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Mechanisms of Semen Cassiae to Treat Hypercholesterolemia Based on Molecular Docking

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Abstract

Hypercholesterolemia is a major risk factor of cardiovascular disease with high prevalence rate. Studies have shown that the traditional Chinese medicine: Semen Cassiae can reduce blood cholesterol and lipids, but the functional components and molecular mechanisms need further elucidation. In this study, molecular docking technology is used to explore the mechanism of Semen Cassiae in the treatment of hypercholesterolemia. A total 88 active ingredients and 196 isomers of Semen Cassiae were obtained from the traditional Chinese medicine database: HERB. 6 hypercholesterolemia-related drug targets: proprotein convertase subtilisin/kexin type 9 (PCSK9), thyroid Hormone Receptor β (THRB), peroxisome proliferator-activated receptors (PPARd), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), insulin receptor (IR) and squalene monooxygenase (SQLE) were obtained from the Therapeutic Target Database. After data preprocessing, molecular docking analysis was conducted using the LibDock tool in Discovery Studio software, and the main "active ingredient-target" combinations with high scores were screened. The structure results showed high binding affinity of the active ingredients to the hypercholesterolemia-related targets. Gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis showed the involved pathways, including cholesterol and lipid metabolism, lipoprotein particle formation, cholesterol and lipid transfer and lipoprotein particle receptor binding processes. This study revealed that the anti-hypercholesterolemia activity of Semen Cassiae is a multicomponent, multitarget, and multipathway effects, and provides evidence for further development the clinical application value of Semen Cassiae on hypercholesterolemia.

Keywords: Semen Cassiae, hypercholesterolemia, molecular docking

1. Introduction

Cardiovascular diseases are the leading cause of morbidity and mortality in the world, and threaten millions of individuals' lives every year (Roth GA, Mensah GA & Fuster V., 2020; Zhang Y Q, 2021). Hypercholesterolemia, as a major risk factor of cardiovascular disease, is typically caused by mutations in genes of proteins responsible for removing low density lipoprotein (LDL) from the circulation (Ference BA, Ginsberg HN, Graham I & et al., 2017; Sharifi M, Futema M, Nair D & et al. 2019; Sjouke B, Kusters DM, Kastelein JJ & et al., 2011). The death and disease burden of hypercholesterolemia increased significantly from 1990. In 2012, 40.40% of Chinese adults had dyslipidemia, the prevalence rate of hypercholesterolemia was 4.9%, which meant about 66 million people suffered from this disease (Zhao S P, 2016). In recent years, hypercholesterolemia has been improved by lipid-lowering therapies combined with proper diet and exercise program (Tokgozoglu L & Kayikcioglu M., 2021). However, there are still many patients unable to achieve the recommended target levels of LDL. Therefore, it is of great significance to develop novel drugs and therapy strategies that can effectively treat hypercholesterolemia.

The traditional Chinese medicine Semen Cassiae (Jue Ming Zi in Chinese) is the dried and mature seeds of Cassia obtusifolia L. or Cassia tora L, which are leguminous plants prevalent in China, Korea, India, and the

western tropical regions. It has long been used as a traditional medicine to treat eye inflammation, photophobia, dizziness, headache, and constipation in China, Korea, and Japan (Ju MS, Kim HG, Choi JG & et al., 2010; Shrestha S, Paudel P, Seong SH & et al., 2018). Semen Cassiae has several pharmacological properties, including fat and cholesterol-lowering, hypoglycemic, anti-inflammatory, antimicrobial, neuroprotection, anti-Alzheimer's disease and anti-cancer effects (Shrestha S, Paudel P, Seong SH & et al., 2018; Jung HA, Ali MY, Jung HJ & et al., 2016; Mei L, Tang Y, Li M & et al., 2015; Tzeng TF, Lu HJ, Liou SS & et al., 2013; Drever BD, Anderson WG, Riedel G & et al., 2008). There are more than one hundred ingredients of it have been identified. Among them, anthraquinones, naphthopyrones and flavonoids are considered the primary functional components (Shrestha S, Seong SH, Paudel P & et al., 2017; Jung HA, Ali MY & Choi JS., 2016; Pang X, Li NN, Yu HS & et al., 2019). It has been reported that Semen Cassiae has effects on reducing blood lipids. Studies have found that Anthraquinones could regulate LDL-C metabolism, increase the content of HDL-C in serum, and reduce the absorption of cholesterol by the intestine (Li XE & Guo BJ., 2002; Luo X, Xu X, Huang C & et al., 2011). However, the functional components and molecular mechanisms of Semen Cassiae's anti- hypercholesterolemia effects need further exploration to develop its medicinal value.

