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Role of compounds from *Terminalia chebula* exhibiting Anti-Cholesterol property

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Abstract: Cardiovascular diseases are the leading cause of deaths on the globally. Although cardiovascular disease usually affects older adults, the antecedents of cardiovascular disease, notable atherosclerosis, begin in early life, making primary prevention efforts necessary from childhood Dos. This is extremely important considering that 1 in 3 people die from complications attributable to atherosclerosis. Cholesteryl esterase is the key enzyme involved in the hydrolysis of lipids and transport of free cholesterol. The hydrolysis of dietary cholesterol ester into cholesterol by cholesteryl esterase in the intestinal lumen is an essential process for its absorption. The reduction of cholesterol absorption by inhibiting cholesteryl esterase is a new target site of intervention for the treatment of hyperlipidaemia. Elevated levels of lipids such as cholesterol and triglycerides in the blood cause hyperlipidaemia, which is an established risk factor of atherosclerosis and coronary heart disease. Although several drugs are generally well tolerated and effective, they have been found to cause many adverse side effects. It necessitates the need to evolve alternative therapies, such as herbal therapy. The results from the molecular docking of cholestryl esterase with gallic acid and ellagicacid from*Terminalia chebula* seeds have provided insight to the use of plants as drugs for the treatment of hypercholesterolemia. As revealed in this study, the compound ellagic acid has the highest potential to regulate the cholesterol level in the blood.
Keywords: Ellagic acid; gallic acid; cholesteryl esterase.

Introduction

The hydrolysis of cholesterol esters in the lumen of the small intestine is catalyzed by pancreatic cholesterol esterase, which liberates free cholesterol. Moreover, it enhances the incorporation of cholesterol into the mixed micelle and aids transport of free cholesterol to the enterocyte[1]. Inhibition of cholesterol esterase is expected to limit the absorption of dietary cholesterol, resulting in delayed cholesterol absorption[2] reduction of cholesterol absorption by inhibiting cholesterol micellization in the intestinal lumen is a new target site of intervention for the treatment of hyperlipidaemia [3].The result of this action is an attenuated postprandial hypertriacylglycerolaemia and hypercholesterolaemia, and consequently, reduced risk of the progression of micro- and macrovascular complications including microangiopathy, cardiovascular, and cerebrovascular diseases [4]. For example, the long-term efficacy of the administration of pancreatic lipase inhibitor has been reflected in the improvements in blood pressure, insulin resistance, weight loss, and serum lipid levels [5].Cholesterol is a multifunctional molecule, which servesas an essential membrane component and as a precursor to bile acids (in the liver), steroid hormones(in the adrenal, testis, and ovaries), and vitamin D [6]. Hypercholesterolemia is a well characterized cardiovascular risk factor that is known to initiate inflammatory and thrombogenic responses in the micro vascular [7].Hydrolysis of cholesterol ester in the lumen of the small intestine is catalyzed by cholesterol esterase (CEase), EC3.1.1.13, which liberates free cholesterol.

Phytosterols and their fatty acyl esters have been known for decades to lower LDL cholesterol, making them powerful nutraceuticals in lowering cardiovascular disease risk. The mechanisms by which phytosterols lower cholesterol, though, have been incompletely characterized. Cardiovascular diseases were collectively the number one cause of death in the United States in 2007 (the latest year in which data were available), accounting for one third ofdeaths [2]. Most of these deaths were attributable to ischemic heart diseases, in which aportion of the muscle in the heart does not receive adequate blood flow. A major cause of ischemia is atherosclerosis, which is characterized by inflammation and lipid accumulation in the lumen of arteries, which decreases blood flow by narrowing the artery and can eventually lead to thrombosis, thereby completely blocking the artery with a blood clot. Phytosterols have been known for almost 60 years to lower blood cholesterol in humans, with initial hypotheses pointing to a disruption of cholesterol absorption [8]. Since then, many studies have been done to quantify the effects of phytosterols on blood cholesterol, with one meta-analysis indicating that phytosterols can lower LDL-C (which is considered atherogenic) by approximately 10% [9]. Phytosterols are therefore a powerful nutraceutical for lowering an atherosclerotic risk factor. In the decades since the initial discovery, a number of mechanisms have been proposed and investigated toexplain the cholesterol-lowering properties of phytosterols.

