Comparison of Pure Palm Olein Oil, Hydrogenated Oil-Containing Palm, and Canola on Serum Lipids and Lipid Oxidation Rate in Rats Fed with these Oils

Seyed-Asadollah Amini PhD¹, Keihan Ghatreh-Samani PhD², Arash Habibi-Kohi PhD¹, Laleh Jafari PhD¹

Abstract

Background: Due to increased consumption of canola oil and hydrogenated oil containing palm and palm olein, and their possible effects on serum lipoproteins, the present study was conducted to determine the effects of these oils on lipids and lipid oxidation level.

Methods: In this experimental study, 88 Wistar rats were randomly assigned to four groups. Control group (A) was on a normal diet. Groups B, C, and D, in addition to normal diet, were fed with hydrogenated oil-contained palm oil, pure palm olein oil, and canola oil, respectively for 4 weeks. Serum biochemical factors [total cholesterol (TC), triglyceride (TG), LDL, HDL, LDL/HDL ratio, oxLDL, paraoxonase-1 (PON1), and malondialdehyde (MDA)] were measured.

Results: The lowest mean serum TC was seen in the control group and the highest in the group B. There were differences in TC, TG, LDL, and PON1 between the control group and other groups (P<0.001). The lowest and highest LDL/HDL ratios were observed in the group C and the control group, respectively. Significant differences were seen in OxLDL and PON1 between the control group and other three groups (P<0.05), while there were no significant differences in oxLDL and PON1 among the other three groups (P>0.05). MDA was higher in groups C and D.

Conclusion: Canola oil, hydrogenated oil-containing palm and palm olein may increase atherosclerosis risk through decreasing PON1 activity and elevating oxLDL. Palm olein oils in rats’ diets cause a considerable decrease in LDL and help to increase HDL.

Keywords: HDL, malondialdehyde, oxidized LDL, palm olein, paraoxonase1

Cite this article as: Amini SA, Ghatreh-Samani K, Habibi-Kohi A, Jafari L. Comparison of Pure Palm Olein Oil, Hydrogenated Oil-Containing Palm, and Canola on Serum Lipids and Lipid Oxidation Rate in Rats Fed with these Oils. Arch Iran Med. 2017; 20(2): 96 – 100.

Introduction

Atherosclerosis is a main cause of mortality worldwide. Low high-density lipoprotein (HDL) is an independent risk factor for atherosclerosis.¹ HDL carries cholesterol from tissues to liver.² ³ This lipoprotein also contributes to the inhibition of low-density lipoprotein (LDL). Oxidized LDL (oxLDL) is thought to play the main role in development and progression of atherosclerosis.⁴ The antioxidant role of HDL is mainly attributed to paraoxanase-1 (PON1) which is a main component of HDL. PON1 can inactivate the toxic products resulting from oxidizing LDL and lipid components. In vitro studies have indicated that PON1 prevents the accumulation of peroxide lipids and suppresses LDL conversion into oxLDL.⁵ ⁶ In recent years, use of palm oil has been on the rise despite debates over its unhealthy effects. It is argued that palm oil is potentially unhealthy as it contains relatively high amounts of saturated fatty acids (SFAs), particularly palmitic acid, and can therefore increase the risk of coronary heart disease and some tumors.⁷ ⁸ Narong et al. studied the effect of palm oil-containing diet on oxidative stress and indicated that palm olein oil contributed to lowering blood pressure and venous thrombosis through preventing oxidative stress because of containing large amounts of fatty acids with a double bond, antioxidants, and vitamins.⁹

