Separation and partial purification of lecithin: cholesterol acyltransferase from serum of obese women with a study of the effect of oily and nano-extract of Castanea fruit in activating the enzyme

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Background: The Chestnut plant is one of the important plants for treating many diseases. Therefore, the oil extract of the Chestnut plant was taken to know its effect on activating an enzyme of lecithin: cholesterol acyltransferase that decreases in obese patients, by isolating it from the blood and using nanotechnology methods.

Methods: The research included the isolation lecithin: acyltransferase cholesterol (LCAT) from the blood of 5 obese women, whose BMI was between 25-30 kg/m², ages ranged from 40-45 years, and they were healthy without any diseases. The oil extract of Chestnut fruit was isolated. Fatty acids present in the oil extract were identified using capillary gas chromatography technique CGC, as the extract contained a percentage of unsaturated fatty acids and a lower percentage of saturated fatty acids. The enzyme was precipitated with ammonium sulfate, dialysis technique, and the gel filtration chromatography technique was used in purification. Finally, effect of the oil extract of the Chestnut fruit was studied at a concentration of (0-100) mg/ml and the nanoparticles-oil extract also on the activity of the LCAT (0-20) mg/ml. The nanoparticles of silver nitrate were prepared at a concentration of 1 mM with the oil extract of the Chestnut fruit and heated at a temperature of 60°C.

Result: The highest peak was obtained from the gel filtration chromatography, which was highly effective. The number of purification times reached 91.72, and the recovery rate was 283.53%, and the effectiveness was qualitative 457.69 U/mg. Oil nanoparticles extraction was confirmed when the solution was transformed by changing its colors from colorless, yellow, orange, and finally coffee in color. The results showed an increase in the activity of the LCAT when treated with the two extracts, also the increase in the activity had a higher effect on the activity of the enzyme.

Conclusion: We concluded during the study that the extract of the fruit of the oily nanoparticles was more effective in increasing the activity of an enzyme lecithin: cholesterol acyltransferase in healthy women with obesity.
Introduction
Lecithin Cholesterol Acyl transferase (LCAT) works to esterify particles on a surface of lipoprotein [1,2], especially HDL-C [3,4].

The enzyme is affected by gender, age, type of food, smoking and alcohol intake [5,6]. It changes the process of transporting free cholesterol and their esterification within the body, as it works to transfer the acyl group at the site Sn°2 of phosphor choline to cholesterol to obtain esterified cholesterol [7,8]. Medicinal plants are considered one of the most important modern methods of treatment to get rid of diseases because they contain many effective compounds [9]. Therefore, the current study focused on using the Chestnut plant for its great importance as it contain nutritional content a high percentage of unsaturated fatty acids that are estimated at (85%) of linoleic acid, as well as a lower percentage of oleic acid and linoleic acid, which is one of the most important fatty acids rich in Omega 3. As the Chestnut fruit is a food for humans and animals and it works to regulate the level of fats in the heart and blood vessels while enhancing the work of insulin in sugar regulation and nerve cell development [10,11].

Obesity is considered one of the diseases of the modern era and it has negative effects on the organs of the body because it causes of high pressure on heart and arteries [12,15]. As an enzyme rises during obesity to collect fats (cholesterol) in blood and slow process of storing it in the liver and converting it to the esterified form [1]. To combat and treat obesity by inhibiting fat-building pathways through enzymes related to reducing body fat-building such as LCAT through increasing free cholesterol [14].

Nanoparticles technology refers to objects ranging in size from 1-100 nanoparticles meters that are made of metal oxides. In recent years, scientists have turned their attention to using nanoparticles to treat obesity [15]. Nanopit has become more widely used in medical and therapeutic applications, as it works to precipitate fat and increase protein and fiber content in muscles [16].

The current study is the first of its kind in terms of linking nanoparticles with enzymes that inhibit fat building. This study aims at the possibility of using the nanoparticles-extract of the Chestnut fruit in activating the activity of an enzyme in obese bodies by separating the enzyme from the blood using several techniques while separating the oil extract from the plant and converting it into nanoparticles.

Methods
Isolation and identification of oil extract

Oil extracted by Soxhlet method for two days using liquid petroleum ether (60-80)°C, then the solvent was evaporated using rotary evaporator [17].