Computational approaches are useful tools to interpret and guide experiments to expedite the drug design process. Currently, by produce atomic level structure-activity relationships (SAR), computer aided drug design (CADD) has been widely used to facilitate the drug design process thereby minimizing time and costs (Yu W & MacKerell AD, Jr., 2017; Macalino SJ, Gosu V, Hong S & et al., 2015). Molecular docking is one of the most frequently used methods in CADD. It enables the identification of novel compounds of therapeutic interest, predicting ligand-target interactions at molecular level, and producing SAR (Ferreira LG, Dos Santos RN, Oliva G & et al., 2015; Pinzi L & Rastelli G., 2019). There are a variety of docking algorithms are available, understanding the advantages and limitations of each method is critical to strategies selection and results generation.

In this study, molecular docking was used to systematically analyze the active components in Semen Cassiae and important drug target genes of hypercholesterolemia to screen out potential active pairs. GO and KEGG pathway analysis were also carried out to reveal relative pathways. These findings can provide foundations for further mechanism study of Semen Cassiae in treatment of hypercholesterolemia, and also reference for using CADD methods to conduct traditional Chinese medicine research.

2. Literature Review

2.1 Acquisition of Active Ingredients of Semen Cassiae

The traditional Chinese medicine database: HERB (http://herb.ac.cn/) was used to search the active ingredients of Semen Cassiae (Fang S, Dong L, Liu L & et al., 2021). Discovery Studio software 2.5 (Accelrys Software Inc, San Diego, CA, USA) was used for further screen and processing. Initial screen was conducted using the Filter by "Lipinski and Veber Rules". Lipinski rule is a method to evaluate the chemical and physical properties of compounds with pharmacological activities and the Veber rule is its extension (Veber DF, Johnson SR, Cheng HY & et al., 2002). Then the screened ingredients were optimized for the MerckMolecular Force Field (MMFF) by using the Change Forcefield function and hydrogenated by the Prepare Ligands function (Yan P, Zheng C, Ye H & et al., 2016).

2.2 Access to Hypercholesterolemia-Related Drug Targets

In the Therapeutic Target Database (https://db.idrblab.org/ttd/), using "hypercholesterolemia" as the search term to access identified drug target genes. Information of targets were collected through Uniport and literature review. The experimentally determined 3D structure of the targets were downloaded from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do) and processed by method described before (Yang X, Liu H, Liu J & et al., 2016). Further pretreatments include Water Molecule and ligands deletions, incomplete amino acid residues supplement, hydrogenation, and remove multi conformation of the targets by the "Clean Protein" function in Discovery Studio software.

2.3 Molecular Docking

The processed active ingredients of Semen Cassiae were molecularly docked to hypercholesterolemia-related drug targets by the LibDock tool in Discovery Studio software. The LibDock score and corresponding active sites were obtained. The higher the LibDock score is, the stronger their binding affinity. By comparing with the original ligands and set the LibDock Score > 100 as the screening threshold, the active substances that can effectively binding with hypercholesterolemia-related drug targets were screened, and related structure were generated.

2.4 GO Analysis and KEGG Pathway Analysis

The intersecting targets between the Semen Cassiae and hypercholesterolemia were imported into the Database

for Annotation, Visualization and Integrated Discovery database (http://david. ncifcrf.gov/). Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted. Related results were presented as bar graphs and bubble plots respectively.

3. Results

3.1 Screening of Potential Active Ingredients of Semen Cassiae

The HERB database was used to collect the potential active ingredients of Semen Cassiae. After further screen and processing, eighty-eight active ingredients and one hundred and ninety-six isomers were obtained (Supplementary table 1).

3.2 Disease Target Screening

The Therapeutic Target Database were used to access to hypercholesterolemia-related drug targets. After information collection and literature review, 6 hypercholesterolemia targets were found (Table 1). These targets were standardized by gene names using the UniProt database.

