Evaluation of the Anti-lipidemic Effect of Polyoxyethylenated Cholesterol on Rats Fed High Fat Diet

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Abstract: Epidemiological and experimental studies have suggested that a high-fat diet might induce oxidative stress or lipid oxidation and subsequently contribute to the high risk of some diseases such as cardiovascular and cerebrovascular ones. The objective of this study was to evaluate the hypolipidemic activity of polyoxyethylenated cholesterol (PEO-Chol) in rats fed high fat diet. Rats were grouped as: group 1 - control, group 2 - treated with PEO-Chol, group 3 - fed on high fat diet for 21 days, group 4 - treated with PEO-Chol simultaneously with high fat diet and group 5 - rats were treated with PEO-Chol after 7 days of high fat feeding. Plasma glucose and lipid profile including total cholesterol (Chol), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), total lipid (TL), and phospholipid (PL) were estimated in both plasma and liver tissue. Lipid peroxidation was measured as malonaldehyde (MDA) in both plasma and liver tissue. Plasma and liver fatty acids were evaluated. Histopathological studies on liver tissue were also performed. The results showed that PEO-Chol supplementation ameliorated significantly the disturbances in plasma glucose, lipid profile and fatty acids. PEO-Chol modulated the MDA levels in both liver tissue and plasma. Also, results of the histopathological investigation for liver tissue showed some normalization. It can be concluded from these results that PEO-Chol may be a contributing factor in the scavenging of free radicals in hyperlipidemic rats and might exert a beneficial role against some metabolic disorders.

Key words: PEO-Chol, lipid peroxidation, glucose, lipid profile, fatty acids.

INTRODUCTION

Lipid and lipoprotein abnormalities play a major role in the development and progression of coronary artery disease. Low levels of high density lipoprotein cholesterol and high levels of low density lipoprotein cholesterol have been identified as independent coronary risk factors [1].

Medical studies show that about 70% of adults over 50 years old suffer atherosclerosis. Hyperlipidemia is a well known risk factor for atherosclerosis [2]. To prevent this disease a number of hypolipidemic drugs have been administrated in clinic. However, they all have obvious or potential side effects to some extent [3]. The search is in continuity for safe, dependable, and effective drugs.

Dietary fat intake has been shown to be important in the development of human obesity and there are also experimental studies showing that high-fat diet can be associated with increased oxidative stress in mammals [4] and more recently literature data have indicated that high-fat diet may increase the incidence of diabetes, hypertension and other degenerative diseases [5-7]. Of particular importance, patients with cardiovascular and cerebrovascular diseases have been reported to eat a diet higher in fat than the general population [8], suggesting that high-fat diet may accelerate the development of these diseases.

Polyoxyethylenated cholesterol (PEO-Chol) is a group of nonionic amphipathic molecules has been used as a solubilizer and as an enhancer on the intestinal absorption of peptides. PEO-Chol with larger numbers of units has been used to introduce modifications on the liposomal surfaces. They exhibited a markedly longer blood-circulation time and a lower accumulation to the reticuloendothelial system [9]. In the present study, the hypolipidemic efficiency of prepared PEO-Chol was evaluated in rats fed with high fat diet.

MATERIALS AND METHODS

Preparation of PEO-Chol: PEO-Chol having an average number (n=10) of oxyethylene units (EO), was prepared by reacting ethylene oxide with cholesterol in two-stage reaction (Fig. 1) using an apparatus similar to that reported in previous work with little
modifications \[^{9,10}\]. Ethylene oxide was fed from cylinder with recommended valve (Fluka). Pulverized sodium hydroxide, as a base catalyst, was employed in the second-stage to get PEO-Chol adduct. The prepared nonionic is more than 90% active, containing less than 10% of free polyoxyethylene glycols. The method of separation and determination of these secondary reaction products, were carried out using the modified Weibull technique \[^{11}\].

