Effect of consumption of unheated and thermally-modified sesame and coconut oils on inflammation mediated metabolic disorders in Wistar rats

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Coconut oil and sesame oil are commonly used in South India for frying foods. On heating, edible oils form hazardous chemicals. This study explores the effect of consumption of unheated and thermally-altered sesame and coconut oils on coronary artery disease (CAD) risk factors in Wistar rats. Thirty Wistar albino rats were randomly divided into five groups (n=6/group). Group I (Control) was fed only chow, Group II: chow + unheated sesame oil, Group III: chow + heated sesame oil, Group IV: Chow + unheated coconut oil, Group V: chow + heated coconut oil. After eight weeks of treatment, serum lipid profile, hs-CRP, leptin, glucose, insulin, HOMA-IR, TNF-α, IL-6 and plasma homocysteine and fibrinogen levels were estimated. Rats in Group II showed a significant decrease in serum cholesterol, LDL-c, TNF-α, hs-CRP, insulin, and HOMA-IR but a significant increase in HDL-c, Group III showed opposite effects on these parameters, except that it decreased serum triglycerides level. Group IV and V did not show any significant effect on stated parameters. We conclude that consumption of unheated sesame oil gives protective effects against the CAD. Thermally altered sesame oil increases the CAD risk. Unheated and thermally altered coconut oil did not show any significant effect. Hence, we recommend that sesame oil better be used for dressing the food and coconut oil for frying.

Keywords: Coconut oil, Coronary artery disease, Insulin resistance, Lipid peroxidation, Sesame oil

Coronary artery disease (CAD) is one of the major causes of morbidity and mortality in developed and developing countries. Prevalence of CAD is increasing in India¹,². Diet, lifestyle and genetic factors contribute to the pathogenesis of CAD. Insulin resistance per se increases the risk of a CAD. High-caloric, oily, low-fiber containing and cholesterol-rich diet are often incriminated for the development of CAD. Oily and high-fat diet increases the chance of obesity and alters blood lipid profile to enhance atherosclerosis that leads to CAD³.

Indians are fond of eating fried food. Different edible oils are used for frying food in different parts of India. In the present study, we selected sesame oil (an unsaturated oil) and coconut oil (a saturated oil) being the two oldest oil seeds known to man⁴ and commonly used in Tamil Nadu and Kerala, respectively. Traditionally sesame oil is considered as health promoting. In Tamil Nadu, sesame oil is commonly known as ‘Nal(la)Ennai’, meaning good oil and unheated sesame oil is commonly used for dressing salads, preserving pickles and added as a taste enhancer to “idli” powder (a side dish for “idli”) and occasionally used in frying. In Kerala, coconut oil is widely used for cooking and frying food. Sesame oil is highly unsaturated (>80%) whereas, coconut oil is saturated (>90%). Coconut oil contains medium chain fatty acids, unlike sesame oil, which contains long chain fatty acids. The effect of coconut oil consumption on health is often debated because of the presence of saturated fatty acids which is assumed to be bad for health.

During frying, food is cooked for few min in oil at its boiling point. Usually, many batches of food are fried in the same oil, thus the frying oil is being heated for a few hours. Heating of oil induce oxidation of fatty acid and produces other changes in oil⁵. Many edible oils are naturally rich in antioxidants. They contain vitamin E, other vitamin E like substances, polyphenols etc⁶. Food dressed with these unheated oils become rich in these health promoting nutrients. But on heating, antioxidants content of oils decrease or are lost completely⁷. A study has shown that consumption of thermally altered oils affects lipid profile⁸. Besides the lipid risk

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factors, many non-lipid factors (commonly known as novel risk factors) also contribute to the pathogenesis of CAD. The influence of consumption of thermally altered oils on most of these novel risk factors is yet to be defined clearly. If thermal alteration of Sesame oil and Coconut oil brings out a similar effect on lipid and non-lipid risk factors predisposing to CAD is not clearly known. To explore all these aspects, the present study is designed.

Materials and Methods

Oil treated diet

Standard pellet diet (Chow) was purchased from Indian Immunological Ltd. (Telangana, India). Coconut and sesame oils were extracted by hand operated mini oil expeller that was procured from Rajkumar Agro Engineers Pvt. Ltd. (Nagpur, India) using coconut kernel and sesame seeds purchased from local market in Tamil Nadu. The oils were stored in a jar at 4°C. Oils were thermally altered by heating at 180±5°C for 2 h. Thermally-altered oils were used within 8 days. The standard food pellets were pounded a little and ground in a mixer. The oil-treated diet made afresh, just before feeding, by mixing grounded chow with respective oils at 20% w/w, water at 5% w/w (to facilitate re-pelleting) and grinding thoroughly in the mixer and re-pelletized by manual compression.

