Network pharmacology investigation of the mechanism underlying the therapeutic action of Shikang granules in retinal ischemia-reperfusion injuries

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Author contributions
Xiaoxuan Wang: designed the study, performed the main experiments, drafted the manuscript. Congying Wang and Chi Zhang: participated in some experiments. Fangyuan Zheng: statistic analysis, drew charts. Longhui Han, Minglian Zhang: revised the manuscript. All authors provided final approval and agree to be accountable for all aspects of the work.

Competing interests
The authors declare no conflicts of interest.

Acknowledgments
This work was supported by the S&T Program of Xingtai (2023ZC178).

Peer review information
Integrative Medicine Discovery thanks all anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations
I/R, ischemia-reperfusion; TCM, traditional Chinese medicine; MoA, mechanism of action; SKG, Shikang granules; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; PPI, protein-protein interaction.

Citation

Executive editor: Xin Yun Zhang.

Received: 13 March 2024; Accepted: 09 May 2024; Available online: 16 June 2024.
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Background: Retinal ischemia/reperfusion (I/R) injury often results in vision loss, and effective clinical management options are currently lacking. Shikang granules (SKG) are traditional Chinese medicine-based preparations commonly used in clinical practice for treating optic atrophy. Methods: Despite decades of clinical use, the precise mechanism of action (MoA) of SKG remains elusive. Here, we employ a network pharmacological approach to elucidate its MoA by identifying active ingredients and relevant targets using the Traditional Chinese Medicine System Pharmacology Database and Analytical Platform. Targets associated with retinal I/R injury were sourced from GeneCards, Online Mendelian Inheritance in Man, and DisGeNET. Venny software facilitated the identification of intersecting targets, which were then subjected to gene ontology functional analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis. To validate the protective effect and explore the MoA of SKG in retinal I/R injuries, we conducted experiments using rat models. Results: Our animal experiments demonstrated that SKG mitigated apoptosis following retinal I/R injury by upregulating the expression of the anti-apoptotic protein Bel-2 and downregulating the expression of BAX, Caspase-9, Caspase-3, PARP, and cytochrome C. Additionally, SKG was found to increase the expression of PI3K and AKT. Conclusions: SKG may exert its protective effects by inhibiting apoptosis through modulation of pro-apoptotic and anti-apoptotic protein expression, as well as activation of the PI3K/AKT pathway.

Keywords: retinal ischemia-reperfusion injury; Shikang granules; apoptosis; PI3K/AKT pathway
Introduction

Retinal ischemia-reperfusion (I/R) injury is a pathophysiological condition commonly associated with various ocular diseases, including retinal vascular obstruction and glaucoma [1, 2]. Triggering factors include the accumulation of reactive oxygen species, leukocyte aggregation, and inflammation of retinal cells [3]. In such situations, reduced blood flow and obstruction lead to malnutrition and oxygen deficiency across multiple retinal areas. However, more severe cellular damage tends to occur following the restoration of blood flow at obstructed sites, leading to retinal neurodegeneration. While the underlying pathological mechanism of this process remains unclear, mainstream opinions attribute these severe retinal injuries to factors such as reactive oxygen species-related damage, cellular apoptosis, and inflammatory factors [4].

From the perspective of traditional Chinese medicine (TCM), retinal ischaemia-reperfusion injury falls under the category of “diseases of the pupil”. The history of TCM is extensive. As a treatment modality, TCM features unique multicomponent regimens that target multiple pathways through multiple targets [5]. Shikang granules (SKG) is a compound drug prepared from Angelicae Sinensis Radix, Paeoniae Alba Radix, Portia, Cassiae Semen, Dioscoreae Rhizoma, Atractylodi Macrocephali Rhizoma, Bupleuri Radix, Chrysanthemi Flos, Lycii Fructus, Erictoacali Flos, Glycyrrhizae Radix, and Acori Tatarinowii Rhizoma.

It is indicated for tonifying the liver and kidney, detoxifying, alleviating blood stasis, and improving blood circulation. Clinical experiments have demonstrated the effectiveness of this drug in managing nervous damage caused by trauma or ischemia [6–8]. However, its mechanism of action (MoA) remains unclear. The present study attempted to elucidate the MoA and explore its potential as a new therapy for retinal I/R injuries using a network pharmacological approach by constructing a drug-disease-target network to predict the active ingredients, core targets, and key pathways of SKG in treating retinal I/R injuries.

