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Exploring the mechanism of active ingredients of *Smilacis Glabrae Rhizoma* in treating migraine headache through network pharmacology and animal experiments

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Author contributions

Dao-Xian Shu: Developing and designing experimental methods and creating models to conduct experiments. Applying statistical, mathematical, computer, or other forms of technology to analyze or integrate research data. Finally, finalizing and revising manuscripts. Jiao-Rong Wang and Ying Wang: Collecting and integrating data. Yan-Yan Chen and Yong-Fang Gu: Providing research materials, reagents, experimental samples, animals, instruments, computational resources, and other analytical tools. Yan-Xin Wang: Helping team members to solve problems encountered during the research process and provide professional advice to promote the progress of the research. Revising papers to ensure quality.

Competing interests

The authors declare no conflicts of interest.

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Peer review information

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Abbreviations

CGRP, calcitonin gene-related peptide; SGR, *Smilacis Glabrae Rhizoma*; TCMSP, Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform; OB, oral bioavailability; DL, drug-likeness; PPI, protein-protein interactions; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; IL-6, interleukin-6; IL-1β, interleukin-1β.

Citation

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Abstract

Background: To explore the potential mechanism of action of the active ingredients of *Smilacis Glabrae Rhizoma* (SGR) in the treatment of migraine using network pharmacology and in vivo experiments. **Methods:** Through the search of Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform, Genecards, Drugbank and other databases, we obtained active ingredients, targets of SGR and related disease targets of migraine, and took the intersection for protein-protein interactions analysis. After constructing the network diagram, network topology analysis was performed to derive the core targets and key active ingredients, and Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed. Finally, molecular docking was performed and validated by in vivo experiments. In vivo experiments, 18 male BALB/c mice were selected, and the SGR group was fed with SGR drinking tablet concentrate, and nitroglycerin injection was used to construct a mouse model of migraine. Enzyme-linked immunosorbent assay test was used to detect the levels of TNF-α, IL-1β, IL-6, and AKT1 in plasma. **Results:** The results showed that the core targets of SGR for the treatment of migraine were TNF-α, IL-1β, IL-6, and AKT1. These core targets and key active ingredients had better binding ability. Compared with the blank group, the number of head scratching in the model group increased. Compared with the model group, there was a significant reduction of the number of head scratching in the SGR group. In comparison with the blank group, the protein level in the plasma in the model group was markedly higher. Compared with the model group, the protein level in the SGR group was significantly lower. **Conclusion:** SGR has the characteristics of improving migraine based on multi-targets, multi-components and multi-pathways, and the mechanism of action may be related to the inhibition of the release of inflammatory factors, neuron protection, and interference with apoptosis and other processes.

Keywords: migraine; *Smilacis Glabrae Rhizoma*; network pharmacology; molecular docking; mechanism of effect

Background

Migraine is the second most common neurological disorder, clinically characterized by recurrent, mostly unilateral, moderate-to-severe throbbing headache, with co-morbidities with anxiety and depression [1, 2]. Epidemiologic investigations have shown that the annual prevalence of migraine in China reaches 9.3%, and it has a certain family aggregation, with a heritability of 46%–52.1% [3, 4]. Migraine attacks are accompanied by numerous vegetative nervous system symptoms, such as pallor, increased respiration, and gastrointestinal disorders. Headache attacks are often preceded by aura manifestations such as blurred vision, flashes of light, hemianopsia, hemifacial and limb numbness, lasting several minutes or half an hour [5]. At present, the pathogenesis of migraine is still unknown, and various theories have tried to elucidate its mechanism, such as vascular disorders, aseptic inflammation of the dura mater, and the low magnesium theory. Among these, the trigeminal vascular disorders theory has gained a high degree of acceptance nowadays. In addition, most scholars agree that the theory of cortical diffusion inhibition is related to migraine with aura. Regarding the recommended therapeutic drugs for acute attacks of migraine, the more widely used ones are non-steroidal anti-inflammatory drugs, triptans, ergotamines, etc. The prophylactic drugs for the remission period are calcium channel blockers, β-blockers, antiepileptic drugs, antidepressants, etc. [6]. However, these drugs also have shortcomings, such as non-steroidal analgesics may have adverse effects such as irritation of the gastrointestinal mucosa, causing abnormalities in liver and kidney function, and increasing the risk of bleeding; vasoconstriction induced by the action of tretinoin may increase cardiovascular disease episodes; as well as ergotamines lead to inaccuracies in efficacy, poor tolerability, and cardiovascular safety issues, which make the use of these drugs in long-term applications or in special populations are limited. More seriously, medication overuse headaches and dependence are becoming more common in migraine patients, and these problems seriously affect the quality of life of patients. Currently, novel migraine drugs including calcitonin gene-related peptide (CGRP) receptor antagonists, monoclonal antibodies to CGRP, and 5-HT1F agonists have been put into clinical use, of which CGRP receptor antagonists and CGRP monoclonal antibodies are hot research topics. A large number of studies have also attempted to treat migraine by injecting botulinum toxin type A, using peripheral nerve blocks, and pulsed transcranial magnetic stimulation. However, their safety and efficacy need to be clinically observed, and some methods are expensive, which puts some pressure on the economy of patients with long-term treatment, so it is extremely necessary to find more advantageous long-term treatment programs. Traditional Chinese medicine is characterized by outstanding efficacy, high safety, and multi-targeted therapy, and it plays a unique advantage in the treatment of migraine [7].

Migraine is not specifically discussed in Chinese medicine, but it is covered by headache, which is mentioned in *Suwen Fenglun* (206 B.C.E.–220 C.E.) and also referred to as “headache” and “brain disorder”. With clinical application and research, the efficacy of Chinese medicine in migraine treatment and prevention has been confirmed, avoiding the toxic side effects of Western medicine treatment, alleviating the pain of patients and in the meantime allowing the development of Chinese medicine to go further. In clinical application, it is found that *Smilacis Glabrae Rhizoma* (SGR) has good efficacy in the treatment of migraine. SGR is the derived from the dried rhizome of *Smilax glabra Roxb*., a genus of plants in the *Smilacaceae* family, a commonly used medicine in Chinese medicine clinics. Its properties is sweet, bland, and neutral flavour, to the liver and stomach meridian, with clear heat, detoxication, dampness relief, and easing joint movement. Its clinical application is more widely used in the treatment of chronic renal failure, gouty arthritis, tumors and many other diseases. Modern research also shows that it has anti-tumor, anti-inflammatory, immunomodulatory, antibacterial, antioxidant and other rich pharmacological activities, suggesting that SGR may exert its clinical efficacy mainly by acting on the inflammatory response process, tumor development and the body’s self-protection and regulation of the ability to play the clinical efficacy.

Network pharmacology is a multidisciplinary field based on biology, computer science, and pharmacology. It explains the complex biological network relationships among drugs, diseases, genes, and targets through genomics, network analysis, and visualization techniques, to anticipate the pharmacological mechanisms by which drugs treat diseases [8]. This study utilized network pharmacology and molecular docking techniques to explore the potential therapeutic targets and pathways of action of SGR for migraine. Based on the analytical results, we conducted in vivo experimental validation to reveal its mechanism of effect from a multi-omics and multi-target perspective (Figure 1).