Materials and Methods

Preparation of plant materials

Fresh fruits of *Terminalia chebula* were collected from Tiruchirappalli during the month of December, 2012.The freshly collected fruits were spread to dry under shade at normal room temperature for seven days in the shade. Upon drying, the leaves were pounded using mortar and pestle into smaller particles and then blended to powder and thepowder was stored in airtight containers and kept under normal room temperature (28 ± 2°C) until required. The plant was identified and authenticated at, St. Joseph's College Trichy, Tamilnadu, India and was given the Voucher Specimen No. VEA/001/2013.

Extraction procedure

10 g of powdered sample was soaked in 100 ml solvent contained in a 500 ml sterile conical flask. The flask was covered with cotton plug and then wrapped with aluminum foil and shaken vigorously at 3 h intervals for 48 h at room temperature. The crude extract was then filtered using muslin cloth and then Whatman no.1 filter paper. The filtrate was evaporated to dryness using rotary evaporator (Model 349/2, Corning Limited) maintained at 40°C and the dried substance was stored in airtight bottles until required. The acetone extracts of the plant was used for GC-MS analysis.

GC –MS Analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising aAOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Results & Discussion

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

The Structure of the enzyme Cholesterol esterase with the PDB ID 1F6W was retrieved from the Protein Data Bank. It is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. After obtaining the structure from Protein Data Bank, the possible binding sites of cholesterol esterase were searched using Q-site Finder.

These include pockets located on protein surfaces and voids buried in the interior of proteins. Q-site Finder includes a graphical user interface, flexible interactive visualization, as well as on-the-fly calculation for user uploaded structures. The inhibitor and target protein was geomentrically optimized and docked using the docking engine Argus Dock. (http://www.arguslab.com/). ArgusLab consists of a user interface thatsupports OpenGL graphics display of molecule structures and runsquantum mechanical calculations using the Argus compute server.

Interference with cholesterol-related enzymes

The interaction of phytosterols with cholesterol-metabolizing enzymes can dramatically affect their cholesterol lowering properties. One such enzyme, pancreatic cholesterolesterase (PCE), is involved in hydrolyzing sterol esters into unesterified sterols. Specificity of the enzyme depends on the sterol moiety, with cholesterol esters more rapidly hydrolyzed than phytosterol esters, and the fatty acyl moiety, with saturated esters being less well hydrolyzed than unsaturated and di-hydroxystearate inhibiting the enzyme. The importance of hydrolyzing esters is described above micellarization). Because a relatively small amount of intestinal cholesterol is esterified, competition for pancreatic cholesterol esterase is likely not a large contributing mechanism for decreased cholesterol absorption.