![Cholesterol](image)

**Fig. 1:** Polyoxyethylenated cholesterol (n=10, n is the average number of oxyethylene units, EO)

### Doses Determination:
In order to determine a non toxic dose of the PEO-Chol, the doses that caused acute toxicity have been identified. Hence, adult male Swiss albino rats were subjected to series of different concentrations ranged from 10 to 100 mg/kg b. wt. dissolved in distilled water. These concentrations were administrated to 10 animals orally for each concentration and each dose repeated five times. Acute toxicity doses were calculated using probability analysis according to Ghosh \[^{12}\]. Thus a non toxic dose of 50 mg/kg b. wt. *in vivo* anti-lipidemic experimental administered as five repeated doses.

### Animals and Diet:
The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland, and according to guidelines for care and use of laboratory animals controlled by the National Research Centre, Egypt were followed. Thirty adult male Swiss albino rats weighing 100-150 g obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo) were used in this study. The animals were housed in plastic cages (6/cage) in a room kept at 23 ± 1°C with a 12-h light:dark cycle. After acclimatization period on a commercial standard pellet diet, and tap water (free access) for 1 week, rats were randomly assigned to one of five groups:

- **Control group:** The rats fed on a commercial standard pellet diet.
- **PEO-Chol group:** The rats were orally administrated with PEO-Chol dissolved in distilled water at a dose of 50 mg/kg b.wt. / 5 days per week for 21 days.
- **High fat diet group:** The rats were placed on a hyperlipidemic diet (89% standard diet, 1% cholesterol and 10% lard) for 21 days.
- **High fat diet rats + PEO-Chol group (simultaneous):** Animals received PEO-Chol treatment in the same day of feeding high fat diet.
- **High fat diet rats + PEO-Chol group (post):** High fat diet rats received (PEO-Chol), 7 days post hyperlipidemic diet supplementation.

All administrations were given directly by using stomach tube. Control and (PEO-Chol) groups were allowed for free access to tap water and standard diet. Rats were fasted overnight before the time of sacrificing. Blood samples were collected in sterile heparinized tubes by heart puncture. Livers were excised out, washed, weighed and homogenized in ice-cold 0.1 M potassium phosphate buffer (pH 7.4) to yield 10% homogenates. Part of the excised liver was used for histopathological examination.

### Experimental Parameters:
Lipid peroxidation (LP) indicated by the formation of malondialdehyde (MDA) was assessed in plasma and liver homogenates after the method described by Ohkawa et al. \[^{13}\]. Lipid profile including plasma and liver total cholesterol (Chol), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), total lipid (TL), and phospholipid (PL) levels were monitored spectrophotometrically according to the manufacturer's instructions, using reagent kits obtained from BioMerieux (France). Plasma glucose level activity was determined using a commercial kit obtained from Roche Diagnostics (Mannheim, Germany).

In order to determine percentage of plasma and liver fatty acids, lipids of plasma and liver were extracted by the method of Fisher et al. \[^{14}\]. A known weight of samples was extracted with diethylether/ethanol (2:1 v/v) for 3 times, after that the solvent was evaporated at 55 °C. The values were calculated per 1 ml or 1 gram weight sample and saponified according to the method of Ashour \[^{15}\]. The fatty acids were esterified by methanol and purified according to the method of Kinsella \[^{16}\].
by Hp 6890 gas chromatography. The chromatograph includes an innowax-crossline polyethylene glycol column 30 mm; i.d. 0.32 mm; 0.5 µm film thickness. The assays were carried out with programmed oven temperature, increases of oven temp as followed: 150°C for 1 min, then elevated to 235°C with rate of 17°C/min, then raised to 245°C for 5 min. Carrier gas was nitrogen injected by 1.5 ml/min Detector: FID, 275°C. Peak areas were measured with an integrator connected to a computer, which expressed the data automatically. Peaks were identified by comparing their equivalent chain lengths with those of authentic fatty acid methyl esters.

