EFFECT OF REUSED PALM OIL ON SERUM LIPID PROFILE IN EXPERIMENTAL RATS

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ABSTRACT

The study was conducted in rat model to determine the effects of repeatedly heated palm oil on factors related to cardiovascular disease, particularly, lipid profile in the study subjects. We postulated that thermally oxidized palm oil which generates free radicals, enhances the oxidative stress and abnormality in lipid profile. For that, we have analyzed the effect of recycled edible oils on body weight gain, lipid profile of experimental rats. Palm oil was chosen in this study because it is widely used in Ethiopia as cooking oil. The oil is often used repeatedly for deep frying in many food outlets in order to lower costs. Our results showed that reused palm oil has deleterious effect on serum lipid profile and it may promote the progression and development of cardiovascular disorders.

Key words: reused palm oil; lipid profile; experimental rats

INTRODUCTION

In Ethiopia both the imported palm oil and repeatedly reused oils are used for different purposes especially as a diet. Even if there are some claims from the community about the health impacts of these reused oils, we haven’t seen any research in Ethiopia designed to assess the effects of these oils on the health of the society.

Cooking oil are vegetable oils, which are obtained from coconut, ground nut, sunflower seeds, palm, cottonseeds etc, are extensively used in the preparation of diet. These fats and oils are the esters of glycerol and various straight chained mono carboxylic acids, known as fatty acids. These fatty acids may be saturated, monosaturated or polyunsaturated [1]. Consumption of foods, which have been subjected repeatedly to deep-frying using various edible oils, is a common feature in Ethiopia, although the type and composition of edible oil commonly used for cooking and frying in the Ethiopian kitchens vary greatly from region to region. These used cooking oils constitute a waste generated from activities in the food sector (industries and restaurants), which has greatly increased in recent years. The main use of reused oil at present is in animal feed and in a much smaller proportion in the manufacture of soaps, biodegradable lubricants or for combustion (recovery of energy in industrial plants). As a consequence, the use of recycled cooking oils in animal feed must be studied from the point of view of safety as the fats and oils which are heated at high temperature during deep frying (which is a popular food preparation method in Ethiopia) which may generates high levels of cytotoxic
products. It may promote the induction, development and progression of cardiovascular diseases [2-3].

Therefore, the main objective of this study is to assess the effects of reused oils on changes in body weight and plasma lipid profile in experimental animals.

MATERIALS AND METHODS

**Materials**
Kits for biochemical analysis of serum total lipids, triglycerides, and total cholesterol, HDL-C were purchased. Potassium dihydrogen phosphate and formalin were purchased. And all other chemicals were of analytical grade and used as received. The imported palm oil was obtained from local market, Addis Ababa, Ethiopia. Reused Palm oil was collected from the fast food joints and restaurants located in Addis Ababa, Ethiopia. (Recycled for 5 times).

**Animals**
Healthy male Wistar rats, 6-8 weeks of age and weighing about 150-180g were used. The animals were procured from Central Animal House Facility, Addis Ababa University, Ethiopia. Rats were acclimatized to laboratory condition with 12 hrs light/dark cycle under constant temperature and humidity and were given *ad libitum* access to balanced diet. The Institutional Animal Ethical Committee of Addis Ababa University approved this Research work.

**Experimental design**
Experimental animals were divided into four groups of six rats each as follows:
- Group I: Rats fed with standard diet and pure drinking water (normal control)
- Group II: Animals supplemented with fresh palm oil diet (Oil control)
- Group III: Rats fed with reused palm oil diet (recycled once)
- Group IV: Rats fed with reused palm oil diet (recycled 5 times)

The test diet was prepared by mixing palm (fresh and reused) oils with normal commercial rat pellet to contain 15% of the palm oils. The 15% diet was prepared by adding 15 g palm (fresh and reused) oils to 85 g rat pellet, and mixed manually and the diets were then left to absorb the vegetable oils at room temperature overnight and stored at 20°C before the feeding trial was conducted.

At the end of the experimental period (6 weeks) their body weights were determined in every two weeks, diets withheld from experimental rats for 12-h and then rats were sacrificed. Blood samples were collected from the portal vein into dry clean centrifuge tubes. For serum separation, blood samples were left at room temperature to get clot and then were centrifuged for 15 minutes at 3000 rpm. Serum was carefully aspirated using a needle and transfers into dry clean test tubes and was kept frozen at -10°C until chemical analysis.