Few studies have been conducted to specifically investigate this effect. Despite containing large amounts of palmitic acid, palm oil has been shown to have a neutral effect on plasma cholesterol concentrations compared to olive oil.¹⁰ A study demonstrated that palm oil caused an increase in myocardial antioxidant enzymes which protect against isoproterenol-induced myocardial necrosis.¹¹ Mozaffarian’s study on trans fats and cardiovascular diseases indicated that use of trans fats increased the risk of cardiovascular diseases because of diminishing the activity of serum paraoxynagenase.¹² Canola is one of the most important oil seeds worldwide. Research has indicated that canola oil fatty acid has health benefits. Canola is rich in essential fatty acids and antioxidants and has the lowest (7%) SFA of all vegetable oils.¹³ Canola-containing diets have been demonstrated to lower cholesterolemia compared to diets with higher levels of SFAs. Studies on the effect of canola oil on heart coronary disease, insulin sensitivity, lipid peroxidation, inflammation, energy metabolism, and growth of cancer cells have indicated a considerable decrease in total cholesterol (TC) and LDL-C caused by use of canola oil compared to other diets containing fats.¹⁴ Van Jaarsveld et al. demonstrated that use of palm olein oil in a diet with moderate fat and cholesterol decreased the risk of atherosclerosis compared to lard oil and soybean oil included in a similar diet.¹⁵ Since canola oil has been recently recommended to people

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Accepted for publication: 21 December 2016

Original Article

Archives of Iranian Medicine, Volume 20, Number 2, February 2017
because of its optimal properties, many people are currently using hydrogenated and partly hydrogenated palm oil and/or palm olein oil, which are less expensive than other oils, and the effects of these oils on health, especially serum lipids and lipid oxidation rate, are being challenged due to lack of conclusive evidence, the present study was conducted to investigate this issue for the first time in Iran.

**Materials and Methods**

This experimental study was conducted in Shahrekord University of Medical Sciences in 2014–2015. The required sample size was estimated at 20 for each group, according to the sample size formula, to compare two means, considering 95% confidence interval and 80% test power. To deal with potential attrition, we assigned 22 rats to each group. In this study, male Wistar rats weighing 150–200 g were used. In order to adapt to the new environment, the rats were kept under appropriate temperature, moisture, and light conditions for two weeks, and then randomly assigned to four groups of 22 each, as follows:

Control group (A) received normal diet; treatment 1 group (B) received hydrogenated oil containing palm oil + normal diet; treatment 2 group (C) received pure palm olein oil + normal diet; and treatment 3 group (D) received canola oil + normal diet.

Rats that were used in all experiments were housed under controlled environmental conditions (16-h Light: 8-h Dark). The animals were permitted unlimited access to water and commercial standard laboratory chow (Pars Dam Co., Iran) which contains 46% NFC, 25% NDF, 19% protein and 10% lipid.

The three experimental diets contained similar amounts of chow and varied only in that one diet contained 25% crude palm oil, another contained 25% hydrogenated palm, and the third contained 25% canola oil in addition to a control group fed with chow alone.

The diets in different groups were similar for ingredients except the type of fat. By a dietitian’s calculation, fat comprised 25% of the total calories received per day. The diet was administered for four weeks. Blood samples were collected from rats’ heart at the end of the experiment and serum biochemical factors were measured.

Serum biochemical factors such as oxLDL, PON1, MDA, TC, LDL, HDL, and TG, were measured by auto analyzer (BT3000) and a commercially available kit (Pars Azmoon Co.) and LDL/HD ratio was calculated. oxLDL was measured by ELISA (Mercodia, Sweden). Data such as rats’ weight and biochemical factors were recorded before and after treatment. The data were analyzed using one-way ANOVA and LSD in SPSS 18.

**Results**

The findings for different parameters in the four groups were obtained as follows:

1- TC:
ANOVA results indicated that the mean TC concentration was significantly different among the four groups ($P < 0.001$) (Table 1). Furthermore, least significant difference (LSD) test indicated that the mean TC was different only in the group B, fed with hydrogenated oil-containing palm oil.

2- TG:
ANOVA indicated that the mean TG was significantly different between the control group and other groups ($P < 0.01$). LSD indicated that mean TG was significantly different between the groups C and D and the other two groups ($P < 0.05$). The mean TG was significantly different between the groups C and D, but no significant difference was seen in TG between the group B and the control group.

3- HDL:
The group B and the control group had the lowest HDL. ANOVA indicated that the mean HDL was significantly different among the four groups ($P < 0.01$). LSD indicated that the mean HDL was significantly different between the three case groups and the control group. The difference was more marked between the group B and other groups ($P < 0.001$).