Materials and methods

Network pharmacology studies

Screening of active ingredients and targets of Shikang granules. Shikang granules consist of Angelicae Sinensis Radix, Paeoniae Alba Radix, Portia, Cassiae Semen, Dioscoreae Rhizoma, Atractylodi Macrocephali Rhizoma, Bupleuri Radix, Chrysanthemi Flos, Lycii Fructus, Erictoacali Flos, Glycyrrhizae Radix, and Acori Tatarinowii Rhizoma. The active ingredients of the drug were screened using the Traditional Chinese Medicine System Pharmacology Database and Analytical Platform (TCMSP) database [9]. We used the above-mentioned herbs as keywords to retrieve their chemical components, with the filter conditions set to oral bioavailability ≥ 30% and drug-drug similarity ≥ 0.18 [9]. The results were utilized to search for relevant targets of the active ingredients. Finally, the identified targets underwent querying in the UniProt database (http://www.uniprot.org/), with the “taxonomy” condition set to “human”, to retrieve the genes pertinent to each target [10].

Identification of retinal I/R related targets. GeneCards (https://www.genecards.org/), Online Mendelian Inheritance in Man (http://www.omim.org/), and DisGeNET (https://www.disgenet.org) were searched using the keyword “retinal ischemia-reperfusion injury” to identify targets associated with the disease. Duplicated results were eliminated to produce the final list of targets [9].

Identification of intersecting targets. Targets related to Shikang granule components and retinal ischemia-reperfusion injuries were interactively mapped using [11] Venny 2.1.0 (https://bioinfogp.cab.csi.es/tools/venny) to identify intersecting targets. The results are presented as Venn diagrams [11].

Establishing protein-protein interaction (PPI) network. The STRING database (https://string-db.org/) was queried with multiple proteins comprising the intersecting targets of Shikang granules and the disease. Organisms were set to “Homo sapiens” and other settings were left as defaults to establish the PPI network [12]. The PPI network was downloaded as a TSV file and imported into Cytoscape 3.10.1. Centiscape 2.2, a Cytoscape centrality calculation application, was employed to calculate the values of parameters such as degree centrality, betweenness centrality, and closeness centrality of the nodes to screen core targets [13].

Establishing a network of compound drug-active ingredient-disease-target interactions. We constructed an interaction network encompassing compound drug-active ingredient-disease-target relationships using Cytoscape 3.10.1 [13].

Enrichment analysis of biological functions and pathways of the molecules. Enrichment analyses for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were conducted on the intersecting targets using the Metascape database, with the species and background set to “human”. Subsequently, we identified the biological processes and key signaling pathways through which the drug molecules exert their effects on diseases by executing the following enrichment analysis commands: “GO Molecular Functions”, “GO Biological Processes”, “GO Cellular Components”, and “KEGG Pathway” [14, 15].

In-vivo experiment

Animal grouping and drug preparation. Animal experiments were approved by the Animal Ethics Committee of Hebei Medical University (approval number: 2024LW02) and strictly adhered to the guidelines set forth by the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research. Male clean-grade Sprague-Dawley rats (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) (license number: SCXK (Jing) 2021-0006), weighing 200–250 g, 6 to 8 weeks of age, were utilized as the animal subjects for this experiment. The rats were acclimatized in a 12-hour light/dark cycle environment with access to adequate water and food for one week prior to the commencement of the procedure.

In a previous study, a rat retinal I/R model was employed, and both flash visual evoked potential and electoretinography were conducted 48 hours post-reperfusion. The findings revealed a superior therapeutic effect of medium-dose Shikang granules compared to both low and high doses. Therefore, a medium dose of Shikang granules was selected to investigate the underlying mechanism.

The preparation of Shikang granules involved the following steps: dry herbal ingredients, including Angelicae Sinensis Radix (10 g), Paeoniae Alba Radix (15 g), Portia (10 g), Cassiae Semen (15 g), Dioscoreae Rhizoma (15 g), Atractylodi Macrocephali Rhizoma (10 g), Bupleuri Radix (10 g), Chrysanthemi Flos (10 g), Lycii Fructus (10 g), Erictoacali Flos (10 g), Acori Tatarinowii Rhizoma (10 g), and Glycyrrhizae Radix (6 g), was soaked in distilled water at a volume-weight ratio (V/W) of 15:1 for 30 minutes. Subsequently, the herbs underwent two decocction cycles, the first lasting 1.5 hours and the second 2 hours, at a V/W ratio of 10:1. Following filtration, the solution was evaporated under reduced pressure to yield suspensions with a final density of 2.5 g/mL (medium dose of Shikang granules) and stored at 4 °C.

