± 0.68</td>
</tr>
<tr>
<td>Luminal diameter of distal tubule (µm)</td>
<td>19.51 ± 1.30</td>
<td>18.80 ± 0.58</td>
<td>19.18 ± 0.41</td>
<td>18.20 ± 0.48</td>
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No significant difference among the groups was noted.
FIGURE CAPTIONS

Fig. 1. Systolic blood pressure in rats fed five-time-heated palm oil (5HPO) and treated with virgin coconut oil (VCO, 1.42 ml/kg, orally) for 16 weeks. Bars represent mean ± SEM (n=8). *vs the control (p < 0.05), #vs 5HPO (p < 0.01).

Fig. 2. Lipid peroxidation content (TBARS) in the kidney of rats fed five-time-heated palm oil (5HPO) and given virgin coconut oil (VCO, 1.42 ml/kg, orally) for 16 weeks. Bars represent mean ± SEM (n=8). *vs the control (p < 0.05), #vs 5HPO (p < 0.05).

Fig. 3. Renal nitric oxide (NO) content in rats fed five-time-heated palm oil (5HPO) and treated with virgin coconut oil (VCO, 1.42 ml/kg, orally) for 16 weeks. Bars represent mean ± SEM (n=8). *vs the control (p < 0.05), #vs 5HPO (p < 0.05).

Fig. 4. Renal heme oxygenase (HO) activity in rats fed five-time-heated palm oil (5HPO) and treated with virgin coconut oil (VCO, 1.42 ml/kg, orally) for 16 weeks. Bars represent mean ± SEM (n=8). *vs the control (p < 0.05), #vs 5HPO (p < 0.05).

Fig. 5. Sections in the renal cortex of control (A), virgin coconut oil (B), five-time-heated (5HPO) without (C) and virgin coconut oil (5HPO+VCO) (D) groups after 16 weeks of treatment, showing a renal corpuscle with glomerulus (G) surrounded by Bowman’s space (Bs) and capsule (yellow arrow), together with distal tubules (DT) and proximal tubules (PT) at 200X magnification.
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296x419mm (300 x 300 DPI)
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210x148mm (300 x 300 DPI)