Table 1. Target Protein for Treatment of Hypercholesterolemia

Targets	PDB ID
Proprotein convertase subtilisin/kexin type 9 (PCSK9)	6U2N
Thyroid Hormone Receptor β(THRB)	3GWS
Peroxisome proliferator-activated receptors (PPARd)	1GWX
3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR)	3CCT
Insulin receptor (IR)	4IBM
Human squalene epoxidase (SQLE, squalene monooxygenase)	6C6N

3.3 Molecular Docking Analysis

The 6 target proteins (PCSK9, THRB, PPARd, HMGR, IR and SQLE) were docking with the 88 active components by LibDock tool in Discovery Studio software. The 10 screened docking results were shown in Table 2, and related diagrams were illustrated in Figure1. The key amino acids in binding sites of target proteins and interactions including van der Waals force, carbon hydrogen bond, Amide-Pi Stacked, Alkyl, Pi-Alkyl, Pi-Sigma and Pi-Cation were all showed.

Table 2. Docking pairs above threshold

Targets	Active components in Semen Cassiae	Lib dock score	Lib dock score with original ligands
THRB	Stigmasterol	161.673	110.11
	Campest-5-en-3beta-ol	157.859	110.11
PPARd	Ochrolifuanine a	152.757	156.313
PCSK9	Ochrolifuanine a	146.785	149.669
	Stigmasterol	129.921	149.669
SQLE	Ochrolifuanine a	145.141	165.62
	gamma-sitosterol	138.036	165.62
HMGR	Ochrolifuanine a	127.247	136.238
	Vitetrifolin c	112.813	136.238
IR	Vitetrifolin c	112.635	114.816

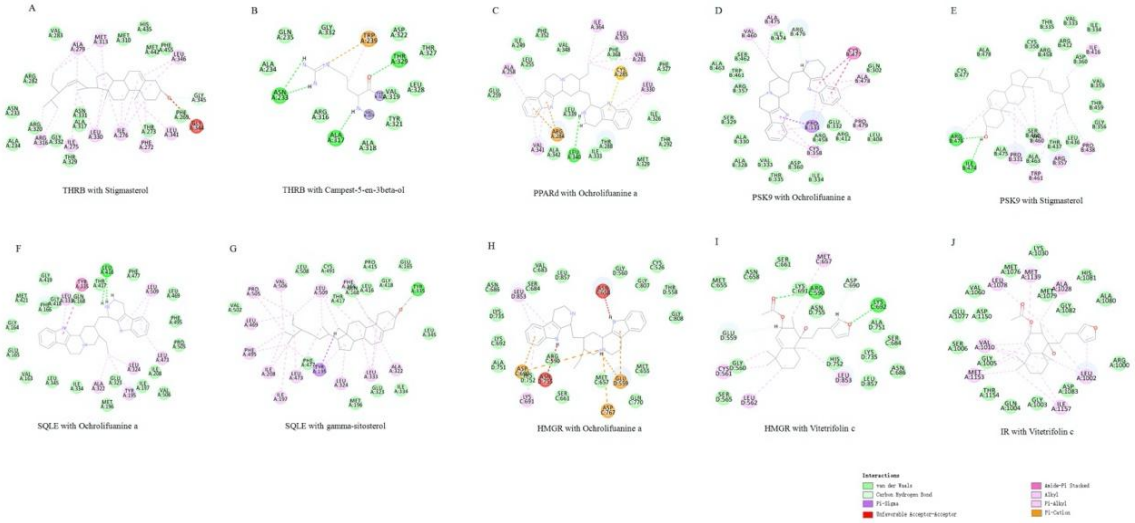

Figure 1. The molecule docking diagrams of active components in Semen Cassiae to hypercholesterolemia-related drug targets

3.4 GO Analysis and KEGG Pathway Analysis

The intersecting targets of Semen Cassiae and hypercholesterolemia were imported into the Database for Annotation, Visualization and Integrated Discovery database for GO analysis and KEGG pathway enrichment analysis. As shown in Figure 2, the obtained biological processes were mainly related to the cholesterol and lipid metabolism and alcohol metabolism. The obtained cell composition pathways were mainly related lipoprotein (LDL, HDL, etc) particle formation, endoplasmic reticulum and endocytic vesicle lumen functions. The obtained molecular function pathways were about cholesterol and lipid transfer and lipoprotein particle receptor binding processes. As illustrated in Figure 3, KEGG enrichment analysis shown that pathways related to hypercholesterolemia were screened, including cholesterol and terpenoid backbone metabolism, fat and vitamin digestion and absorption, AMPK signaling pathway and TCA cycle. These results show that the Semen Cassiae could treat hypercholesterolemia through multiple targets and multiple pathways.

