Investigation of the Mechanisms of Neuroprotection Mediated by Lobelia Species via Computational Network Pharmacology and Molecular Modeling

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Research

Keywords: Neuroprotection, Lobelia, Network Pharmacology, Docking, Traditional Chinese Medicine, Alzheimer's disease

DOI: https://doi.org/10.21203/rs.3.rs-61537/v1

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Abstract

Background

Several species of the medicinally valuable genus *Lobelia* (Campanulaceae) exhibit neuroprotection. While the neuroprotective mechanisms of some components (e.g. lobeline, lobelanine, and lobelanidine) belonging to the *L. nicotianaefolia* or *L. inflata* are extensively characterized, there remains the need to study and elucidate the mechanism of action of other species and their active components. In this work, we have studied the neuroprotective mechanism of the pharmacokinetically favorable active compounds of 17 *Lobelia* species.

Methods

Network pharmacology approach and molecular modeling were employed. We have conducted drug-likeness evaluation, oral bioavailability prediction followed by the Gene Ontology (GO) terms and pathways enrichment analysis, protein-protein and protein-compound interaction network construction and analysis, and molecular docking studies. Five neurodegenerative diseases viz. Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, epilepsy, and Amyotrophic lateral sclerosis along with the common neuroprotection mechanism-associated genes were evaluated.

Results

We revealed the neuroprotective mechanism of the active ingredients of *Lobelia* species. Our study strongly indicates that 12 unique active ingredients viz. luteolin, kaempferol, acacetin, chryseriol, norlobelanine, lobelanine, 2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone, hydroxygenkwanin, lobelanidine, quercetin, and diosmetin regulates 31 targets within multiple signaling pathways. The nitric oxide synthase, brain (NOS1), androgen receptor (ANDR), sodium-and chloride-dependent GABA transporter 1 (SC6A1), apoptosis regulator Bcl-2 (BCL2), RAC-alpha serine/threonine-protein kinase (AKT1), cellular tumor antigen p53, apoptosis regulator BAX, and tumor necrosis factor (TNFA) were identified as the majorly regulated genes. A majority of these target proteins act via several cancer-related pathways proven to have cross-talks with the pathogenesis of neurodegenerative diseases.

Conclusions

This study explains how the active ingredients of the *Lobelia* species exhibit their neuroprotective actions and provide a reference basis to investigate their pharmacological effects in detail.

Background

Neuronal injury is a pathological hallmark of some of the most commonly-known neurodegenerative diseases. In general, the severity of the neuronal damage determines the consequences such as neuronal degeneration or death (1). The etiology and progression of the common neurodegenerative and neuronal
disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), epilepsy, and Amyotrophic lateral sclerosis (ALS) are some well-known examples of the severe consequences of the neuronal damages. The oxidative stress, excitotoxicity, neuroinflammation, mitochondrial dysfunction, and protein aggregations in the brain have been reported to play the central role in the neuronal damages (2).

Neuroprotective strategies and mechanisms that aim to limit the neuronal loss and/or rescue the neuronal damage progression and/or regenerate the neuronal structural and functional integrity are commonly used in several neurodegenerative diseases. Several investigations are also currently underway to find novel ways to protect the nervous system from injury and damage (3). The concept of intervening the neurotransmission receptors by the agonist or antagonist of the natural neurochemical modulators to exhibit the neuroprotection effects has been long-established (4). For example, caffeine, an A2 receptor antagonist was found to protect dopaminergic neurons against the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neuronal toxicity in the experimental model of PD (5). Other neurotransmitters such as serotonin, gamma-aminobutyric acid (GABA), and glutamate are also associated with regulating the inhibition and excitation of motor neurons. For example, drugs like flumazenil interact with the GABAergic system and alter motor behavior (6). Other pharmacological interventions are also known to provide neuroprotection as well as disease-modifying activities.