Sprague-Dawley rats were selected as the experimental species and randomly allocated to the normal control, disease model, or SKG groups. Rats in the SKG group received the herbal suspension via gavage once daily for seven days. Retinal ischemia was induced on the 5th day following the initiation of treatment.

Animal model preparation. The rats were fully anesthetized and administered 0.5% oxybuprocaine eye drops for topical anesthesia of the eyes. The conjunctival sacs were then cleaned with 0.5% chloramphenicol dexamethasone eye drops, and the periorbital areas were disinfected with iodine. Following this, the pupils were dilated using compound tropicamide eye drops, and saline was infused into the anterior chamber via a 25G needle positioned at a height of 149 cm (110 mmHg) [16]. As a result, the eyeballs hardened, and the bulbar conjunctiva quickly became opaque. After 60 minutes, the infusion vial was gradually lowered to the level of the rat's eyeball to
slowly relieve intraocular pressure and restore retinal blood supply. Subsequently, the infusion set was turned off, and the perfusion needle was removed from the anterior chamber. It was observed that the color of the iris and the bulbar conjunctiva quickly returned to normal, and the fundus retina exhibited a reddish hue, indicating the unoccluding of obstructed vessels and the formation of reperfusion, confirming the successful construction of the I/R model. Gatifloxacin ophthalmic gel was topically applied during surgery to protect the cornea, and postoperatively, tobramycin-dexamethasone ophthalmic ointment was administered to the eyes twice daily to prevent infection. The left eye of each rat was designated for the experimental procedures.

The rats were euthanized 48 hours after the induction of I/R injuries, and tissue sections were collected and preserved for further analysis. Three independent in vivo experiments were conducted.

Western blotting. Protein quantification was conducted utilizing a bicinchoninic acid assay (BI-WB005, Nanjing SenBeiJia Biological Technology Co., Ltd., Nanjing, China). Proteins underwent separation via gel electrophoresis and subsequent transfer onto a support membrane within a wet tank. Subsequently, the membrane was precluded from non-specific binding by blocking with a 5% non-fat milk solution prior to primary antibody probing. The primary antibodies listed below were employed for overnight incubation at 4 °C: PARP1 rabbit polyclonal antibody (Proteintech, 1:500, 13371-1-AP), Caspase 3 rabbit polyclonal antibody (Proteintech, 1:500, 19677-1-AP), Caspase 9/P35/P10 rabbit polyclonal antibody (Proteintech, 1:500, 10380-1-AP), BAX rabbit polyclonal antibody (Proteintech, 1:500, 50599-2-lg), Bcl2 rabbit polyclonal antibody (Proteintech, 1:500, 26593-1-AP), Cytochrome rabbit polyclonal antibody (Proteintech, 1:500, 10993-1-AP), PI3 Kinase p85 alpha monoclonal antibody (Proteintech, 1:500, 60225-1-lg), AKT polyclonal antibody (Proteintech, 1:500, 10176-2-AP), and Beta Actin monoclonal antibody (Proteintech, 1:1000, 66009-1-lg). After primary antibody incubation, membranes were probed with horseradish peroxidase-conjugated secondary antibodies (1:5000) for 60 minutes at room temperature. Finally, a chemiluminescent reagent was applied to the membrane, followed by incubation for 5 minutes at room temperature. Fluorescence signal analysis was conducted using a GelView6000ProII automatic gel imaging system.

Statistical analysis. Experimental data were analyzed utilizing GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Single-factor analysis of variance was employed to compare differences in experimental data among groups, revealing statistical significance across all groups (P < 0.05).

