Uncovering The Mechanism of Shiwuwei Luodimingmu Wan On Cataract Via Network Pharmacology

Zhanhao Zhang
Xizang Minzu University

Yuliang Wang
Xizang Minzu University

Hongyan Lu
Xizang Minzu University

Yongjun He
Xizang Minzu University

Li Wang
Xizang Minzu University

Jianwen Zheng
Xizang Minzu University

Tianbo Jin (✉ tianbojin2017@163.com)
Xizang Minzu University

Research Article

Keywords: Shiwuwei Luodimingmu Wan, cataract, multiple targets and pathways, molecular docking, network pharmacology

Posted Date: October 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-936880/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Cataract is one of the most common eye diseases. The purpose of this study was to screen the active components and potential targets of Shiwuwei Luodimingmu Wan, then to explore the mechanism of Shiwuwei Luodimingmu Wan in the treatment of cataract through network pharmacology. Information about the chemical constituents and their targets of herbs in Shiwuwei Luodimingmu Wan was collected from traditional Chinese medicine system pharmacology (TCMSP) database. Cataract related genes were searched in Pubmed-Gene database and Genecards database. Then, the possible protein-protein interactions (PPIs) were derived from STRING database. Next, compound-target-diseases network was constructed using Cytoscape software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using DAVID. Furthermore, we evaluated binding potential of key targets and compounds through molecular docking. There were 102 active components including quercetin, baicalein and kaempferol in Shiwuwei Luodimingmu Wan and 28 potential targets related to cataract. More importantly, these genes are involved in several pathways associated with cataract. Docking results showed that core compound (quercetin) had certain affinity with MAPK14. This study revealed that the ability of Shiwuwei Luodimingmu Wan to produce clarity of vision may was achieved through quercetin to affect multiple targets and pathways.

1. Introduction

Cataract is a common blinding eye disease [1], which has the highest rate of blinding in China. Epidemiological investigations and experimental studies have confirmed that long-term and chronic ultraviolet radiation is closely related to the occurrence of cataract [2][3][4][5][6]. There are many factors affecting the intensity of ultraviolet radiation, such as latitude, altitude, surface reflection, outdoor exposure time and occupation [7]. Tibet is a region with high altitude, low latitude and perennial snow cover [8]. Its ultraviolet radiation is very strong, so the incidence of cataract is significantly higher than that of other regions [9]. However, in recent years, due to changes in the environment [10], the prevalence of cataract and the rate of blindness outside Tibet are increasing year by year, which seriously affects the vision and life of patients [11][12]. We know that some patients choose surgery and phacoemulsification is an important way to treat cataract [13][14][15]. Nevertheless, from the perspective of clinical practice, there are inevitably some postoperative complications after surgery [16]. Corneal edema is a common postoperative complication [17], and studies showed that postoperative adjustment by combining traditional Chinese medicine can improve the effect of cataract treatment [18].

Some plateau herbs have been found to be effective in treating cataract. The commonly prescribed for the treatment of cataract is Tibetan medicine - Shiwuwei Luodimingmu Wan, composed of Luodi, Gypsum Rubrum (Hanshuishi), Carum carvi (Zanghuixiang), Shihuihua, Glycyrrhiza uralensis Fisch (Gancao), Chebulae Fructus (Hezi), Zhaxungao, Caryophylli Flos (Dingxiang), Jinqianbaihuashe, Phyllanthi Fructus (Yuganzi), Tiexie, Hematite (Daizheshi), Carthami Flos (Honghua), Maohezi and Lvronghao. It has the function of clearing liver and improving eyesight. In the prescription, Luodi plays a major role in the treatment of cataract. Luodi hydrolyzed by proteolytic enzyme can dissolve and
dissipate the denaturetic protein and make the crystal transparent; Zanghuixiang, Gancao, Hezi, Lvronghao have anti-inflammatory and anti-oxidation effects [19], so the crystal damaged by ultraviolet radiation can be repaired in time; Honghua can dilate blood vessels and improves circulation [20]; In addition, Jinqianbaihuashe provides protein and fat nutrition, which can promote blood circulation and remove blood stasis, as well as disperse and open knots. The combination of various drugs can effectively improve eye circulation, provide crystal nutrition, effectively dissolve crystal denaturated protein, and restore crystal transparency. However, the molecular mechanism of Shiwuwei Luodimingmu Wan in treating cataract remains unclear. Network pharmacology is a comprehensive research method integrating chemical informatics, bioinformatics, network biology and traditional pharmacology [21], which can systematically and comprehensively reveal the bioactive components and the mechanism of action of traditional Chinese medicine prescriptions, and reflect the relationship between multiple components and multiple targets of traditional Chinese medicine [22]. Therefore, we used the research method of network pharmacology to explore the mechanism of Shiwuwei Luodimingmu Wan in the treatment of cataract, and used molecular docking technology to simulate and predict the interaction of disease-related targets and related components.