4- LDL:
ANOVA indicated that the mean LDL was significantly different among the four groups ($P = 0.003$). LSD indicated that there were significant differences in LDL levels between the groups B and C and the control group, between the groups C and D and the group B, and between the group D and the group C ($P < 0.05$), but no significant difference was seen in LDL between the group D and the control group ($P = 0.292$).

**Table 1. Biochemical parameters in groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical parameters</th>
<th>Total cholesterol (mg/dL) Mean ± SD</th>
<th>Triglycerides (mg/dL) Mean ± SD</th>
<th>HDL (mg/dL) Mean ± SD</th>
<th>LDL (mg/dL) Mean ± SD</th>
<th>LDL/HDL ratio Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group: normal diet (group A)</td>
<td></td>
<td>72.5 ± 8</td>
<td>116.7 ± 35.1</td>
<td>34.8 ± 5.7</td>
<td>15.5 ± 7.3</td>
<td>0.46 ± 0.25</td>
</tr>
<tr>
<td>Hydrogenated oil-containing palm oil + normal diet (group B)</td>
<td></td>
<td>90.3 ± 16.2</td>
<td>113.7 ± 34.9</td>
<td>47.6 ± 9.2</td>
<td>19.9 ± 11.2</td>
<td>0.43 ± 0.27</td>
</tr>
<tr>
<td>net palm olein oil + normal diet (group C)</td>
<td></td>
<td>77.3 ± 9.9</td>
<td>173.6 ± 40.7</td>
<td>39 ± 4.9</td>
<td>9.5 ± 6.5</td>
<td>0.24 ± 0.16</td>
</tr>
<tr>
<td>canola oil + normal diet (group D)</td>
<td></td>
<td>76.9 ± 10.5</td>
<td>136.3 ± 31.8</td>
<td>38.4 ± 4.7</td>
<td>12.6 ± 8</td>
<td>0.32 ± 0.19</td>
</tr>
<tr>
<td>$P$-value **</td>
<td></td>
<td>0.001</td>
<td>0.005</td>
<td>0.006</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Standard deviation; **ANOVA test ($P < 0.05$: significant difference)
Effect of Palm Oil, and Canola on Serum Lipids

Table 2. PON1, MDA and oxLDL levels in groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>oxLDL (pg/dL)</th>
<th>MDA (mg/dL)</th>
<th>PON1 (μ/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group: normal diet (group A)</td>
<td></td>
<td>4.5 ± 1.4</td>
<td>0.59 ± 0.12</td>
<td>250.9 ± 122.2</td>
</tr>
<tr>
<td>Hydrogenated oil–containing palm oil + normal diet (group B)</td>
<td></td>
<td>9.2 ± 3.9</td>
<td>0.69 ± 0.14</td>
<td>115.8 ± 83.1</td>
</tr>
<tr>
<td>Pure palm olein oil + normal diet (group C)</td>
<td></td>
<td>7.8 ± 1.6</td>
<td>0.77 ± 0.07</td>
<td>120.5 ± 81.8</td>
</tr>
<tr>
<td>Canola oil + normal diet (group D)</td>
<td></td>
<td>7.5 ± 1.8</td>
<td>0.79 ± 0.14</td>
<td>131.4 ± 74.8</td>
</tr>
<tr>
<td>Statistical analysis &quot;&quot;</td>
<td></td>
<td>P = 0.008</td>
<td>P = 0.0004</td>
<td>P = 0.001</td>
</tr>
</tbody>
</table>
<pre><code>                                                                               | F = 4.24       | F = 6.69      | F = 10.67      |
</code></pre>

* Standard deviation; "" One-way ANOVA (P < 0.05: significant difference)

5- LDL/HDL ratio:
ANOVA indicated a significant difference in LDL/HDL ratio among the groups (P < 0.001). LSD indicated that LDL/HDL ratios of the group C and the other groups were significantly different (P < 0.001), but there was no significant difference in LDL/HDL ratio between the groups B and D, and the control group (P > 0.05).