**Histopathological Studies:** Sections of liver were fixed in 10% formalin in saline and embedded in paraffin wax then processed routinely for Hematoxylin and Eosin (H & E) staining method [17].

**Statistical Analysis:** The SPSS/PC computer program was used for the statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test for multiple comparisons. The data were expressed as mean ± Standard error (SE). Differences were considered significant at p < 0.05.

**RESULTS AND DISCUSSION**

Figure (2) shows that the levels of plasma TG, Chol, PL and TL distinctly increased in high fat diet group, compared to those of control and PEO-Chol groups. While, HDL-C was significantly decreased in high fat diet group compared to PEO-Chol group. In high fat diet + PEO-Chol (simultaneously) group TG, Chol, PL and TL were significantly decreased compared to high fat diet group, while HDL-C was insignificantly increased. In high fat diet + PEO-Chol group (post) TG, Chol, PL and TL were significantly decreased compared to high fat diet group, while TG, PL and TL were significantly increased compared to PEO-Chol group, and HDL-C was significantly decreased.

Results shown in Figure (2) demonstrated that liver TG, Chol, and PL contents in rats fed high fat diet increased as compared to control and PEO-Chol groups, while HDL-C was significantly decreased compared to PEO-Chol group. Also, high fat diet + PEO-Chol (simultaneously) group TG, Chol, and PL were significantly decreased compared to high fat diet group, while HDL-C was insignificantly increased compared to high fat diet group. As regards to the results of high fat diet + PEO-Chol group (post), TG, Chol, and PL showed significant increase as compared to PEO-Chol group, while HDL-C was insignificantly decreased.
Feeding on high fat diet induced significant elevation in LP level of plasma and liver of rats as compared to control and PEO-Chol groups. On the other hand, when hyperlipidemic rats were treated with PEO-Chol, a significant decrease was observed in LP level in their plasma and liver as compared to high fat diet group (table 1). Also the plasma glucose level showed a significant increase in high fat diet group compared to control and PEO-Chol groups while, a decrease was recorded in plasma glucose in the high fat rats + PEO-Chol (simultaneously) and high fat diet + PEO-Chol (post) groups compared to the values of high fat diet group (table 1).

Tables (2, 3) illustrate alterations in the distribution profile of fatty acids in the plasma and liver in the different groups. The percentage of saturated fatty acids increased by high fat diet group while the percentage of unsaturated fatty acids decreased. The administration of PEO-Chol decreased saturated fatty acids and increased unsaturated fatty acids compared to high fat diet group.

Histopathological examination of liver tissues in control and PEO-Chol groups showed no pathological changes (fig. 3, 4). The livers of high fat diet rats (fig. 5, 6) showed pronounced hepatic fatty changes (steatosis) in the form of intracytoplasmic fat vacuoles in most of the hepatic cells. There are abundant mononuclear inflammatory cells in the groups of high fat diet + PEO-Chol (simultaneously) and high fat diet + PEO-Chol (post) with markedly decreased the fatty changes (fig. 7, 8). In addition there is no pathological changes between high fat diet + PEO-Chol (simultaneously) and high fat diet + PEO-Chol (post).

Discussion: Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is a major risk factor for the development of cardiovascular diseases [18]. The abnormal metabolism and elevation of plasma cholesterol and lipoproteins are well-documented risk factors for the development of atherosclerosis. Evidence from clinical trials indicates that reducing plasma cholesterol by dietary and/or pharmacological means leads to reductions in the incidence of death from cardiovascular disease [19,20]. Hyperlipidemia in experimental rats was evidenced by a significant enhancement in the level of triglyceride and phospholipid in blood and liver [21].

Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in different animal models [22]. The data obtained in the present study revealed that rats received high fat diet showed significant increase in plasma Chol, TG, PL and TL which agreed with others [23,24]. The increased blood cholesterol up to 25%, seems to be the result of liver lipid deposition, which provides acetyl coenzyme A to liver cells for cholesterol synthesis [25]. The excessive liver lipid deposition leads to steatosis [26], which represents an imbalance between triglyceride synthesis in the liver and its secretion [27].

