Evaluation of Anti-Atherosclerotic Activity of Virgin Coconut Oil in Male Wistar Rats Against High Lipid and High Carbohydrate Diet Induced Atherosclerosis


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Abstract
The aim of this study was to analyze the effect of virgin coconut oil (VCO) on high lipid diet (HLD) and high carbohydrate diet (HCD) induced atherosclerosis in male Wistar rats. Spectrophotometer was used to determine the lipid parameters by enzymatic endpoint method. The plasma total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL) levels were measured using commercial enzymatic kits. The results showed that feeding with normal and VCO diet significantly decrease (p<0.05) body weight when compared to the control group rats. Body weight and lipid profile were estimated after 8 weeks of treatment. VCO showed a significant (p<0.05) reduction in TC, TG, Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) levels and significant (p<0.05) increase in HDL in HCD /HLD group rats. There was significant decrease in atherogenic index (AI) (p<0.05) of all VCO treated groups when compared to the Control group. Hence, there was increase in the percentage of protection in VCO treated animals after 8 weeks. The decrease of lipid profiles in the experimental rats showed that VCO possesses anti-atherosclerotic activity.

Keywords: Virgin coconut oil, Atherosclerosis, Diet, Atherogenic index

1 Introduction
Atherosclerosis is an arterial disease caused by chronic inflammatory response of white blood cells. The atheroma contains remnants of cellular debris in the form of dead cells, triglycerides, cholesterol and calcium in the crystallized form. LDL carrying triglycerides and cholesterol promote the atherogenesis, while the functional HDL prevents the hardening of arteries by reducing the formation of arterial atheromatous plaques. Atherosclerosis remains asymptomatic for many decades; hence, it is called clinically silent disease, as the person suffering from this arterial disorder does not know about any drastic changes in arteries. Atherosclerosis is the leading cause of myocardial infarction, stroke, heart failure and coronary artery disease (CAD). CAD and atherosclerosis causes many deaths in developed countries. Atherosclerosis develops progressively in elastic and muscular arteries, of medium and large sized characterized by focal intimal lesions called atheromas or atherosclerotic plaques that protrude into vessel lumen and eventually leading to various complications. There are a number of environmental, genetic and metabolic factors involved in the formation and evolution of the atherosclerotic plaque.

Practicing regular exercise and stopping smoking alleviates cardiovascular diseases including atherosclerosis. Diet change helps to prevent the development of atherosclerosis. It is suggested that Mediterranean diet containing fiber food and unsaturated oils improves cardiovascular outcomes and is better than a low fat diet. Studies show that herbs, spices and diets rich in fruits and vegetables result in less number of cardiovascular diseases and deaths.

Virgin coconut oil consists of 92% of saturated, 6% of monounsaturated and 2% of polyunsaturated fatty acids. 10% long chain and 90% medium chain saturated fatty acids constitute the virgin coconut oil. These fatty acids are easily absorbed and used as energy for metabolism, thus increasing the metabolic activity, hence, it can help protect the body from disease and accelerate healing.
The purpose of this study was to investigate the effectiveness of VCO, and to analyze its protective effect against the risk factors of atherosclerosis i.e., TC, HDL, LDL, VLDL, TG in rats, fed with HCD and HLD.

2 Material and Methods

2.1 Experimental animal

The study was approved by animal ethics committee of the management and science university (MSU), Shah Alam, Malaysia. VCO was purchased from Organic Gain Sdn. Bhd., Bandar Baru Bangi, Selangor, Malaysia. The pellets BR-II were used as the animal food. Sixty male Wistar rats weighing 200-220 g of 2 months old were obtained from the Laboratory Animal House of the University. Animals were randomly divided equally into six experimental groups comprising of ten animals each (n =10 per group). After 2 weeks of acclimatization, each group of rats was fed on the following diets: Group 1 was fed the normal pellet diet (control), Group 2 was administered normal diet with VCO (1 ml/day), Group 3 named as HCD received bread with pellet, Group 4 animals received HCD bread and pellet with VCO (1 ml/day), Group 5 called as HLD animals received cheese with pellet, Group 6 named HLD animals received cheese and pellet with VCO (1 ml/day). All groups had free access to diets and water ad libitum for 10 weeks.

2.2 Determination of dosage and VCO delivery

VCO was administered by oral gavage at a dose of 1.42 ml/kg according to the minimal recommended dose of 10ml/per day in humans. VCO given to human therapy is 3 tablespoons or equal to 45 ml/day. When converted to rat, it was 0.018 X 45 = 0.81 ml/200 g BW/day or equal to 1.09 ml/270 g BW/day. In this study dose was 1 ml/day/270 g BW.

2.3 Plasma lipid analyses

Spectrophotometer was used to measure the blood cholesterol levels by Enzymatic Endpoint Method. The plasma TC, TG and HDL levels were measured using commercial enzymatic kits (Human Gesellschaft fur biochemical und Diagnostic ambh, Germany). LDL was estimated by Friedewald equation:

\[ \text{LDL (mg/dl)} = \text{TC−HDL−TG}/5 \]

VLDL level was calculated using the following equation:

\[ \text{VLDL (mg/dl)} = \text{TG}/5 \]

Atherogenic Index was calculated by using the formula. Atherogenic Index (AI) = Total Cholesterol – HDL/HDL

Percentage of protection was calculated based on Dhandapani R.