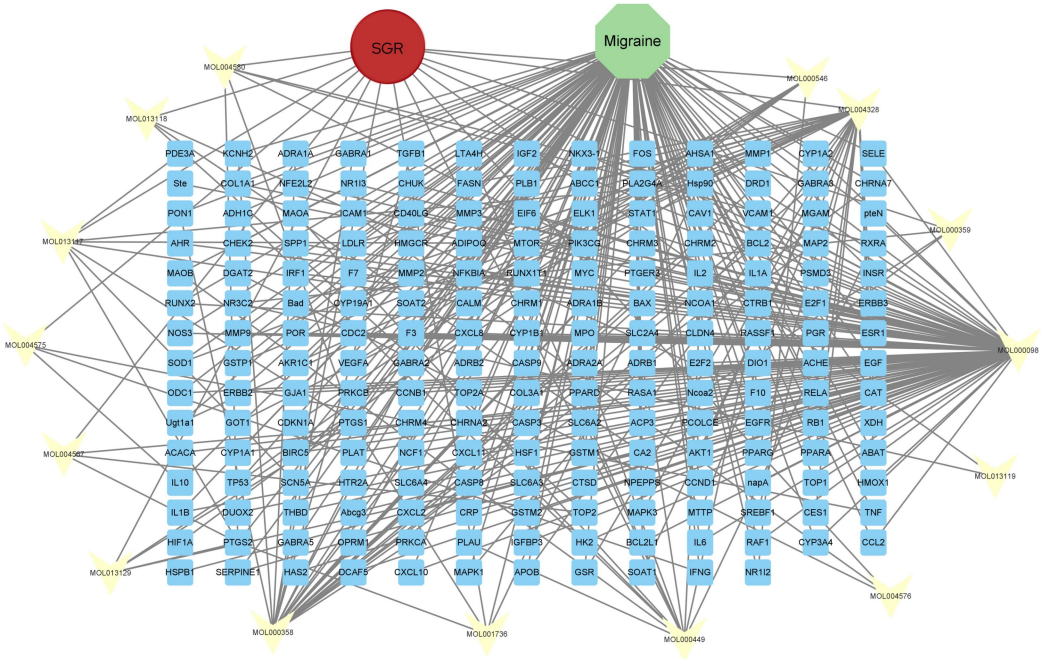

Figure 1 Network model of *Smilacis Glabrae Rhizoma*-chemical components-target-migraine. SGR, *Smilacis Glabrae Rhizoma*.

Materials and methods

Methods of network pharmacology analysis

SGR composition and target acquisition. Based on the retrieval of SGR using Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform (TCMSP), all the active ingredients of SGR were obtained. The active ingredients of SGR were screened by setting the screening conditions of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. OB and DL were set to screen the active ingredients of SGR. The relevant targets of each effective active ingredient were retrieved using the reverse pharmacophore matching database PharmMapper platform (http://lilab-ecust.cn/pharmmapper/index.html), and the retrieved ingredient targets were transformed.

Acquisition of migraine-related targets. The Genecards database (https://www.genecards.org/) and DrugBank database (https://go.drugbank.com/) were searched for migraine-related disease targets using "migraine" as the keyword.

Venn diagram construction of migraine and SGR targets. The targets of migraine and effective active ingredients of SGR were input into Venny 2.1 software to obtain the intersecting targets and plot the Venn diagram.

Construction of "drug-active ingredient-target" network diagrams. We used Cytoscape 3.9.1 software to calculate the topology of SGR target points and to draw the network graph, in which different nodes of the network graph represent "drug, active ingredient, target".

Constructing a "SGR-migraine target" protein-protein interactions (PPI) network. Using the STRING database (https://cn.string-db.org/), we imported shared targets of SGR and migraine, and mapped the shared target PPI network. We exported the data to visualize and analyze the PPI network with Cytoscape_v3.9.1 software. The CytoHubba and CentiScaPe 2.2 plug-ins were used to calculate the topological parameters of each target in the PPI network, and the total targets were screened to identify the pivotal targets of SGR for the treatment of migraine.

Shared target Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The shared targets of SGR and migraine were input into the Metascape database (https://metascape.org/gp/index.html#/main/step1), and a threshold value of $P < 0.05$ was set to analyze the GO and KEGG pathway enrichment of the shared targets. The top 10 entries of GO and KEGG analysis, as well as the involvement of functions and access information, were plotted as bar charts and bubble charts for visualization of the data results, respectively.

Molecular docking of core key targets and key active ingredients. Based on a "drug - active ingredient - target" analysis, the top three active ingredients and the top four key targets with the highest level of selection, as selected by the PPI network, were targeted. We downloaded the MOL2 files of the key active ingredients from PubChem database (https://pubchem.ncbi.nlm.nih.gov/search/search.cgi) and used ChemBioDraw software for energy minimization and other treatments. The PDB files of the core key targets were downloaded from the PDB database and pretreated using PyMOL software to separate original ligands and remove water molecules. Target proteins were pretreated by separating ligands and removing water molecules using PyMOL software. Separate ligands and remove water molecules. Pretreated small molecules and target protein molecules were imported into AutoDockTools 1.5.6, hydrogenated and charge-distributed, and recorded in PDBQT format. After hydrogenation and charge distribution, molecular docking was performed with key targets and key active proteins. Using AutoDock vina software, molecular docking was carried out between the core key targets and key active ingredients to obtain the binding energy (affinity, kcal/mol) and binding position of the core key targets and key active ingredients. The PDBQT format file of the docking results was then exported, and the corresponding file was imported into PyMOL software again for visualization and display.

Experimental study of SGR active ingredients on migraine mice

Experimental subjects. There were 18 male BALB/c mice of specific-pathogen-free grade, with a body weight of 40 (± 10) g, eight weeks of age, provided by Beijing SBF Biotechnology Co., Ltd. (Beijing, China) (Laboratory Animal Production License: No. SCXK (Beijing) 2019-0010). The above animals were kept in the specific-pathogen-free grade animal room, with a temperature of 22–25 ˚C, humidity of 40%–70%, and free access to water and food. The animals were acclimatized for 1 week before the start of the experiment. The experiment was approved by the Experimental Animal Ethics Committee of Anhui University of Traditional Chinese Medicine, the animal number is AHUCM-mouse-2023003. All experimental protocols related to animals obeyed the Care and Use of Laboratory Animals published by the US National Institutes of Health and the Consensus of Authors' Guidelines on Animal Ethics and Welfare proposed by the International Association of Veterinary Editors.

Grouping and drug administration. Eighteen mice were randomly divided into 6 mice in the blank group, 6 mice in the model group, and 6 mice in the SGR group. In the SGR group, the drug concentration was 420 mg/kg, which was converted to the human clinical equivalent dose, and the mice were gavaged at a volume of 10 mL/kg for 10 days, once a day. The blank group and model group were given 10 mL/kg of distilled water in equal volume every day.

Model preparation and behavioral observation. One hour after the last administration, mice in all groups except the blank group were subcutaneously injected with 10 mL/kg of nitroglycerin at the head and neck, and the blank group was given an equal volume of saline. Behavioral observations were performed and recorded 15 minutes after subcutaneous administration of nitroglycerin, and the observation time was 60 minutes. The success criteria for modeling were persistent reddening of both ears, irritability, an increased number of cage-climbing instances, and frequent scratching of the head by the limbs of the mice.