The neurotransmitter receptors modulation, anti-inflammatory responses, and anti-oxidative stress are well-studied pharmacological intervention approaches to protect the neurons. Due to the complex pathways associated with the pathogenesis of neurodegenerative diseases, the paradigm of multi-target-directed ligands (MTDLs) has been long warranted and has been well-researched in recent years (7–9). Several novel MTDLs with synthetic optimizations have been published in recent years (10, 11). In this respect, the herbal medicine or natural products provide an unprecedented advantage of exerting multiple effects on different biological targets (12–14). For example, *Crocus sativus, Nigella sativa, Coriandrum sativum*, and *Ferula assafoetida, Curcuma longa*, to name a few, showed neuroprotective effects by regulating multiple disease-associated targets and signaling pathways (15). Curcumin, the major constituent of *Curcuma longa*, exerts a neuroprotective role in PD via multiple mechanisms including the restoration of the GSH decreased levels, mediation of the overexpression of BCl-2 (inducible nitric oxide synthase (iNOS) antagonist) (16), reduction of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, total nitrite generation, and decreased activation of NF-κB (17, 18).

*Lobelia* (Campanulaceae) is a genus of flowering plant natives to the temperate and warmer regions with 450 species currently known. Several *Lobelia* species were traditionally used for medicinal purposes and several species have been continuously evaluated for their distinct pharmacological activities (19). *Lobelia chinensis* is reported to have anti-oxidative (20), anti-inflammatory (21), anti-viral (21), anti-cancer (22, 23) and anti-diabetic properties (24). *Lobelia inflata*, also known as Indian tobacco, has a long history of use in the treatment of severe breathing problems including asthma, whooping cough, and bronchitis. Amongst the chemical constituents of the *Lobelia* species, a majority of the research has been performed on pyridine alkaloids such as lobeline, lobelanine, and lobelanidine (25–27). For example, Li et
al., (28) reported the dopaminergic neuroprotective effects of lobeline against the MPTP-induced dopaminergic neuron death. The lobeline extracted from the leaf of Lobelia nicotianaefolia was shown to have antiepileptic activity by modulating the GABAergic mechanism (29).

Several other chemical classes such as glycosides, lignans, flavonoids, flavonoid, and amino acids endowed with diverse pharmacological effects were revealed (30–34). In a recent study, Ge et al., (24) studied the anti-diabetic mechanism of the metabolites extracted from Lobelia chinensis using network pharmacology approaches. Their study revealed 5-hydroxymethylfurfural and acacetin as two major active ingredients modulating the insulin resistance signaling pathway and diabetic pathway. Moreover, their study also provided a basis for the further study of the active constituents of the Lobelia chinensis in other diseases and their pharmacological mechanisms.

In this study, we performed network pharmacology and molecular modeling analysis of the active ingredients and corresponding targets of 17 herbs of the genus Lobelia. The aim was to decipher their neuroprotective mechanism of action and provide a rationale for future pharmacological studies. The relationships between the active ingredients and potential targets/pathways were established and presented systematically.

**Materials And Methods**

**Active Ingredients and Biological Targets**

A total of 17 herbs of the genus Lobelia were studied. A total of 233 known active ingredients were retrieved from the Natural Product Activity and Species Source Database (NPASS) (35). The chemical structures and the corresponding targets were mapped to the ChEMBL database (36). Additionally, TCM systems pharmacology database and analysis platform (TCMSP) (37) was also used for the mapping. Three ADME-related properties including OB (oral bioavailability) ≥ 30%, drug-likeness (DL) ≥ 0.18, and half-life (HL) ≥ 4 were used to screen the active constituents. The open-source cheminformatics package RDKit (http://www.rdkit.org) was used to standardize the chemical structures and determine the pharmacokinetic parameters (whenever necessary). After that, 49 distinct chemical ingredients and their 411 corresponding targets were selected. Finally, 12 targets that belonged to the cytochrome 450 families were discarded.

**Neuroprotection and Neurodegenerative Disease-Related Gene**

The associated-genes of five neurodegenerative diseases viz. AD, ALS, epilepsy, HD, and PD along with the genes associated with the common neuroprotection mechanism were collected. The gene databases viz. GeneCards (38), The Comparative Toxicogenomics Database (CTD) (39), Human Genome Epidemiology (HuGE) Navigator (40) and The Online Mendelian Inheritance in Man (OMIM) (41) were used. Many custom filtering criteria were applied. For example, only the genes annotated to have ≥ 20 publications associated with them were retrieved from the HuGE navigator database. Only the genes
annotated with the “protein-coding” function were retrieved from the GeneCards database. Only the genes annotated with the label “M” and/or “T” were retrieved from the CTD database. Finally, the total genes collected from the various databases were filtered using the NCBI Gene database and only the “protein-coding” genes were selected.

