Exploring the Pharmacological Mechanism of Cassiae Semen a Cting on Cataracts Based on a Network Pharmacology Approach

Ying Zhong

Shaoxing Shangyu People's Hospital

Youfa Fang (✉ youfafang@126.com)

Shaoxing Shangyu People's Hospital

Research

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Abstract

Background

Cassiae Semen (CS) is one of the most well-known herbs used in the treatment of cataracts in China. However, the potential mechanisms of its anti-cataracts effects have not been fully explored.

Method

The active compounds of CS were obtained from TCMSP database, and their targets were retrieved from the TCMSP, STITCH and DrugBank databases. Cataracts related target genes were identified from the GeneCard, Malacard, and OMIM databases. GO and KEGG analysis were performed using DAVID online tools, and Cytoscape were used to construct compound-targets network and protein-protein interaction (PPI) networks, cluster analysis were carried out using MCODE plugin for Cytoscape.

Results

We obtained 13 active compounds from CS and 105 targets in total to construct a compound-target network, which indicated that emodin, stigmastero, and rhein served as the main ingredients in CS. A total of 238 cataracts related targets were identified from public databases. PPI networks of compound targets and cataract-related targets were constructed and merged to obtained the central network, enrichment analysis showed 50 key targets in the central network enriched in several important signaling pathways, such as thyroid hormone signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway. The top 4 genes with higher degree in the central network were TP53, HSP90, ESR1, EGFR, indicating their important roles in the treatment of cataracts.

Conclusions

The present study systematically revealed the multi-target mechanisms of CS on cataracts using network pharmacology approach, and provided indications for further mechanistic studies and also for the development of CS as a potential treatment for cataracts patients.

Background

Cataract is currently the main cause of visual impairment and blindness globally, accounting for 46 percent of blind people [1,2]. Visual impairment leads to a series of difficulties in patients' daily life and social problems, which would contribute to an extensive economic burden to the society [3]. Up to date, surgery is the main method for the treatment of cataracts. Nevertheless, in developing countries, owing to the limited access to surgery caused by higher prevalence of blindness due to cataracts [4] and lack of medical resources [4], it is urgent to develop pharmacological strategies to management of cataracts. Based on the mechanism of cataracts' formation, herbal, minerals, amino acids, and antioxidants were developed to treat cataracts. Meanwhile, there are other available approaches by inhibiting glycation, phase separation, matrix metalloproteinase and modulating the TGF- β pathway [6].

Cassiae semen (CS), the seed of *Cassia obtusifolia* L. or *Cassia tora* L. of the family Leguminosae, was initially recorded in the earliest book of Chinese materia medica “Shennong Bencao Jing” and described for treating dizziness and headache, improving vision, and nourishing the liver [7]. Modern pharmacological studies reported the therapeutic potential of *Cassia tora* leaves in preventing cataract [8,9]. It has been revealed that anthraquinone compounds, including obtusin, emodin, aloe-emodin, are the main bioactive components in CS [10-12]. In addition, recent study suggested that emodin could serve as a potential therapeutic agent for cataract [13], and the antioxidant activity of active ingredients from CS has also been confirmed in many studies [13], which may be used as antioxidants for cataracts. However, although many studies confirmed the CS showed noticeable anti-cataracts effects, the underlying mechanisms against cataracts have not been fully explored yet.

In this study, we aimed to systematically elucidate the the pharmacological mechanisms of CS against cataracts based on a network pharmacology approach. Firstly, we screened for active ingredients of CS on the basis of oral bio-availability (OB) and drug-likeness (DL) parameters, and obtained the targets of the active ingredients. In addition, cataracts related targets were identified through three databases (OMIM, Malacards and Genecards). PPI data were obtained and used to constructed PPI network, and GO and KEGG enrichment analyses were carried out to find the potential mechanism of CS against cataracts.

Materials And Methods

Data preparation

Active compounds and their targets in CS

The active compounds in CS were identified and obtained from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) (<https://tcmospw.com/tcmosp.php>) [17]. It gathered the information of herbs, compounds, compound targets, compound related diseases, and pharmacokinetic properties of each compounds. In this study, the compounds with $OB \geq 30\%$ and $DL \geq 0.18$ were identified as active ingredients. The adopted threshold values for OB and DL indicated good oral absorption and suitable characteristics for drug development of the compounds [17,18].