6- oxLDL:
One-way ANOVA indicated that mean oxLDL was significantly different among the four groups (P = 0.008, F = 4.24) (Table 2). LSD indicated that oxLDL levels of the control group and the other three groups were significantly different (P < 0.05), and there were no significant difference in oxLDL among the other three groups (P > 0.05).

7- MDA:
One-way ANOVA indicated a significant difference in MDA among the four groups (F=6.69; P < 0.001). Furthermore, LSD indicated that MDA levels of the control group and group B were not significantly different (P = 0.15), but MDA levels of the groups A and C were significantly different (P < 0.001). In addition, there was a significant difference in MDA between the groups B and C (P = 0.027) and between the groups B and D (P = 0.024).

8- PON1:
One-way ANOVA indicated a significant difference in PON1 among the four groups (F = 10.67; P < 0.001). LSD indicated that the control group was the source of difference from the other three groups (P < 0.001), but no significant difference was seen in PON1 among the other three groups (P > 0.05).

**Discussion**

The main purpose of this study was to investigate the effect of pure palm olein oil and hydrogenated oil-containing palm and canola on serum lipids in rats fed with these oils. TC in the rats fed with normal diet (control group) was lower than the other three groups. Although the difference was significant among the four groups of the study, TC was significantly higher in the group fed with hydrogenated oil-containing palm oil compared to the other groups. As previously proposed, since saturated acids comprise 50% of palm oil-containing hydrogenated oil, it causes an increase in cholesterol.16 However, a lower TC level was seen in rats fed with pure palm olein oil. As demonstrated by different studies conducted worldwide, palm olein oil, due to its high levels of unsaturated fatty acids, does not lead to increased TC and even causes an increase in serum HDL in healthy people, which is effective on blood pressure and venous thrombosis through preventing oxidative stress.9,11

TG was higher in the group fed with pure palm olein oil than the other three groups. In this study, use of hydrogenated oil-containing palm oil had no considerable effect on serum TG, but contributed positively to HDL which is a beneficial lipoprotein, and the highest HDL was observed in this group. HDL in all groups fed with oil was higher than the control group, which is a useful finding on the combined oils used in human diets.

In the group fed with pure palm olein oil, the lowest LDL level was observed while the highest LDL was seen in the group fed with hydrogenated oil-containing palm oil. This is the most important finding of the present study as it represents that use of pure palm olein oil can considerably lower LDL, which is very useful particularly for patients with or predisposed to atherosclerosis.

In addition, use of pure palm olein oil may help to lower LDL level, which is one of the most important effective components of atherosclerosis.

In this study, the LDL level decreased in the group fed with palm olein by approximately two-folds compared to the group fed with hydrogenated oil-containing palm oil. Some studies have obtained similar findings on reduction of serum LDL in laboratory animals, especially rats.15,17

The lowest LDL/HDL ratio was observed in the group fed with palm olein and the highest LDL/HDL in the control group with a significant difference between the control group and the three case groups. However, several studies conducted worldwide have reported inconsistent findings. For example, Bosch et al. studied the variations in plasma lipoprotein after use of palm oil at various proportions, including 50% olein in margarine and mayonnaise, by a healthy population and 100% pure palm olein in control groups, and indicated no significant difference in serum lipids, with only the group using 100% palm oil exhibiting a partial increase in VLDL-C, which could be due to individual differences.

Furthermore, some studies have shown that all saturated vegetable oils such as coconut oil and palm olein affect serum lipids in different ways.18 In contrast, Marzuki et al. studied the variations in plasma lipoprotein after feeding with a diet with palm oil and soybean oil, and indicated that in healthy young people, the diet with palm olein oil or soybean oil caused no variations in serum cholesterol, LDL-C or HDL-C, while soybean oil-containing diet
caused a partial increase in LDL-C compared to the palm olein-containing diet in young people with hypercholesterolemia.\textsuperscript{19}

The study by Idris \textit{et al.} on a diet containing cholesterol and saturated trans fats and lipoproteins in monkeys indicated that use of trans fats caused an increase in LDL-C/HDL-C ratio and a decrease in apoA1 trans fats, leading to an increase in apoB/apoA1. However, palm olein caused an increase in apoA1.\textsuperscript{20}