2. Material And Methods

2.1 Screening of active compounds in herbs

We found that Shiwuwei Luodimingmu Wan is a prescription drug for treating cataract on the reputable Tibetan medicine website (http://www.xyzyw.cn/). Chemical compositions of herbs in Shiwuwei Luodimingmu Wan were collected from TCMSP database. Then the constituents were filtered by integrating Oral bioavailability (OB) and drug-likeness (DL), and chemical components that cannot satisfy both OB ≥ 30% and DL ≥ 0.18 were deleted as suggested by the TCMSP database [23].

2.2 Identification of target genes associated with compounds

The corresponding target proteins were found according to the compounds from TCMSP. Then, the multiple proteins were converted into corresponding genes using STRING (https://string-db.org/cgi/input.pl) database with the “Homo sapiens” species selecting.

2.3 Potential target genes for cataract

In Pubmed-Gene (https://www.ncbi.nlm.nih.gov/gene/?Term) and Genecards (https://www.genecards.org/) database, inputting "cataract" to lookup cataract related genes.

2.4 Protein–protein interactions network

The target genes of the compounds were mapped to the cataract genes, and the common targets were collected in Venny2.1.0. After that, Protein–protein interactions (PPIs) were derived from STRING
database. Then, the network was built by Cytoscape software, and the core targets were selected by degree values.

2.5 Construction of compounds-targets network

To understand the potential effect of Shiwuwei Luodimingmu Wan in the treatment of cataract, compounds-targets network was performed using Cytoscape software.

2.6 Function and pathway enrichment analysis

In order to explore the signal transduction pathway and function of targets, we carried out GO function analysis and KEGG pathway enrichment analysis by using DAVID (https://david.ncifcrf.gov/). R-package software was used to compare and verify the retrieval results with DAVID database, and the visualization was performed by using bubble graph and bar graph. Key pathways and their associated genes and components were analyzed using Cytoscape software to know the mechanism of action of Shiwuwei Luodimmingmu Wan for curing cataract.

2.7 Molecular docking

Molecular docking is performed to simulate the action mode and intensity of potential drug and target in the treatment of diseases [24]. According to the number of nodes, the proteins and chemical components of the docking were screened. The 3D structures of candidate protein targets were downloaded from the SWISS-MODEL (https://swissmodel.expasy.org/interactive). The structures of predicted monomer small molecular were obtained from the ChemSpider database (https://www.chemspider.com/). Then AutoDockTools-1.5.6 was applied to not only remove water molecules and pro-ligand small molecules, but also hydrogenate and charge [25]. Finally, molecular docking calculations were performed using Autodock Vina 1.1.2 and docking results were analyzed by Discovery Studio 2016 Client.

3. Results

3.1 Active ingredients filtering

A total of 157 components of seven herbs in Shiwuwei Luodimingmu Wan were extracted from TCMSP, including 1 ingredient in Luodi, 92 in Gancao, 8 in Hezi, 6 in Dingxiang, 18 in Yuganzi, 22 in Honghua and 10 in Lvronghao (Fig. 1). Due to duplication, fourteen of these compounds were deleted, resulting in 143 effective active ingredients. Basic information of these constituents in Shiwuwei Luodimmingmu Wan is listed in Supplementary Table 1. We found that Gancao had the most chemical constituents among the herbs. And among other ingredients, quercetin is found in six other herbs except Hezi.