In addition, to identify the corresponding targets of CS active compounds, the TCMSP database, STITCH (<http://stitch.embl.de/>) and the Drugbank database (<https://www.drugbank.ca/>) were used to find potential targets. Eventually, 13 active compounds of CS were obtained (**Table 1**), with a total of 105 targets after removing duplicates.

Potential target genes of cataracts

The cataracts-related targets were identified from three public databases, including the GeneCards (<https://www.genecards.org/>) database, Online Mendelian Inheritance in Man (OMIM, <https://www.omim.org/>) database, and the MalaCards ([https:// www.malacards.org/pages/info](https://www.malacards.org/pages/info))

database [19-21]. Then we obtained the standard gene names of the identified targets from the UniprotKB (<https://www.uniprot.org/help/uniprotkb/>) database.

Protein-protein interaction (PPI) data

We obtained the PPI data using the plugin Bisogenet [22] of Cytoscape 3.5.1 software, which collected PPI data from six databases, including the Database of Interacting Proteins (DIP™), Biological General Repository for Interaction Datasets (BioGRID), Human Protein Reference Database (HPRD), IntAct Molecular Interaction Database (IntAct), Molecular INTERaction database (MINT), and biomolecular interaction network database (BIND), and visualized the PPI network of compound targets and disease targets with Cytoscape software.

Network construction and analysis

Network analysis can scientifically interpret the complex relationships among herbs, compounds, diseases, and genes [23,24]. In the study, the compounds-targets network and the PPI networks of CS compound targets and cataracts-related targets were generated by Cytoscape (version 3.7.1) [25]. MCODE Cytoscape plugin was used to carry out module analysis. The key targets and the central network was screened using a topological method, which adopts six topological parameters, including degree centrality (DC), closeness centrality (CC), betweenness centrality (BC), Eigenvector Centrality (EC), Local average connectivity-based method (LAC), and Network Centrality (NC), to assess the central attributes of all nodes in a network with the Cytoscape plugin CytoNCA. Specifically, nodes which value are greater than the mean value for all six parameters were identified as key targets, and the central network composed of these key nodes and the edges between them was also depicted using Cytoscape software.

Enrichment analysis

In this study, we used online tools of the Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov>, v6.8) to perform gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis [26]. Functional categories and pathways with significant changes of $p < 0.05$ were identified. The top 10 GO functional categories and the top 20 pathway categories were used to plotting.

Results

In our study, a total of 13 active compounds in CS were identified using ADME model, including [rhein](#), [toralactone](#), [stigmasterol](#), [aloe-emodin](#), campesterol, rubrofusarin gentiobioside, [rubrofusarin](#), [aurantio-obtusin](#), [obtusin](#), [gluco-obtusifolin](#), [9,10 - dihydroxy - 7 - methoxy - 3- methylene - 4H- benzo\[g\]isochromen - 1 - one](#), [quinizarin](#), and [CLR](#). Detailed information is presented in **Table 1**. Among these compounds, [rubrofusarin](#) was failed to get targets information in public databases.

CS compound-target network

The compound-target network consisted of 117 nodes (12 active compounds and 105 targets) and 152 edges, as shown in **Fig. 1A**. The top 3 compounds in the network with more targets were MOL000471 ([aloe-emodin](#), degree=32), MOL000449 ([Stigmasterol](#), degree=31), and MOL002268 ([rhein](#), degree=20), indicating their important role in treating cataracts. Furthermore, it showed that many targets were connected and affected by multiple compounds. Prostaglandin-Endoperoxide Synthase 2 (PTGS2), Nuclear Receptor Coactivator 2 (NCOA2) and Prostaglandin-Endoperoxide Synthase 1 (PTGS1) were the top three targets with higher number of connected compounds. The PPI network of the compound targets were depicted in **Fig. 1B**, and the characteristics of CS targets were clarified by GO analysis and KEGG pathway analysis. It revealed that the majority of the potential targets existed on the nucleus with the function of protein binding, and highly enriched in the regulation of transcription, signal transduction, response to drug, apoptotic process and oxidation-reduction process (**Fig. 1C**). In addition, ninety-five significantly enriched pathways ($p < 0.05$) were identified and the top 20 pathways mainly contained cancer-related pathways, signal transduction pathway and virus-related pathways (**Fig. 1D**).