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**Legend as in Figure 2.**

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**Table 1:** Effect of PEO-Chol on the levels of plasma and hepatic lipid peroxidation (LP) and plasma glucose in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma LP (µmol/ml)</th>
<th>Hepatic LP (µmol/g tissue)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.65 ± 0.32</td>
<td>112.50 ± 4.43</td>
<td>124.57 ± 4.65</td>
</tr>
<tr>
<td>PEO-Chol</td>
<td>14.66 ± 0.51</td>
<td>118.05 ± 5.85</td>
<td>123.41 ± 3.59</td>
</tr>
<tr>
<td>High fat diet</td>
<td>19.81 ± 0.67</td>
<td>167.47 ± 6.71</td>
<td>139.60 ± 1.82</td>
</tr>
<tr>
<td>High fat diet + PEO-Chol (simultaneously)</td>
<td>16.85 ± 0.20</td>
<td>122.89 ± 5.21</td>
<td>121.04 ± 3.37</td>
</tr>
<tr>
<td>High fat diet + PEO-Chol (post)</td>
<td>17.48 ± 0.55</td>
<td>141.11 ± 5.76</td>
<td>116.81 ± 4.57</td>
</tr>
</tbody>
</table>

Legend as in Figure 2.
Table 2: Effect of PEO-Chol on fatty acids composition in plasma of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fatty acid</th>
<th>Control</th>
<th>PEO-Chol</th>
<th>High Fat diet</th>
<th>High fat diet + PEO-Chol (simultaneous)</th>
<th>High fat diet + PEO-Chol (post)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14</td>
<td>0.71 ± 0.02</td>
<td>0.87 ± 0.01</td>
<td>1.02 ± 0.02</td>
<td>0.70 ± 0.01</td>
<td>0.91 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>21.91 ± 0.23</td>
<td>21.61 ± 1.20</td>
<td>33.39 ± 0.36</td>
<td>23.46 ± 0.50</td>
<td>27.51 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>C18:2</td>
<td>4.12 ± 0.09</td>
<td>4.22 ± 0.05</td>
<td>6.92 ± 0.16</td>
<td>4.29 ± 0.22</td>
<td>5.22 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>C18:3</td>
<td>10.90 ± 0.10</td>
<td>10.64 ± 0.15</td>
<td>18.92 ± 0.12</td>
<td>14.05 ± 0.40</td>
<td>17.30 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>C20:1</td>
<td>3.91 ± 0.54</td>
<td>39.65 ± 0.20</td>
<td>21.05 ± 0.50</td>
<td>35.00 ± 0.31</td>
<td>28.42 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>C20:4</td>
<td>14.91 ± 0.15</td>
<td>14.12 ± 0.19</td>
<td>7.27 ± 0.83</td>
<td>13.67 ± 0.21</td>
<td>9.97 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>0.57 ± 0.01</td>
<td>0.60 ± 0.02</td>
<td>0.67 ± 0.03</td>
<td>0.55 ± 0.02</td>
<td>0.64 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>31.67 ± 0.21</td>
<td>31.65 ± 1.17</td>
<td>48.52 ± 0.12</td>
<td>33.65 ± 0.88</td>
<td>59.58 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>MUFA</td>
<td>12.98 ± 0.19</td>
<td>12.70 ± 0.03</td>
<td>22.72 ± 0.32</td>
<td>16.22 ± 0.31</td>
<td>21.02 ± 0.12</td>
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</tr>
<tr>
<td>PUFA</td>
<td>55.39 ± 0.63</td>
<td>55.58 ± 0.35</td>
<td>29.07 ± 0.72</td>
<td>50.04 ± 0.33</td>
<td>39.38 ± 0.25</td>
<td></td>
</tr>
</tbody>
</table>

Legend as in Figure 2.
SFA: Saturated fatty acid.
MUFA: Mono-unsaturated fatty acid.
PUFA: Poly-unsaturated fatty acid.