Malondialdehyde (MDA) estimation

Malondialdehyde (MDA) estimation from thermally modified oils was done by assaying thiobarbituric acid reactivity as described by Niehaus WG Jr. Oil (0.5 mL) was added and mixed to 5 mL of TBA-TCA-HCl solution containing 15% Trichloroacetic acid, 375 mg Thiobarbituric acid and 0.25 N HCl. The content was incubated at 100°C (boiling water bath) for 30 min. After incubation, the lower aqueous layer was aspirated and absorbance was read at 536 nm by a spectrophotometer (Hitachi U3900, Japan) after setting zero with control. Concentration of MDA was calculated by using its molar extinction coefficient (1.56×10^5 M^-1 cm^-1). Results were expressed in µM/L.

Experimental design

Thirty Wistar albino rats (Rattus norvegicus) weighing 180±30 g of body weight (8 weeks old) of either sex were procured from Jamia Hamdard, Hamdard Nagar, New Delhi after taking permission from the institutional animal ethics committee (IAEC) of Maulana Azad Medical College, New Delhi. The rats were kept under standard laboratory conditions (at 25±2°C, 12-12 h dark-light cycle) according to Guidelines for use of Laboratory Animals in Medical Colleges, published by Indian Council of Medical Research, New Delhi, India. The rats were acclimatized for 7 days, then randomly divided into five groups of six animals each (3:3– male:female) in well-ventilated cages. Male and female rats were kept in separate cages. The groups were given food and water ad libitum for fifty-six days (8 weeks) as follows: Control group (Group –I), Standard chow only; Unheated sesame oil group (Group –II), chow mixed with unheated sesame oil (20% w/w); Thermally altered Sesame oil group (Group –III), chow mixed with thermally-altered sesame oil (20% w/w); Unheated coconut oil group (Group –IV), chow mixed with unheated coconut oil (20% w/w); Thermally altered Coconut oil group (Group –V), chow mixed with thermally-altered coconut oil (20% w/w).

Collection and preparation of blood samples

After eight weeks of treatment, rats were over night fasted, and then anesthetized by intraperitoneal (i.p.) thiopental sodium (50 mg/kg) and 5-6 mL of blood was collected by cardiac puncture. Two-milliliter aliquots of blood samples were mixed in sodium citrate for fibrinogen and homocysteine estimation and rest collected in plain vials. Serum was separated by centrifugation (3000 rpm for 5 min) within 20 min of the collection of blood and stored in 0.5 mL aliquots in micro-centrifuge vials at −80°C for further analysis.

Biochemical analysis

Serum cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglyceride (TAG) and hs-CRP were measured by using commercially available kits adapted to fully automated random access clinical chemistry analyzer (Randox Imola) from Randox Pvt. Ltd (Crumlin, UK). The low-density lipoprotein-cholesterol (LDL-c) level was calculated from the values of serum total cholesterol, HDL-c, and triglyceride level as described by Friedwald. Fibrinogen and homocysteine levels were estimated in ACL Elite Pro coagulation analyzer using plasma and the kit provided by Instrumentation Lab, Bedford, USA. Serum leptin (rat) was measured by using a commercially available ELISA kit (Sincere Biotech Co., Ltd., Beijing, China), according to the
manufacturers protocol. Serum TNF-α, IL-6, and Leptin were estimated using commercially available ELISA kits (Wuhan Boster Biological Technology, Ltd., Wuhan, China), according to the manufacturers protocol. Absorbance was measured by a microplate reader according to the manufacturer's instructions (Biorad 680 XR, CA, USA).

**Statistical analysis**

Data are expressed as mean ± S.D. of six rats in each group. Statistical evaluations were done using one-way analysis of variance (ANOVA) followed by post-hoc (Tukey HSD) test. All statistical analyses were performed utilizing the IBM SPSS version 17 software. The level of statistical significance was set at \( P < 0.05 \).

**Results**

The MDA level in coconut and sesame oil after 2 h of heating was 11.8 and 236.6 µM/L, respectively, which was raised from respective basal levels (i.e., of unheated oils) of 0.38 and 16.67 µM/L.

Table 1 shows that consumption of unheated sesame oil significantly decreased serum total cholesterol (by nearly 24%) and LDL-c levels (by nearly 40%), whereas HDL-c levels are significantly increased (by 17%) as compared to those in control rats. However, it had no significant effect on triglycerides, plasma homocysteine, and fibrinogen levels. Consumption of thermally-altered sesame oil significantly increased serum cholesterol (by 16%), LDL-c (by 28%) but significantly decreased HDL-c (by 29%) and TAG (by 32%) as compared to controls.