3.2 The main therapeutic targets predicted

In pubmed-gene and genecards databases, 221 and 3,712 disease-related genes were found, respectively. We finally collected a total of 219 coincidence genes by taking intersection, and 28 genes were cross-
identified with 331 drug target genes (Fig. 2). The target names and gene symbols of these 28 genes are shown in Supplementary Table 2.

3.3 PPIs network analysis

There are 27 interacting targets in the network (PDE10A are not involved in protein interaction). The darker the color, the more proteins it interacts with. The darkest is TP53, then VEGFA, MAPK14, ESR1, SOD1, CAT (Fig. 3). These genes are functionally classified into oxidase, cytogenic, protease, and apoptotic inhibitors. They may play a key role in the network. The top ten genes are presented in Supplementary Table 3 according to the degree values.

3.4 Compounds-targets network analysis

As you can see from Fig. 4, we found that different active ingredients could act on the same targets and on different targets, which fully reflected the mechanism of action of the multi-component and multi-target of the Tibetan medicine Shiwuwei Luodimingmu Wan. According to the relatively high number of nodes, three small molecules were screened and corresponding targets were found.

3.5 Analysis of gene functions and target pathway

GO enrichment analysis in DAVID resulted in 148 GO items (P < 0.05), among which 105 were biological process (BP) items, 16 were cellular component (CC) items, and 27 were molecular function (MF) items, accounting for 71%, 11% and 18% respectively. We listed the top 10 items in each category, as shown in Fig. 5. Biological processes involved cell aging, cellular response to hypoxia, negative regulation of cell growth, positive regulation of gene expression, positive regulation of peptidyl-tyrosine phosphorylation, etc. Cellular component involved extracellular space, nuclear chromatin, cytoplasm, cytosol, peroxisome, mitochondrion, etc. Molecular function involved enzyme binding, identical protein binding, transcription factor binding, protein homodimerization activity, RNA polymerase II transcription factor activity, etc.

According to the enrichment analysis of KEGG pathways by DAVID, 20 signaling pathways were screened with P < 0.05. We showed the top 20 KEGG pathways in Fig. 6. The schematic diagram of the path is shown in Fig. 7(TOP 5). Analysis of the targets-pathways network (Fig. 8) revealed that TP53, BCL2 and MAPK14 are involved in a large amount of cataract-related pathways.

3.6 Molecular docking

Molecular docking analysis provided a visual explanation of the interaction between key component and protein targets associated with contract. Here, molecular docking for Shiwuwei Luodimingmu Wan ingredient (quercetin) and TP53, BCL2 and MAPK14 proteins were analyzed, and the results showed that
quercetin had strong affinity with MAPK14 proteins (Fig. 9). We found Van der Waals force and π-π stacking were the main forms of interaction.

4. Discussion

In this study, based on the network pharmacology method, we identify bioactive compounds, potential targets and the pathways modulated by these compounds in Shiwuwei Luodimingmu Wan treatment of cataract. 102 active components and 28 key targets were confirmed. Interestingly, we found that quercetin has relatively high number of nodes and the number of related genes was also relatively more. This observation indicated that quercetin is a key ingredient in the prescription for treating cataracts. Quercetin is a flavonoid with anti-oxidation, anti-viral and antibacterial effect, and can inhibit inflammatory cells activation. Previous studies have suggested that quercetin involve in the prevention of cataract. The involved mechanisms in these findings include mitigating the production of reactive oxygen species, inhibiting vascular endothelial growth factor pathways, suppressing tumor suppressor gene and apoptosis, and suppressing the production of inflammatory markers [26–29].

