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Author's Affiliation:
1. Department of Sciences, College of Basic Education, University of Mosul - Iraq
2. College of Dentistry, University of Mosul - Iraq

***Corresponding Author:**
Intisar Ghanim Taha
Email: intsarghanim@uomosul.edu.iq

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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

Intisar Ghanim Taha[1]*, Eman Salem Mahmoud[2], Shemaa Abass Ayob[2]

Abstract

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/m^2, 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 283.33%, 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 (83%) 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 [12,13]. 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: 120-280º 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/m^2.

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 quino 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.

Fatty acids	The percentage of acid / gram of the plant
Oleic	22.4
Linoleic	40.4
Palmitic	10
Linolenic	27
Behenic acid	3
Stearic	3.7
Elaidic	2.1
Eicosenoic	5.2
Heptadecanoic	1.4

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

Purification procedure of LCAT

The substrate preferred (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.

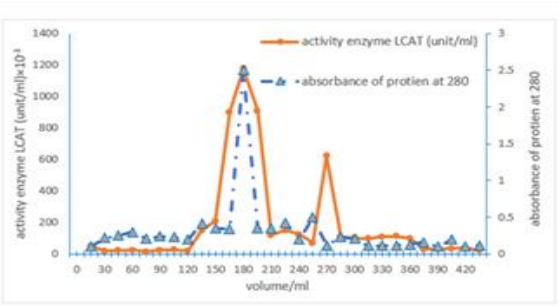

Figure 1: Elution profile of LCAT purification from serum.

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).

Figure 2. Stages of formation of nanoparticles with oil extract by changing colors.

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).

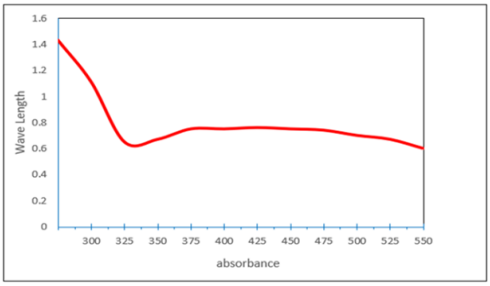

Figure 3: Absorption spectrum of silver particles with Chestnut oil extract.

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.

Oil extraction (mg\ml)	Activity of enzyme LCAT (U\ml)	Oil extraction+ nanoparticles practical (mg\ml)	Activity of enzyme LCAT (U\ml)
0	1100	0	1100
10	1106	2	1108
20	1112	4	1114
30	1120	6	1119
40	1122	8	1125
50	1125	10	1130
60	1130	12	1138
70	1134	14	1140
80	1139	16	1147
90	1141	18	1150
100	1143	20	1155

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 s).

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) 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.

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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

1. Lee RG, Kelley KL, Sawyer JK, Farese Jr RV, Parks JS, Rudel LL. Plasma cholesteryl esters provided by lecithin: cholesterol acyltransferase and acyl-coenzyme a: cholesterol acyltransferase 2 have opposite atherosclerotic potential. Circulation research, (2004); 95(10): 998-1004.
2. Ossoli A, Pavanello C, Calabresi L. High-density lipoprotein, lecithin: cholesterol acyltransferase, and atherosclerosis. Endocrinology and metabolism, (2016); 31(2): 223.
3. Ali R, Baban R, Ali S. Evaluation of lipid metabolizing enzymes: Paraxonase1 (PON1) and lecithin cholesterol acyltransferase (LCAT) activities in children with nephrotic syndrome. Baghdad Journal of Biochemistry and Applied Biological Sciences, (2021); 2(01): 48-59.
4. Gao H, Sun Z, Lu K, Qian D, Zhao Y, Xue B. Influence of lecithin cholesterol acyltransferase alteration during different pathophysiologic conditions: a 45 years bibliometrics analysis. Frontiers in Pharmacology, (2022); 131062249.
5. Lemberg A, Schreier L, Romay S, Fernández MA, Rosello D, et al. Involvement of serum apolipoprotein AI and B100 and lecithin cholesterol acyl transferase in alcoholic cirrhotics. Annals of hepatology, (2007); 6(4): 227-232.
6. Turri M, Conti E, Pavanello C, Gastoldi F, Palumbo M, et al. Plasma and cerebrospinal fluid cholesterol esterification is