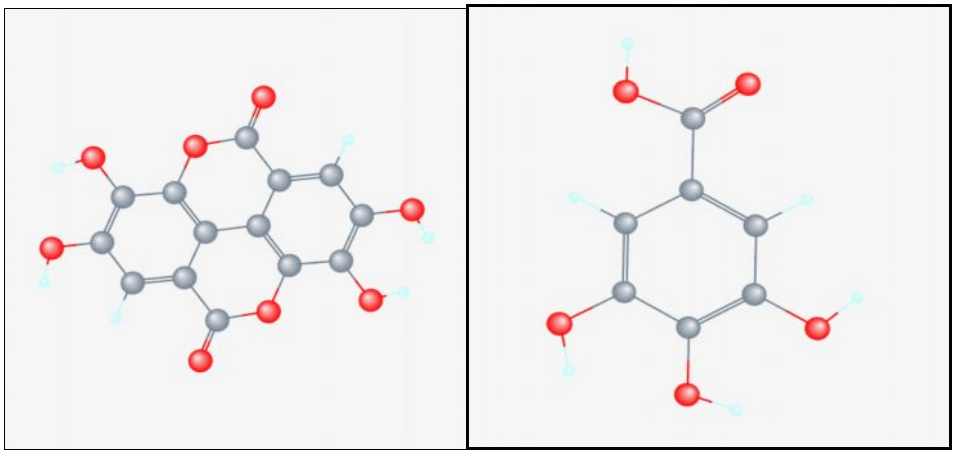


Fig.1: 3D Structures of Ellagic acid and Gallic acid

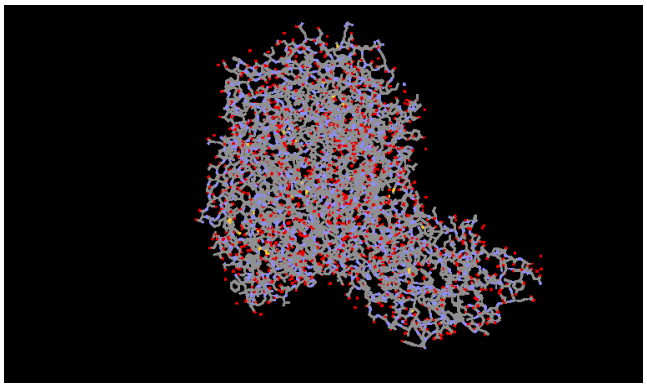


Fig.2: Structure of Cholesteryl Esterase (2BCE, DOI: 10.2210/pdb2bce/pdb)

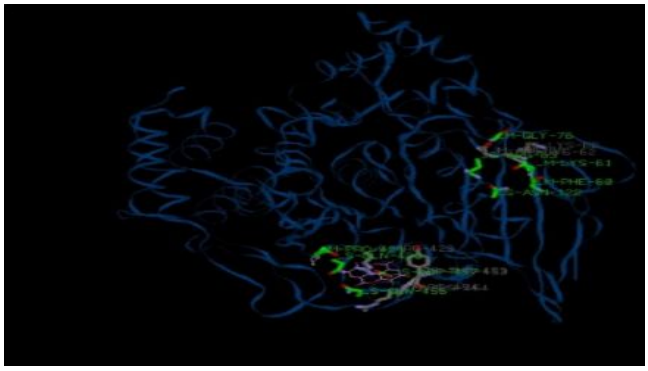


Fig.3 Ligand 2BCE withElagic acid, Gallic acid, (CID_5281855, CID_370)