Behavioral observations included: the number of times the forelimbs or hindlimbs scratched their heads and the number of times they threw their heads (scratching and face-patting were counted as one event), climbing up the cage (observed during the experimental process), and ear redness (other symptoms were excluded from the record). One point was recorded for each occurrence of the first 2 symptoms. The total behavioral score of each mice was recorded.

Specimen collection. After 3.5 hours of nitroglycerin injection, mice in each group were anesthetized by intraperitoneal injection of 1% pentobarbital (50 mg/kg). Blood was collected from the abdominal aorta, placed in an anticoagulated vacuum blood collection tube, gently shaken, and centrifuged at 4 ˚C, 3000 r/min (centrifugation radius 7.5 cm) for 10 minutes. Then, plasma was separated and stored at –80 ˚C for future use. The levels of AKT1, interleukin-6 (IL-6), TNF-α, and interleukin-1β (IL-1β) in mouse plasma were detected by enzyme-linked immunosorbent assay test.

Statistical analysis

Data were expressed as $\bar{x} \pm s$ and analyzed using SPSS 26.0 software and GraphPad Prism 9.0 software. When the data in each group conformed to a normal distribution, the One-way analysis of variance was used for comparison between groups. If the variance was homogeneous, the least significant difference method was used for comparison of differences between groups; if the variance was not homogeneous, the Tamhane method was used for comparison of differences between groups. Independent samples *t*-test was used for comparison between two groups. When the data in each group did not conform to a normal distribution, a non-parametric test was used for comparison between groups, and $P < 0.05$ was considered statistically significant.

Results

Network pharmacology analysis

Screening of active ingredients and targets in SGR. Based on the screening of all the effective active ingredients of SGR and their related targets in the TCMSP database, 15 active ingredients of SGR were obtained by setting OB ≥ 30% and DL ≥ 0.18 as screening conditions (Table 1). Using the MOLID of SGR as the keyword, the PharmMapper platform was utilized to identify the targets related to the active ingredients, with a fitting criterion set to ≥ 0.8. A total of 193 SGR active ingredient targets were obtained after standardizing the data through sorting and de-weighting.

Network diagram of "SGR - active ingredient - target - migraine". Cytoscape_v3.9.1 was used to construct the "SGR - active ingredient - target" network for the 15 active ingredients and 210 drug targets of SGR, with different nodes indicating different active ingredients and corresponding targets obtained from the screening. The network includes 209 nodes and 225 edges. The analysis results showed that SGR has the characteristics of being multi-component and multi-target (Figure 1).

Acquisition of targets and PPI network construction of SGR for migraine treatment. Through the TCMSP database, 193 active component targets of SGR were mined, and 3,548 targets related to migraine were retrieved from the Genecards and Drugbank databases. Using Venny2.1 online software, 121 common genes of SGR active components-migraine were identified (Figure 2). The PPI network was constructed using the STRING database, setting the combined_score to 0.4 to obtain the PPI network graph (Figure 3). In this graph, the nodes represent the role of the target, and the node size, color, and transparency indicate the magnitude of the degree value, with the importance of the target being positively correlated with the degree value. The nodes are connected to each other by edges, which represent the connections between the targets. There are 121 nodes and 1956 edges. The topological parameters of PPI network targets were analyzed by the CytoHubba plug-in of Cytoscape_v3.9.1 software and the CentiScaPe 2.2 plug-in, using the constraints of degree, betweenness, and closeness. By filtering the analyzed results based on the threshold value, 22 targets were obtained and sorted according to the parameter values. The information of the top 20 targets shows that AKT1, IL-6, TNF-α, IL-1β, etc. are the core key targets of SGR for the treatment of migraine (Table 2).

GO annotation and KEGG pathway enrichment analysis of shared targets. In the GO enrichment analysis of shared targets, the top 20 entries of biological progress, cellular component, and molecular function were analyzed (Figure 4). Biological progress contains 662 entries, and the targets are mainly involved in the processes of response to drugs, response to xenobiotic stimuli, etc.; cellular component has 78 entries, including integral component of plasma membrane, neuron projection, etc.; molecular function contains 126 entries, with the targets mainly playing roles in transcription factor binding, enzyme binding, and so on. KEGG pathway enrichment analysis of the total targets involved 172 pathways, with the main pathways being pathways in cancer, PI3K-Akt signaling pathway, TNF signaling pathway, IL-17 signaling pathway, and pathways of neurodegeneration-multiple diseases, etc.

Table 1 Effective active ingredients of *Smilacis Glabrae Rhizoma*

MOLID	Effective active ingredient	OB (%)	DL
MOL000098	quercetin	46.43	0.28
MOL013129	(2R, 3R)-2-(3, 5-dihydroxyphenyl)-3, 5, 7-trihydroxychroman-4-one	63.17	0.27
MOL013119	Enhydrin	40.56	0.74
MOL013118	Neoastilbin	40.54	0.74
MOL013117	4, 7-Dihydroxy-5-methoxyl-6-methyl-8-formyl-flavan	37.03	0.28
MOL004580	cis-Dihydroquercetin	66.44	0.27
MOL004576	taxifolin	57.84	0.27
MOL004575	astilbin	36.46	0.74
MOL004567	isoengelitin	34.65	0.70
MOL004328	naringenin	59.29	0.21
MOL001736	(-)-taxifolin	60.51	0.27
MOL000546	diosgenin	80.88	0.81
MOL000449	Stigmasterol	43.83	0.76
MOL000359	sitosterol	36.91	0.75
MOL000358	beta-sitosterol	36.91	0.75

OB, oral bioavailability; DL, drug-likeness.

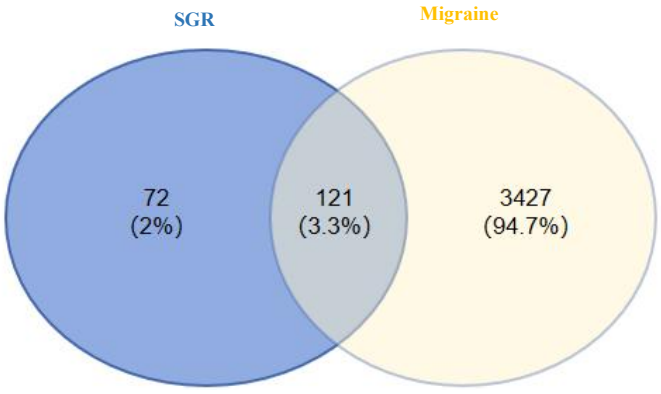

Figure 2 Veen diagram of key target points. SGR, *Smilacis Glabrae Rhizoma*.