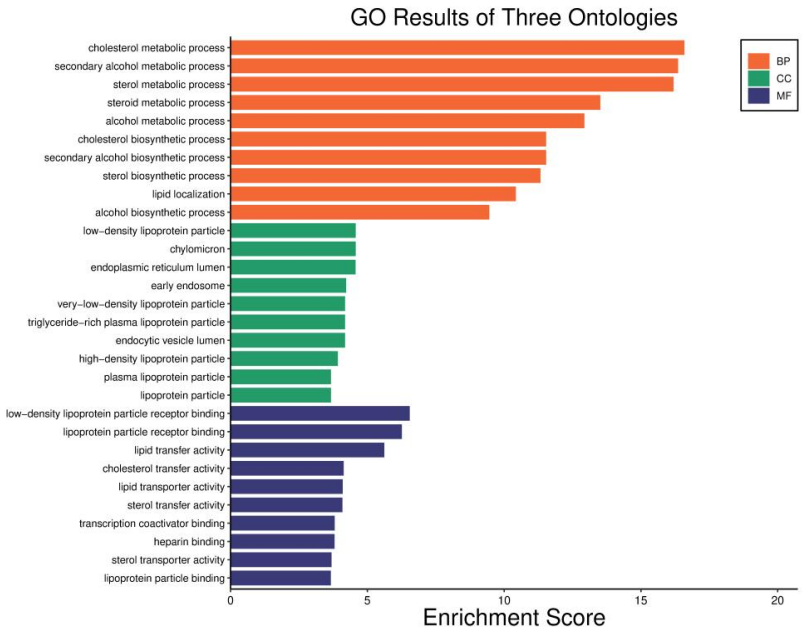

Figure 2. Go enrichment analysis

BP: biological process, CC: cell composition, MF: molecular function

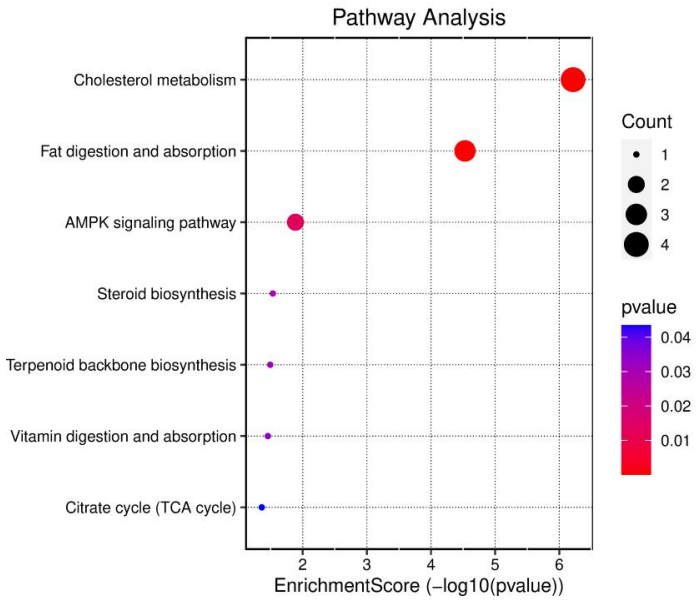

Figure 3. KEGG pathway analysis

AMPK: AMP-activated protein kinase, TCA cycle: tricarboxylic acid cycle

4. Discussion

Pharmacological studies on Semen Cassiae have shown its wide spectrum of medicinal functions, including fat and cholesterol-lowering. A series of putative active compounds were also isolated and identified by separation-based methods such as TLC, HPLC, high-speed counter-current chromatography and column chromatography (Ali MY, Park S & Chang M., 2021). Among them, anthraquinones and naphthopyrone are the primary functional components, and studies suggesting some compounds might have great medical significance in the future. However, despite there are *in vitro* and *in vivo* studies to substantiate its bioactivities to disease, most action mechanisms are still unknown. More studies are required before Semen Cassiae and its components can be considered for clinical use.

For exploring the action mechanisms of Semen Cassiae to hypercholesterolemia, molecular docking was conducted in this study. More than 100 active ingredients of Semen Cassiae were screened from HERB Database, which consistent with other studies. Key target proteins for treating hypercholesterolemia were obtained from Therapeutic Target Database, which mainly focused on disease-related genes that have been used as drug targets. There are 6 targets screened. After function scoring and threshold screening, the highest active ingredients of Semen Cassiae to hypercholesterolemia include Ochrolifuanine a, Stigmasterol, Campest-5-en-3beta-ol and Vitetrifolin c. Reported active substances of Semen Cassiae such as Anthraquinone and naphthopyrones were not with high scores during our analysis. This implies that they may not direct binding on target genes but through other mechanisms. These results illustrated the multicomponent and multitarget characteristics of Semen Cassiae for hypercholesterolemia treatment.

