Effect of excessive intake of thermally oxidized sesame oil on lipids, lipid peroxidation and antioxidants' status in rats

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Fresh and thermally oxidized sesame, groundnut and coconut oils were fed to different groups of rats, as high fat diet (20%). Feeding fresh and thermally oxidized oils increased the levels of total cholesterol, low density lipoprotein cholesterol (LDL-C), and phospholipids but high density lipoprotein cholesterol (HDL-C) decreased in all the experimental animals. The levels of very low density lipoproteincolesterol (VLDL-C) and triacylglycerol increased only in groundnut and coconut oils-fed groups and decreased in sesame oil-fed group when compared with the control. When fresh and the corresponding thermally oxidized oils-fed groups were compared with the control, total cholesterol and LDL-cholesterol alone increased while triacylglycerol, VLDL-cholesterol, HDL-cholesterol, HDL/LDL ratio and phospholipids decreased. Thiobarbituric acid reacting substances increased in all the experimental animals and more so in corresponding thermally oxidized oils. It was less pronounced in sesame oil-fed groups when compared with the corresponding other oils-fed groups. Feeding of thermally oxidized oils decreased the levels of vitamin E, vitamin C and reduced glutathione when compared with fresh oils. Among the three thermally oxidized edible oils, sesame oil exhibited lesser risk for hyperlipidemic disorders.

Deep fat frying is one of the most commonly used procedure for the preparation of foods. During such process the oil is exposed to elevated temperature and air. Thermal oxidation of edible fats is known to alter its nutritional properties. Repeated deep frying of oils has been reported to cause accumulation of various oxidation and heat degradation products such as hydroperoxides, toxic aldehydes and carbonyl compounds. Saturated fats play an important role in the pathogenesis of atherosclerosis while vegetable oils containing polyunsaturated fatty acids undergo the process of lipid peroxidation generating free radicals and toxic aldehydes that aggravate atherosclerosis. Vegetable oils contain varying amounts of vitamin E and carotenoids which can act as antioxidants to detoxify the hydroxy and peroxy radicals thus controlling lipid peroxidation.

Coconut, groundnut and sesame oils are widely used in South India for cooking. These oils are used either fresh or fried for the sake of flavour in various food preparations. Of these oils, sesame oil is highly unsaturated followed by groundnut oil, while coconut oil is highly saturated. Though reports are available on the effect of feeding various fresh and thermally oxidized edible oils, no report is available on the effect of feeding of thermally oxidized sesame oil, especially as high fat diet on serum lipids, lipid peroxidation and antioxidants' status. In this study we have fed the above three oils as fresh and thermally oxidized high fat diet to albino rats, evaluated and compared lipid, lipid peroxidation and antioxidants' status.

Materials and Methods

Male Wistar rats (80-120g), bred in the Animal House of Experimental Medicine, Rajah Muthiah Medical College, were randomly divided, into seven group (6 animals / group). They were maintained at room temperature with a 12 hr light:dark cycle. Diet (Hindustan Lever Ltd., Mumbai) and water were available ad libitum.

Coconut, groundnut and sesame oils were purchased from the local market. The oils were heated at 185°C for half an hour per day for two consecutive days. After frying, samples of heated oils, along with remains of fresh oils were stored in jars at 4°C. The control animals received the commercial diet. The fat content of the commercial diet was 8%. The experimental groups received totally 20% (as high fat diet) of either fresh or thermally oxidized oils mixed along with the diet. The experimental diet was given to animals by gradually increasing the fat percentage to 20% in 2 weeks and then feeding was continued for 2 months.

The various groups include:
Group 1, Control animals

* Correspondent author
Group 2, Fresh sesame oil (SF)
Group 3, Thermally oxidized sesame oil (ST)
Group 4, Fresh groundnut oil (GF)
Group 5, Thermally oxidized groundnut oil (GT)
Group 6, Fresh coconut oil (CF)
Group 7, Thermally oxidized coconut oil (CT)

Blood samples were collected by sinoocular puncture, allowed to clot at room temperature for 30 min. The serum was separated by centrifugation at 1300 g for 15 min and stored at 4°C until analysis. All the chemicals used for the study were of analytical grade. Food intake was similar in all the groups.