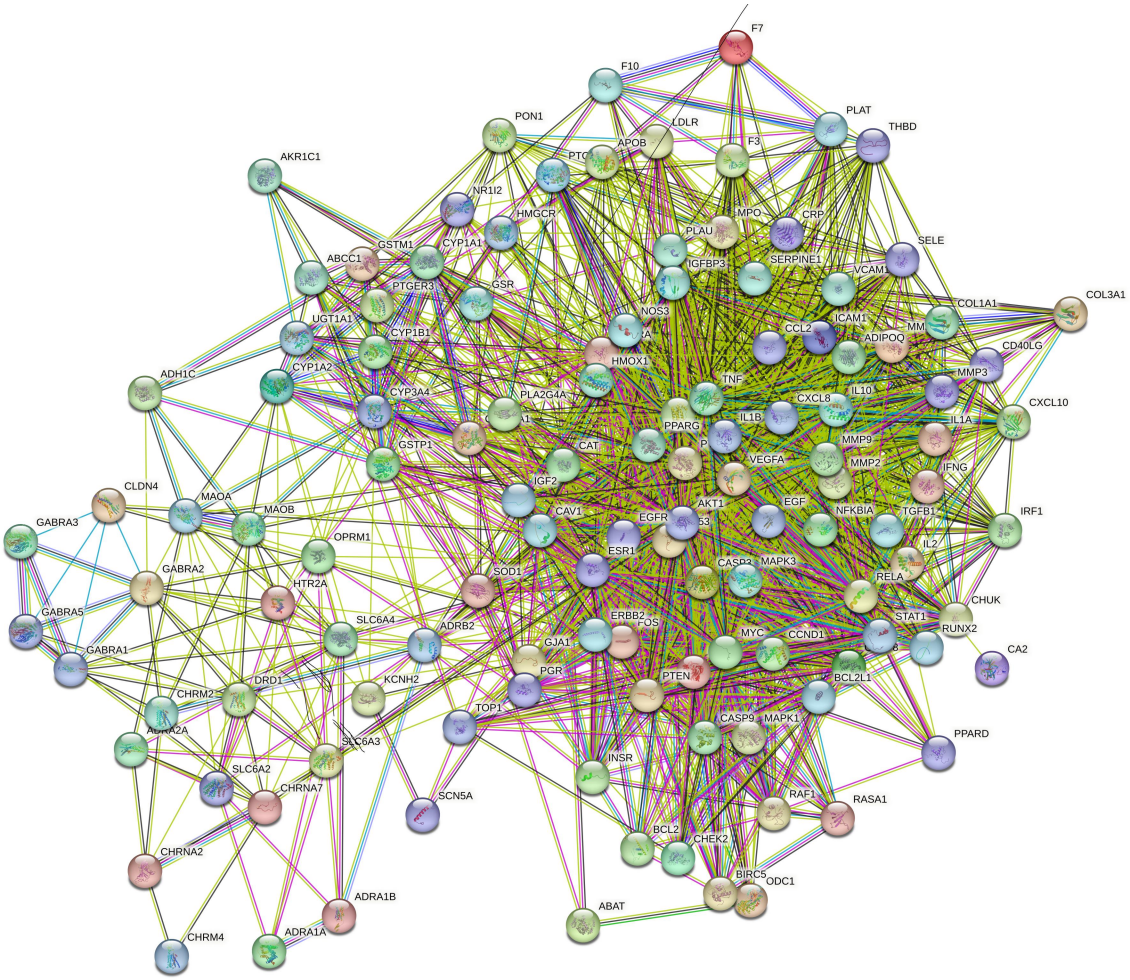

Figure 3 Protein-protein interactions network of targets between *Smilacis Glabrae Rhizoma* and migraine

Table 2 Top 20 target information of PPI network

Name	Degree	Name	Betweenness	Closeness
AKT1	83	AKT1	1156.15976	0.754717
IL-6	82	FOS	642.440466	0.736196
TNF-α	81	IL-6	641.936699	0.722892
TP53	74	TNF-α	502.040359	0.697674
IL-1β	73	CAT	480.202940	0.681818
VEGFA	73	ESR1	478.582720	0.674157
PTGS2	70	EGFR	477.554376	0.674157
CASP3	70	IL-1β	368.724321	0.674157
EGF	67	MMP9	325.827689	0.666667
MAPK3	67	CASP3	315.786575	0.666667
EGFR	66	ERBB2	305.243286	0.662983
ESR1	64	TP53	295.192414	0.652174
PPARG	64	CAV1	246.995521	0.648649
MMP9	64	VEGFA	244.616581	0.648649
CXCL8	62	PTGS2	215.625133	0.641711
FOS	59	EGF	206.611115	0.641711
MYC	57	HMOX1	202.518953	0.625000
CAT	56	MAPK3	183.425929	0.621762
ERBB2	53	PPARG	174.835764	0.618557
HMOX1	53	PTEN	147.373592	0.618557

PPI, protein-protein interactions; IL-6, interleukin-6; IL-1β, interleukin-1β.

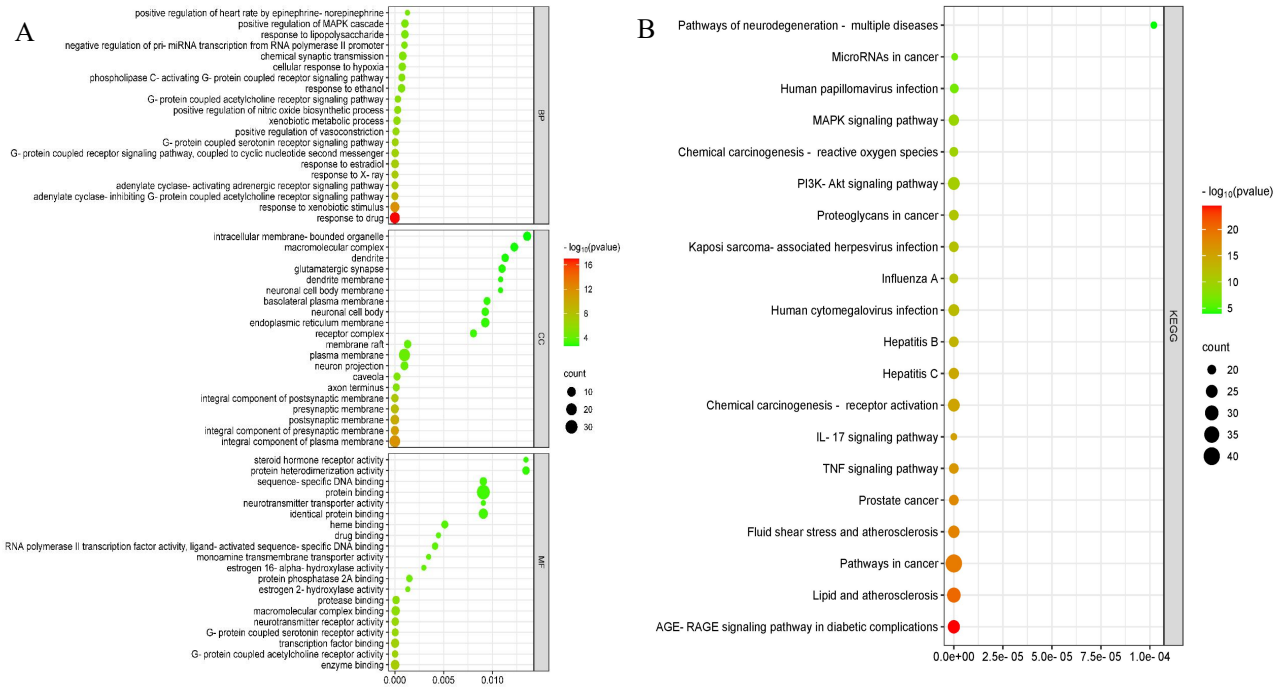

Figure 4 GO enrichment and KEGG pathway enrichment analysis. (A) GO enrichment analysis. (B) KEGG pathway analysis. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological progress; CC, cellular component; MF, molecular function.

Molecular docking results of core key targets and key active ingredients. Molecular docking results of core key targets and key active ingredients. By analyzing the degree values of the active ingredient nodes in the network diagram of "SGR - active ingredient - target - migraine", the results showed that the top 5 active ingredients in the degree rankings were quercetin, naringenin, loksinosideand, sterol and β-sterol, suggesting that these three active ingredients may be the potential key active ingredients of SGR in the treatment of migraine. The binding activities between the 3 potential key active ingredients and the core key targets AKT1 (PDB ID: 1UNQ), IL-6 (PDB ID: 1A1U), TNF-α (PDB ID: 5UUI), and IL-1β (PDB ID: 5R85) were validated using the molecular docking method. The binding energy between each component and the target is generally considered to be less than –4.25 kcal/mol (1 kcal = 4.2 J) (Table 3), indicating some binding activity between the two, less than –5 kcal/mol indicating good binding activity, and less than –7 kcal/mol indicating strong binding activity between the two [9]. The results of molecular docking showed that the docked components had some binding activities with the targets, and most of them had better binding activities with the targets, with strong binding activities between IL-1β and quercetin, loksinoside, naringenin, and TNF-α and naringenin. The docking results were visualized for components with binding energies less than –5 kcal/mol, with good binding activity, and targets (Figure 5).

Animal experiment

Behavioral. The number of head scratches and behavioral scores in the model group were significantly higher than those in the blank group. The number of scratches in the SGR group was significantly reduced compared with the model group. Although the number of scratches in the SGR group was significantly lower than in the model group, there was no statistically significant difference in the number of scratches between the groups. There was no statistically significant difference between groups in the number of scratches in the cages (Table 4).

Effect of SGR on plasma AKT1, IL-6, TNF-α, IL-1β protein expression in migraine mice. Compared with the blank group, plasma AKT1, IL-6, TNF-α, and IL-1β levels were significantly higher in the model group mice. Compared with the model group, these levels were significantly decreased in the SGR group (Figure 6).

Discussion

SGR is the dried rhizome of *Smilax glabra Roxb*, a light-leaved sarsaparilla of the *Smilacaceae* family, which contains a variety of chemical components such as sugars, organic acids, phenylpropanoids, flavonoids and flavonoid glycosides, sterols, saponins, and volatile oils [10]. Modern pharmacological studies have proved that SGR has a wide range of biological activities, and its active ingredients have pharmacological effects such as analgesic, anti-inflammatory, antibacterial, antioxidant, and immunosuppressive properties [11–13], which have good medicinal value in clinical practice.

In this study, 15 active ingredients of SGR for the treatment of migraine and 121 migraine-SGR common targets were obtained based on the network pharmacology method, and a network model was constructed. This network model was analyzed, and the top-ranked active ingredients were quercetin, naringenin, and β-sitosterol.

Quercetin is a special subclass of flavonoids, which are biologically active natural compounds built on the structure of flavonoids [14]. Some studies have already demonstrated that quercetin has anti-inflammatory, anti-infective, anti-tumor, neurogenic protection, antihypertensive, and hypoglycemic effects, mainly exerted through the regulation of cellular enzymes [15–17]. The release of inflammatory mediators acting on the sensory nerve endings causes the onset of pain [18]. Quercetin inhibits inflammation by decreasing neuronal hyperexcitability and injury receptor activation, thereby reducing pain.

In an in vitro study, it was shown that quercetin can suppress inflammation by inhibiting lipopolysaccharide-induced TNF-α production in macrophages, which reduces microglia activation-induced apoptotic neuronal death [19]. This process is inhibited by the production of cyclooxygenase and lipoxygenase associated with inflammation, and inflammatory pathway activation is limited, thus blocking inflammation. NF-κB is activated in the TLR signaling pathway, thereby regulating inflammation. TLR triggers MyD88 to interact with IRAK4, which later transmits signals to NF-κB and MAPK to activate inflammatory factor expression via the MyD88-dependent pathway [20].

Table 3 Binding energy between core key targets and key active ingredients

MOLID	Molname	Binding energy (kcal/mol)			
		AKT1	IL-6	TNF-α	IL-1β
MOL000098	quercetin	–6.1	–6.3	–6.8	–7.4
MOL004328	naringenin	–6.5	–6.3	–7.0	–7.4
MOL000358	beta-sitosterol	–6.8	–4.9	–5.8	–5.2

IL-6, interleukin-6; IL-1β, interleukin-1β.