Table 3.

Targets	PDB ID
Proprotein convertase subtilisin/kexin type 9 (PCSK9)	6U2N
Thyroid Hormone Receptor β(THRB)	3GWS
Peroxisome proliferator-activated receptors (PPARd)	1GWX
3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR)	3CCT
Insulin receptor (IR)	4IBM
Human squalene epoxidase (SQLE, squalene monooxygenase)	6C6N

The screened targets for treating hypercholesterolemia were PCSK9, Thyroid hormone receptor β, PPARd,

HMGR, insulin receptor and SQLE. PCSK9 is a serine protease involved in a protein-protein interaction with the LDL receptor that has both human genetic and clinical validation. Blocking this protein-protein interaction prevents LDL receptor degradation and thereby decreases LDL cholesterol levels (Petrilli WL, Adam GC, Erdmann RS & et al., 2021; Cunningham D, Danley DE, Geoghegan KF & et al., 2007). Thyroid hormone receptor β (THRB) is critical for the regulation of thyroid hormones on cholesterol and bile acid metabolism (Angelin B & Rudling M., 2010). Studies have showed that drugs such as macrothyroxine could reduce the LDL level by interaction with the THRB (Jakobsson T, Vedin LL & Parini P., 2017). The PPARs are nuclear receptors for fatty acids that regulate glucose and lipid homeostasis. Reports have proved that PPARd is related to cholesterol metabolism, and participates in lipid homeostasis (Pettersson I, Ebdrup S, Havranek M & et al., 2007; Skogsberg J, McMahon AD, Karpe F & et al., 2003). HMGR catalyze the generation of mevalonateis, and is a rate-limiting enzyme in the process of cholesterol synthesis in liver cells. Statins are a class of known effective HMGR inhibitors, and inhibition of HMGR could reduce cholesterol synthesis (Sarver RW, Bills E, Bolton G & et al., 2008; Wang Z, Cheng L, Kai Z & et al., 2014). Studies have found that insulin receptor could bind with LDL receptors in the absence of insulin, further inhibit the function of the latter and result in the accumulation of extracellular LDL. Drugs could regulate the LDL level by interfering this combination (Chandra NC., 2021; Anastassiadis T, Duong-Ly KC, Deacon SW & et al., 2013). Squalene monooxygenase (SQLE) is a cholesterol synthesis flux control enzyme, which catalyzes the conversion of squalene to 2,3(S)-oxidosqualene. This reaction is a step of the biosynthetic process of lanosterol, and further affects the synthesis of cholesterol. SQLE inhibition is targeted for the treatment of hypercholesteremia, cancer, and fungal infections (Gill S, Stevenson J, Kristiana I & et al., 2011; Padyana AK, Gross S, Jin L & et al., 2019). Our docking analysis illustrate that the binding of active ingredients of Semen Cassiae could affect the structure of these targets. These could further affect their catalytic activity or normal functions, and then achieve lipid-lowering effects. The GO and KEGG analysis results also consistence with these functions.

As the understanding of the pathogenesis of disease is not deepened, it is recognized that most diseases are complex systemic diseases with multiple factors and links, which has greatly impacted the traditional concept of drug research and development, and turned to drug research with multiple components, multiple targets and affinity (Yang X, Liu H, Liu J & et al., 2016). At present, the research and development of new drugs is becoming more and more difficult. Many pharmaceutical enterprises have turned to the development of natural drugs. In recent years, it is generally believed that the mechanism of action of traditional Chinese medicine is multi-component and multi target (Ali MY, Park S & Chang M., 2021), which coincides with the current concept of drug research and development. It has a good prospect for the development of traditional Chinese medicine, especially computer assisted research and development of traditional Chinese medicine

There are limitations of our study. First, the research data are obtained from related literature and database, the integrity and authenticity of these results need be further determined and improved. In addition, further systematic pharmacology and biology studied are needed to conduct to confirm the prediction reactions and treatment effects.

5. Conclusion

In this study, the active substances in cassia seed which interact with important target genes of hypercholesterolemia were analyzed by means of simulation and mathematical model, providing a basis for further elucidating the mechanism of action and developing new drugs. Semen Cassiae is an edible medicinal plant that is important to the food industry and has a wide range of potential pharmacological uses. This study provides an explanation for the anti- hypercholesterolemia activity of Semen Cassiae, and also evidences for further development of this traditional Chinese medicine.

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