**Food Intake and Body Weight Gain Assay**
Food intake (FI) was calculated every other day. The biological value of the different diets was assessed by the determination of its effect on body weight gain (BWG) at the end of the experimental period using the following formulas: BWG = Final Body Weight - Initial Body Weight

**Lipid Profile and Lipoprotein Cholesterol Assay**
Triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) concentrations were determined using enzymatic methods using commercially available kits. Low density lipoprotein cholesterol (LDL-C) concentration was
calculated by using the method of Wilson and Spiger [4]. LDL-Cholesterol = Total cholesterol - (HDL-C+ TG/5)

Table 1: Effect of body weight in control and experimental animals

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td><strong>Initial body weight (g)</strong></td>
<td>243.67 ± 22.48</td>
<td>253.33 ± 22.1</td>
<td>262.33 ± 23.36</td>
<td>275.67 ± 24.79</td>
</tr>
<tr>
<td><strong>Final body weight (g)</strong></td>
<td>284.83 ± 25.83</td>
<td>293.17 ± 28.67</td>
<td>315.33 ± 27.39</td>
<td>355.33 ± 25.01</td>
</tr>
<tr>
<td><strong>Weight gain (g)</strong></td>
<td>41.17 ± 5.12</td>
<td>39.83 ± 10.47</td>
<td>53.00 ± 9.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.67 ± 3.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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Results are expressed as mean ± S.D for six rats in each group. Statistical significance at $P<0.05$, as compared with <sup>a</sup>Group I.

Table 2: Lipid profile in control and experimental animals

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>133.67 ± 12.87</td>
<td>253.33 ± 22.1</td>
<td>262.33 ± 23.36</td>
<td>312.23 ± 30.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>168.83 ± 17.83</td>
<td>193.17 ± 18.67</td>
<td>243.33 ± 23.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>315.33 ± 34.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>HDL-cholesterol</strong></td>
<td>66.17 ± 5.92</td>
<td>49.83 ± 4.47</td>
<td>31.00 ± 3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.67 ± 2.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>LDL-cholesterol</strong></td>
<td>35.6 ± 3.26</td>
<td>39.81 ± 3.84</td>
<td>44.22 ± 4.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.61 ± 6.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at $P<0.05$, as compared with <sup>a</sup>Group I. Units: mg/dL.

**DATA ANALYSIS**

Data are presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to detect the significant changes between the groups. The student least significant difference (LSD) method was used to compare the means of different groups and the significance was denoted by ‘P’ value. A commercial software SPSS version 10.0 was employed to find out the statistical significance between the groups.

**RESULTS**

The body weights of rats were calculated during the study and shown in Table 1. After six weeks of treatment, their weight gain was calculated by deducting the weight at the end of the study with their respective initial weight. The results show that there was a significant increase in body weight at the end of the study for all groups ($P < 0.05$). The highest body weight increase was observed in group IV animals.

Table 2 shows the lipid profile total cholesterol, triglycerides, LDL and HDL. The animals fed with reused palm oil showed significantly increased levels ($P<0.05$) in total cholesterol, triglycerides and LDL with decreased HDL levels when compared to the control groups.

**DISCUSSION**
Nutrition is a very important aspect of health in general. Nutrition plays major roles in preventing infectious diseases. The existence of nutrition related problems in a community means much more than the problem itself. Like many other health related issues, nutritional problems are also common in Ethiopia. In different countries, there are nutritional policies and programs which are designed to tackle the problem. This shows that nutrition is a public health important issue.

The analysis of the physical change in the rats showed similar increase in their body weight in all the treatment groups, except group IV (5 times reused palm oil) which observed higher increase in body weight gain compared to other groups. The reason for this is not well understood but it might be due to increase in triglyceride level. Similar finding also seen in studies carried out by Shastry C.S et al in India who reported that all the treatment groups showed a significant increase in body weight gain but the higher increase was seen in group IV animals [5].

The lipid profile study shows increase in cholesterol, triglyceride and LDL levels and decrease in HDL levels in the animals treated with reused oils. Increase cholesterol level in reused oil, ingestion of oxidized lipids rich in linoleic acid causes profound alteration in membrane composition fluidity and function. This alteration is likely to be associated with an enhanced cholesterol turn over, as indicated by the greater cholesterol excretion observed in the experimental group [6]. Increase in triglyceride level after oil ingestion may be due to the increase availability of substrate free fatty acids for esterification.

From the present study we conclude that repeatedly heated palm oil appears to increase triglyceride level with loss in protective effect which aggravates the development of atherosclerosis. Therefore, it is important that we should utilize protective nutritional value of palm oil in full and discourage the usage of repeated heating oil in our daily diet to reduce the risk of cardiovascular disorders.

REFERENCES