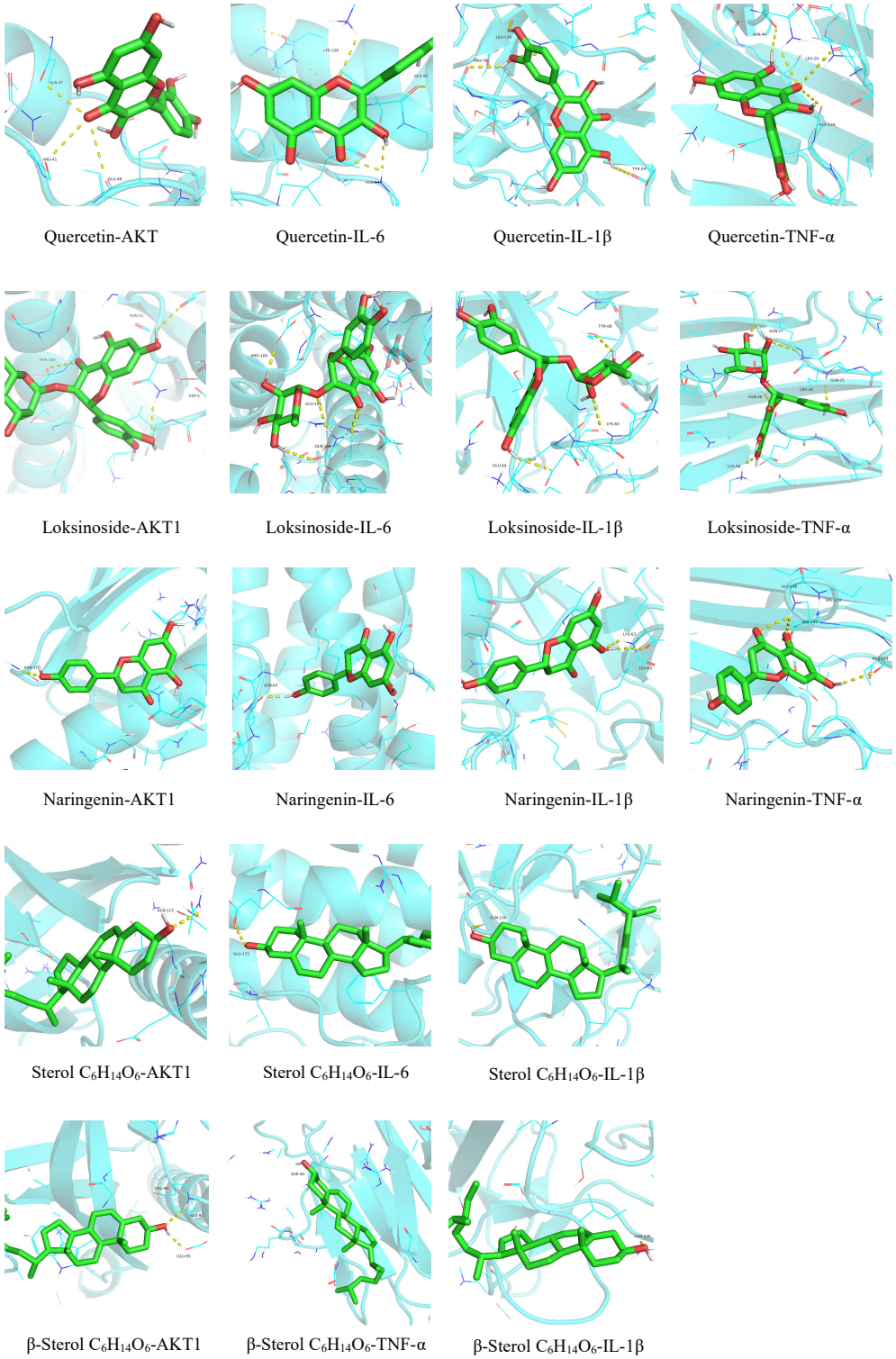

Figure 5 Molecular docking pattern between core key targets and key active ingredients. IL-6, interleukin-6; IL-1β, interleukin-1β.

Table 4 Comparison of the behavioral results of mice in each group ($\bar{x} \pm s$, n = 6)

Group	Behavioral score	Number of scratching
Blank group	2.16 ± 0.81	1.83 ± 0.75
Modeling group	48.66 ± 6.12*	47.16 ± 6.30*
Smilacis Glabrae Rhizoma group	16.16 ± 2.92#	14.50 ± 3.39#

Statistical differences are expressed as follows: compared with the blank group, $^{*}P < 0.01$; compared with the model group, $^{\#}P < 0.01$

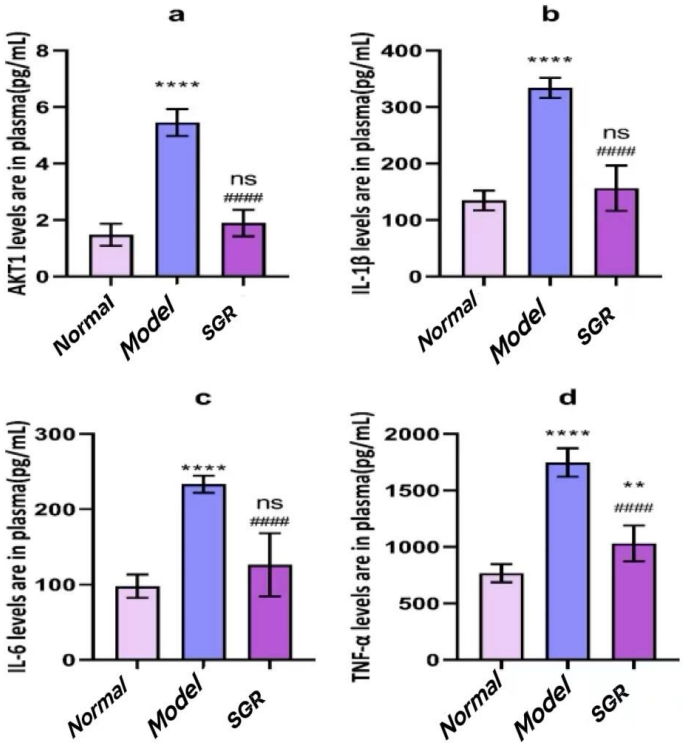

Figure 6 Effects of *Smilacis Glabrae Rhizoma* on plasma AKT1, IL-6, TNF-α, and IL-1β levels in migraine mice ($\bar{x} \pm s$, n = 6). The statistical differences are expressed as follows: compared with the blank group, $^{****}P < 0.0001$, $^{**}P < 0.01$, ns is not statistically significant; compared with the model group, $^{\#\#\#\#}P < 0.0001$. SGR, *Smilacis Glabrae Rhizoma*; IL-6, interleukin-6; IL-1β, interleukin-1β.

In the study of quercetin on cognitive function in double transgenic mice, it was found that quercetin may have inhibited the TLR4/NF-κB signaling pathway in the mouse hippocampus, thereby blocking the expression of TLR4 and NF-κB p65 proteins and inhibiting the expression of TNF-α, IL-1β, and IL-6. This, in turn, improved cognition and modulated inflammatory responses in mice [21]. The migraine attack process itself is an energy-consuming process [22], and neuronal energy depletion subsequently triggers apoptosis. Quercetin can activate the PI3K/Akt pathway and inhibit the ERK1/2 signaling pathway, thereby weakening the autophagy and apoptosis of neuronal cells [23].

Akt1, a serine/threonine-specific protein kinase, is a key protein in the PI3K/Akt signaling pathway, known for its role in regulating cell proliferation and growth and involved in cellular processes including apoptosis and glucose metabolism [24]. In the study of post-injury treatment of traumatic brain injury rats with quercetin, it was found that the level of phosphorylated Akt in brain tissues increased within 48 hours, suggesting that the Akt pathway was involved in the process of brain injury. In contrast, the level of p-ERK1/2 was significantly decreased in the quercetin-treated group compared with the blank-treated group, indicating that quercetin could control and ameliorate neurological function by attenuating neuronal apoptosis and autophagy of neuronal cell impairment [25].

Monoamine oxidase depletion during migraine attacks is followed by cerebral vasodilation, which leads to headaches. Naringenin is a natural flavonoid, and studies have shown that naringenin is a potential monoamine oxidase inhibitor with antioxidant, anti-inflammatory, and neuroprotective properties [26, 27]. In the inflammatory pathway, SIRT1 can inhibit NF-κB transcription by deacetylating NF-κB and suppressing NF-κB downstream inflammatory factors to reduce tissue inflammation [28]. In a study of naringenin on hypoxic-ischemic brain injury in neonatal rats, it was found that the cognitive ability of naringenin-treated Sprague-Dawley rats was higher than that of the model group, and the levels of IL-1β and TNF-α were significantly reduced. This indicated that naringenin could control inflammation and protect neurons by activating SIRT1/NF-κB, thereby improving headache symptoms [29].

β-Sitosterol is a phytosterol found in cereal plants, as well as in many traditional Chinese medicines. Modern pharmacology has found that β-sitosterol has a variety of medicinal properties, such as anti-inflammatory, analgesic, cholesterol-regulating, anti-tumor, and antidepressant effects [30]. A study of the anti-inflammatory ability of β-sitosterol found that β-sitosterol reduced the expression of NLRP3, a key component of the NLRP3 inflammasome, and inhibited the activation of caspase-1, successfully inhibiting the secretion of inflammatory factors [31].