Results

Analysis of active ingredients and targets of Shikang granules

Utilizing Traditional Chinese Medicine System Pharmacology Database and Analytical Platform queries, we screened 195 molecules as the active constituents of Shikang granules. These molecules were associated with various botanical sources, including Angelicae Sinensis Radix (2), Paoniae Alba Radix (7), Poria (6), Cassiae Semen (13), Dioscoreae Rhizoma (12), Arracoidis Macrocephalae Rhizoma (4), Bupleuri Radix (8), Chrysantheni Flos (18), Lycii Fructus (33), Eriocauli Flos (6), Glycyrrhizae Radix (82), and Acori Tatarinowii Rhizoma (4). After eliminating duplicates, the list of active ingredients comprised 176 molecules. UniProt was utilized to discern genes associated with the target proteins of these active constituents. Subsequently, results were deduplicated, and active ingredients lacking any gene targets were excluded. Ultimately, 266 genes were identified as pertinent to the drug.

Identification of retinal I/R related targets

A search on GeneCards yielded 2136 genes relevant to I/R injuries. Additionally, the Online Mendelian Inheritance in Man database identified 163 related targets, while no known targets associated with I/R were found in DisGeNET. Following deduplication, a total of 2263 I/R-related targets were identified.

Identification of intersecting targets

Targets related to SKG and the disease were illustrated on a Venn diagram generated using Venny 2.1.0. A total of 110 intersecting targets were identified (Figure 1A).

PPI network

The PPI network of targets associated with SKG and the disease was established via the STRING database (Figure 1B). Subsequently, the PPI data were imported into Cytoscape 3.10.1 to generate the visualized PPI network diagram, which was further analyzed using Centiscape 2.2. Consequently, 28 core targets were identified: AKT1, TNF, IL6, VEGFA, TP53, CASP3, PTGS2, MMP9, PPARG, CAT, IL10, EGF, MYC, NOS3, IL4, HMOX1, VCAM1, FOS, PTEN, CASP8, SERPINE1, SIRT1, PPARG, ADIPOQ, APP, CAV1, NR3C1, and SOD1 (Figure 1C).

Figure 1 Results of network pharmacology study. (A) Venn diagram showing intersection genes between Shikang granules and retinal ischemia-reperfusion injury. (B) Protein-protein interaction network diagram. (C) Selection of core targets. I/R, ischemia-reperfusion.

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Drug-active ingredient-disease-target interaction network
The interaction network of drug-active ingredient-disease-target was established using Cytoscape 3.10.1 (Figure 2). The network analysis command was executed, and nodes were sorted based on their degree values in descending order. The five most prevalent compounds identified were quercetin, luteolin, kaempferol, naringenin, and 2-methylchromone (Table 1).

KEGG enrichment analysis results
The core targets associated with SKG treatment for retinal I/R injuries were inputted into Metascape for KEGG signaling pathway enrichment analysis. A total of 258 signaling pathways were identified in this investigation. The top 20 pathways were selected based on their P-values (Figure 3A). The most significant pathways included those related to cancer, lipid metabolism, atherosclerosis, and diabetic complications, notably the AGE-RAGE, PI3K/AKT, HIF-1, VEGF, and cAMP signaling pathways. Of these, the PI3K/AKT pathway plays a crucial role in regulating cell proliferation and apoptosis. AKT1, TP53, and CASP3 were identified as the main targets of SKG within the PI3K/AKT pathway for treating retinal I/R injuries.

GO enrichment analysis results
The core targets associated with SKG treatment for retinal I/R injury were analyzed using Metascape for GO enrichment analysis. A total of 6113 results were generated, comprising 4980 biological processes, 711 molecular functions, and 422 cellular components. The top 20 entries across the three categories were examined (Figure 3B). The findings revealed that the active components of SKG exhibited sensitivity to oxygen levels, bacterial-origin molecules, inorganic compounds, upregulation of cytokine production, modulation of apoptotic signaling pathways, and upregulation of programmed cell death.

Effect of Shikang granules on apoptosis markers in the I/R models
Compared to the control group, the expression levels of BAX, CASP9, CASP3, PARP, and Cyt C were significantly upregulated in the model group. Conversely, the expression level of BCL-2 protein was significantly downregulated in the model group. In contrast, the expression levels of BAX, CASP9, CASP3, PARP, and Cyt C were significantly downregulated in the SKG group. Moreover, the level of BCL-2 protein expression was significantly upregulated in the SKG group (Figure 4).