Table 3: Effect of PEO-Chol on fatty acids composition in liver tissue of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fatty acid</th>
<th>Control</th>
<th>PEO-Chol</th>
<th>High Fat diet</th>
<th>High fat diet + PEO-Chol (simultaneous)</th>
<th>High fat diet + PEO-Chol (post)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14</td>
<td>1.06 ± 0.03</td>
<td>1.32 ± 0.08</td>
<td>1.47 ± 0.01</td>
<td>1.42 ± 0.05</td>
<td>1.41 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>20.40 ± 0.77</td>
<td>20.55 ± 2.67</td>
<td>26.97 ± 0.10</td>
<td>22.65 ± 0.42</td>
<td>23.81 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>C18:2</td>
<td>0.99 ± 0.08</td>
<td>1.10 ± 0.07</td>
<td>2.19 ± 0.05</td>
<td>1.21 ± 0.06</td>
<td>1.95 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>C18:3</td>
<td>19.67 ± 0.69</td>
<td>18.68 ± 0.22</td>
<td>21.35 ± 1.27</td>
<td>20.54 ± 0.39</td>
<td>22.36 ± 0.82</td>
<td></td>
</tr>
<tr>
<td>C20:1</td>
<td>14.71 ± 0.57</td>
<td>14.16 ± 0.18</td>
<td>25.00 ± 0.83</td>
<td>22.67 ± 0.24</td>
<td>24.37 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>C20:4</td>
<td>22.07 ± 0.40</td>
<td>24.52 ± 0.65</td>
<td>11.86 ± 0.32</td>
<td>16.68 ± 0.22</td>
<td>14.27 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>0.55 ± 0.03</td>
<td>0.51 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>0.76 ± 0.02</td>
<td>0.78 ± 0.02</td>
<td>1.00 ± 0.05</td>
<td>0.89 ± 0.03</td>
<td>0.99 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>MUFA</td>
<td>0.38 ± 0.02</td>
<td>0.31 ± 0.01</td>
<td>0.11 ± 0.03</td>
<td>0.25 ± 0.01</td>
<td>0.18 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>PUFA</td>
<td>16.56 ± 0.22</td>
<td>15.36 ± 0.45</td>
<td>5.97 ± 0.21</td>
<td>9.35 ± 0.27</td>
<td>6.87 ± 0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.38 ± 0.29</td>
<td>2.12 ± 0.09</td>
<td>1.09 ± 0.02</td>
<td>1.62 ± 0.04</td>
<td>1.33 ± 0.03</td>
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</tr>
<tr>
<td>C24</td>
<td>0.43 ± 0.02</td>
<td>0.59 ± 0.01</td>
<td>2.81 ± 0.22</td>
<td>2.37 ± 0.03</td>
<td>2.25 ± 0.05</td>
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</tr>
<tr>
<td>SFA</td>
<td>42.32 ± 1.09</td>
<td>81.92 ± 2.54</td>
<td>53.51 ± 1.19</td>
<td>47.87 ± 0.10</td>
<td>50.94 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>MUFA</td>
<td>16.08 ± 0.54</td>
<td>15.57 ± 0.11</td>
<td>27.29 ± 0.81</td>
<td>24.13 ± 0.18</td>
<td>26.50 ± 0.31</td>
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</tr>
<tr>
<td>PUFA</td>
<td>43.56 ± 0.62</td>
<td>42.53 ± 1.13</td>
<td>19.05 ± 0.55</td>
<td>27.97 ± 0.51</td>
<td>22.68 ± 0.47</td>
<td></td>
</tr>
</tbody>
</table>

Legend as in Figure 2.
The main effect of fat–cholesterol enriched diet was accumulation of cholesterol and triglycerides in the serum and tissues, mainly in the liver. The lipid lowering effect of PEO-Chol (as a hydrophobic surfactant) on rats receiving fat–cholesterol enriched diet could be result from various mechanisms such as inhibition of intestinal absorption of dietary lipids, inhibition of de novo synthesis or increased catabolism of cholesterol and fatty acids in the liver. This explanation agreed with the finding of other investigations who mentioned that a hydrophobic surfactant, markedly influenced the cholesterol homeostasis of intestinal mucosa, however, it was not the specified whether this effect was due to the direct surfactant – cholesterol interaction, or due to the result of other not well known biochemical or biophysical processes [28].