Table 2 shows that the consumption of unheated sesame oil decreased serum hs-CRP (by nearly 24%), TNF-α (by nearly 25%), and IL-6 (by nearly 37%). Plasma glucose level did not alter, but insulin level and HOMO-IR decreased (by nearly 13). Consumption of thermally-altered sesame oil significantly increased hs-CRP (by 33%), TNF alpha (by 39%), IL-6 (by 34%), insulin (by 9%) and HOMA-IR (by 16%).

Unheated and thermally-altered coconut oil did not show any significant effect on the parameters discussed above (Table 1 & 2).

**Discussion**

Unheated sesame oil significantly decreased serum total cholesterol and LDL-c levels, whereas HDL-c levels are significantly increased. This indicates that lipid risk factors for CAD are decreased by the consumption of unheated sesame oil, whereas it had no effect on plasma homocysteine and fibrinogen, the two novel CAD risk markers in rats. Serum hs-CRP, TNF-α, and IL-6 levels have decreased. These parameters are markers for evaluation of these effects.

### Table 1 — Effect of consumption of unheated and thermally altered sesame and coconut oils (20% w/w) on serum lipid profile, homocysteine and fibrinogen in albino rats (n = 6/group). Results are expressed as Mean ± SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Unheated sesame oil)</th>
<th>Group III (Thermally altered sesame oil)</th>
<th>Group IV (Unheated coconut oil)</th>
<th>Group V (Thermally altered coconut oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum total cholesterol (mg/dL)</td>
<td>92.0 ± 6.0</td>
<td>70 ± 2 *</td>
<td>107 ± 2 * ‡</td>
<td>87 ± 6</td>
<td>92 ± 2</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mg/dL)</td>
<td>35 ± 3</td>
<td>42 ± 2 *</td>
<td>25 ± 2 * ‡</td>
<td>39 ± 4</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mg/dL)</td>
<td>43 ± 6</td>
<td>26 ± 3 *</td>
<td>55 ± 2 * ‡</td>
<td>40 ± 5</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>Serum triacylglycerol (mg/dL)</td>
<td>43 ± 5</td>
<td>44 ± 4</td>
<td>29 ± 4 * ‡</td>
<td>48 ± 3</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>Plasma homocysteine (µmol/L)</td>
<td>1121 ± 1089.56</td>
<td>0.59 12.8 ± 0.76</td>
<td>102.22 ± 0.63 93.97 ± 0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma fibrinogen (mg/dL)</td>
<td>180 ± 13</td>
<td>178 ± 7</td>
<td>170 ± 8</td>
<td>176 ± 4</td>
<td>180 ± 4</td>
</tr>
</tbody>
</table>

*P < 0.001 in comparison to controls and ‡P < 0.05 in comparison to unheated oil group by one way ANOVA followed by Tukey’s post-hoc HSD

### Table 2 — Effect of consumption of unheated and thermally altered sesame and coconut oils (20% w/w) on pro-inflammatory parameters (hs-CRP, TNF-α and IL-6), insulin, glucose, HOMA-IR, and leptin in albino rats (n = 6/group). Results are expressed as Mean±SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Unheated sesame oil)</th>
<th>Group III (Thermally altered sesame oil)</th>
<th>Group IV (Unheated coconut oil)</th>
<th>Group V (Thermally altered coconut oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum hs-CRP (mg/dL)</td>
<td>4.2 ± 0.2</td>
<td>3.2 ± 0.2 *</td>
<td>5.6 ± 0.3 * ‡</td>
<td>3.8 ± 0.4</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>45.8 ± 0.8</td>
<td>34.4 ± 1.4 *</td>
<td>64.0 ± 2.9 * ‡</td>
<td>45.8 ± 0.8</td>
<td>48.4 ± 2.0</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>114.3 ± 112.718</td>
<td>1.9 *</td>
<td>153.3 ± 5.2 * ‡</td>
<td>108.7 ± 25.4</td>
<td>103.3 ± 40.8</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.9 ± 0.5</td>
<td>6.1 ± 0.3</td>
<td>6.3 ± 0.3</td>
<td>6.3 ± 0.2</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>68.1 ± 3.2</td>
<td>57.6 ± 2.1 *</td>
<td>74.5 ± 4.7 * ‡</td>
<td>66.1 ± 3.7</td>
<td>71.3 ± 3.0</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>18.0 ± 1.7</td>
<td>15.7 ± 0.8 *</td>
<td>20.8 ± 0.7 * ‡</td>
<td>18.6 ± 1.1</td>
<td>19.6 ± 1.4</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>13.1 ± 2.6</td>
<td>11.9 ± 1.4</td>
<td>11.5 ± 0.5</td>
<td>13.2 ± 2.2</td>
<td>12.6 ± 1.7</td>
</tr>
</tbody>
</table>
inflammatory state\textsuperscript{13}. A decrease in these parameters indicates that consumption of unheated sesame oil decreases the inflammatory state. Pro-inflammatory state favors atherogenesis and contributes to the pathogenesis of CAD\textsuperscript{14,15}.

Plasma glucose level did not alter, but insulin level and HOMO-IR decreased following the consumption of unheated sesame oil. This is suggestive of an insulin-sensitizing effect of unheated sesame oil. Insulin resistance is now considered to be a risk factor for CAD\textsuperscript{16}. Based on the observed results, we conclude that consumption of unheated sesame oil gives protection against CAD.

Sesame oil is found to contain several antioxidants and chemo-preventive agents such as tocopherol, sesamolin and sesaminol\textsuperscript{17-19}. These compounds are not only antioxidants but are known to have an effect on lipid metabolism. Sesame lignans which are present in heated sesame oil lower the cholesterol concentration in serum due to its content of tocopherol, which inhibits the absorption of cholesterol from the intestine and suppresses its synthesis in the liver\textsuperscript{20}. Several reports suggest that PUFA decreases Fatty acid synthesis and increases fatty acid oxidation in birds and mammals\textsuperscript{21,22}. All these factors probably contribute to the beneficial effects, due to the consumption of unheated sesame oil.

On the contrary, consumption of thermally-altered sesame oil was found to increase the serum cholesterol, and LDL-c but decreased triglycerides and HDL-c. Increase in serum cholesterol and LDL-c and decrease in HDL-c indicates an increase in the CAD risk on consumption of thermally-altered sesame oil. There was no change in plasma homocysteine and fibrinogen level. Although, euglycemia was maintained, serum insulin level and HOMA-IR were increased indicating impairment of insulin-resistant state due to consumption of thermally-altered sesame oil. At the same time, hs-CRP, TNF-\(\alpha\), and IL-6 were increased indicating a pro-inflammatory state. From these findings, we conclude that consumption of thermally-altered sesame oil increases the risk of a CAD. The increase in MDA levels indicates that there was lipid peroxidation on heating of oils. As coconut oil contains mostly the saturated fatty acids, the extent of rise in MDA level on heating for two hours was comparatively lesser than that of sesame oil. During heating, oils and fats suffer thermal oxidation and produce compounds such as peroxides. The peroxides turn into aldehydes, ketones, epoxides, dimers, and polymers, undermining the quality of food\textsuperscript{23}. These products are known to have deleterious effects because of their pro-oxidative and pro-inflammatory nature\textsuperscript{24,25}. In addition, they also destroy the beneficial components of the oils. Hence, when thermally-altered sesame oil is consumed, the beneficial effects found during the consumption of unheated sesame oil are not observed. The pro-oxidants are known to induce the insulin resistance state contributing to CAD\textsuperscript{26,27}.

A decrease in serum triglycerides level, which is seen after the consumption of thermally-altered sesame oil might be because of the inhibition of absorption of fatty acids, due to the deleterious products generated during heating of oils\textsuperscript{28}.

The impact of serum triglycerides on the pathogenesis of CAD is lower than that of serum cholesterol\textsuperscript{29} Unheated and thermally-altered coconut oil did not show any significant effect on the parameters discussed above (Table 1 & Table 2). Coconut oil is a saturated oil. It was expected to affect the lipid parameters. But that was not observed. This is probably because coconut oil is metabolically very active and undergoes beta-oxidation very fast, as it is rich in medium chain fatty acids. It is known that Carnitine Palmitoyal Transferse-I, that regulates the entry of long-chain fatty acids into mitochondria for beta-oxidation is bypassed by the medium chain fatty acids\textsuperscript{29-31}. The antioxidant content of coconut oil is also very low in comparison to sesame oil. Probably because of this, unheated coconut oil does not produce any beneficial effect on CAD risk parameters. On heating, coconut oil is found to produce less malondialdehyde\textsuperscript{32} because of its saturation. This lower heat-induced changes might be responsible for the difference in the effect of consumption of thermally-altered sesame and thermally-altered coconut oils.

Hence, we conclude that unheated sesame oil should be used for the dressing of salads and preservation of pickles. Consumption of unheated sesame oil with “idli” powder as a taste enhancer is probably beneficial and should be promoted. For cooking and frying food, unsaturated oils should preferably be avoided, particularly when heating is required for a long duration. Coconut oil is highly saturated and undergoes less heat-induced alterations. Hence for frying and cooking food, the use of coconut oil is beneficial and should be preferred.
References