Serum cholesterol was estimated by the method of Allain, and triacylglycerol by the method of Fossate et al using reagent kit. HDL-cholesterol was estimated by the method of Lopes and phospholipids by the method of Zilversmit and Davis. Thiobarbituric acid reactive substances (TBARS) were estimated by the method of Yagi, vitamin C by the method of Roe and Kuether, vitamin E by the method of Baker and Frank, and reduced glutathione (GSH) by the method of Ellman. Statistical analysis was done by analysis of variance technique and means were compared using Duncan's multiple range test.

The values were expressed as means ± SEM.

**Results and Discussion**

Table 1 presents serum lipid profile of animals fed with control, fresh and thermally oxidized oils. Serum total cholesterol and LDL cholesterol increased, as saturated fatty acids' contents in the oils increased; thermal oxidation of oils, further increased total cholesterol and LDL-cholesterol while fresh as well as thermally oxidized sesame oil showed a less increase when compared with the other two oils. The decrease in the cholesterol levels of sesame oil when compared with the other two oils could be attributed to the presence of lignans which were reported to inhibit cholesterol absorption and also synthesis in rats by reducing the activity of HMG-CoA reductase. Reduction of cholesterol in sesame oil group may also be due to the presence of large amounts of linoleic acid. Naturally occurring oils that contain a high proportion of linoleic acids are beneficial in lowering plasma cholesterol while a high proportion of saturated fatty acids raise the level.

Triacylglycerol and VLDL-cholesterol showed a significant increase in both fresh and thermally oxidized coconut and groundnut oils fed groups while significant decrease in sesame oil group when compared with control. TG clearance seems to be better in the case of both fresh and thermally oxidized sesame oils not only than other oils but even that of control. Hypertriglyceridemia was associated with an increased risk of coronary heart disease because of the increased production of atherogenic lipoproteins, chylomicron and VLDL remnants. The concentrations of plasma cholesterol, triacylglycerol and phospholipid levels tended to decrease with an increase in dietary lignan levels in the rats fed with evening primrose oil as the fat source. Lignans present in the sesame oil may be responsible for the reduction of triacylglycerol, phospholipid and

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL/C (%)</th>
<th>Phospholipids (mg/dl)</th>
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<tbody>
<tr>
<td>Control</td>
<td>73.55</td>
<td>41.15</td>
<td>8.23</td>
<td>55.98</td>
<td>9.34</td>
<td>6.20</td>
</tr>
<tr>
<td>SF</td>
<td>91.45</td>
<td>35.51</td>
<td>7.10</td>
<td>48.25</td>
<td>36.10</td>
<td>1.44</td>
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<td>61.48</td>
<td>0.67</td>
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<td>12.25</td>
<td>43.80</td>
<td>56.23</td>
<td>0.80</td>
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<td>GT</td>
<td>137.99</td>
<td>49.81</td>
<td>9.96</td>
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<tr>
<td>CF</td>
<td>128.71</td>
<td>69.95</td>
<td>13.97</td>
<td>41.83</td>
<td>72.90</td>
<td>0.60</td>
</tr>
<tr>
<td>CT</td>
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<td>11.61</td>
<td>29.48</td>
<td>118.13</td>
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**Table 1—Serum lipid profile in control, sesame, groundnut and coconut oils-fed animals.**

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**Table 1**—Serum lipid profile in control, sesame, groundnut and coconut oils-fed animals. [Values are means of 6 animals/group]
cholesterol when compared with other oils. The decrease in TG and VLDL-cholesterol in sesame group than control may be due to high clearance of TG that requires further investigation. The decrease in the level of triacylglycerol in heated oil groups could be due to the fact that repeated deep frying of oils causes thermal degradation of triacylglycerol. Decrease in the levels of phospholipids on feeding heated oils may be due to the oxidative degradation of phospholipids on heating, since phospholipids are susceptible substrates for peroxidation.

The remarkable decrease in HDL/LDL ratio is due to high fat. High fat content decreased HDL/LDL ratio thereby increasing the risk but thermal oxidation caused about 50% further reduction in the ratio thereby further aggravating the risk. Among the three high fat groups, sesame oil group seems to be less risky for lipid disorders.