Enrichment Analysis of Genus *Lobelia* Targets

The overlapping targets of genus *Lobelia* related to the neuroprotection and neurodegenerative diseases were mapped into Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGGs) and REACTOME Pathway (42). The GO functional annotations were carried for the biological process (BP), molecular function (MF), and cellular components (CC) terms (43).

Compound-Target Network Analysis

The Cytoscape v3.7.2 (44, 45) was used to build and analyze the compound-target network. The ECFP4 fingerprints (46) was used to evaluate the chemical diversity of the compounds.

Pharmacology Network Analysis

The STRING v11 (47) was used to construct the protein-protein interaction (PPI) networks of the overlapping targets related to the genus *Lobelia*, common neuroprotection, and neurodegenerative diseases. The analysis and modularization were performed using Cytoscape v3.7.2. The MCODE algorithm (48) was used to determine highly interconnected regions in the PPI network. The degree cutoff, node density cutoff, and node score cutoff were kept to 2, 0.1, and 0.2, respectively.

Molecular Docking

The docking studies of the representative compounds and targets were performed with AutoDock 4.2 (49) using the UNIX automation script. The BIOVIA Discovery Studio (San Diego: Dassault Systèmes) was used for pre-processing the chemical structures and biomolecules. The metal ions and/or substrate molecules (if any) were kept in the binding pocket of the targets. The Lamarckian genetic algorithm search was employed for the docking. The key residues of the binding pocket were kept flexible. The center of the binding pockets of the individual targets was selected for the grid placement. A total of 60 runs along with 2.5 million energy evaluation steps were employed. The representative pose selection was done based on the cluster analysis of the docked poses. The PyMOL Molecular Graphics System (Version 1.8.4.0, Schrödinger, LLC) was used for the visualizations and graphics generations.

Results

*Lobelia* herbs, Active Ingredients, and Known Targets

In this study, a total of 17 herbs belonging to the genus *Lobelia* were selected. The criteria of their inclusion in this study were based on available information related to the metabolites and their biological
targets. For the herbs are known to have at least one experimentally characterized metabolite and one corresponding target was included. The collected herbs are listed in Supplementary Table S1. Initially, the chemical constituents collected from the NPASS database were mapped to the ChEMBL database and the corresponding bioactivity data were retrieved. A total of 194 compounds were successfully mapped in this manner. Additionally, the chemical constituents and corresponding biological targets of *Lobelia chinensis* were also retrieved from the TCMSP database. A total of 71 chemical constituents were retrieved from the TCMSP database. The redundant chemical structures between the *Lobelia chinensis* collected from both databases were merged into one and the final selection was based on the TCMSP database. Finally, a total of 233 molecules were subjected to the ADME-filtering. In this study, we used three ADME-filtering criteria; \( \text{OB} \geq 30\% \), \( \text{DL} \geq 0.18 \), and \( \text{HL} \geq 4 \). The aim of this filtering was to select the molecules with good absorption, slow metabolism after oral administration, and suitability for the drug-development. A similar approach has been used in other studies (50). In this work, we did not consider the blood-brain barrier (BBB) permeability as filtering criteria. The reason was that certain natural products (e.g. quercetin) with the theoretical prediction of poor BBB permeability when tested experimentally exhibited satisfactory permeability (51). Following the ADME-filtering, 49 unique chemical compounds associated with 411 corresponding targets were selected. Out of that, 12 targets related to the cytochrome 450 families were discarded from further study. Finally, a total of 153 non-reductant targets were studied.

**Neuroprotection and Neurodegenerative Disease-Related Gene**

The candidate genes of the five most common neurodegenerative diseases *viz.* AD (180 genes), ALS (121 genes), epilepsy (2667 genes), HD (65 genes), and PD (127 genes) along with the genes commonly associated with the neuroprotection (NP, 101 genes) mechanism were collected. A series of gene databases (see Material and Methods) were used. Initially, the genes related to each disease and NP mechanism were compared and analyzed individually with the *Lobelia* genes (153 genes). For the sake of simplicity, this set of genes which includes individual diseases will be called Set I. The overlapped genes of the Set I are shown in Supplementary Figures S1. In the final selection, all the genes associated with five neurodegenerative diseases mentioned above and those of NP mechanisms were overlapped with the *Lobelia* genes. For the sake of simplicity, this set of genes will be called Set II. To be noted that all the subsequent studies reported in the main paper were performed on Set II. The reason for the selection of Set II for the detailed analysis was because of the fact that different neurodegenerative diseases including those mentioned in this study broadly share the common pathogenesis and signaling pathways related to neuronal damages and protection. Therefore, the genes of the Set II best represented the neuroprotective mechanism associated with different neurodegenerative diseases. Moreover, the analysis related to Set I was also performed (see Supplementary Information).