Cataracts related target genes

A total of 238 target genes related to cataracts were identified from the OMIM (48), Malacard (8) and Genecard (232), after removing the duplicates. The PPI network (removing nodes without any connection) of these targets were constructed (**Fig. 2A**), which included 148 nodes and 290 edges. The data of GO analysis and KEGG pathway analysis were shown in **Fig. 2B**. It revealed that 373 GO terms were significantly enriched ($P < 0.05$), with 281 in biological process, 43 in cellular component, and 49 in molecular function. In addition, a total of 67 pathways ($P < 0.05$) were affected by cataracts, and the top 20 enriched pathways were shown in **Fig. 2B**, mainly included cancer-related pathways, signal transduction pathway and virus-related pathways.

In addition, module analysis obtained a cluster of 6 targets with score=5.60 from the PPI network of cataracts target genes (**Fig. 2C**). Enrichment analysis showed these targets were enriched in protein processing in endoplasmic reticulum, and involved in visual perception and response to stimulus (**Fig. 2D**), indicating the important role of this cluster in the pathogenesis of cataracts.

CS anti-cataracts targets analysis

We generated the PPI network of potential anti-cataracts targets of CS, as shown in **Fig. 3A**. It consisted of 335 nodes and 704 edges, and fifty key targets with 251 interactions were screened from the network (**Fig. 3B**). In addition, GO analysis showed that two hundred and seventy-nine GO terms were significantly enriched, and the top 10 terms were shown in (**Fig. 3C**). These results indicated that various biological processes were involved in the anti-cataracts effects of CS. Moreover, we identified 87 significantly enriched pathways in total and the top 20 pathways were shown in **Fig. 3D**.

Discussion

Cataracts, the major cause of blindness, is characterized by blurry vision. It has been reported be associated with various risk factors, including smoking, hypertension, steroid consumption, diabetes, and ionizing radiation [27,28]. CS is a classical herb used to remove “liver fire” for improving eyesight. It has been clinically used to treat ophthalmic disease, such as cataracts, myopia and dry eye symptoms, for thousands of years in China. In this study, a network pharmacology approach was applied to comprehensively elucidate potential mechanisms of the beneficial effects of CS on cataracts. In this study, we identified 13 active compounds in CS and 105 potential targets of these active compounds in total, and 238 cataracts-related targets were also obtained from the three public databases. Four genes, including ESR1, MAPK14, CASP3, AKR1B1, were shared between CS compound targets and cataracts’ targets, indicating their possible anti-cataracts action. Central network analysis obtained a central network with 50 key targets, which significantly enriched in the pathways correlated with cataracts, such as thyroid hormone signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway. The potential mechanisms of CS against cataracts were for the first time comprehensively investigated in the present study, which laid a theoretical foundation for the clinical application of CS in the treatment of cataract and for further research. The candidate CS targets and pathways involved in cataracts progression were summarized in Fig. 4. Among the active compounds in CS, the top three active ingredients with most targets were aloe-emodin, stigmasterol and rhein, indicating their potential role in the treatment of cataracts. Aloe-emodin is an anthraquinone derivative, which possesses the antiangiogenic effect on laser induced choroidal neovascularization by inhibiting of the HIF-1 α /VEGF signaling pathway and has the potential to be developed for the prevention and treatment of diabetic retinopathy [29]. In addition, aloe-emodin metabolites could regulate cell’ energy, antioxidation and the phosphorylation of ERK kinases to decrease NMDA-induced apoptosis of retina ganglion cells [30]. Stigmasterol is a steroid alcohol with immune-modulatory properties either alone or as a component of phytosterol mixtures [31]. It was reported to attenuate both innate and adaptive immune responses, and inhibit inflammatory cell recruitment and oxidative stress as well [15,32]. Rhein a major component of many medicinal herbs with various properties, including anti-inflammatory, antioxidant and anticancer activities [33-35]. Oxidative stress has been observed in the onset and progression of cataractogenesis [36,37], and antioxidants and free radical scavengers have been suggested as potential drugs for the management of cataracts. Hence, the therapeutic effect of CS on cataracts may, at least in part, result from the antioxidant activity of compounds. Network analysis suggested that four shared targets may play crucial roles in the treatment of cataracts, including aldose reductase (AKR1B1), caspase-3 (CASP3), mitogen-activated protein kinase 14 (MAPK14) and estrogen receptor (ESR1). AKR1B1, an NADPH-dependent aldo-keto reductase, has been shown to be involved in diabetic cataract and retinopathy [38]. Previous study reported that elevated AKR1B1 can increase AcSOD2 and RAGE-induced epithelial-mesenchymal transition (EMT) in epithelial human lens of DM cataracts via decreasing AMPK activation[39], and the significance of AKR1B1 in the mediation of sugar-induced lens opacification has also been confirmed [40], indicating the potential use of AKR1B1 inhibitors in preventing cataractogenesis. CASP3 is one of the central mediators of apoptosis, has been revealed to be associated with the pathogenesis of cataract [41]. MAPK14 plays an important role in cataract formation, owing to the activation of MAPK14 can lead to the induction of cataract [42]. Estrogen-therapies showed