From the above studies, it is clear that SGR may be involved in migraine through the oxidative stress response, the release of inflammatory factors in the inflammatory response, apoptosis, and neuronal protection.

In GO enrichment analysis, it was found that the targets of SGR active ingredients were mainly enriched in the response to exogenous

stimuli, plasma membrane components, and enzyme binding. These processes were associated with the occurrence of inflammatory responses. Among the pathways enriched by SGR for migraine treatment, the pathway in cancer was more significant. The pathway in cancer includes the processes of signaling pathways such as PI3K/Akt, IL-6, and NF-κB. The inflammatory response is an important pathological process in migraine, and inhibiting the inflammatory pathway can effectively reduce the inflammatory response and thus improve migraine [32]. Liu et al. found that PI3K/AKT may be activated in the rat migraine model. PTEN is an upstream regulator of the pathway, and its inhibition enhances the activation of phosphatidylinositol-3, 4, 5-trisphosphate, which inhibits the expression of GSK-3β to achieve the inhibition of migraine [33]. TNF-α, IL-1β, and IL-6 are important pro-inflammatory factors involved in inflammatory responses. Studies have shown that inhibition of the TLR4/MyD88/NF-κB signaling pathway can alleviate the apoptosis of neuronal cells due to inflammation [34]. Molecular docking of key active ingredients and core targets by software revealed that conformations were all stable and the binding energies were less than –5 kcal/mol, except for β-sitosterol and IL-6, which were poorly bound. This suggests that SGR acts on the targets through active ingredients such as quercetin, naringenin, and β-sitosterol for the treatment of migraine headache. Based on the results of network pharmacology analysis, in vivo experiments were performed. The final results showed a striking difference in behavioral scores in the SGR group compared with the model group, indicating that SGR had a relieving effect on migraine. Meanwhile, enzyme-linked immunosorbent assay test results showed that the level of inflammatory factors was significantly reduced in the SGR group, suggesting that in the mouse migraine model, SGR reduced the expression of inflammatory factors TNF-α, IL-1β, and IL-6, and that SGR may exert its pharmacological effects through the modulation of inflammation. Additionally, AKT1 expression levels were reduced in the SGR group, suggesting that AKT1 is involved in the cellular damage process of migraine occurrence, and SGR may affect the occurrence of migraine by limiting the activation of the PI3K/AKT pathway.

Conclusion

In the present study, we used network pharmacology techniques, along with in vivo experiments, and found that SGR may improve migraine through the action of quercetin, naringenin, β-sitosterol, and other components with the targets of TNF-α, IL-1β, IL-6, and AKT1. SGR mainly acts by regulating the release of inflammatory factors, providing neuronal protection, and interfering with apoptosis.

References

1. Wu, XH, Liu Y, Chen FC, et al. Exploration of the effective components and mechanism of action of Angelica dahurica formula in migraine rats based on network pharmacology and experimental validation. *Asia-Pac Tradit Med* 2024:1–7. (Chinese) Available at: https://link.cnki.net/urlid/42.1727.r.20240510.0916.003
2. Headache and Sensory Disorders Specialized Committee of the Chinese Society of Research Hospitals, Neurologists Branch of the Chinese Medical Doctors' Association. Chinese migraine diagnosis and treatment guidelines (2022 edition). *Chin J Pain Med.* 2022;28(12):881–898. (Chinese) Available at: https://doi.org/10.3969/j.issn.1006-9852.2022.12.001
3. Wang X, Yin Z, Lian Y, et al. Premonitory symptoms in migraine from China: A multi-clinic study of 4821 patients. *Cephalalgia.* 2021;41(9):991–1003. Available at: http://doi.org/10.1177/0333102421997850
4. He M, Yu S, Liu R, et al. Familial occurrence of headache disorders: A population-based study in mainland China. *Clin Neurol Neurosurg.* 2016;149:143–146. Available at: http://doi.org/10.1016/j.clineuro.2016.08.010
5. Zhang LH, Yang YX. Progress of research on the clinical manifestations and pharmacologic control of migraine. *Chin Community Physician.* 2004;22:6–8. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=l4t8J_Kk_A8Sy153InBFrX9j1F1HwHkW-UNqs2lYUMnxEfGgNMnI9k4rrDcjAA1IzRKHsBcs9leiTfn5dZcQRSGMhd2ofw2S_L3jhP1eJnJStjOxRsmdQYxivDpA_a4U&uniplatform=NZKPT&flag=copy
6. Wang L, Yin XH, Yang TJ, Yang DZ, Hao B. Pathogenesis of migraine and new targets for its treatment. *Chin J Pract Intern Med.* 2023;43(07):594–598. (Chinese) Available at: http://doi.org/10.19538/j.nk2023070113
7. Li HW. Discussion on the application of Poria in clinic. *Med Forum* 2013;17(11):1441–1442. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=l4t8J_Kk_A-R_Nw4PEFH7IHaTiylQX6LicJCcezYUbG6aVUAiIbryAOfqxyeUx3gOXljXMn7Z5cCVzdUxgult6Kl7nFFHjDjc8u8FKUG-jq0YXfVdxBcfFS3NPq9SqEL&uniplatform=NZKPT&flag=copy
8. World Federation of Chinese Medicine Societies. Guidelines on Methods for Evaluating Network Pharmacology. *World Chin Med.* 2021;16(04):527–532. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=WfYi3ZLogAZi2V_UZ4sasFnQSEP-hXvLZxk_msG5N5W1ZO3CN_OEWfTdbtUJ4sq2pEWOajNmvsUgH7m1SET3Ahw5EfPKMeId_J4gNk--CMUr10QImws0eChN9WyIIU63Z-09pX_KLJw=&uniplatform=NZKPT&flag=copy
9. Ravala SK. Molecular Docking: Bioinformatics Tool. *J Microbiol Pathol.* 2021;5(5):134. Available at: http://doi.org/10.37421/jmbp.2021.5.134
10. Tang YZ, Liu JY, Jiang ZZ, Jiang T, Peng HY, Zhang LY. Study on the chemical composition of Glabrous Greenbrier Rhizome total glycosides. *Chin Tradit Herb Drugs.* 2022;53(22):6977–6984. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=lQz6UQjnwp9WidW8pPIOSpLH-a9o1M5kHDCTugry0CfcW_Hb8CElvAv-Ps9Wu1MfqG1cXsmQfmKNe0YenzMiI3Bc7HGQNS5tIej_JCMLtoCLVDhj-gz6R1kbR16JcMABsqO3wHAXo3Y=&uniplatform=NZKPT&language=CHS
11. Yang CK. Progress of clinical application of Glabrous Greenbrier Rhizome. *Asia-Pac Tradit Med.* 2017;13(17):77–78. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=lQz6UQjnwp-L3tzImaH6lBZZitP5eU5gBijx1LS2n9Rc2-R1WX36oTftbbbHF0ZIUXIkMdNaDdAcm_m-I6st_MillSWBn8MLPQbG2ykk07Pi0JI2NYF7i8HxSaY5ASYgxuFZ1I8uMBI=&uniplatform=NZKPT&language=CHS
12. Zhao X, Chen R, Shi Y, Zhang X, Tian C, Xia D. Antioxidant and Anti-Inflammatory Activities of Six Flavonoids from Smilax glabra Roxb. *Molecules.* 2020;25(22):5295. Available at: http://doi.org/10.3390/molecules25225295
13. Liang QJ, Liang WP, Li Q, Guo HW, Zhu D. Progress of anti-gout effects of Glabrous Greenbrier Rhizome. *World Chin Med.* 2018;13(11):2937–2940. (Chinese) Available at: http://doi.org/10.3969/j.issn.1673-7202.2018.11.063
14. Singh P, Arif Y, Bajguz A, Hayat S. The role of quercetin in plants. *Plant Physiol Biochem.* 2021;166:10–19. Available at: https://doi.org/10.1016/j.plaphy.2021.05.023
15. Zhao Q, Wei J, Zhang H. Effects of quercetin on the pharmacokinetics of losartan and its metabolite EXP3174 in rats. *Xenobiotica.* 2018;49(5):563–568. Available at: http://doi.org/10.1080/00498254.2018.1478168
16. Marunaka Y, Marunaka R, Sun H, et al. Actions of Quercetin, a Polyphenol, on Blood Pressure. *Molecules.* 2017;22(2):209. Available at: http://doi.org/10.3390/molecules22020209
17. Haddad P, Eid H. The Antidiabetic Potential of Quercetin: Underlying Mechanisms. *Curr Med Chem.* 2017;24(4):355–364. Available at: http://doi.org/10.2174/0929867323666160909153707
18. Huang WW. Advances in the study of migraine-associated