In hypercholesterolemic diet, liver the primary organ to metabolise the cholesterol ingested in excess, is affected by oxidative stress. It results from an imbalance between the production of free radicals and effectiveness of antioxidant defense system [29]. Increased oxidant stress, appears to play an important role in the chronic inflammatory responses to hypercholesterolemia and atherosclerosis [30]. Thus oxidative stress leads to cell damage related to free
radicals and lipid peroxidation, including the destruction of the cell membrane structure \[^{[31]}\]. An increase of lipid peroxidation, in animals fed with a high cholesterol diet has been previously reported \[^{[32]}\]. Increased levels of lipid peroxidation in both plasma and liver tissue of hyperlipidemic rats indicated the enhancement of the oxidative stress status in rats. As a result of increased lipid profile, lipid peroxidation increased which is well correlated to previous studies \[^{[32,33]}\]. Sevanian and Hochstein \[^{[34]}\] mentioned that, PUFAs are readily oxidized by free-radical chain mechanisms, as their allylic hydrogen atoms are easily removed to produce a radical site subject to O\(_2\) addition, which is followed by decomposition reactions with loss of unsaturated lipids. This process is well known as auto-peroxidation and is thought to play a leading role in membrane damage and degenerative reactions. It has been well established that nutrition plays an important role in the etiology of hyperlipidemias and atherosclerosis. Several animal and human studies have confirmed the hypercholesterolemic properties of saturated fatty acids and cholesterol and altering lipoprotein pattern \[^{[35]}\]. Administration of PEO-Chol modulated lipid peroxidation in the plasma and liver of hyperlipidemic rats. This can be explained by Cserháti \[^{[36]}\] who mentioned that the hydrophilic ethylene oxide chain probably has two functions: it regulates the insertion depth of hydrophobic moiety (longer ethylene oxide chain draws the hydrophobic moiety toward the aqueous outer phase), or it binds to the polar head group of phospholipids. As the long ethylene oxide chain can contact more than one head group, it can stabilize the membrane organization.

The present detailed analysis of fatty acid composition provides evidence that specific fatty acids are affected by high fat diet in rats and they may be involved in the development of increased levels of triglycerides, cholesterol, phospholipids, and total lipids in plasma and liver tissue. From these, specifically palmitoleic acid (16:1) and oleic acid (18:1) for MUFA and linoleic acid (18:2) and arachidonic acid (20:4) for PUFA. In fact, after 3 weeks of hyperlipidemia, the levels of 16:1 and 18:1 fatty acids increased, whereas the levels 18:2 and 20:4 fatty acids decreased in plasma and liver tissue. These observations are in accordance with the results of earlier study \[^{[37]}\] who mentioned that there is an increase in serum 16:1 fatty acid levels and a decrease in serum 18:2 fatty acid levels in patients with type 2 diabetes.

The increase in 16:1 and 18:1 fatty acids and the decrease in 18:2 and 20:4 fatty acids in the liver and plasma that preceded the other changes in the present study appear to be important factors in the development of fatty acid imbalances. There is evidence that circulating fatty acids increase hepatic glucose output, probably by increasing gluconeogenesis or glycogenolysis \[^{[38]}\].

In conclusion, our results demonstrated hypolipidemic effect of PEO-Chol in rats. However, additional studies on PEO-Chol are needed for better understanding of the mechanism of action.

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