We further focused our attention on the target genes and related pathways. Functional enrichment analysis predicted multiple cataract-related pathways. These pathways are closely associated with apoptosis and senescence and the occurrence of cataract is related to lens epithelial cell apoptosis, nutrition and oxidation [30, 31]. The observation is in agreement with the reported. Moreover, according to the comprehensive analysis of PPI, compound-target network and pathway-target network, TP53, BCL2 and MAPK14 was screened out, which may participate in integrated regulation of Shiwuwei Luodimingmu Wan through protein interactions and cataract-related pathways. In human genes, TP53 is a famous tumor suppressor gene, which plays an important regulator of cell growth, proliferation damage repair [32][33][34]. The most prominent feature of p53 is that it is a transcription factor, which affects the occurrence and development of diseases by targeting many genes and pathways related to apoptosis or cell cycle regulation. Another key target, Apoptosis regulator Bcl-2 transcribed by the BCL2, regulated mitochondrial outer membrane permeability, then activated downstream caspase cascades for apoptosis and inhibits p53-mediated apoptosis [35]. It is reported that the apoptosis of lens epithelial cells was negatively regulated by BCL2 [36]. It is noteworthy that MAPK14 is the most highlight targeted gene in prescription-disease interactions. The protein encoded by this gene is a member of the MAP kinase family, as integrators of multiple biochemical signals, involved in cell proliferation, differentiation, transcriptional regulation and development. To further analysis the interactions between quercetin with TP53, BCL2 and MAPK14, we performed the molecular docking, and suggested the effective bindings and stable complex with low energy between the ligand and receptors. Vina score indicated strongest relationship between quercetin and MAPK14.

Out of all the analysis, MAPK14 seems to be the most interesting target. The effect of quercetin on key genes MAPK14 may be a crucial factor of Shiwuwei Luodimingmu Wan in the treatment of cataract. Nevertheless, the specific mechanism has not been studied, and needs to be verified in combination with experiments such as mouse models.
5. Conclusions

In summary, this study first preliminarily verified the pharmacological mechanism of Shiwuwei Luodimingmu Wan in the treatment of cataract by using network pharmacology and molecular docking, which laid a good foundation for the follow up in depth discussion. We hope it will be helpful for the development of new drugs and the treatment of cataract.

Abbreviations

SLW: Shiwuwei Luodimingmu Wan; TCMSP: Traditional Chinese Medicine Systems Pharmacology; PPIs: protein-protein interactions; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; OB: Oral bioavailability; DL: drug-likeness; BP: biological process; CC: cellular component; MF: molecular function.

Declarations

Acknowledgements

We thank all the participants in this study.

Funding

There was no funding for the study.

Statement of Ethics

The paper is exempt from ethical committee approval. TCGA and GEO belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Author Contributions

Zhanhao Zhang and Tianbo Jin designed the study and wrote the manuscript. Yuliang Wang conducted component analysis. Hongyan Lu, Yongjun He, Li Wang, Jianwen Zheng offered some advices on this research and handled the figure. All authors have reviewed and approved the final manuscript.

Availability of data and materials

All relevant data are within the manuscript.

Consent for publication

Not applicable
Conlicts of Interest

There are no conlicts of interest to declare.