A total of 31 overlapping genes in Set II were retrieved and the IUPHAR classification (52) was performed on them. The categorical distribution of the overlapped gene is shown in Fig. 1A. As expected, a majority
of the genes belonged to the enzyme class (56.3%) followed by ion transporter (12.5%), nuclear hormone receptors (12.5%), other protein (12.5%), and catalytic receptor (6.3%).

We have also performed a clustering analysis to determine the chemical diversity of the collected chemical constituents. The 49 unique compounds could be represented into 10 well-defined clusters as shown in Fig. 1B. The representative chemical structure of each cluster is shown in Fig. 1C. The aglycone part of the flavonoid such as diosmetin and 18 other aglycones constituted the most populated cluster (Cluster 1).

**Enrichment Analysis of the Candidate Genes**

All the overlapped genes belonging to Set I and Set II were tested for functional enrichment with three GO terms (BP, CC, and MF) and KEGG/REACTOME pathways. The result of the GO terms and KEGG/REACTOME pathways enrichment of genes in Set I are shown in Supplementary Figures S2-S7. The result of the GO enrichment of the genes in Set II is shown in Fig. 2A. The description is provided in the following section.

The “positive regulation of transcription from RNA polymerase II promoter”, “positive regulation of transcription, DNA-templated”, “response to drug”, and “negative regulation of apoptotic process” were the most significantly enriched terms in the BP. Interestingly, the “response to gamma radiation (GO:0010332)” was also found to be within the top ten most enriched BP terms. The ionizing radiation has been shown to have a debilitating impact on neurodegenerative diseases. Several studies reported that radiation inhibits neurogenesis through different mechanisms such as neuroinflammation, elevate reactive oxygen and nitrogen species, oxidative stress, protein degradation, and mitochondrial dysfunctions, among others (53–56). The major cellular components such as nucleus, cytosol, cytoplasm, mitochondria along with synapse were indicated as the location of the overlapped genes in Set II.

The KEGG/REACTOME pathways analysis of overlapped genes were analyzed with BH-corrected P-values < 0.05 (Fig. 2B). The enrichment of the overlapped genes was mostly found in the pathways in cancer, hepatitis B, Akt signaling pathway, proteoglycans in cancer, MAPK signaling pathway, HTLV-1 infection, prostate cancer, colorectal cancer, influenza A, thyroid hormone signaling pathway, tuberculosis, Chagas disease, endometrial cancer, Amyotrophic lateral sclerosis, and bladder cancer, among other. Indeed, a number of studies supported the intriguing cross-talks between cancer and neurodegeneration (57–59). The key candidate pathways-targets interaction network is shown in Fig. 2C.

**Compound-Target Networks**

The compound-target network was constructed to establish the role of the active ingredients of the genus *Lobelia* and the overlapped targets found in Set I and Set II. The compound-network diagram diagrams of the overlapped genes in AD, ALS, epilepsy, HD, NP, and PD are shown in Supplementary Figures S8-S13 and for Set II is shown in Fig. 3.
According to the analysis, 12 unique compounds viz. quercetin (MOL000098), luteolin (MOL000006), kaempferol (MOL000422), acacetin (MOL001689), chryseriol (MOL003044), norlobelanine (MOL012216), lobelanine (MOL012208), 2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone (MOL012209), hydroxygenkwanin (MOL005530), lobelanidine (MOL012207), and diosmetin (MOL002881) were found associated with the 31 target proteins in Set II. Among these compounds, quercetin (degree: 26), luteolin (degree: 10), kaempferol (degree: 7), acacetin (degree: 5), and chryseriol (degree: 4) were found to be a high-degree association (≥ 4 proteins). Among the targets, nitric oxide synthase, brain (NOS1, degree: 5), androgen receptor (ANDR, degree: 5), sodium- and chloride-dependent GABA transporter 1 (SC6A1, degree: 4), BCL2 (degree: 3), AKT1 (degree: 3), P53 (degree: 3), BAX (degree: 3), and TNFA (degree: 3) were found to be associated with at least three compounds. The candidate compounds and target relationships are listed in Supplementary Table S2-S8.

Certain species of Lobelia, such as *Lobelia inflata* and *Lobelia cardinalis* are extensively characterized and the pharmacological properties of their chemical constituents are well-studied (60). Among them, the pyridine alkaloids lobeline and lobinaline were thoroughly investigated (27, 61–63). However, due to the structural complexity of the glycosidic components, the evaluation of their bioactivities has been continuing to prove highly challenging. Thus, our study establishing the relationship between the active ingredients of the *Lobelia* species to their potential targets should aid a tremendous value in elucidating their mechanism of actions in neuroprotection.

**Construction and Analysis of Target Proteins PPI Network**

The PPI network was constructed to understand the interrelation between the neuroprotection associated candidate genes of the genus *Lobelia*. The constructed PPI network is shown in Fig. 4. The network edges were first created based on the molecular interaction by keeping the interaction score to ≥ 0.400. A total of 31 nodes and 249 edges were found in the network with an average node degree of 16.1. As expected, AKT1, TP53, MYC, TNF, EGF, EGFR were amongst the central targets in the PPI network. In the next step, the highly interconnected regions in the PPI network were analyzed using the MCODE algorithm. A well-organized and highly interconnected hub region with 20 nodes were retrieved. The targets ESR1, MYC, IL1B, IFNG, CXCL8, CASP9, IL2, CCL2, EGF, EGFR, FOS, MMP2, HSPB1, AKT1, TP53, BCL2L1, AR, HIF1A, TNF, and CCND1 constituted the cluster. Interestingly, all the targets associated in this cluster had an association score ≥ 0.9, which in turn suggests the high confidence in their interactions. The topological parameters of the PPI network are shown in Table 1.
Table 1
The topological parameters of the PPI network.

| Genes   | Degree | Betweenness Centrality | Average Shortest Path Length | Closeness Centrality |
|---------|--------|------------------------|-------------------------------|----------------------|
| BCL2L1  | 24     | 0.02054442             | 1.20689655                    | 0.82857143           |
| TP53    | 24     | 0.02054442             | 1.20689655                    | 0.82857143           |
| AKT1    | 28     | 0.10602706             | 1.03448276                    | 0.96666667           |
| GSK3B   | 13     | 0.00616809             | 1.5862069                     | 0.63043478           |
| EGFR    | 21     | 0.01242899             | 1.31034483                    | 0.76315789           |
| EGF     | 22     | 0.01542304             | 1.27586207                    | 0.78378378           |
| BAX     | 9      | 0.00049759             | 1.72413793                    | 0.58                 |
| CASP9   | 19     | 0.01023331             | 1.37931034                    | 0.725                |
| CXCL8   | 19     | 0.00330769             | 1.37931034                    | 0.725                |
| IL1B    | 19     | 0.02195186             | 1.34482759                    | 0.74358974           |
| MYC     | 24     | 0.02054442             | 1.20689655                    | 0.82857143           |
| BCL2    | 11     | 0.00269961             | 1.65517241                    | 0.60416667           |
| ESR1    | 21     | 0.01045047             | 1.31034483                    | 0.76315789           |
| CCND1   | 20     | 0.01077983             | 1.34482759                    | 0.74358974           |
| TNF     | 25     | 0.04919786             | 1.13793103                    | 0.87878788           |
| HIF1A   | 17     | 0.00428457             | 1.44827586                    | 0.69047619           |
| AR      | 17     | 0.00730507             | 1.44827586                    | 0.69047619           |
| FOS     | 23     | 0.05947999             | 1.20689655                    | 0.82857143           |
| CCL2    | 17     | 0.00290018             | 1.44827586                    | 0.69047619           |
| IL2     | 16     | 0.00504994             | 1.48275862                    | 0.6744186            |
| IFNG    | 14     | 0.00054133             | 1.55172414                    | 0.64444444           |
| SOD1    | 16     | 0.02218413             | 1.44827586                    | 0.69047619           |
| CTSD    | 11     | 0.0013186              | 1.65517241                    | 0.60416667           |
| HSPB1   | 17     | 0.00420332             | 1.44827586                    | 0.69047619           |
| PRKCB   | 9      | 0.00992863             | 1.68965517                    | 0.59183673           |
| NOS1    | 7      | 0.00356263             | 1.75862069                    | 0.56862745           |
### Molecular docking

Molecular docking was carried out to elucidate the binding modes of the 12 active ingredients to the 15 targets (NOS1, BCL2, AR, AKT1, AChE, IL2, EGFR, ER, MAOB, PRKCB, CTSD, MMP2, GSK3B, SOD1, and HIF1A) for which crystal structures were known. Interestingly, most of the *Lobelia* compounds showed a relatively much higher binding affinity against targets such as MAOB (monoamine oxidase B), PRKCB (protein kinase C beta type), AR (androgen receptor), AChE (acetylcholinesterase), and ER (estrogen receptor) than the other 10 targets. For example, the pyridine alkaloid lobelanidine interacted with MAOB with a docking score of -11.12 kcal/mol. (2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone) interacted with AChE with a docking score of -11.911, which should be deemed as a potent binding. The docking score of the best-ranked molecules against the selected targets is shown in Table 2. The molecular interactions and binding mode of the selected molecules are shown in Figs. 5–7.
Table 2
Docking results of the best-ranked candidate compounds.

| Protein | PDB ID | Compound ID | Docking score (kcal/mol) |
|---------|--------|-------------|--------------------------|
| MAOB    | 2BK3 (119) | MOL012207 (lobelanidine) | -11.12 |
|         |        | MOL012209 (2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone) | -10.506 |
|         |        | MOL002881 (diosmetin) | -10.488 |
|         |        | MOL000098 (quercetin) | -10.164 |
|         |        | MOL012208 (lobelanine) | -10.006 |
| PRKCB   | 2I0E (120) | MOL005530 (hydroxygenkwanin) | -8.264 |
|         |        | MOL000098 (quercetin) | -8.189 |
|         |        | MOL000006 (luteolin) | -8.141 |
| AR      | 2PIU (121) | MOL000006 (luteolin) | -9.38 |
|         |        | MOL003044 (chryseriol) | -9.224 |
|         |        | MOL000098 (quercetin) | -9.101 |
|         |        | MOL000422 (kaempferol) | -9.012 |
| AChE    | 4EY7 (122) | MOL012209 (2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone) | -11.911 |
|         |        | MOL012207 (lobelanidine) | -11.756 |
|         |        | MOL012216 (norlobelanine) | -10.967 |
|         |        | MOL001689 (acacetin) | -9.233 |
| ER      | 5TOA (123) | MOL000006 (luteolin) | -9.985 |
|         |        | MOL000098 (quercetin) | -9.948 |
|         |        | MOL012207 (lobelanidine) | -9.782 |

Interestingly, the inhibitory effects of some of the compounds revealed in this study against their targets were reported. For example, quercetin and diosmetin were reported to have MAO inhibitory IC$_{50}$ values of 90 µM ((64) and 2.10 µM (65), respectively. Both quercetin and luteolin were reported to have inhibitory activity on protein kinase C (66, 67). Luteolin, quercetin, and kaempferol were reported to suppress the
function of the AR receptor in different cancer cells (68–70). Likewise, acacetin as well several acacetin derivatives such as linarin (acacetin-7-O-β-d-rutinoside), acacetin-7-O-methyl ether Mannich base derivatives and acacetin-7-O-β-D-galactopyranoside were reported to exhibit AChE inhibition (71–73). Quercetin was reported to stimulate cancer cell proliferation via the estrogen receptor (74).

Discussion

Neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, epilepsy, and Amyotrophic lateral sclerosis are some of the most pressing burdens on the global healthcare system. The high rate of the mortality and morbidity associated with these diseases, particularly in the elderly population, demands novel therapies. Unfortunately, to date, not much success has been achieved in developing effective therapeutics. However, tremendous progress has been made in unraveling the causative mechanism and pathogenesis of these diseases.

One very critical causative mechanism of the most neurodegenerative diseases is the neuronal damage (1). It is now well-established that acute as well as chronic neuronal damage initiates and helps in the progression of neurodegenerative diseases. Thus newer therapeutic strategies to protect the neurons from the damages are needed to be developed (3). In this direction, the use of multi-component herbal products offers a great alternative. Several herbal medicines or natural products are known to exhibit neuroprotection and provide beneficial effects in the treatment of neurodegenerative diseases (12–14). However, there have been several clinical concerns that hinder a wide-adoption of herbal/natural products in the prevention and therapy of neurodegenerative diseases. The lack of scientific evidence or support for patient safety and their efficacy has been often touted as the main reason. Thus, studying the rationale for the explanation of the molecular mechanism of actions of the pharmacokinetically suitable herbal components are of paramount importance.