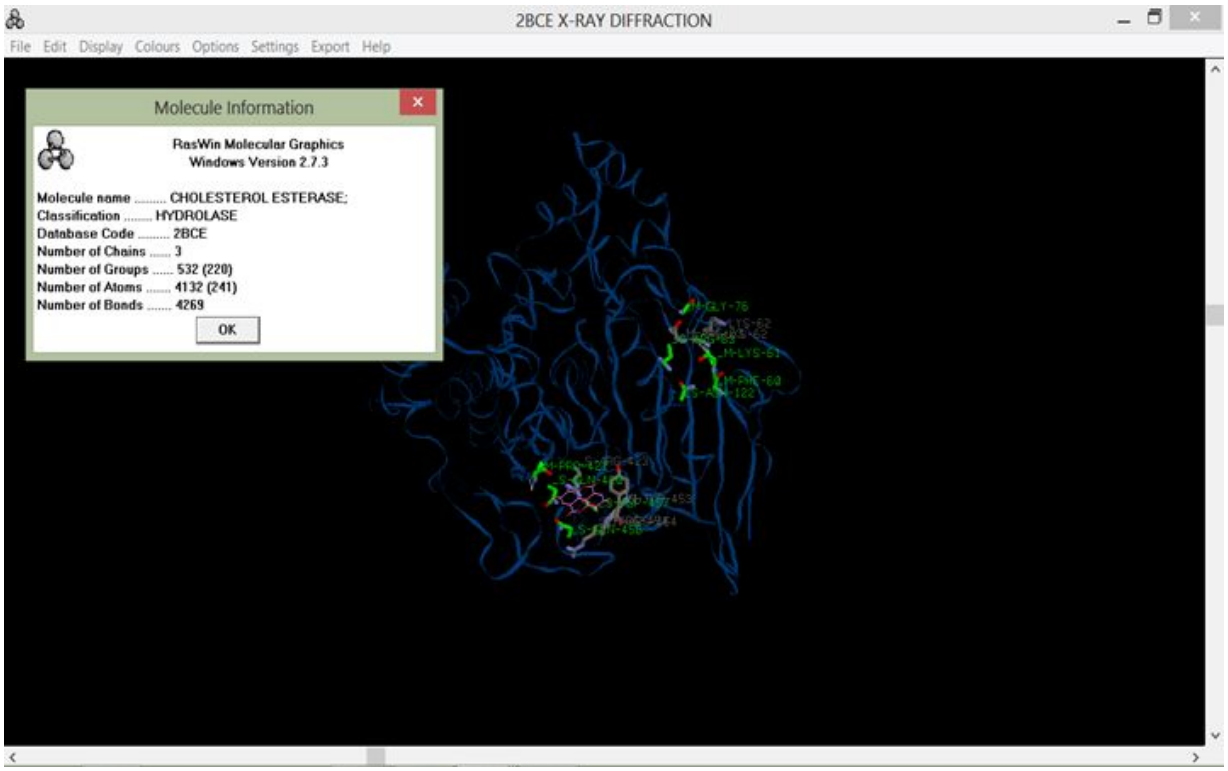
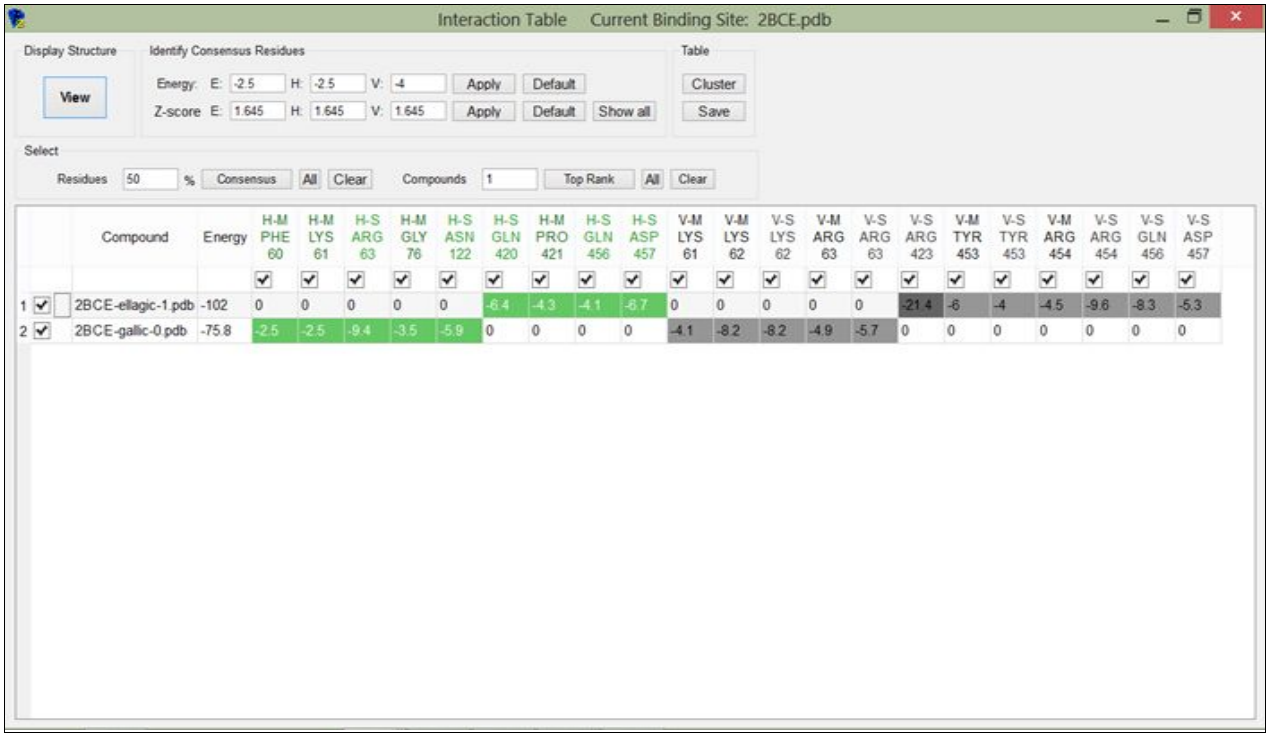