References

1. Thompson J, Lakhani N. Cataracts (2015) Primary care 42:409–423
2. Wegener A, Heinitz M, Dwinger M (2002) Experimental evidence for interactive effects of chronic UV irradiation and nutritional deficiencies in the lens. Dev Ophthalmol 35:113–124
3. Xhauaire G, Uhoda E, Rakic JM (2005) [Eye and ultraviolet light]. Revue medicale de Liege 60(Suppl 1):99–102
4. Lucas RM (2011) An epidemiological perspective of ultraviolet exposure–public health concerns. Eye contact lens 37:168–175
5. Abraham AG, Cox C, West S (2010) The differential effect of ultraviolet light exposure on cataract rate across regions of the lens. Investig Ophthalmol Vis Sci 51:3919–3923
6. Löfgren S (2017) Solar ultraviolet radiation cataract. Exp Eye Res 156:112–116
7. Izadi M, Jonaidi-Jafari N, Pourazizi M, Alemzadeh-Ansari MH, Hoseinpourfard MJ (2018) Photokeratitis induced by ultraviolet radiation in travelers: A major health problem. Journal of postgraduate medicine 64:40–46
8. Wang X, Tseng ZJ, Li Q, Takeuchi GT, Xie G. From 'third pole' to north pole: a Himalayan origin for the arctic fox. Proceedings Biological sciences 2014;281
9. Javitt JC, Taylor HR (1994) Cataract and latitude. Documenta ophthalmologica Advances in ophthalmology 88:307–325
10. Bais AF, McKenzie RL, Bernhard G, Aucamp PJ, Ilyas M, Madronich S, Tourpali K (2015) Ozone depletion and climate change: impacts on UV radiation. Photochemical photobiological sciences: Official journal of the European Photochemistry Association the European Society for Photobiology 14:19–52
11. Lou L, Wang J, Xu P, Ye X, Ye J (2017) Socioeconomic Disparity in Global Burden of Cataract: An Analysis for 2013 With Time Trends Since 1990. Am J Ophthalmol 180:91–96
12. Song P, Wang H, Theodoratou E, Chan KY, Rudan I (2018) The national and subnational prevalence of cataract and cataract blindness in China: a systematic review and meta-analysis. Journal of global health 8:010804
13. Zhu DC, Shah P, Feuer WJ, Shi W, Koo EH (2018) Outcomes of conventional phacoemulsification versus femtosecond laser-assisted cataract surgery in eyes with Fuchs endothelial corneal dystrophy. J Cataract Refract Surg 44:534–540
14. Scott WJ, Tauber S, Gessler JA, Ohly JG, Owsiank RR, Eck CD (2016) Comparison of vitreous loss rates between manual phacoemulsification and femtosecond laser-assisted cataract surgery. J Cataract Refract Surg 42:1003–1008
15. Nystrom A, Almarzouki N, Magnusson G. Phacoemulsification and primary implantation with bag-in-the-lens intraocular lens in children with unilateral and bilateral cataract. 2018;96:364–70

16. Chen X, Yu Y, Song X, Zhu Y, Wang W, Yao K (2017) Clinical outcomes of femtosecond laser-assisted cataract surgery versus conventional phacoemulsification surgery for hard nuclear cataracts. J Cataract Refract Surg 43:486–491

17. Sharifipour F, Panahi-Bazaz M, Ildani E, Hajizadeh M, Saki A (2015) Oxygen therapy for corneal edema after cataract surgery. J Cataract Refract Surg 41:1370–1375

18. Zhang D, Li M (2019) Puerarin prevents cataract development and progression in diabetic rats through Nrf2/HO1 signaling. Mol Med Rep 20:1017–1024

19. Wu TY, Khor TO, Saw CL, Loh SC, Chen Al, Lim SS, Park JH, Cai L, Kong AN (2011) Anti-inflammatory/Anti-oxidative stress activities and differential regulation of Nrf2-mediated genes by non-polar fractions of tea Chrysanthemum zawadskii and licorice Glycyrrhiza uralensis. AAPS J 13:1–13

20. Zhou X, Tang L, Xu Y, Zhou G, Wang Z (2014) Towards a better understanding of medicinal uses of Carthamus tinctorius L. in traditional Chinese medicine: a phytochemical and pharmacological review. J Ethnopharmacol 151:27–43

21. Shu Z, Wu T, Shahen M, Guo Z, Shu J, Wang HE, Shar AH, Farag MR, Alagawany M. System-Pharmacology Dissection of Traditional Chinese herbs SINI Decoction for Treatment of Cardiovascular Diseases. 2019;91:e20180424

22. Song X, Zhang Y, Dai E, Wang L, Du H (2020) Prediction of triptolide targets in rheumatoid arthritis using network pharmacology and molecular docking. Int Immunopharmacol 80:106179

23. Tao Y, Tian K, Chen J, Tan D, Liu Y, Xiong Y, Chen Z. Network Pharmacology-Based Prediction of the Active Compounds, Potential Targets, and Signaling Pathways Involved in Danshiliuhao Granule for Treatment of Liver Fibrosis. 2019;2019:2630357