In a review study by Fattore \textit{et al.}, the negative role of SFAs as a risk factor for cardiovascular diseases was reported to deserve further investigation and it was demonstrated that not only the type of fat but also triglyceride structure contributed to cholesterolemia. The role of diets containing palmitic acid and palm oil in developing the cancer is small, with no convincing evidence.\textsuperscript{7}

In the study by Sundram \textit{et al.}, feeding with palm oil, palm olein, and palm stearin caused an increase in HDL-C. Moreover, cholesterol was not significantly different between the rats fed with corn oil, palm oil and palm olein but was lower in the rats fed with soybean oil compared to those fed with corn oil and palm oil, palm olein, and palm stearin.\textsuperscript{17}

In Gullesserian’s study, use of canola oil in 17 children and adolescents caused a decrease in TG, TC, and LDL-C, but no significant changes in HDL-C and LP (a).\textsuperscript{21} The highest total mean concentration of TC can explain the inconsistency of the findings of Gullesserian’s study and the present study.

The effects of hyperlipidemia, cholesterol, and atherogenic lipoproteins (LDL and VLDL) have been established on development of atherosclerosis,\textsuperscript{22} and their dysfunction is a risk factor for cardiovascular diseases, diabetes, hypertension, and gastrointestinal diseases, which are due to these factors, are considered important public health issues.\textsuperscript{23} In the recent two decades, large studies have been conducted on use of antioxidants to prevent atherosclerosis and associated complications, and therefore it is considered an important health priority in many countries.\textsuperscript{24,25}

The second aim of this study was to investigate the effects of the studied oils on plasma lipids oxidation, oxLDL, paraoxonase-1, and MDA in rats.

At the completion of intervention, a significant difference was seen in oxLDL among the four groups, which was significantly higher in the groups fed with canola oil and pure palm olein. The findings indicated that feeding with palm olein oil and other oils cause an increase in oxLDL. Oil consumption increases oxidative stress, reduces antioxidant capacity and antioxidant enzyme activity like paraoxonase 1. Because oxLDL contributes to producing toxic compounds in vessels wall and atherosclerosis development,\textsuperscript{4} use of such oils may exacerbate atherosclerosis.

Moreover, there was a significant difference in MDA level between the control group and specific palm olein + canola-fed group. Serum MDA is an index to determine the oxidation rate of unsaturated fats in serum. Besides that, MDA can be used as an index to determine the risk of cardiovascular diseases. In the present study, MDA was higher in diets with unsaturated oils such as palm olein, which may be due to oxidation of unsaturated fatty acids.

The mean level of PON1 decreased significantly in the experimental groups compared to the control group, but no significant difference was seen among the three groups, which may be due to diet and no use of large amounts of fat in the control group.

Transmission of PON1 gene to rats causes a two- to four-fold increase in enzyme activity and diminishes susceptibility to vascular diseases.\textsuperscript{28} Mackness \textit{et al.} demonstrated that PON1 activity decreased in cardiovascular disease patients, which was inversely correlated with atrium-induced thickening of heart vessels.\textsuperscript{26}

In conclusion, given the inconsistent findings on the effect of palm olein oil and other oils, individual and social characteristics, nutritional habits, and most importantly genetic background of people and communities and many other unknown factors may affect lipidemia level.

In this study, canola oil, hydrogenated oil-containing palm and palm olein, reduced PON1 activity and elevated oxLDL; therefore, they may increase atherosclerosis risk. Since the findings of the present study on plasma lipids oxidation are consistent with those of other studies, use of palm olein oils in rats’ diets can considerably decrease LDL and help to increase HDL, and since use of oils in diets is inevitable, saturated and high trans fats should not be used as much as possible, and instead, non saturated oils, particularly palm olein, should be used.

Similar studies on humans with an appropriate sample size and length of intervention, and observance of research ethics are recommended. An interventional project seems necessary to reduce consumption of solid oils and to make community aware of the health effect of different oils.

Acknowledgment

This research project was supported by the deputy of research and technology of Shahrekord University of Medical Sciences, Shahrekord, Iran.

References