2.4 Statistical analysis

Statistical analysis was performed using SPSS for Windows version 21. Mean values of the findings were compared among and between groups. Analysis of variance (ANOVA) and unpaired “t” test was performed to assess the significance among the groups and between groups respectively. Pearson correlation coefficient test was performed to evaluate the correlation of biochemical parameters with the severity of atherosclerosis. ‘p’ value <0.05 was considered significant.

3 Results

3.1 Body weight in albino Wistar rats

There was significant decrease in body weight of the (Normal +VCO) treated group (188.32 g±12.08) when compared to the normal control group (266.80 g ± 18.51) at week 8 (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wt (g) Week 8</th>
<th>(AI) Week 8</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N)</td>
<td>266.55±11.34</td>
<td>3.19±0.05</td>
<td>--</td>
</tr>
<tr>
<td>N+VCO</td>
<td>187.45±7.33*</td>
<td>0.50±0.15*</td>
<td>84.32%</td>
</tr>
<tr>
<td>HCD</td>
<td>274.61±2.87</td>
<td>2.54±0.26</td>
<td>--</td>
</tr>
<tr>
<td>HCD+VCO</td>
<td>218.43±4.38</td>
<td>0.63±0.15*</td>
<td>59.87%</td>
</tr>
<tr>
<td>HLD</td>
<td>285.50±2.84</td>
<td>3.13±0.15</td>
<td>--</td>
</tr>
<tr>
<td>HLD+VCO</td>
<td>196.16±34.48</td>
<td>1.17±0.14*</td>
<td>64.26%</td>
</tr>
<tr>
<td>F</td>
<td>7.759</td>
<td>56.067</td>
<td>--</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.000</td>
<td>&lt;0.000</td>
<td>--</td>
</tr>
</tbody>
</table>

Atherogenic index in ratio

3.2 Lipid profile

Plasma TC, TG, VLDL and LDL levels were found to be significantly increased in rats fed with normal diet, HCD and HLD at the end of 8 weeks. A significant reduction in TC, TG, VLDL and LDL levels was observed with all treated VCO (1mg/day) used groups i.e. N+VCO, HCD+VCO and HLD+VCO. Data also show that rats fed with N, HCD and HLD diet had significant decrease in plasma level of HDL at UK J Pharm & Biosci, 2015: 3(2); 11.
p<0.05 as compared to the rats fed with N+VCO, HCD+VCO and HLD+VCO (Table 2).

3.4 Atherogenic index (AI)

Tabulated results demonstrated that the VCO treated all animal groups showed the significantly decrease (p<0.05) in the AI when compared to the control groups and increase in the percentage of protection in all VCO treated groups after week 8 (Table 2).

4 Discussions

So far there had been no solid foundation and scientific evidence suggesting that VCO possessed any anti-atherosclerotic activity. In present study, the anti-atherosclerotic activity of VCO was evaluated in rats receiving HLD and HCD. Determination of body weight in experimentally induced atherosclerosis is considered to be a positive factor to find out the prognosis of disease. Results indicated increase in body weight of animals from the beginning to the end of the experiment in all six groups, but at the end, increase in body weight is low in VCO treated groups as compared to control group. In this study, it was found that food intake in the VCO diet was lower compared to the control group. However, we observed that there was no significant difference in body weight between the HCD, HLD diet and the normal control group at 8 weeks. Leong et al showed the same result, although the food intake of VCO and control diet was same, yet, the VCO diet group was found to have decreased body weight compared to the control group at 8 weeks. HLD+VCO diet group experienced reduced body weight compared to the control group at 8 weeks. This illustrates that VCO administration decreases the body weight. Studies show that VCO reduces the abdominal obesity in human beings.

Table 2: Effect of Virgin coconut oil on plasma lipid profile in diet-induced atherogenic albino Wistar rats after 8 weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl) week 8</th>
<th>TG (mg/dl) week 8</th>
<th>HDL(mg/dl) week 8</th>
<th>VLDL(mg/dl) week 8</th>
<th>LDL(mg/dl) week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N)</td>
<td>80.25±1.30</td>
<td>213.40±43.56</td>
<td>11.02±0.40</td>
<td>42.67±8.71</td>
<td>11.86±1.87</td>
</tr>
<tr>
<td>N+VCO</td>
<td>53.99±7.96</td>
<td>78.85±13.06</td>
<td>36.51±3.76</td>
<td>15.76±2.61</td>
<td>2.40±0.03</td>
</tr>
<tr>
<td>HCD</td>
<td>103.34±1.16*</td>
<td>212.08±8.58</td>
<td>29.16±0.31*</td>
<td>44.61±0.64</td>
<td>42.99±1.35*</td>
</tr>
<tr>
<td>HCD+VCO</td>
<td>75.41±3.07</td>
<td>115.44±4.30</td>
<td>46.08±3.61*</td>
<td>23.08±0.86</td>
<td>11.07±5.52</td>
</tr>
<tr>
<td>HLD</td>
<td>110.41±26.83</td>
<td>223.97±69.40</td>
<td>22.82±1.18*</td>
<td>44.79±13.88</td>
<td>51.74±3.90*</td>
</tr>
<tr>
<td>HLD+VCO</td>
<td>75.78±4.56</td>
<td>109.75±28.67</td>
<td>36.30±1.72*</td>
<td>21.94±5.73</td>
<td>32.07±9.29</td>
</tr>
<tr>
<td>F</td>
<td>3.119</td>
<td>3.191</td>
<td>28.300</td>
<td>3.415</td>
<td>17.157</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.049</td>
<td>&lt;0.046</td>
<td>&lt;0.000</td>
<td>&lt;0.038</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN ± SEM; n=5 P < 0.05, * Significant difference from control group at P<0.05