Diagnosis of fatty acids in the oil product
Fatty acids of the oil isolated from the plant were identified using a capillary gas chromatography device in the laboratories of the Ministry of Science and Technology / Department of Environment and Water / Baghdad, which used a flame ionizing detector (FID).

Device type: Shimadzu 2010
Shaft type: SE-30
Column dimensions: 30 m x 0.25 mm x 0.25 mm
Column temperature: 120-280° C
Gas used: inert nitrogen gas.

Extraction of Lecithin cholesterol acyl transferase enzyme
In this study the serum was chosen from fat women aged 40-45 year with BMI 25-30 kg/m².

Crude extraction
Drawn was 25 ml of blood from the subjects, and the blood was left to coagulate for 15 minutes, then the precipitate was separated from the filtrate by a centrifuge at a speed of 1100g. The filtrate was taken to conduct enzyme purification from the serum at a temperature 37°C [18].

Precipitation with ammonium sulfate
The protein particles in the blood were precipitated using ammonium sulfate (67) percent weight/volume within 20 seconds with continuous stirring. Leaving it overnight at 4°C then centrifuged at 6000g. Finally, dissolved in buffer phosphate [19] and stored at -20°C.

Dialysis
After obtaining the precipitate from the previous step, the dialysis process was performed on it through addition phosphate buffer with change every 2hr [20].

Gel Filtration Chromatography
The crude solution resulting from the previous step (dialysis) was taken and then placed in a separation column with dimensions of (2.2 x 45) cm which contained gel (G-100) and phosphate buffer solution pH= 1.6. The resulting solution was collected from the purification column at a rate of 5 ml/5 min.

Determination Lecithin: Cholesterol acyl transferase activity in serum
The method included esterification of the cholesterol substrate to cholesterol ester catalysis by the enzyme, LCAT, and the method depended on measuring the absorbance intensity quinon amine by resulting from free cholesterol remaining from the reaction at wavelength of (545) nm by spectrophotometer [21].
Optimal activity concentration of oil extraction (LCAT)
The concentration of oil extraction was 10-100 mg/ml added to enzyme and nanoparticles-solution oil extraction then reached to 2-20 mg/ml [22].

Biosynthesis of silver nanoparticle with oil extraction
The oil extract of the Chestnut fruit was used in the preparation of silver nanoparticles. An aqueous solution of silver nitrate was prepared at a concentration of 1 mM. The solution was heated at 60°C, then the oil extract was stored with stirring for two hours. The central sediment was taken, dried [23,24] and diagnosed with an ultraviolet and visible spectroscopy device. The diagnosis was made in the laboratories of the College of Pharmacy, University of Nineveh.

Results
The current study was conducted for the purpose of obtaining natural products such as oils by separating them from the fruits of plants and studying their components and their biological effects. LCAT activity, diagnosis of Chestnut sativa oil extracts, diagnosed in the GC technique. It contained many fatty acids. The amount of these compounds was the percentage of fatty acid / gram of the plant fruit as shown in Table 1.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>The percentage of acid / gram of the plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic</td>
<td>22.4</td>
</tr>
<tr>
<td>Linoleic</td>
<td>40.4</td>
</tr>
<tr>
<td>Palmitic</td>
<td>10</td>
</tr>
<tr>
<td>Linolenic</td>
<td>27</td>
</tr>
<tr>
<td>Behenic acid</td>
<td>3</td>
</tr>
<tr>
<td>Stearic</td>
<td>3.7</td>
</tr>
<tr>
<td>Elaidic</td>
<td>2.1</td>
</tr>
<tr>
<td>Eicosenic</td>
<td>5.2</td>
</tr>
<tr>
<td>Heptadecanoic</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 1: Percentage of fatty acid / gram of the plant in Chestnut sativa fruit.

Purification procedure of LCAT
The substrate referred (cholesterol) was used to detect the activity of the enzyme LCAT because it had a high specificity towards the enzyme. The enzyme worked to esterify cholesterol. Figure (1) showed the steps of purification of the enzyme LCAT, separated from the blood serum of obese women, as it was noted that the activity of the enzyme LCAT increased from 350 U / ml in the first step of purification to 1190 U / ml. The last step of purification, while the percentage of enzyme LCAT recovery reached 283.33, and the specific activity increased from 4.99 U/mg to 457.69 U/mg. The number of enzyme purification times increased from 1 to 91.72.

Identification and characterization silver nanoparticles color change
It was made using an aqueous solution of silver nitrate with the oil extract of Chestnut fruit as reducing, covering and stabilizing agents for the nanoparticles at a temperature of 60°C. The initial diagnosis was done by observing changes in the color of the particles through the appearance of several colors at different times referring to silver nitrate solution from colorless to the colors shown in the figure (2).

Identification of silver nanoparticles using visible and ultraviolet spectroscopy
The results indicated the possibility of oil extract of the Chestnut fruit to produce nanoparticles, by measuring the absorption spectrum of visible and ultraviolet rays within the range (300-550) nm for solution. This technique is one of the most important methods in which nanoparticles are identified due to irritation. The rotation of the vibrations (electron, hole), as show in figure (3).
Effect of oily product on enzyme activity

The results shown in the table (2) showed the effect of the oil extract of the Chestnut fruit by activating the enzyme for the two extracts (oil alone + oil with silver particles), but the effect was higher in the oil extract.

<table>
<thead>
<tr>
<th>Oil extraction (mg/ml)</th>
<th>Activity of enzyme LCAT (U/ml)</th>
<th>Oil extraction+ nanoparticles practical (mg/ml)</th>
<th>Activity of enzyme LCAT (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1100</td>
<td>0</td>
<td>1100</td>
</tr>
<tr>
<td>10</td>
<td>1106</td>
<td>2</td>
<td>1108</td>
</tr>
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<td>1125</td>
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<td>14</td>
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<tr>
<td>200</td>
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<td>20</td>
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</tbody>
</table>

Table 2: The effect of the oil extract of the Chestnut fruit by activating the LCAT enzyme for the two extracts (oil alone + oil with silver particles).

Discussion

This enzyme lecithin: cholesterol acyltransferase is one of the most important enzymes that work to reduce cholesterol through its esterification, and thus reduces low-density lipoprotein levels and increase high-density lipoprotein, as it was noted that the enzyme did not show significant activity except at the highest peak by Gel Filtration Chromatography, as the second peak did not show any activity. Even when using the ion exchange technique, when separating the enzyme from pig blood serum and heart patients, it was found that it possesses one homologue at the upper peak with high efficiency [25,26].

It was also noted that the oily extract of the Chestnut fruit contains many unsaturated fatty acids that worked to reduce cholesterol in the blood, and the oil silver particle-extract during its preparation had a change in color. The change in color is clear evidence of the formation of silver nano-particles [27].

We have obtained silver nano-particles within the range (1-100 nm). It is that the most important characteristic that one can develop, as they were not within the size of quantum particles or quantum dots (1-15 nm) particle that cause problems when used in life technologies [28].

Also, it was observed that the absorption spectrum of the silver nanoparticles harmful to the presence of the oil extract of the Chestnut fruit. Many studies indicated that the wavelength of aqueous extracts or fungal or even bacterial extracts falls within the range [29]. The property of diffusion and adsorption of the particles due to the small size and their penetration of the active site of the enzyme, thus activating it through their attachment to the site N-glycosylation make them interesting candidates for further research. These particles increase affinity of the enzyme towards the substrate and thus increase the effectiveness of the enzyme or the speed of the enzymatic reaction [30]. As the results indicated that there was an increase in the effective of the enzyme when it was treated with the oil extract of the Chestnut and nanoparticles-extract of the oil of the Chestnut, the increase was higher in the effective of the enzyme (when the enzyme was treated with the silver particle s-oil extract, reason for this rise).

It has high stability, low cost, and important properties such as size, structure-dependent catalytic activities, surface area, and ability to self-assemble [30, 31].

It was concluded during the research that the oil extract of Chestnut fruit and the nanoparticles worked to increase the activity of the enzyme LCAT, but the increase in the activity of the enzyme was higher when treated with combination of silver particles-extract.

Acknowledgement

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Author Contributions

Eman and Dr. Intisar separated the enzyme and studied the effect of the oil extract on activating the enzyme. Assistant teacher Shaima Abbas also separated the oil extract from the plant and performed the rest of the techniques. Dr. Faith contributed in writing research and analyzing the results.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References


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