Effect of Shikang granules on the PI3K-AKT pathway in I/R models
In the I/R model group, the expression levels of PI3Kp85 and AKT were significantly downregulated compared to the control group. Conversely, in the SKG group, the expression levels of PI3Kp85 and AKT were significantly upregulated compared to the model group (Figure 5).

Figure 2 Network of drug-active ingredient-disease-target interactions. SKKL, Shikang granules.

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TCSMP, Traditional Chinese Medicine System Pharmacology Database and Analytical Platform.
Figure 3 Enrichment analysis of biological functions and pathways of molecules. (A) Results of KEGG enrichment analysis. (B) Results of GO enrichment analysis. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Oncology.

Figure 4 Effect of Shikang granules on apoptosis markers in the I/R models. (A & C) Representative Western blot bands. (B & D) Western blot tests revealed that Shikang granules inhibited the expression of BAX, CASP9, CASP3, PARP, and Cyt C, and increased the expression of BCL-2. *P < 0.05, ***P < 0.0001 vs. I/R, I/R, ischemia-reperfusion; SKG, Shikang granules.
Discussion

Retinal I/R injury is commonly associated with several retinal diseases categorized in TCM as “internal ocular disorders,” including ischemic optic neuropathy and vascular obstruction [1, 2, 18]. Retinal I/R injury often presents in conditions described in TCM theories as “sudden blindness,” “sudden blindness due to meridian damages,” “fuzzy eyesight,” or “cloudy vision” [19]. These conditions typically have poor prognoses, potentially leading to irreversible death of retinal ganglion cells [20]. However, within the TCM domain [21], a range of effective treatments for these diseases has been identified, among which is SKG, a preparation indicated for tonifying the liver and kidney, detoxification, alleviating blood stasis, and improving blood circulation. Previous studies conducted by our team revealed that SKG could enhance mesenteric microcirculation in mice [22] and improve visual and clinical outcomes in patients with neuropathy following trauma or ischemia-related injuries [7, 8]. Nonetheless, the pathogenesis of retinal I/R injury remains elusive, involving various modes of cellular death, including apoptosis, pyroptosis, and ferroptosis [18, 23, 24]. The primary objective of this study was to explore whether SKG can reverse cell death processes in retinal I/R injuries and, if so, to elucidate the mechanism through which it exerts its efficacy.

To gain deeper insights into the therapeutic potential of SKG in treating retinal I/R injuries, we utilized a network pharmacology approach to predict and validate pertinent treatment targets. Through the construction and analysis of a drug-disease-target network, we discerned the primary constituents of SKG to be quercetin, luteolin, and kaempferol. Notably, these active ingredients exhibit antioxidant and anti-inflammatory properties [25-28].

Through the PPI network diagram, we identified 28 core targets of SKG in the treatment of retinal I/R injuries, including AKT1, TNF, IL6, VEGFA, TP53, CASP3, PTGS2, MMP9, PPARG, CAT, IL10, EGF, MYC, NO3, IL4, HMox1, VCAM1, FOS, PTEN, CASP8, SERPINE1, SIRT1, PPARA, ADIPOQ, APP, CAV1, NR3C1, and SOD1. The top ten nodes in the PPI network were AKT1, TNF, IL6, VEGFA, TP53, CASP3, PTGS2, MMP9, PPARG, and CAT. These target proteins play pivotal roles in various signaling cascades that trigger the release of inflammatory factors and oxidative stressors following retinal I/R. These stressors attack and activate the endothelial cells of the fundus microvessels, disrupting the vascular barrier and eventually leading to vasogenic edema. AKT1 is a crucial mediator of the PI3K/AKT signaling pathway involved in many biological processes [26, 27]. Studies have demonstrated that the cellular repressor of E1A-stimulated genes exerts a protective effect on retinal ganglion cells in retinal I/R injury by activating the anti-apoptotic AKT signaling cascade [20].

Bulk RNA sequencing of unmyelinated and myelinated optic nerve regions in mice after optic nerve injuries and mice with microbead-induced glaucoma under laboratory conditions has shown a significant increase in the expression of Wnt, Hippo, and PI3K-AKT genes in both conditions [29]. Caspase-8, encoded by CASP8, is the key activating protein in the exogenous apoptotic pathway, while Caspase-3, encoded by CASP3, is essential for the transduction of apoptotic signals in many downstream pathways [30].