Serum levels of TBARS, vitamin C, vitamin E and reduced glutathione of all the animals are presented in Table 2. Increase in the levels of TBARS observed in the experimental groups may be due to increase in fat content of the diet. Thermal oxidation of oils generates certain heat degradation products such as hydroperoxides, carboxyl compounds and toxic aldehydes, that can cause increased peroxidation of lipids. Feeding heated oils resulted in the elevation of TBARS, in our study also. Carbonyl compounds formed by deterioration of oils have a prooxidant action on fatty acids in vitro and when ingested by animals has been reported to participate in in vivo lipid peroxidation. Lipid peroxidation levels in ST, GF and CF oils are similar whereas thermal oxidation of the latter two oils further increased TBARS level. Though sesame oil is highly unsaturated, lipid peroxidation is low in sesame oil fed group. It has been reported that addition of sesamol was found to enhance the antioxidative action of the tocopherol and the antioxidative stability of the oil was mainly due to the synergistic action of these substances. The unroasted crude sesame oil contains 0.43 mg/dl while refined oil contains 1.7 mg/dl sesame. This may be the reason for low lipid peroxidation in sesame oil though vitamin E levels are the same in ST, CT and GF.

Glutathione is widely distributed in animal tissues and provides cellular protection against oxidative damage. Its decrease in our study could be due to increased utilization to trap hydroperoxides and other reactive oxygen species. An inverse relationship is observed between TBARS and GSH levels. In ST, GF and CF groups, as there is no significant variations in TBARS level, GSH level is similar. Vitamin C is an excellent antioxidant both in vitro and in vivo. Decrease in the level of vitamin C could be due to increased utilization to trap the reactive oxygen species or due to decrease in the level of GSH because vitamin C and GSH are synergistic antioxidants. Vitamin E is well accepted as nature's most effective lipid soluble chain breaking antioxidant in the biological system protecting cell membrane from peroxidative damage. Increase in the levels of vitamin E may be due to the presence of high amounts of vitamin E in vegetable oils. Decrease in the level of tocopherols during feeding of thermally oxidized oils may be due to the consumption of tocopherols to quench the free radicals generated on

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Table 2—Levels of TBARS, vitamin C, vitamin E and reduced glutathione (GSH) in control, sesame, groundnut and coconut oils-fed animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS (nmol/ml)</th>
<th>Vitamin C (mg/dl)</th>
<th>Vitamin E (mg/dl)</th>
<th>GSH (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.88</td>
<td>1.28</td>
<td>6.75</td>
<td>35.56</td>
</tr>
<tr>
<td>SF</td>
<td>2.98</td>
<td>0.50</td>
<td>16.46</td>
<td>29.35</td>
</tr>
<tr>
<td>ST</td>
<td>3.83</td>
<td>0.50</td>
<td>11.56</td>
<td>23.41</td>
</tr>
<tr>
<td>GF</td>
<td>3.92</td>
<td>0.70</td>
<td>12.0</td>
<td>24.95</td>
</tr>
<tr>
<td>GT</td>
<td>4.88</td>
<td>0.40</td>
<td>9.23</td>
<td>19.35</td>
</tr>
<tr>
<td>CF</td>
<td>3.40</td>
<td>0.75</td>
<td>13.95</td>
<td>26.46</td>
</tr>
<tr>
<td>CT</td>
<td>4.40</td>
<td>0.49</td>
<td>11.63</td>
<td>20.76</td>
</tr>
</tbody>
</table>

Means following same letter are not significantly different at 5% level

SF- Sesame oil fresh, ST - Sesame oil thermally oxidised
GF- Groundnut oil fresh, GT - Groundnut oil thermally oxidised
CF- Coconut oil fresh, CT- Coconut oil thermally oxidised
heating. Furthermore, the decrease may also be due to exposure of oils to high temperature that has been reported to decompose tocopherols by some oxidation products present in heat treated oils.

Thus our study suggests that sesame oil feeding, even as a high fat diet, can exert significant triacylglycerol lowering effect. Thermal oxidation of these oils had an adverse effect on serum lipids on the whole but the effect produced by thermally oxidized sesame oil is less pronounced when compared with groundnut and coconut oils.

Acknowledgment

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References

14 Roe J H & Kuether C A, Detection of ascorbic acid in whole blood and urine through the 2,4-DNPH derivative of dehydroascorbic acid, J Biol Chem, 147 (1943) 399.
16 Ellman G L, Tissue sulphydryl groups, Arch Biochem Biophys, 82 (1959) 70.