Fig.4: Docking of cholesterol esterase with ellagic acid and galic acid

Table 1:Binding Energy and Hydrogen Bond Interaction for the best compound docked against 2BCE



	Compound	Energy	H-M PHE 60	H-M LYS 61	H-S ARG 63	H-M GLY 76	H-S ASN 122	H-S GLN 420	H-M PRO 421	H-S GLN 456	H-S ASP 457	V-M LYS 61	V-M LYS 62	V-S LYS 62	V-M ARG 63	V-S ARG 63	V-S ARG 423	V-M TYR 453	V-S TYR 453	V-M ARG 454	V-S ARG 454	V-S GLN 456	V-S ASP 457
1	2BCE-ellagic-1.pdb	-102	0	0	0	0	0	-6.4	-4.3	-4.1	-6.7	0	0	0	0	0	-21.4	-6	-4	-4.5	-9.6	-8.3	-5.3
2	2BCE-gallic-0.pdb	-75.8	-2.5	-2.5	-9.4	-3.5	-5.9	0	0	0	0	-4.1	-8.2	-8.2	-4.9	-5.7	0	0	0	0	0	0	0

The present work was taken up to determine the potential efficacy of hypocholesteric activity of bioactive compounds from *T. chebula*. Among the two compounds tested, ellagic acid exhibited lower energy as – 102, while galic acid demonstrated -75.8. Hence, the present study indicates that the herbal species *Terminalia chebula* can be used in the treatment of Hypercholesterolemia, which shows a strong binding affinity towards Cholesterol esterase. This brings a strong focus towards these herbs that, when administered during the treatment of hypercholesterolemia may block cholesterol esterase. Ellagic acid showed the highest affinity towards cholesterol esterase compared to gallic acid. This creates a strong hypothesis that the effects of complex formation by cholesterol esterase and ellagic acid contribute towards combating against hypercholesterolemia. Hence, Cholesterol esterase may become a prospective target for inhibition of hypercholesterolemia and may unlock a strong initiative in developing novel ligand which is specified towards it. The mechanism of action is the compound to inhibit the activity of cholesterol esterase that is involved in cholesterol absorption. So, it regulates the cholesterol level in the blood and reduces hypercholesterolemia.

References

1. Myers-Payne SC, Hui DY, Brockman HL, Schroeder F,Cholesterol esterase: A cholesterol transfer protein, Biochemistry,1995, 34: 3942–3947.
2. Heidrich JE, Contos LM, Hunsaker LA, Deck LM, Vander Jagt DL, Inhibition of pancreatic cholesterol esterase reduces cholesterol absorption in the hamster, BMC Pharmacol.2004, 4: 5.
3. Kirana C, Rogers PF, Bennett LE, Abeywardena MY, Patten GS. Naturally derived micelles for rapid *in vitro* screening of potential cholesterol-lowering bioactives, J.Agric. Food Chem. 2005, 53 : 4623–4627.
4. Neovius M, Narbro K. Cost-effectiveness of pharmacologicalanti-obesity treatments: A systematic review, Int. J.Obes. 2008, 32: 1752–1763.
5. Ioannides-Demos LL, Piccenna L, McNeil JJ. Pharmacotherapies for obesity: Past, current, and future therapies, J.Obes. 2011 Article no. 179674
6. Xu J, Kochanek K, Murphy S, Tejada-Vera B. Deaths: final data for 2007. Natl Vital Stat Rep.2010, 58:1-136
7. Karen Y. Stokes, LeShanna Calahan, Candiss M. Hamric, Janice M. Russell and D. Neil Granger, CD40/CD40L contributes to hypercholesterolemia-induced microvascular inflammation. American J. Physiol. Heart Circ. Physiol. 2009, 296(3): H689–H697.

8. Pollak O. Reduction of blood cholesterol in man. Circulation,1953, 7:702-706.
9. Demonty I, Ras RT, Van der Knaap HCM, Duchateau GSMJE, Meijer L, Zock PL,Geleijnse JM, Trautwein EA. Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr. 2009, 139:271-284.

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