hampered in Alzheimer's disease. Alzheimer's Research & Therapy, (2023); 15(1): 95.
7. Rosales C, Gillard BK, Gotto Jr AM, Pownall HJ. The alcohol–high-density lipoprotein athero-protective axis. Biomolecules, (2020); 10(7): 987.
8. Saeedi R, Li M, Frohlich J. A review on lecithin: cholesterol acyltransferase deficiency. Clinical Biochemistry, (2015); 48(7-8): 472-475.
9. Matejić JS, Stefanović N, Ivković M, Živanović N, Marin PD, Džamić AM. Traditional uses of autochthonous medicinal and ritual plants and other remedies for health in Eastern and South-Eastern Serbia. Journal of Ethnopharmacology, (2020); 261113186.
10. España MA, Rodriguez Galdon B, Diaz Romero C, Rodriguez Rodriguez E. Fatty acid profile in varieties of Chestnut fruits from Tenerife (Spain) Perfil de ácidos grasos en variedades de castañas procedentes de Tenerife (España). CyTA-Journal of Food, (2011); 9(1): 77-81.
11. Mannelli F, Minieri S, Tosi G, Secci G, Daghio M, et al. Effect of Chestnut tannins and short chain fatty acids as anti-microbials and as feeding supplements in broilers rearing and meat quality. Animals, (2019); 9(9): 659.
12. Jones NR, Ordóñez-Mena JM, Roalfe AK, Taylor KS, Goyder CR, et al. Body mass index and survival in people with heart failure. Heart, (2023); 109(20): 1542-1549.
13. Lavie CJ, Kokkinos P, Lin G-M (2023) Obesity paradox is still alive in heart failure. BMJ Publishing Group Ltd and British Cardiovascular Society. pp. 1506-1507.
14. Mc Auley MT. Effects of obesity on cholesterol metabolism and its implications for healthy ageing. Nutrition research reviews, (2020); 33(1): 121-133.
15. Trandafir LM, Dodi G, Frasinariu O, Luca AC, Butnariu LI, et al. Tackling dyslipidemia in obesity from a nanoparticlesparticlestechnology perspective. Nutrients, (2022); 14(18): 3774.
16. Liu S, Yu H, Zhu L, Zhang X, Li P, et al. Dietary nanoparticlesparticles-Se supplementation regulates lipid deposition, protein synthesis and muscle fibre formation in grass carp fed with high-fat diet. British Journal of Nutrition, (2023); 130(10): 1678-1688.
17. Sayyar S, Abidin ZZ, Yunus R. Optimisation of solid liquid extraction of jatropha oil using petroleum ether. Asia-Pacific Journal of Chemical Engineering, (2013); 8(3): 331-338.
18. Bruns DE, Tietz NW, Burtis CA, Ashwood ER Tietz textbook of clinical chemistry and Molecular diagnostics. Chapter: Book Name. 2012 of publication; Elsevier.
19. Robyt JF, White BJ. Biochemical techniques: theory and practice. (No Title), (1987).
20. Miltko R, Bełżecki G, Kasperowicz A, Michałowski T. Isolation and purification of chitinolytic enzymes of rumen ciliates Eudiplodinium maggii. Progress on Chemistry and Application of Chitin and its Derivatives, (2010); (15): 189-196.
21. Manabe M, Abe T, Nozawa M, Maki A, Hirata M, Itakura H. New substrate for determination of serum lecithin: cholesterol acyltransferase. Journal of Lipid Research, (1987); 28(10): 1206-1215.
22. Sezgin AC, Artik N. Determination of saponin content in Turkish tahini halvah by using HPLC. Adv J Food Sci Technol, (2010); 2(2): 109-115.
23. Elbossaty W. Green tea as biological system for the synthesis of silver nanoparticlesparticles. J Biotechnol Biomater, (2017); 7(269): 2.
24. Radhakrishnan K, Rettinaraja T, Mohan A, Jainulabideen SS. Synthesis of silver nanoparticlesparticlesparticles using flavonoid: apigenin and its antibacterial effect. EJPMR, (2017); 4(1): 422-426.
25. Al-Juraisy ATY, Al-Juraisy AAM. Biochemical and Kinetic Study for the partially purified Lecithin: Cholesterol acyltransferase from serum cardiovascular disease. Tikrit Journal of Pure Science, (2020); 25(2): 32-37.
26. Czarnecka H, Yokoyama S. Regulation of lecithin-cholesterol acyltransferase reaction by acyl acceptors and demonstration of its "idling" reaction. Journal of Biological Chemistry, (1993); 268(26): 19334-19340.
27. El-Chaghaby G, Rashad S, Eid H. Antioxidant, antimicrobial and anti-cancer properties of silver nanoparticlesparticlesparticles biosynthesized using artichoke waste extract. Kuwait Journal of Science, (2022); 49(3).
28. Tanase C, Berta L, Coman NA, Roşca I, Man A, et al. Antibacterial and antioxidant potential of silver nanoparticlesparticlesparticles biosynthesized using the spruce bark extract. Nanoparticlesparticlesmaterials, (2019); 9(11): 1541.
29. Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, et al. Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American Heart Association. Circulation, (2017); 135(15): e867-e884.
30. Alizadeh N, Salimi A, Sham T-K, Bazylewski P, Fanchini G. Intrinsic enzyme-like activities of cerium oxide nanoparticlesparticlescomposite and its application for extracellular H2O2 detection using an electrochemical microfluidic device. ACS omega, (2020); 5(21): 11883-11894.
31. Wei H, Wang E. Nanoparticlesparticlesmaterials with enzyme-like characteristics (nanoparticlesparticleszymes): next-generation artificial enzymes. Chemical Society Reviews, (2013); 42(14): 6060-6093.