inflammatory factors. *Nanjing Society of Rehabilitation Medicine. Proceedings of the Third National Conference on Rehabilitation and Clinical Pharmacy Academic Exchange* (II). 2022:5. (Chinese) Available at: https://doi.org/10.26914/c.cnkihy.2022.078668

19. Tang J, Diao P, Shu X, Li L, Xiong L. Quercetin and Quercitrin Attenuates the Inflammatory Response and Oxidative Stress in LPS-Induced RAW264.7 Cells: In Vitro Assessment and a Theoretical Model. *Biomed Res Int.* 2019;2019:7039802. Available at: https://doi.org/10.1155/2019/7039802
20. Kumar V. Toll-like receptors in the pathogenesis of neuroinflammation. *J Neuroimmunol.* 2019;332:16–30. Available at: http://doi.org/10.1016/j.jneuroim.2019.03.012
21. Liu Y, Li Q, Zheng HW, Zhang ZH, Liu YH. Effects of quercetin on cognitive function, inflammatory response in APP/PS1 mice. *J Xuzhou Med Univ*. 2020;40(03):175–179. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=lQz6UQjnwp_yXJTvGNZal4rNnpOnP1aWDbSANYDsiO_dtMAhkTg0i-yyryNwd4fFowTn_xbXApHqwmA7_HxxHjuaVSulwD2o9TGGgbe1mjqs72Fyw7oUrN8U1NyvDYVyi567eXKBaS4=&uniplatform=NZKPT&language=CHS
22. D'Andrea G, Granella F, Leone M, Perini F, Farruggio A, Bussone G. Abnormal Platelet Trace Amine Profiles in Migraine With and Without Aura. *Cephalalgia.* 2006;26(8):968–972. Available at: http://doi.org/10.1111/j.1468-2982.2006.01141.x
23. Miao Z, Miao Z, Wang S, Shi X, Xu S. Quercetin antagonizes imidacloprid-induced mitochondrial apoptosis through PTEN/PI3K/AKT in grass carp hepatocytes. *Environ Pollut.* 2021;290:118036. Available at: http://doi.org/10.1016/j.envpol.2021.118036
24. Vara JÁF, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev*. 2004;30(2):193–204. Available at: http://doi.org/10.1016/j.ctrv.2003.07.007
25. Du G, Zhao Z, Chen Y, et al. Quercetin attenuates neuronal autophagy and apoptosis in rat traumatic brain injury model via activation of PI3K/Akt signaling pathway. *Neurol Res.* 2016;38(11):1012–1019. Available at: http://doi.org/10.1080/01616412.2016.1240393
26. Govindasamy H, Magudeeswaran S, Kandasamy S, Poomani K. Binding mechanism of naringenin with monoamine oxidase – B enzyme: QM/MM and molecular dynamics perspective. *Heliyon.* 2021;7(4):e06684. Available at: http://doi.org/10.1016/j.heliyon.2021.e06684
27. Zou L, Ning M, Wang W, Zheng Y, Ma L, Lv J. Naringenin Prevents Propofol Induced Neurodegeneration in Neonatal Mice Brain and Long-Term Neurocognitive Impacts on Adults. *Drug Des Devel Ther*. 2020;14:5469–5482. Available at: https://doi.org/10.2147/DDDT.S280443
28. Wang W, Wang L, Yang M, et al. Circ-SIRT1 inhibits cardiac hypertrophy via activating SIRT1 to promote autophagy. *Cell Death Dis.* 2021;12(11):1069. Available at: http://doi.org/10.1038/s41419-021-04059-y
29. Yu M, Liu Y, Li MN. Protective effect of naringenin on hypoxic-ischemic brain injury in neonatal rats. *J Harbin Med Univ.* 2022;56(06):541–544. (Chinese) Available at: http://doi.org/10.20010/j.issn.1000-1905.2022.06.0541
30. Jia S, Zhang F, Zhou H, et al. Bioactivity and application of food-borne sterols. *Sci Technol Food Ind* 2019;40(08):310–316. (Chinese) Available at: https://dx.doi.org/10.13386/j.issn1002-0306.2019.08.052
31. Liao P-C, Lai M-H, Hsu K-P, et al. Identification of β-Sitosterol as in Vitro Anti-Inflammatory Constituent in Moringa oleifera. *J Agric Food Chem.* 2018;66(41):10748–10759. Available at: http://doi.org/10.1021/acs.jafc.8b04555
32. Li L, Wang XY, Li N, Zhang H. Status of the mitogen-activated protein kinase (MAPK) and nuclear transcription factor-κB (NF-κB) pathways in the pathogenesis of migraine headache. *Chin J Clin Pharm.* 2019;35(10):1071–1074. (Chinese) Available at: http://doi.org/10.13699/j.cnki.1001-6821.2019.10.039
33. Liu Y-Y, Jiao Z-Y, Li W, Tian Q. PI3K/AKT signaling pathway activation in a rat model of migraine. *Molecular Med Reports.* 2017;16(4):4849–4854. Available at: http://doi.org/10.3892/mmr.2017.7191
34. Hao RJ, Hu YC, Yu GR, Qin XD, Lu YW. Harpagoside attenuates neuroinflammation-induced neuronal damage by inhibiting the TLR4/MyD88/NF-κB pathway. *Chin Tradit Herb Drug.* 2023;54(13):4202–4213. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=lQz6UQjnwp_F_YxEKGoKkaGlQGFiyR2QN7DT8IcFmNNho2I38uyHtYK3zMun4rDh8X1WODy8jwEuXSIWiWeQeRrufiIhVynetXgeAbhCBvjzfT6i8ncRAIj_-VVld00f7RdJs92y1GY=&uniplatform=NZKPT&language=CHS