Results in the present study revealed that rats on HCD and HLD diet showed a significant increase in plasma TC, TG, LDL, VLDL and a significant decrease in HDL compared with normal rats (p < 0.05). Our observation was consistent with the reports of Azonov et al. and Sunder et al. Elevated serum cholesterol, TG and VLDL, LDL and decreased HDL have been implicated in the etiology of cardiovascular diseases (CHD). High serum lipids (TG, and VLDL, LDL) contributed to the development of cardiovascular diseases in various ways. According to Gokkusu and Mostafazadeh, Standard VCO dose of 1 mg/day administered along with normal pellet diet, showed a significant decrease (p < 0.05) in all the lipid parameters while there was a significant (p < 0.05) increase in HDL level. It was also seen that VCO along with HCD and HLD fed animals, showed a significant decrease in all the lipid parameters (p < 0.05) with a significant rise in HDL (p < 0.05) level as compared to HCD and HLD control animals. According to Shah, lauric acid is the main medium chain fatty acid present in the VCO. Small sized medium chain fatty acids are easily absorbed through small intestine without enzymatic process. These fatty acids are carried to the liver blood flow to be metabolized and transported to the mitochondria without carnitine to produce energy quickly and efficiently, so they are not deposited as fat in the tissue. A total cholesterol /HDL ratio of ≤ 3 connotes a low risk, a ratio of around 4.5 an average risk and ratio of ≥8 a high risk of developing coronary artery disease. In our study, atherogenic index is decreased significantly (p < 0.05) in all VCO treated groups when compared with HLD and HCD group animals. A 1% decrease in HDL is associated with a 3-4% increase in the risk of heart disease. Our study showed that HDL increase in plasma results in equivalent increase in percentage of protection in atherosclerosis.

Previous studies also revealed decreased levels of lipids in rats fed with VCO than Copra oil (CO), olive oil and sunflower oil. Total polyphenol content of VCO (84 mg/100 g oil) and CO (64.4 mg/100 g oil) were estimated. VCO supplementation prevents the blood-

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pressure raising effect of five-time heated palm oil (5HPO). The blood-pressure lowering effect of VCO may be attributable to its high polyphenol component.26, 25 Furthermore, another study revealed that phenolic fraction of VCO contains higher levels of caffeic acid, P-coumaric acid, ferulic acid and catechin than CO.29 In addition, we reported previously that VCO contains significantly higher amounts of vitamin E (30.87 µg/100 g oil) than CO (12.76 µg/100 g oil) 30. There is presence of total tocopherols and tocotrienols31 including B carotene in VCO32. Studies show that increased concentration of B carotene increase the fecal secretion of bile acids and decreases the concentration of lipids.33 In addition, we reported previously that VCO contains increased levels of phytosterols30. It is clear that the phytosterols competitively blocks the absorption of cholesterol and increases fecal excretion of bile acids and neutral sterols for improving circulating lipid profiles to reduce the risk for CHD34. All these biologically active micronutrients present in VCO act synergistically and results in beneficial alteration in lipid levels.

5 Conclusions

Our study shows that VCO decreases body weight, TC and LDL, hence it prevents atherosclerosis in HCD and HLD fed male rats. The VCO had anti-obesity potential, although its effect varied among diets as its effect might be relatively lower in HCD induced obesity than in HFD induced obesity. This study indicates dietary VCO beneficially modulates the hepatic lipid metabolism, by regulating the synthesis and degradation of lipids, may be due to the difference in absorption, transport and catabolism of its constituent fatty acids as well as the higher amounts of biologically active un saponifiable minor components present in VCO. Our preliminary data strongly suggests that VCO with its high polyphenol content is capable of maintaining the normal levels of cholesterol and other lipid parameters in plasma and also increased the concentration of HDL cholesterol in male Wistar rats fed HCD and HLD.

6 Competing interests

There is no conflict of interest.

7 Author’s contributions

SB is the research student of masters in biomedicine. ZO and SR are supervisor and the co-supervisor respectively. HK helped in the animal handling. JK helped in dosage preparation. MH helped in biochemical analysis. Sundus helped in sample analysis. SAD and KM helped in the statistical analysis.

8 References

17. Liau KM, Lee YY, Chen CK, Rasool AH G. “An open-label pilot study to assess the efficacy and safety of virgin coconut oil in...