protection against age-related cataracts in humans and rodent models, and ER α overexpression has previously been reported in lens epithelial cells [43], indicated that estrogen protection may result from direct interactions with its receptors in the eye. In addition, TP53 with the highest degree in the central network indicated its important role in the treatment of cataracts, and previous studies also confirmed that p53 involves in the pathogenesis of cataracts and mediates the anti-cataract effect of certain compounds [44]. Module analysis and central network analysis revealed that α B-crystallin (CRYAB) may play an important role in the treatment of cataracts. It is a chaperone that maintain protein stability and preserve lens transparency [45,46] by preventing proteins from aggregating via low-affinity amphipathic interactions [47]. In our study, the PPI data of compound targets and cataracts-related targets were obtained to construct the PPI network. Enrichment analysis of these two set of targets revealed a series of shared pathways, such as PI3K-Akt signaling pathway, MAPK signaling pathway, FoxO signal pathway. To obtained the central network of CS anti-cataracts targets, we merged the PPI network of compounds target and cataracts related targets. KEGG pathways enrichment analysis showed that the key targets of CS against cataracts were mainly enriched in the thyroid hormone signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway, indicated the involvement of these pathways in the treatment of cataracts. The thyroid hormone signaling pathway participants in the regulation of growth, development and glucose metabolism. The modulation of glycolysis and carbon flux reprogramming can increase the glutathione (GSH) syntheses and activate the antioxidant enzymes [48], which are benefit for protecting the lens from oxidative stress leading to opacification. Previous study has reported a decrease lenticular GSH level occurred during formation of most cataracts [49]. As a substrate for glutathione peroxidase, GSH can destroy lipid peroxide (LPO) and hydrogen peroxide, which mediate the hepatic oxidative stress and contribute to cataracts formation [50]. Thence, a possible GSH consuming factor is considered to be cataractogenic. It was believed that the stimulated glycolysis result in the restoration of hepatic ATP by recovering the citric acid cycle, consequently facilitate de novo synthesis of GSH. However, Kosano et al. demonstrated that thyroxine treatment accelerated the GSH-GSSG cycle rather than de novo synthesis of GSH to maintain a certain level of hepatic GSH necessary for reducing elevated LPO [51]. The MAPK signaling pathway is another enriched pathway for CS in the treatment of cataract, which involves in various cellular functions, including cell proliferation, differentiation and migration. Hashida et al. found the association of cataract formation with the upregulation of MAPK cascade protein [52]. In addition, the MAPK/ERK1/2 signaling pathway also participate in the regulation of human lens epithelial cells' function by γ -Klotho gene [53]. Andrographolide is confirmed to be useful in curbing EMT-mediated posterior capsular opacification, because it helps maintain epithelial characteristics by regulating EMT markers and inhibiting the MAPK signalling pathway in lens epithelial cells (LECs) [54]. Peng et al. demonstrated that p-coumaric acid act as a potential therapeutic drug for cataracts by suppressing the apoptosis of human LECs via modulating MAPK signaling pathway [55]. Therefore, the role of MAPK signaling pathway for CS against cataracts should also be validated in the future. Noteably, the PI3K-Akt signaling pathway might be associated with the ingredients of CS and anti-cataracts' activity. It has been demonstrated that PI3K-Akt signaling pathway involved in the pathogenesis of cataracts [56,57]. Meanwhile, a series of compounds exhibited an effect on cataract by modulating the PI3K-Akt signaling pathway, such as alkylphosphocholine erufosine [58], quercetin [59], and andrographolide [60]. Many of