24. Pinzi L, Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. International journal of molecular sciences 2019;20

25. Guo S, Wu J. Investigating the multi-target pharmacological mechanism of danhong injection acting on unstable angina by combined network pharmacology and molecular docking. 2020;20:66

26. Du L, Hao M, Li C, Wu W, Wang W, Ma Z, Yang T, Zhang N, Isaac AT, Zhu X, Sun Y, Lu Q, Yin X (2017) Quercetin inhibited epithelial mesenchymal transition in diabetic rats, high-glucose-cultured lens, and SRA01/04 cells through transforming growth factor-β2/phosphoinositide 3-kinase/Akt pathway. Molecular cellular endocrinology 452:44–56

27. Bungau S, Abdel-Daim MM, Tit DM, Ghanem E, Sato S, Maruyama-Inoue M, Yamane S, Kadonosono K. Health Benefits of Polyphenols and Carotenoids in Age-Related Eye Diseases. Oxidative medicine and cellular longevity 2019;2019:9783429

28. He L, Zhang N, Wang L, Du L, Li C, Li Y, Li X, Zhu X, Lu Q, Yin X. Quercetin inhibits AQP1 translocation in high-glucose-cultured SRA01/04 cells through PI3K/Akt/mTOR Pathway. Current molecular pharmacology 2020
29. Sanderson J, McLauchlan WR, Williamson G (1999) Quercetin inhibits hydrogen peroxide-induced oxidation of the rat lens. Free Radic Biol Med 26:639–645

30. Taylor A (1993) Cataract: relationship between nutrition and oxidation. J Am Coll Nutr 12:138–146

31. Njie-Mbye YF, Kulkarni-Chitnis M, Opere CA, Barrett A, Ohia SE (2013) Lipid peroxidation: pathophysiological and pharmacological implications in the eye. Frontiers in physiology 4:366

32. Pappas K, Xu J, Zairis S, Resnick-Silverman L, Abate F, Steinbach N, Ozturk S, Saal LH, Su T, Cheung P, Schmidt H, Aaronson S, Hibshoosh H, Manfredi J, Rabadan R, Parsons R. p53 Maintains Baseline Expression of Multiple Tumor Suppressor Genes. Molecular cancer research: MCR 2017;15:1051-62

33. Adamsen BL, Kravik KL, De Angelis PM (2011) DNA damage signaling in response to 5-fluorouracil in three colorectal cancer cell lines with different mismatch repair and TP53 status. Int J Oncol 39:673–682

34. Jackson TR, Salmina K, Huna A, Inashkina I, Jankevics E, Riekstina U, Kalnina Z, Ivanov A, Townsend PA, Cragg MS, Erenpreisa J (2013) DNA damage causes TP53-dependent coupling of self-renewal and senescence pathways in embryonal carcinoma cells. Cell cycle (Georgetown Tex) 12:430–441

35. Zheng JH, Viacava Follis A, Kriwacki RW, Moldoveanu T (2016) Discoveries and controversies in BCL-2 protein-mediated apoptosis. FEBS J 283:2690–2700

36. Lee EH, Wan XH, Song J, Kang JJ, Cho JW, Seo KY, Lee JH (2002) Lens epithelial cell death and reduction of anti-apoptotic protein Bcl-2 in human anterior polar cataracts. Molecular vision 8:235–240

Figures
Figure 1

Shiwuwei Luodimingmu Wan herbs-molecules network.
Figure 2

Venn diagram of the relationship between the targets of Shiwuwei Luodimingmu Wan and the cataract related targets.
Figure 3

Protein-protein interactions (PPIs) network.
Figure 4

Potential active compounds-targets network of Shiwuwei Luodimingmu Wan acting on cataract.
Figure 5

Gene Ontology (GO) analyses of major genes.
Figure 6

KEGG enrichment analysis diagram.
Figure 7

Diagram of disease-related signaling pathways.
Figure 8

Targets-pathways network.
Figure 9

The analysis of the interaction between quercetin and MAPK14.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx