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Lignans aid novel therapy for migraine through Network Pharmacology

R RUSHENDRAN

SRM Institute of Science and Technology (Deemed to be University)

Chitra Vellapandian (chitrav@srmist.edu.in)

SRMIST: SRM Institute of Science and Technology (Deemed to be University) https://orcid.org/0000-0001-7927-0983

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Abstract

Most of the women's daily life disturbed due to migraine attack. Till today there were lots off researches going on to treat migraine but the eminent scientists found the specific drugs to minimize the symptoms produced in the migraine attack. Network pharmacology will be the different strategy to treat migraine via networking of compound-target-pathway approach. This research looked at the molecular mechanism by which the herbal combination of *Chrysanthemum indicum* and *Crocus sativus* is effective in relieving migraine symptoms. The biomolecules of CICS were acquired from Dr. Duke's Phytochemical and Ethnobotanical and IMPPAT Databases. Active ingredients were screened by SwissADME tool and toxicity by OSIRIS tool. The migraine associated targets of active ingredients were determined by online databases. The biomolecule-target-pathway network and degree analysis were evaluated by Cytoscape software 3.9.1. Asarinin, Chrysantherol, Chrysanthetriol, Fargesin, and Crocetin were identified as key ingredients. From the 118 common targets specifically AKT1, VEGFA, TNFα, IL6, and PTGS2 were identified as top key targets based on topological parameters to treat migraineurs. Through enrichment analysis, we identified 167 significant signaling pathways. Lignans and Sesquiterpenes showed good binding affinity with more than 7.00kcal/mol in the molecular docking analysis. Pain relievers and anti-inflammatories are the cornerstones of treatment for CICS-related migraine. Multiple paths, targets, and components can be used in conjunction with the two plants. Lignan fraction having antimigraine activity in dose dependent manner, further keen research should be done with the Fargesin which provide a reference for clinical migraine management.

Introduction

Headaches that are either unilateral or bilateral and pulsate at least fifteen days per month are diagnostic of chronic migraine (CM) [1, 2]. According to the World Health Organization (WHO), this condition is among the deadliest forms of chronic dysfunction and has a devastating impact on patients' mental and physical well-being. Widespread consensus now exists that central sensitization, characterized by a sustained elevation in the excitation of neurons in the trigeminal nucleus caudalis (TNC) in response to painful stimuli, is a crucial pathogenic mechanism in CM. Although medical researchers have yet to pinpoint an effective therapy for migraines but present marketing drugs such as CGRP antagonists, although over-the-counter (OTC) medications like serotonin agonists may provide some comfort, they often come with serious cardiac side effects that prevent most individuals from taking them. Despite this, some people continue to rely on OTC options like roll-ons, gels, and pain-relieving sprays etc. [3–5] may provide a more long-term solution on temporary based herbal remedies.

Ayurvedic medicine is one of the India's oldest healthcare systems in the world. Over decades, it has been refined in India. In modern ayurveda, Complementary Alternative Medicine (CAM) therapies including herbal supplements and different diets play an important role in maintaining good health and resolving health issues. Indian medicine has been just as influential in Asia as Greek medicine; it has expanded throughout Indo-China, Indonesia, Tibet, Central Asia, Japan and nowadays greater influence throughout the world [6]. Many herbal plants have not yet been scientifically proven for migraines or other

neurological illnesses, but it is proposed that they may provide a better remedy. After several literature studies, we screened that, *Chrysanthemum indicum* and *Crocus sativus* (CICS) herbal combination may give better relief from migraine.

For thousands of years, the flowering plant *Chrysanthemum indicum* L. has been employed in both Chinese and South Korean medicine. In Asia, *Chrysanthemum* tea is a popular beverage. It is mostly used for medicinal purposes such as hypertension, inflammation, and respiratory illnesses in South Korea due to its higher concentration of active chemical components and superior therapeutic effects. Recently it was identified that this plant having some other therapeutic properties including anti-inflammatory [7–9], adipogenesis [10], prevents neurotoxicity [11], anti-obesity [12]. The inflorescence of *Chrysanthemum indicum*, especially the flower buds, has anti-inflammatory [13], hepatoprotective [14], antimicrobial [15], anti-osteoporosis and antioxidant qualities [16], making it an antiphlogistic for headaches, conjunctivitis, furuncles, and carbuncles.

The Middle Eastern crocus, *Crocus sativus* (Safron), is a member of the iris family (Iridaceae). The stigmas of saffron flowers contain a relatively high proportion of the carotenoid pigment known as crocin. Sugars (including gums, starch, reducing sugars, pectin, dextrins, and pentosans) make up about 63% of saffron's chemical composition (although this can vary depending on the environmental conditions in the country of origin), along with 12% protein, 10% water, 5% minerals, 5% crude fibre, 5% fat and traces of riboflavin and thiamine vitamins [17]. It is having various therapeutic properties such as insomnia, alzheimer's disease [18], neuropsychiatric and age-related diseases [19], antidepressant [20], ovarian dysfunction [21], anti-inflammatory, antioxidant, immunoregulatory [22], anti-tumor [23], diuretic [24], analgesic [25], appetite suppressant, hypnotic, hepatoprotective, and bronchodilator effects. In addition to its usage as a herbal sedative, passionflower has several other medicinal applications, including as an aphrodisiac, antispasmodic, diaphoretic, stimulant, expectorant, stomachic, eupeptic, antitussive, and emmenagogue. Serotonin, norepinephrine, dopamine, glutamate, and gamma-aminobutyric acid type A (GABA-A) are only few of the neurotransmitters that have been shown to be affected by saffron extract and its active components. So we are hoping that migraine can be treated and alleviated with *Chrysanthemum indicum* and *Crocus sativus* (CICS) due to it is having capacity to treat various neuro associated disorders through Network Pharmacological approach.

Network pharmacology is a computational artificial intelligence (AI) technique developed in the 20th century, reduces the amount of time it takes to identify a potential drug discovery or protein/gene target for specific disease. Consistent with the holistic view of traditional medicine, and similar to the mechanism of multiconstituents and multitargeting function of traditional medicine compounds, it will anticipate the mechanism of drug functions in disease therapy as a whole [26–28]. As a new field of study, network pharmacology connects the dots between diseases, genes, and potential therapeutic interventions in order to shed light on the biomolecular basis of drug action. It is quickly emerging into a comprehensive and effective method for characterising the intricate interplay between drugs and biological systems such human organs, illnesses, metabolic pathways, and target proteins [29]. Combining computational prediction and experimental confirmation, the Network pharmacology

paradigm provides a novel means of elucidating the molecular mechanisms at play in drug action [27]. Precision and efficacy in therapeutic intervention are provided by synergistic multi-compound network pharmacology and drug repurposing, which obviates the need for drug discovery and expedites clinical translation [30]. Combining a Network pharmacological approach with herbal medicine could help pinpoint the active natural biomolecule in instantaneously.

Materials and Methods

Predicting and Screening for potential active ingredients and Toxicity analysis

CICS biomolecules were acquired from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/) and the Indian Medicinal Plants, Phytochemistry, and Therapeutics Databases-IMPPAT (https://cb.imsc.res.in/imppat/), respectively. Initially biomolecules were screened using oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. Small molecular compounds are predicted to have druglikeness based on the Lipinski principle, which states that such compounds must meet the following criteria such as a molecular weight ≤ 500, a lipid water partition coefficient ≤ 5, and a rotatable bond count ≤ 10. The obtained biomolecules that were found in the screening process as active ingredients. The Swiss ADME tool and Swiss Target Prediction were used to make predictions about the effects of active substances. OSIRIS was used to test the compound for toxicity once it had been screened. The predicted toxicity risks for screened compounds were measured by their mutagenicity, tumorigenicity, irritancy and reproductive effects are described using Osiris property Explorer.

Assessment of CICS Targets Associated with Migraine and Their Possibility for Use in Migraine Treatment

Searches for "Migraine" in DisGeNet (https://www.disgenet.org/), GeneCard (https://www.genecards.org/), OMIM (https://www.omim.org/), and the Therapeutic Target Database (https://db.idrblab.net/ttd/) obtained list of genes were merged and deduplicated. After normalising the data on the UniProt database, we were able to gain the pertinent targets of migraine treatment. Using a venn diagram constructed with data from Bioinformatics and Evolutionary Genomics, the underlying targets for herb matching for migraine therapy were identified (https://bioinformatics.psb.ugent.be/webtools/Venn/). The underlying targets for herb pairing of CICS in the treatment of migraines were identified and their intersection was exploited.

Constructing Protein-Protein Interaction Networks and Screening for Essential Targets

Analysis of Protein-Protein Interaction (PPI) is crucial for dissecting complex cellular mechanisms and gaining insight into biological processes. The STRING tool was used to build the PPI network. The aforementioned overlapping targets were loaded into STRING with the species set to "homo sapiens," and

the interaction score set to 0.4, yielding the PPI network relationship. CytoScape_v3.9.1 was used to create the PPI network diagram. The network analyzer feature was used to determine the accuracy of the value. Central targets could be defined as the top 10 targets with the highest degree scores.

Gene Ontology Functional Enrichment and Kyoto Encyclopaedia of Genes and Genomes-Based Pathway Analysis

On the Metascape platform, we ran a bioinformatics enrichment analysis on the CICS possible targets for migraine therapy. This included a GO analysis of biological processes, molecular activities, and cellular components, as well as a KEGG pathway enrichment analysis. We used "Homo sapiens" as our species and a significance threshold of $P < 0.01$. After the data was evaluated, it was saved in a bioinformatics platform and the top 10 GO functions and 20 KEGG pathways were chosen.

Biomolecule-Target-Pathway Network Building (B-T-P)

In order to determine CICS for migraine treatment, its active ingredients, possible targets, and top 20 pathways were entered into the software Cytoscape v_3.9.1. Topological parameters for the network including degree, betweenness, and proximity were computed. Network Analyzer was used to identify the principal target and effective active biomolecules of CICS for migraine treatment.

Molecular Docking Assessment

The selected CICS core targets and their matching therapeutic agents were molecularly docked. Core proteins were obtained from the PDB database (https://www.rcsb.org/). The protein for docking studies was selected based on the sequence alignments and activity. The compound structures were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). After minimising the force Celd relevant to ligand energy and its energy using Chem3D Pro 12.0 software, a 3D structure of the major effective ingredients was saved in PDB format for docking analysis. Molecular docking was carried out via AutoDockTool 1.5.6 and the relevant calculation results were acquired after proteins were dewatered; addition of polar hydrogens and Kollman charges; assigned atoms to AD4 type finally converted to the pdbqt format. After detecting root of Torsion tree for specific ligand and saved in pdbqt format for docking analysis. Better integration activity between active ingredients and targets was screened using the docking value affinity, where values of < 4.25 kcal/mol are indicative of binding activity between ligands and targets, < 5.0 kcal/mol are indicative of enhanced binding activity, and < 7.0 kcal/mol have the highest docking activity. At last, the BIOVIA Discovery Studio programme was used to display the molecular docking outcomes.

Experimental Validation

Chrysanthemum indicum Lignan fraction

Chrysanthemum indicum plant were collected at local market, Guduvancheri, Tamil Nadu and it was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Tambaram, Chennai, Tamil Nadu (Reg. No. PARC/20224707). The leaves were separated, shaded dried, and precautions were taken to avoid contamination during leaf drying. Home mixer was used for making coarse powder (1kg) of raw material and defatted twice with hexane. Then the crude extract (200g) were obtained by extracting with hydroalcoholic extract (Ethanol and water; 70:30) using soxhlet setup, stored in desiccator, and used whenever it required. Based on their molecular weight and retention time, biomolecules present in crude extract were identified using GCMS solution-RT analysis software. The analysis were performed in programmed mode with the following parameters: start temperature 50°C, kept for 5 minutes; programming rate °C/min. = 2, 4, 8, and final temperature 300°C, held for 30 minutes. The sample size was 1µl, and the split ratio was 1:80. Helium was used as the transport gas. The temperature of the injector remained constant at 300°C.

The test samples of hydroalcoholic extract (1000 µg/ml) were mixed with 0.5 ml of water and 0.2 ml of Folin-Ciocalteu's phenol reagent (1 : 1). After 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 5 mL with distilled water. The reaction was kept in the dark for 30 min. and the absorbance of blue color from different samples was measured at 765 nm. The phenolic content was calculated as Gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of Gallic acid (2–64 µg/ml), $y = 0.0078x + 0.002$, $R^2 = 0.9997$.

Animals

Male CD1-mice weighing between 25–30 g were randomly split into five groups of six and housed in cages maintained at a constant 21 ± 1°C with a humidity level of 45%. Normal laboratory fare, including filtered water, was readily available. SRM College of Pharmacy, SRMIST's Institute Animal Ethics Committee (IAEC/268/2021) gave its stamp of approval to all animal research, and those experiments were conducted in accordance with the CPCSEA regulations governing the care and use of experimental animals. All of the animals were given a week to settle into their new homes before the experiment begun. The mice were randomly split into five groups using an Excel-generated random number sequence, and the results were analysed using a simple average.

Nitroglycerine induction and drug administration

The introduction of NTG resulted in the development of chronic migraine (CM). From a 5mg/ml stock solution, 0.9% saline was used to make 1mg/ml NTG on a daily basis. For 9 days (total 5 times), mice received intraperitoneal injections of diluted NTG (10mg/kg, b.wt) on alternate days. Mice were randomly assigned to one of five treatment groups: normal (group 1), NTG (group 2–10 mg/kg. b.wt), standard NTG (group 3-Sumatriptan, 600 µg/kg, b.wt), NTG + CILF (group 4-Test I, 200 mg/kg, b.wt), and NTG + CILF (group 5-Test II, 400 mg/kg, b.wt) for 11 days. Mice were slaughtered 24 hours following their final dose, and their rostral spinal cords were removed and dissected for histological analysis and western blot inquiry to determine if the animals met the study's criteria.

Enzyme-linked Immunosorbent assay (ELISA)

Protein levels of CGRP, IL-6, TNFα, and PTGS2 were compared in serum samples. All commercial ELISA kits were used in accordance with the manufacturers' instructions. The tests were performed in duplicate in 96-well plates, and the results were reported in picograms per millilitre. Proteins from the tissue were used for ELISA assays, including CGRP (25-400pg/ml), IL-6 (15.6-500pg/ml), TNF (200-1000pg/ml), and PTGS2 (200-1000pg/ml). Tissue was homogenised at 300C before being diluted 1:9 with phosphate-buffered saline (pH 7.2–7.4, 0.01mol/L). For 15 minutes, the slurry was centrifuged at 5000 rpm to separate the supernatant. Following the protocols, samples were introduced to the appropriate wells of the microenzyme plates and bound with the appropriate antibodies. After covering the plate with a membrane, the HRP-conjugated reagent was incubated at 370 degrees Celsius for 60 minutes. The wells were first cleaned with washing buffer, then chromogen solutions A and B were added. Both A and B chromogen solutions were heated to 370 degrees for 15 minutes in the light. The last step is to measure the OD at 450nm [5].

Western blot analysis

Following a 10-minute centrifugation at 500xg and 400C, the tissue was homogenised in buffer. The cell pellet was suspended in cold PBS and lysis buffer A, both of which were also chilled, and a cocktail of protease inhibitors (4 ml) was added to the latter. After incubation and centrifugation, the supernatant fraction should be carefully collected after the fat top layer has been removed. The mixture was centrifuged at 7000 g for 15 minutes after adding 400 µl of ice-cold lysis buffer B containing 4 µl of protease inhibitor. The bicinchoninate test was used to determine the protein content. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to separate 20 g of protein extract, and the resolved proteins were then transferred to nitrocellulose membranes. Anti-iNOS (D6B6S) Rabbit mAb #13120 and primary anti-CGRP antibody (D5R8F) for cell signalling were treated with each membrane. Management of Loads Dilutions of goat anti-rabbit IgG (1:5000) and anti-beta tubulin antibody (1:1000) (both from Abcam; catalogue number ab21058) were used [31, 32]

Results

Biomolecule Screening and Target Prediction of CICS

A total of 358 biomolecules were obtained for CICS with the help of Dr. Duke's Phytochemical and Ethnobotanical Databases and IMPAAT databases after duplication. In that only 5 biomolecules such as Lignans (Asarinin and Fargesin), Sesquiterpenes (Chrysantherol and Chrysanthetriol), and Crocetin were qualified after keen screening of druglikeness property along with BBB permeability using SwissADME tool mentioned in Table 1 and toxicity of biomolecule with OSIRIS software tool. 421 targets were obtained after the results of target prediction were combined and deduplicated by Swiss target prediction.

Table 1
Active biomolecules of CICS

Plant	Biomolecule	MW	RB	HBA	HBD	TPSA	Drug Score	LogP
Chrysanthemum indicum	Chrysantherol	234.33	1	2	1	29.46	0.46	2.7
Chrysanthemum indicum	Fargesin	370.40	4	6	0	55.38	0.78	2.9
Chrysanthemum indicum	Chrysanthetriol	254.37	1	3	3	60.69	0.45	3.3
Chrysanthemum indicum	Asarinin	354.35	2	6	0	55.38	0.65	3.2
Crocus sativus	Crocetin	328.40	8	4	2	74.6	0.58	5.4

MW-Molecular weight; RB-Rotatable bonds; HBA-Hydrogen bond acceptor; HBD-Hydrogen bond donor; TPSA-Topological surface area; LV-Lipinski violation

Target Screening and Indications for CICS for Migraine Management

DisGeNet (552), Gene Cards (553), OMIM (6), and TTD (35) gene databases were searched for migraine-related targets, yielding a total of 724 targets after merging and deleting duplicates. By comparing the 724 targets associated with migraines to the 421 targets of CICS, 118 identical targets were discovered and further protein-protein interaction analysis was performed which are represented in Fig. 1.

Constructing of Protein-Protein Interaction (PPI) Network

In order to learn more about PPI networks, the STRING database was populated with data from 118 intersection targets. There are 65 proteins represented as nodes in the PPI network and 927 PPI relationships represented as edges were illustrated in Fig. 2. Cytoscape v_3.9.1 was used to visualise the information on protein interactions in the PPI network. After collecting the PPI network, we used the network analyzer to determine the degree value. AKT1, VEGFA, TNF, IL6, and PTGS2 were the top 5 targets according to degree value, with 62, 53, 50, 49, and 43 degrees of separation, respectively. These targets serve as hubs at the core of the PPI network, linking together the various nodes.

Analysis of KEGG Pathways and Enrichment of GO Functions

Using the Metascape system, we analysed 118 CICS therapeutic candidates for migraines in terms of GO and KEGG pathway enrichment. The investigation yielded a total of 1362 GO enrichment analysis comprising cellular component analysis (74), biological process analysis (1161), and molecular function analysis (127).

Results from the top ten GO characteristics were chosen, and the bioinformatics platform was used to preserve and visualize the results. The KEGG analysis yielded a total of 167 pathways; gene ontology of top 20 targets of which were selected for input and visualisation via the bioinformatics platform represented in Fig. 3. The majority of the KEGG pathways involve the binding of ligands to receptors in the nervous system, estrogen signalling pathway, platelet activation, morphine addiction, fluid shear stress and atherosclerosis, endocrine resistance, phospholipase D signalling pathway, AGE-RAGE signalling pathway, cAMP signaling pathway, GABAergic synapse, Prolactin signaling pathway, TNF signaling pathway, Serotonergic synapse, Neurotrophin signaling pathway, ErbB signaling pathway, MAPK signalling pathway, Calcium signaling pathway, Sphingolipid signaling pathway etc.

Biomolecule Target Pathway Network Assessment

The B-T-P network figure of CICS for migraine treatment was created using CytoScape3.9.1. To get constituents and main action targets, network topological properties were investigated by implanted tools. There are 160 nodes in this network, connected by 463 edges were illustrated in Fig. 4. The screened biomolecules such as Chrysantherol, Fargesin, Chrysanthetriol and Asarinin from *Chrysanthemum indicum*; Crocetin from *Crocus sativus* may play a crucial role in the migraine treatment. The top 5 targets for topological parameters were AKT1, VEGFA, TNFα, IL6, and PTGS2. These are considered as potential targets of CICS in migraine treatment.

Molecular docking assessment

The B-T-P network diagram of the CICS for migraine therapy and screened biomolecules like Asarinin, Chrysantherol, Chrysanthetriol, Fargesin, and Crocetin were docked individually to elucidate the binding affinity between target proteins and corresponding biomolecules. It shows that the possible key effective ingredients have high binding activity with the key targets but except safranal shows binding affinity < -7.00 kcal/mol. The interaction between AKT1 (PDBID:6BUU), VEGFA (PDBID:5DN2), IL-6 (PDBID:8D82), TNF (PDBID:1EXT), and PTGS2 (PDBID:5F19) individual ligand was determined. Intermolecular forces must be taken into account throughout the docking process. Hydrogen bonds are the primary intermolecular forces examined. The docking score was illustrated in values of individual ligand and protein (Fig. 5). Asarinin and Fargesin has good binding affinity (-10 kcal/mol) with PTGS2 when compare to others and also inhibited neuroinflammatory biomarkers which is responsible for migraine attack.

Effect of CILF on Nitroglycerin induced mice

Total Lignan content in the test samples using the calibration curve, were found to be (62.99 mg) of Gallic acid equivalents/g dry weight of extract. Reliability of the mouse model was assessed by measuring the protein levels of PTGS2, IL-6, CGRP, and TNFα. For 11 days, mice were given daily doses of Sumatriptan (600μg/kg, b.wt i.p) and CILE (200mg/kg and 400mg/kg, b.wt p.o). NTG was administered to mice on days 1, 3, 5, 7, and 9, and daily sumatriptan and CILE therapy significantly decreased NTG-

induced basal hypersensitivity and acute hyperalgesia. In comparison to the NTG-induced group, Sumatriptan and CIHE dramatically reduced the protein expression levels of TNFα, IL-6, PTGS2, and CGRP shown in illustrated in Fig. 8.

Discussion

The morbidity and recurrence rates of migraine, a frequent clinical primary headache, are high. It takes time to heal from the sickness that severely impacts the patient's life, work, studies and lowers their quality of life. Trigeminovascular theory is accepted by current medicine for migraine pathophysiology. Inflammatory mediators produced by microglia in the caudate nucleus are closely linked to central sensitization in the trigeminal nerve pathway, which is the main pathologic feature of migraine. Migraine treatment is still lacking in efficacy and safety. In this scenario, herbal based treatment may give better solution. Based on literature we have found different herbal plants that may treat migraine. From the list of the plants we have screened two selected plants for further research work. CICS has been shown to have a considerable therapeutic effect on migraines, and its compatibility with migraine pathophysiology is also noteworthy. So we strongly believing that based on the phytochemical constituents present in the CICS may treat migraine and reduce the symptoms rapidly and get relief from the migraine attack.

Chrysanthemum indicum L. a perennial plant in the Compositae family, has been employed in traditional medicine for the treatment of swelling, scrofula, discomfort, and pemphigus for over two thousand years in China. More than 190 different biomolecules, such as flavonoids, phenylpropanoids, terpenoids, and phenolic acids have been extracted and characterised from this plant. Anti-inflammatory, anti-oxidant, anti-pathogenic microorganism, anti-cancer, immune-regulating, and hepatoprotective actions are just some of the pharmacological activity found in this plant extracts or monomeric components, as proven in a plethora of recent investigations [33]. Crocus species are found in Western Asia, Southern Europe, and North Africa, where they are grown for their decorative value. Only *Crocus sativus* L. (saffron) is produced for human consumption. Including its antioxidant, antimicrobial, cytotoxic, neuroprotective, cardioprotective, hypolipidemic, antidepressant, and antihyperglycemic properties as well as its protective effects against retinal lesions, Crocus sativus L. and its phytoconstituents are discussed in this up-to-date review of their biological properties and pharmacological activities. Saffron's natural antioxidant and antibacterial qualities make it an excellent choice for use as a food preservative [34].

Using CICS network pharmacology; we performed a preliminary screening of five biomolecules, including Chrysantherol, Fargesin, Chrysanthetriol, Asarinin, and Crocetin, for the treatment of migraines. Key targets were identified by integrating the PPI network with the B-T-P network, which included AKT1, VEGFA, TNF, IL6, and PTGS2. Asarinin and Fargesin are targeting c-Jun N-terminal kinase 2 (JNKs) through MAPK9 responsible for the migraine therapy by IL1 signaling pathway, T Cell Receptor Signaling Pathway, NAD-dependent deacetylase sirtuin 2, Norepinephrine transporter, IL17 signaling pathway, Sodium/glucose cotransporter 2, Norepinephrine transporter, Dopamine transporter, Inflammasome Activation Pathways, Oxidative Stress, MAPK Signaling, NOD-like Receptor Signaling Pathways and Toll-like Receptor (TLR) Pathway.

Chrysantherol is specifically targeting MAP kinase ERK2 through MAPK1 that could play a role in the management of migraine by EGFR Signaling Pathway, IL-1 Family Signaling Pathways, Cyclooxygenase-2, Voltage-gated potassium channel subunit Kv1.5, Cytochrome P450 11B1, Cytochrome P450 11B2, Dopamine transporter, Caspase-1, Transient receptor potential cation channel subfamily-V member 3, Epidermal growth factor receptor erbB1, MAP kinase ERK2, and Common Cytokine Receptor Gamma-Chain Family Signaling Pathways. Chrysanthetriol is particularly activating PI3-kinase p110-alpha subunit and Protein kinase C alpha which may responsible for treating migraine by TLR pathway, AKT Signaling Pathway, Cyclooxygenase-1, LXR-alpha, Tyrosine-protein kinase JAK1 and JAK2, Voltage-gated potassium channel subunit Kv1.5, and Atypical NFkB pathway. The connectivity of pathways among biomolecules was represented in Fig. 6. The network degree analysis of screened five biomolecules and top 5 targets were mentioned in the Table 2 and Table 3.

Table 2
Network topological parameters of top 10 target genes

Target	Degree	Betweenness Centrality	Closeness Centrality
AKT1	62	0.113355742	0.674698795
VEGFA	53	0.060474219	0.612021858
TNF	50	0.033594163	0.605405405
IL6	49	0.034079376	0.622222222
PTGS2	43	0.037448996	0.583333333

Table 3
Network topological parameters of key biomolecules

Biomolecule	Degree	Closeness Centrality	Betweenness Centrality
Crocetin	108	0.384736	0.1926858
Chrysanthetriol	32	0.310476	0.0402610
Chrysantherol	38	0.315280	0.0525776
Fargesin	29	0.304293	0.0340864
Asarinin	12	0.291940	0.0074344

KEGG enrichment analysis revealed that neuroactive ligand receptor interaction was the primary pathway involved in CICS for migraine treatment [35], morphine addiction, TNF signaling pathway, AGE-RAGE signaling pathway, retrograde endocannabinoid signaling, MAPK signaling [36], cocaine addiction, estrogen signaling pathway, chemical carcinogenesis receptor activation, cholinergic synapse and prolactin signaling pathway. The nerve signal transmission system is tightly bound to neurological processes, and it consists of receptors and ligands on the plasma membrane, as well as intracellular and extracellular signalling channels represented in Fig. 7. These pathways involve the synthesis and release

of transmitters at synapses, as well as the interaction of these transmitters with receptors, to facilitate the transmission of pain signals to the central nervous system [37, 38]. Using a network-pathway based approach; we can gain a deeper understanding of the function of genetic factors and their interactions in migraine processes and discover novel avenues for therapy. Especially Asarinin and Fargesin shown good binding affinity and the amino acid interaction are discussed and revealed that all these novel five biomolecules having capacity to treat migraine which was tabulated in Table 4.

Table 4
Amino acid interaction of ligand and protein

Biomolecule	AKT1	VEGFA	TNF	IL-6	PTGS2
	6BUU	5DN2	1EXT	8D82	5F19
Asarinin	LYS-356	ARG-232	ARG-104, ARG-77, CYS-96	GLN-169	TRP-387
Chrysantherol	GLN-352, ASN-351	PRO-230	GLN-82, ASN-110	LEU-88	ALA-199
Chrysanthetriol	GLN-352, ASN-351, TYR-350	ARG-231, PRO-230, LYS-229	ASN-110, PHE-112, LYS-75	VAL-110, LEU-88	ALA-199, THR-206
Crocetin	GLN-352, TYR-350, LYS-356	ARG-231, ASP-228	ARG-104, ARG-77, ASN-110, CYS-96	ALA-66, SER-91, LEU-142	GLN-203, TRP-387
Fargesin	GLN-352	CYS-225	TYR-106	LEU-142	HIS-207, HIS-388

To elicit various migraine-like symptoms in animals that are similar to those caused by CGRP infusion in humans, including pain-like symptoms, CGRP is one of the most well-defined migraine triggers can assist in producing neuroinflammatory molecules. A typical migraine symptom, mechanical hypersensitivity, can be replicated in rodents after CGRP injection [39]. IL-6 is a pro-inflammatory cytokine that is upregulated in the serum and blood of migraine patients during an attack [40, 41]. TNF-α activates the transcription of calcitonin gene-related peptide (CGRP) and plays a key role in migraine pathophysiology [42]. PTGS2 is inducible and typically produces prostanoids that mediate responses to physiological stresses and inflammation [43].

Additionally, the antimigraine action was assessed using an *in vivo* (on living animals) paradigm induced by NTG. The biomarkers TNF-α, PTGS2, CGRP, and IL-6 were shown to be significantly raised at the outset of treatment using ELISA and protein expression, but were dramatically reduced after treatment with Sumatriptan and CILF inhibiting CGRP synthesis and other proinflammatory molecules like TNF-α, PTGS2, and IL-6 in a dose-dependent manner suggests that CILF may have antimigraine efficacy.

A current target molecule to treat migraineurs in the future may be found in the Lignan fraction of Chrysanthemum indicum, which has been revealed to have antimigraine activity. In particular, Fargesin

displays good binding affinity compared to other biomolecules. Studies on the safety, efficacy in animals, and efficacy in humans of the natural phytocompound Fargesin are needed.

Conclusion

Overall, the analgesic and anti-inflammatory effects of CICS make it a good treatment option for migraineurs. The results of this investigation employing a network pharmacology strategy and molecular docking technology have shown the cellular mechanism of CICS for migraine treatment through multitarget, multicomponent, and multibiological pathway control. Lignans and Sesquiterpenes are the novel components of CICS that may responsible for its efficacy in treating migraines by inhibiting the production of neuroinflammatory molecules when compare to other biomolecules. Active compounds of CILF that act on respective targets, like TNFα, IL-6, CGRP, and PTGS2, may be effective in treating migraines by altering neuroactive ligand-receptor interaction, calcium signalling routes, cAMP signalling pathways, TLR, NLRP3, and PI3K pathways. The key conclusion of the study was that CILF pharmacological mechanism in migraine therapy can be anticipated using existing datasets. Using network pharmacology and molecular docking technology introduces the potential mechanism of CILF in treating migraine, which not only offers a reference for clinical management of migraine but also provides some theoretical and data support for the development of novel biomolecules to treat migraine without any side effects. To assure the dependability and validity of forecast outcomes, nevertheless, further pharmacological investigation emergence of CICS and additional tests on animals are required with isolated biomolecule from Lignans such as Fargesin.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Rapuru Rushendran; Interpreted, data analysis, supervised, validation, and data compilation by Vellapandian Chitra. The first draft of the manuscript was written by Rapuru Rushendran and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

Data availability

All information gathered or deduced from this study, along with any relevant online appendices, can be found in this report. If you have any additional questions, please contact the author who wrote the article and the data are available from the corresponding author on reasonable request

Ethics approval

All animal experiments performed were approved by the Ethics Committee for Animal Experimentation of SRM Institute of Science and Technology and conducted in accordance with the CPSCEA guidelines. The ethical approval reference number is IAEC/268/2021.

Consent to Participate

Not applicable

Consent to Publish

Not applicable

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Figures

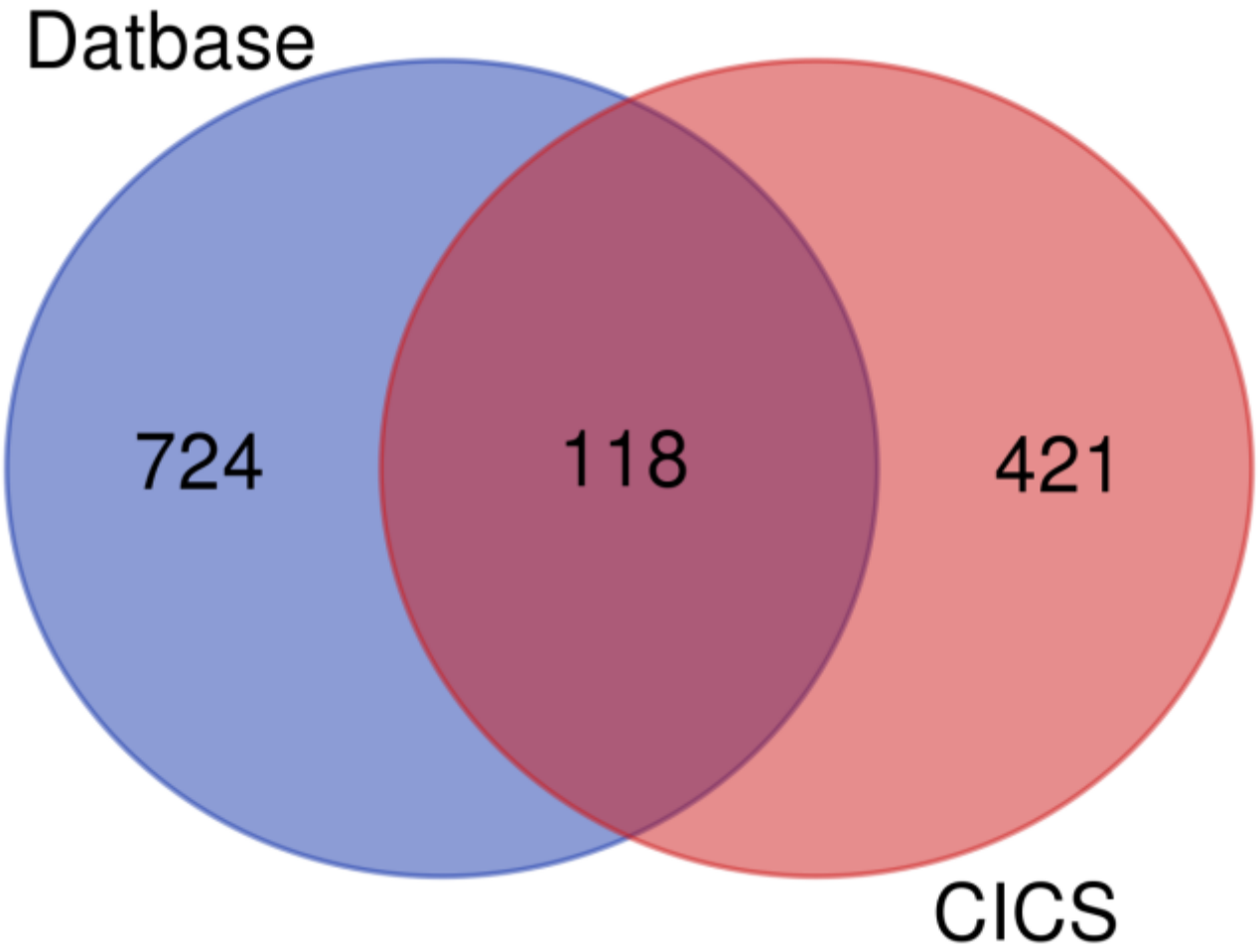

Figure 1

Common genes of CICS and databases

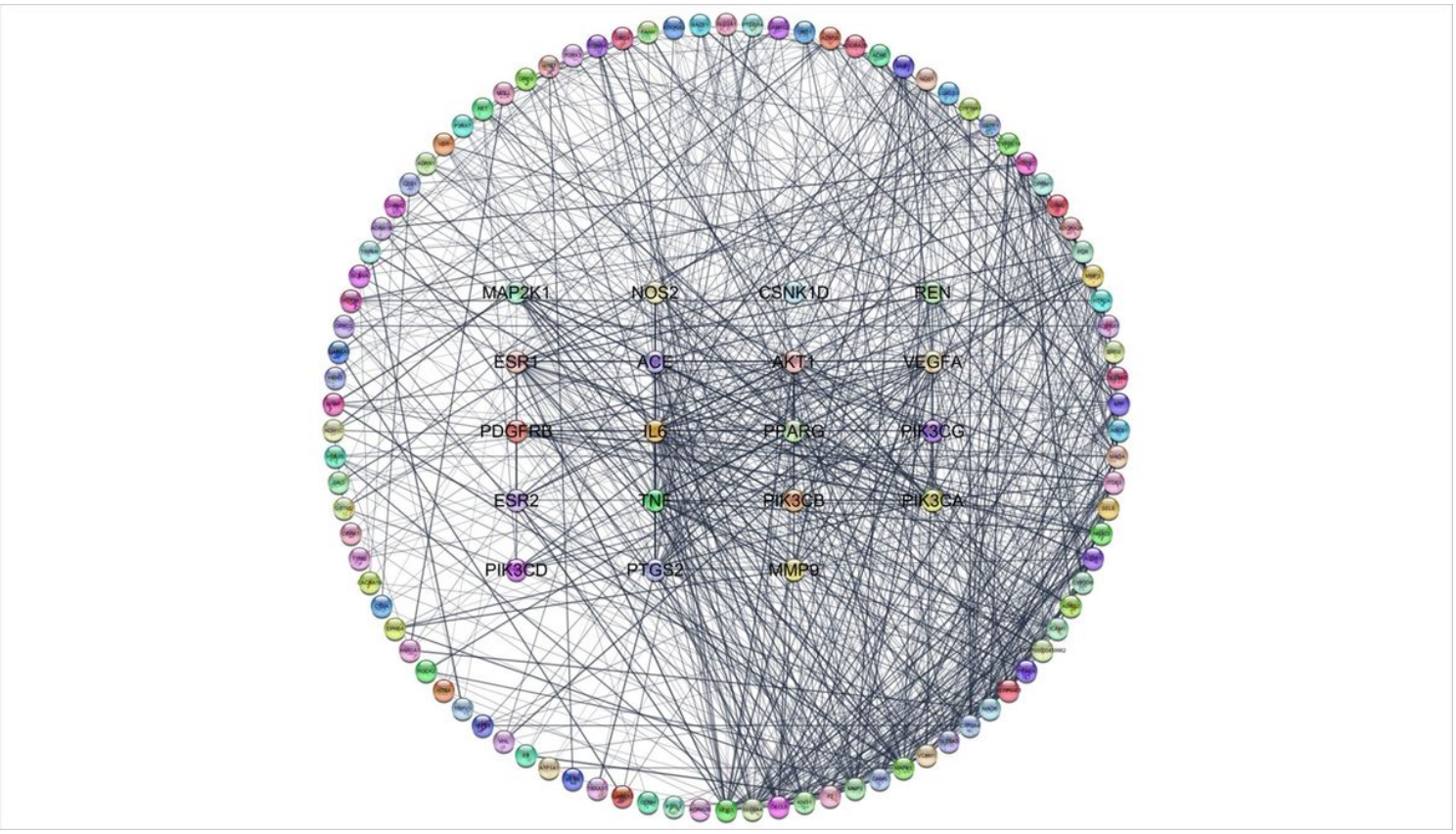

Figure 2

Interactions between proteins encoded by genes

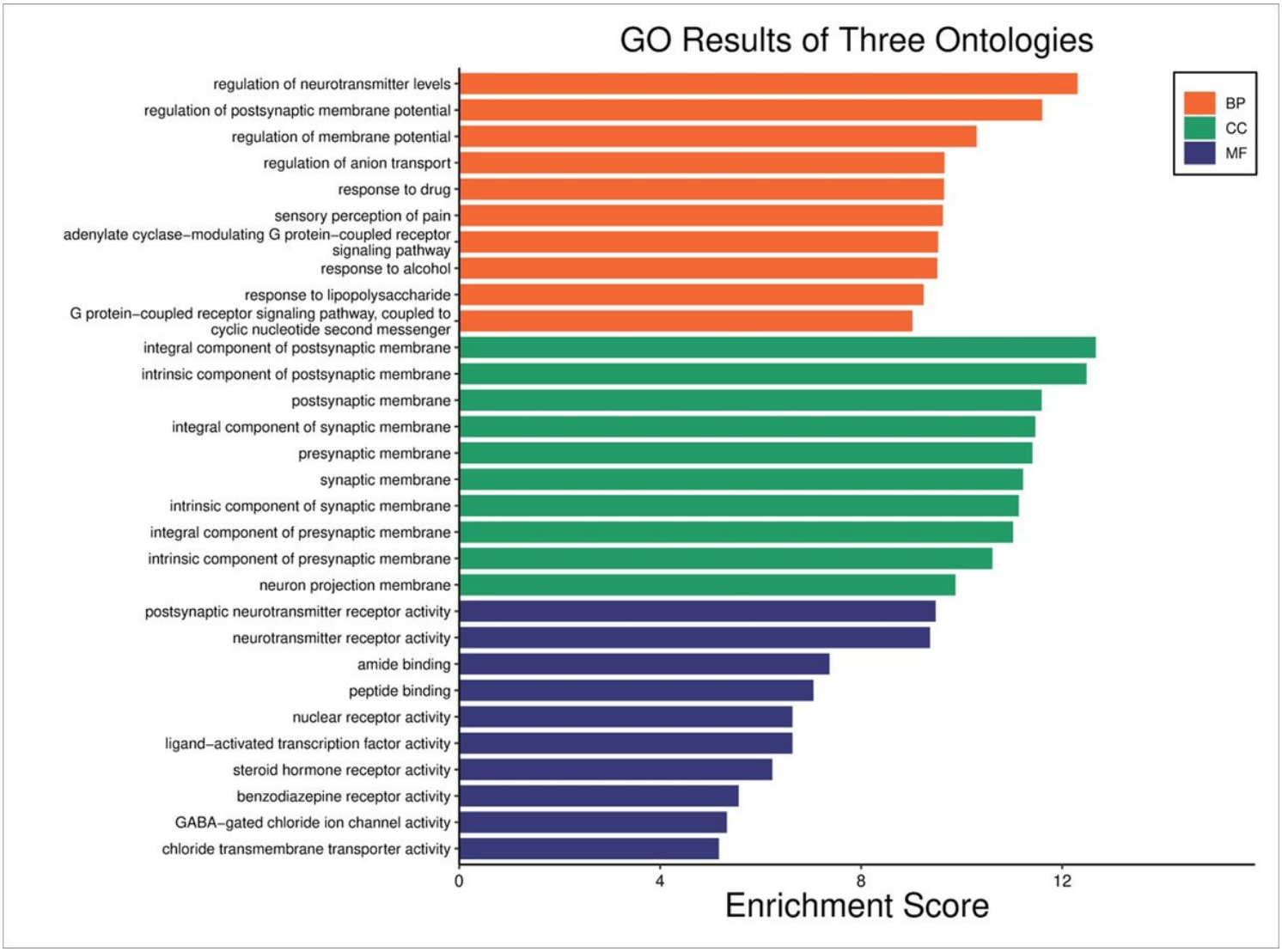

Figure 3

Gene ontology enrichment analysis (BP-Biological process, CC-Cellular components, and MF-Molecular function)

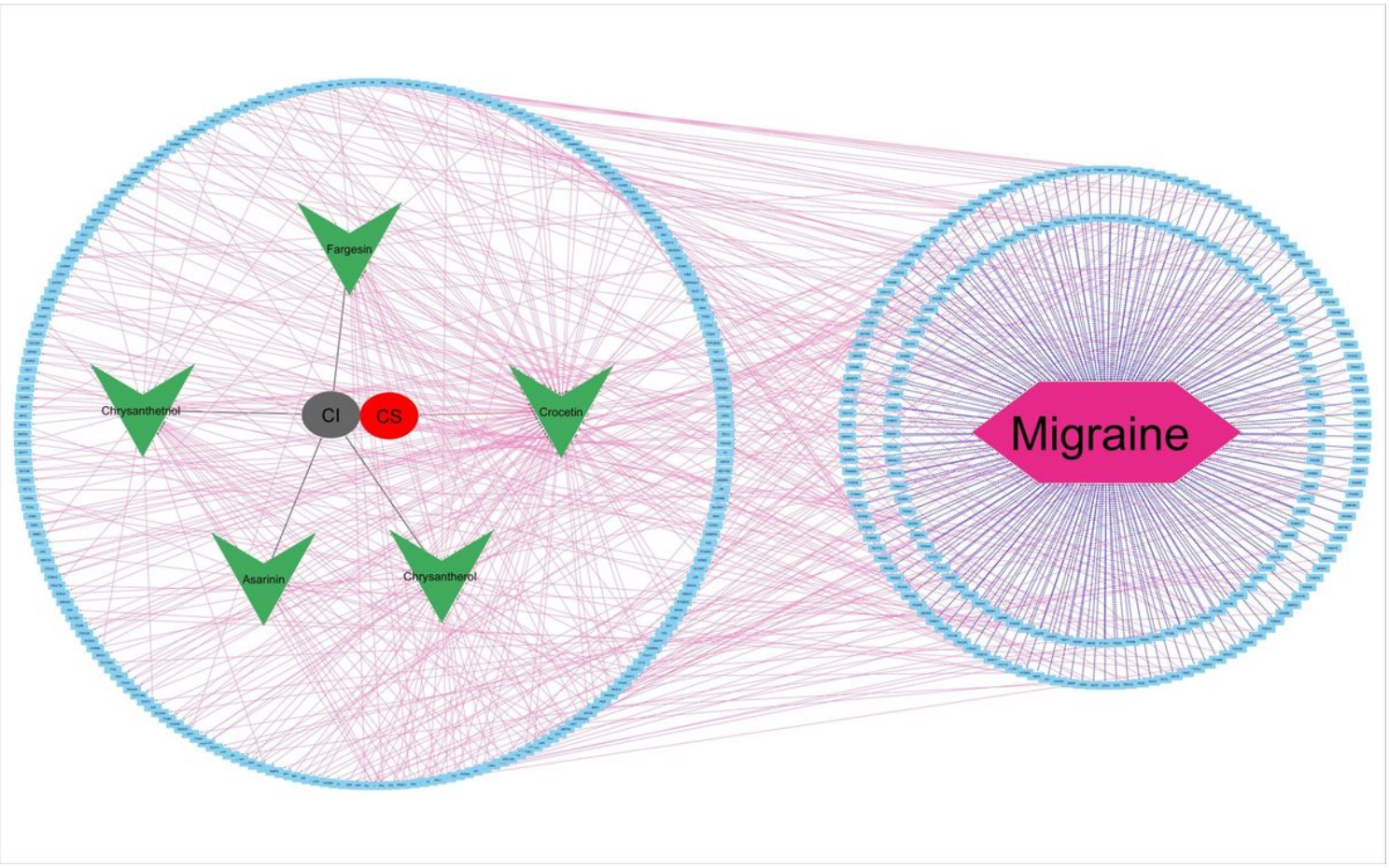

Figure 4

Biomolecule-Target-Pathway of network

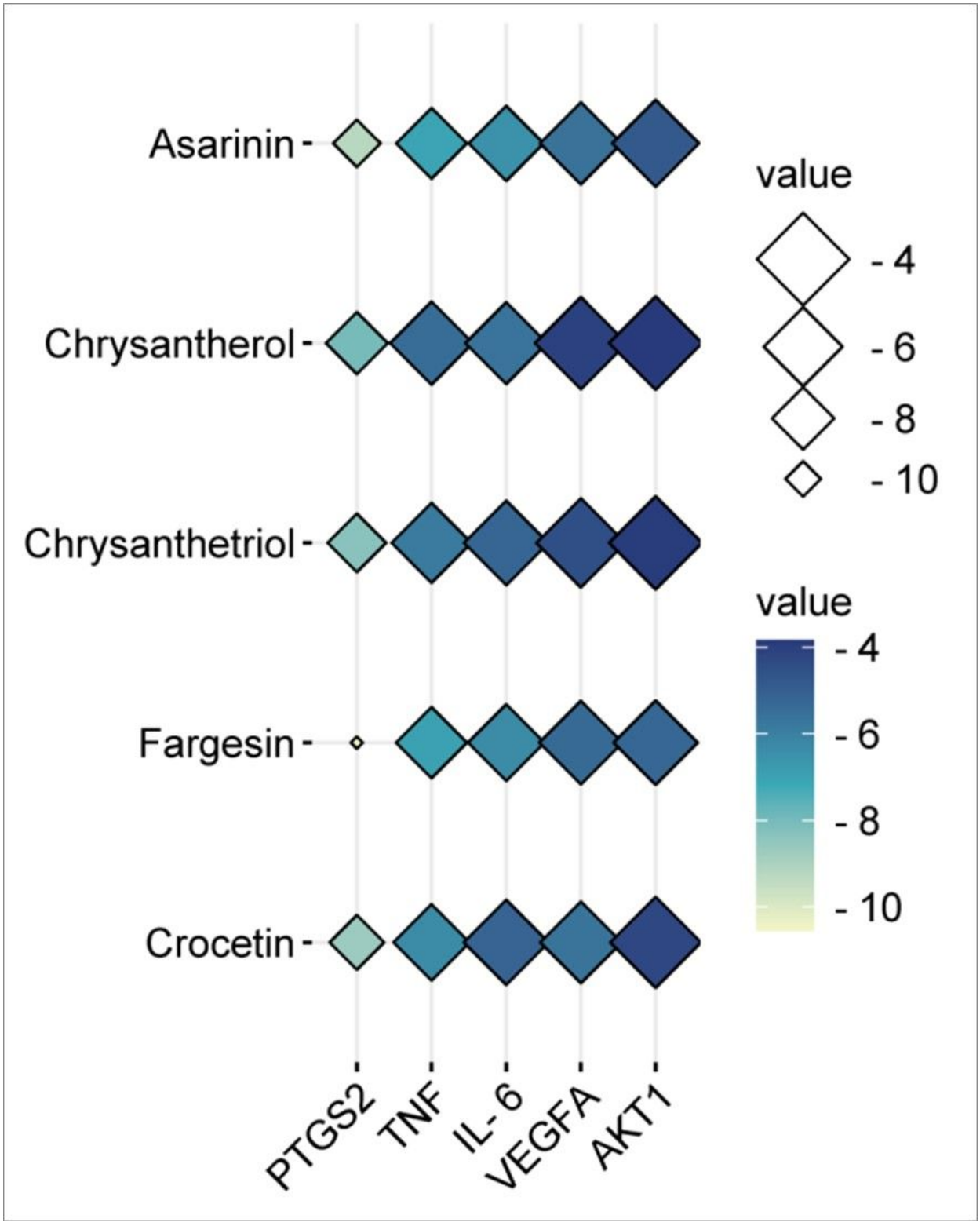

Figure 5

The molecular docking analysis evaluated that Fargesin and Asarinin may inhibit the specific gene activity such as PTGS2, TNFα, IL-6, VEGFA, and AKT1 which are initiators of neuroinflammation.

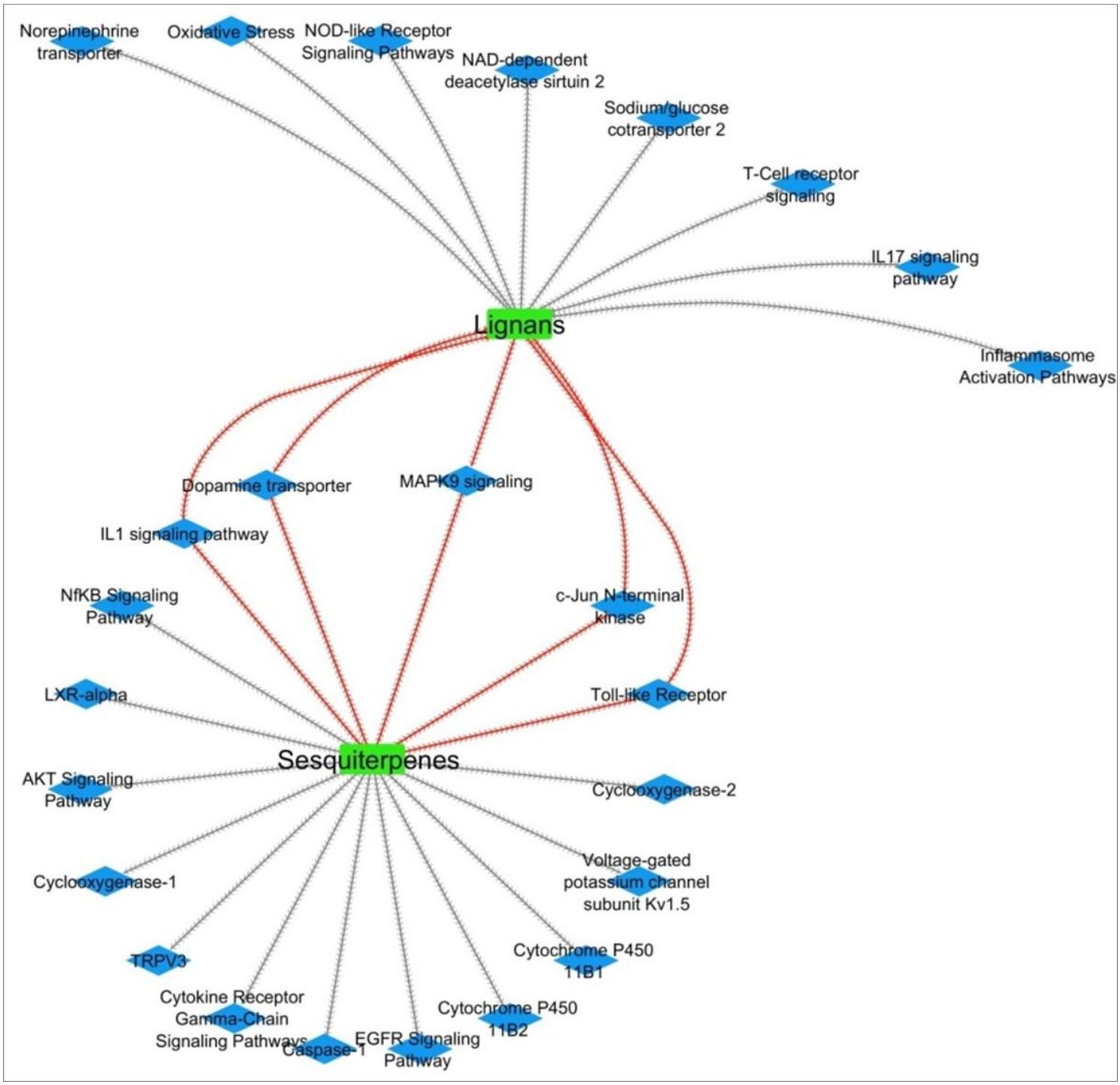

Figure 6

Lignans and Sesquiterpenes common pathway shows that are interlinked with MAPK9 signaling, c-Jun N-terminal kinase, Toll-like receptor, IL1 signaling pathway which could be specific targets in neuroinflammation.



Figure 7

KEGG enrichment analysisreveals that enrichment score was more in neuroactive ligand receptor interaction which gave an idea that Lignans and Sesquiterpenes are playing a major role for alleviating neuroinflammation. Lignans and Sesquiterpenes reported good binding affinity convincible for further *in vitro* and *in vivo*studies.

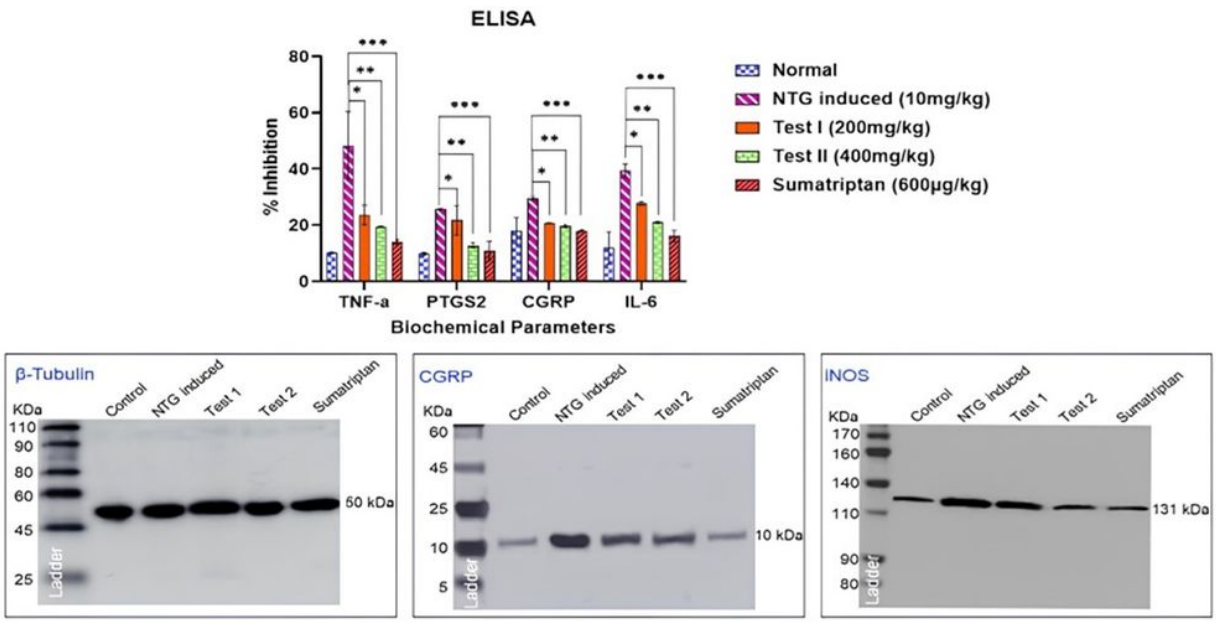

Figure 8

Expression of CGRP, IL-6, TNFα, and PTGS2 were reduced by the indicated percentages. The respective protein expressions are elevated after repeated NTG injection. After therapy, all of the predicted biomarkers were lower than in the NTG-inducing group. Western blotting was used to examine changes in CGRP and iNOS protein expression following chronic CILF administration following repeated NTG induction (n=6 mice per group). When compared to NTG-induced and β-Tubulin expression, which is regarded a house-keeping gene, the protein expression was dramatically reduced in the treatment groups (Test-I, Test-II, and Sumatriptan). Statistical analyses were performed by one-way ANOVA, followed by a Multiple t-test; ***$p < 0.001$, **$p < 0.002$, *$p < 0.33$ vs. NTG induced group (n = 6 mice per group).