The GO enrichment analysis unveiled that both SKG and retinal I/R injury participate in modulating oxidative stress and apoptosis signaling cascades. Additionally, the KEGG pathway enrichment analysis demonstrated that the shared targets of SKG and retinal I/R injury were significantly enriched in signaling pathways associated with cancer, lipid metabolism, atherosclerosis, and notably, the PI3K-AKT signaling pathway. Based on these network pharmacology analysis findings, we infer that the therapeutic effect of SKG on retinal I/R injury may be attributed to the PI3K/AKT signaling pathway, crucial for cell apoptosis and proliferation. Furthermore, previous studies have indicated that salicin can regulate apoptosis in gastric cancer cells by inhibiting the PI3K/AKT/mTOR pathway [11].

Classical apoptotic pathways consist of both endogenous and exogenous routes [31]. The exogenous pathway also referred to as the death receptor pathway, is characterized by a caspase cascade initiated upon the binding of specific death receptors with their ligands. This interaction activates downstream signals via Caspase-8. Furthermore, Caspase-8 can trigger the endogenous apoptotic pathway by interacting with Bid [32], initiating the mitochondrial pathway of apoptosis. Upon interaction, the Bax family proteins bind with the Bcl-2 family proteins, forming aggregates on the outer mitochondrial membrane. This event leads to the alteration of mitochondrial somatic pressure and the loss of transmembrane potential, which prompts the release of Cyt C from the mitochondria into the cytoplasm. Cyt C then binds to Apaf-1 to form apoptotic complexes. These complexes activate the precursor of Caspase-9, subsequently triggering the activation of Caspase-3 and Caspase-7, culminating in the initiation of the caspase cascade and ultimately resulting in cell death [31, 33]. PARP is a Zn²⁺-dependent eukaryotic DNA-binding protein recognized as an important DNA repair enzyme due to its specific affinity for free DNA ends. In the early stages of apoptosis, the competitive binding of Caspase-3 with PARP neutralizes the repair effect of the latter, thereby accelerating the process of cell death [34].

Recent studies suggest that quercetin regulates the phosphorylation of the PI3K/Akt/FoxO3a signaling pathway, inhibits oxidative stress, reduces ovarian tissue damage, improves ovarian response, and restores ovarian reserve function [35]. Research has demonstrated that luteolin inhibits apoptosis and enhances cardiomyocyte...
contractile function, partly through the PI3K/Akt pathway, under simulated I/R conditions [36]. Kaempferol can protect the hearts of diabetic rats by modulating Nrf2, NF-xB, and PI3K/Akt/GSK-3β signaling pathways [37]. These studies indicate that a single component of TCM can exert therapeutic effects by regulating the PI3K/Akt pathway; however, there is no therapeutic effect of combined medication. Single herbs are seldom used in TCM; instead, they are typically combined to enhance drug efficacy and improve clinical outcomes.

We conducted additional tests to validate the network pharmacological findings indicating that SKG inhibits cell apoptosis in retinal I/R injury by activating the PI3K/AKT pathway in rat models of induced retinal I/R injury. Our animal experimental results suggest that SKG inhibits apoptosis following retinal I/R injury by upregulating the expression of Bcl-2 protein and downregulating the expression of BAX, Caspase-9, Caspase-3, PARP, and Cyt C. Furthermore, we observed an increase in the expression of PI3K and AKT upon SKG treatment, indicating that SKG inhibits retinal cell apoptosis through modulation of the PI3K/AKT signaling pathway.

In conclusion, this study systematically identified potential targets of SKG in treating retinal I/R injuries using a visual network pharmacological approach and elucidated the MoA of SKG in treating retinal I/R injury. Our findings offer a novel approach for the clinical utilization of SKG in the prevention and treatment of ischemic ophthalmopathies. This study highlights the involvement of apoptosis regulation, oxidative stress, and the PI3K/AKT signaling pathway in the treatment of retinal I/R injuries with SKG. Specifically, in animal experiments, SKG inhibits apoptosis by downregulating pro-apoptotic proteins, upregulating anti-apoptotic proteins, and activating the PI3K/AKT pathway. However, due to the inherent complexity of the components in a TCM-based regimen like SKG, and the multitude of targets involved, further research is necessary to fully elucidate the pharmacodynamic mechanism of SKG.

References