the active ingredients in CS have been proven to regulate the PI3K-Akt signaling pathway, including rhein [16], aloe-emodin [61], and rubrofusarin [62], indicating that CS acted on cataracts possibly through the PI3K-Akt signaling pathway.

Conclusion

In conclusion, this study used a network pharmacology approach to explore the potential mechanisms of CS acted on cataracts. Key targets and pathways involved in the treatment of cataracts using CS were identified, which provided an evidence for the clinical application of CS in cataract treatment and for further studies. However, from a critical point of view, further experiments (*in vivo* and *in vitro*) are required to validate our findings. This study also provided clues to evaluate the synergy of herbs in the treatment of other complex diseases.

Abbreviations

CS: Cassiae Semen ; PPI: protein-protein interaction ; OB: bio-availability ; DL: drug-likeness ; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database ; DIP™: Database of Interacting Proteins ; BioGRID: Biological General Repository for Interaction Datasets ; HPRD: Human Protein Reference Database ; IntAct: IntAct Molecular Interaction Database ; MINT: Molecular INTERaction database ; BIND: biomolecular interaction network database ; DC: degree centrality , CC: closeness centrality , BC: betweenness centrality , EC: Eigenvector Centrality , LAC: Local average connectivity-based method ; NC: Network Centrality ; GO: gene Ontology ; KEGG: Kyoto Encyclopedia of Genes and Genomes ; PTGS2: Prostaglandin-Endoperoxide Synthase 2 ; NCOA2: Nuclear Receptor Coactivator 2 ; PTGS1: Prostaglandin-Endoperoxide Synthase 1 ; AKR1B1: aldose reductase , CASP3: caspase-3 , MAPK14: mitogen-activated protein kinase 14 ; ESR1: estrogen receptor ; EMT: epithelial-mesenchymal transition ; CRYAB: α B-crystallin ; GSH: glutathione ; LPO: lipid peroxide ; LECs: lens epithelial cells

Declarations

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Not applicable.

Author contributions

YZ and YFF participated in the design of this project; YZ and YFF analyzed the experimental data; YZ and YFF contributed to drafting the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

Ethics approval and consent to participate

Not applicable

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

The characteristics of active compounds in CS and their targets. (A) the network of active compounds and their targets; (B) the PPI network of active compounds' targets; (C) Top 10 enriched GO terms of compounds' targets; (D) the top 20 enriched pathways of compounds' targets.

Figure 2

The characteristics of cataracts-related targets. (A) The PPI network of the cataracts-related targets; (B) KEGG and GO analysis of the cataracts-related targets; (C) A sub-network from module analysis with score=5.60; (D) GO and KEGG results of the sub-network from module analysis.

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Figure 3

The central network analysis and bioinformatic analysis. (A) The merged PPI network of compound targets and cataract-related targets; (B) central network obtained from the merged network; (C) Top 10 enriched GO terms of the key targets from the central network; (D) the top 20 enriched pathways of the key targets from the central network.

Figure 3

The central network analysis and bioinformatic analysis. (A) The merged PPI network of compound targets and cataract-related targets; (B) central network obtained from the merged network; (C) To 10 enriched GO terms of the key targets from the central network; (D) the top 20 enriched pathways of the key targets from the central network.

Figure 4

The candidate CS targets and pathways involved in cataracts progression, the blue quadrilateral represent CS targets.

Figure 4

The candidate CS targets and pathways involved in cataracts progression, the blue quadrilateral represent CS targets.

Supplementary Files

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