In this study, through integrated network pharmacology and molecular modeling approach, we aimed to provide an explanation for the neuroprotective mechanism of the chemical constituents of a medically valuable genus called Lobelia. We studied a total of 17 herbs belonging to this genus and their impact on the five neurodegenerative diseases viz. Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, epilepsy, and Amyotrophic lateral sclerosis. Since the neuronal damages associated with the initiation and progression of these diseases share the common mechanism, the overlapped genes associated with these diseases and those related to the genus Lobelia were studied and presented.

After filtering the compounds with favorable ADME-properties, 49 compounds associated with 153 unique target proteins were selected and studied. The results of the network pharmacology (Fig. 3) indicated that 12 compounds viz. quercetin, luteolin, kaempferol, acacetin, chryseriol, norlobelanine, lobelanine, 2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone, hydroxygenkwanin, lobelanidine, and diosmetin were majorly associated with 31 unique target proteins related to the neuroprotective mechanism. The major targets among them were found to be nitric oxide
synthase, brain (NOS1), androgen receptor (ANDR), sodium- and chloride-dependent GABA transporter 1 (SC6A1), apoptosis regulator Bcl-2 (BCL2), RAC-alpha serine/threonine-protein kinase (AKT1), cellular tumor antigen p53, apoptosis regulator BAX, and tumor necrosis factor (TNFA).

KEGG/REACTOME pathways analysis results showed that the candidate genes involved in enriched pathways were related to the relevant pathways in cancer (Fig. 2). Several studies established the cross-talks between the etiology of cancer and neurodegenerative diseases. For example, the role of the cancer-associated enriched targets in this analysis such as the AKT serine/threonine kinase (AKT1) (75, 76), BCL2 associated X apoptosis regulator (BAX) (77), apoptosis regulator (BCL2) (78–80), C-X-C motif chemokine ligand 8 (CXCL8) (81–84), Fos proto-oncogene, AP-1 transcription factor subunit (FOS) (85), androgen receptor (AR) (86, 87), caspase 9 (CASP9) (88–90), cyclin D1 (CCND1) (91, 92), epidermal growth factor receptor (EGFR) (93, 94), epidermal growth factor (EGF) (93, 94), glycogen synthase kinase 3 beta (GSK3B) (95–98), hypoxia inducible factor 1 alpha subunit (HIF1A) (99, 100), matrix metalloproteinase 2 (MMP2) (101–104), protein kinase C beta (PRKCB) (105–107), and tumor protein p53 (TP53) (108, 108–110) in neurodegenerative diseases are well-established. These results suggested that the active ingredients of genus Lobelia included in this study may act by targeting the signaling pathways that are common to cancer. Interestingly, the nitric oxide synthase 1 (NOS1) (111, 112), tumor necrosis factor (TNF) (113–115), superoxide dismutase 1 (SOD1) (116–118) associated with ALS pathways have their role also associated with neurodegenerative diseases. The analysis of the PPI network (Fig. 4 and Table 1) further confirmed that the majority of these target proteins have molecular interactions with each other and participate in the signaling cascade. Further, the molecular docking studies (Figs. 5–7) performed on the 15 target proteins provide a rationale for the molecular mechanism of the binding of the compounds.

Conclusions

Neurodegenerative diseases have complex mechanisms with several intertwined signaling pathways and multiple targets associated with it. Therefore, targeting based on multi-ingredient–multi-target–multi-pathway is highly desirable. In this work, we provide the neuroprotective mechanisms of the 17 herbs belonging to the genus Lobelia. We establish the relationship between their active ingredients and target proteins and signaling pathways. We further provided theoretical validation of the binding mechanism of the compounds with the target proteins. Although our theoretical findings are consistent with other biological reports, further experimental validation of the pharmacological effects would be highly valuable. Moreover, this study provides adequate background and confidence to do so.

Declarations

Acknowledgements
Not applicable.

**Funding**

The financial support for the work was provided by the China Hunan Provincial Science & Technology Department (HHN2017SC10, 2015NK2008) and Innovation Project for Graduate Students of Hunan University of Chinese Medicine (2019CX48).

**Contributions**

QZ performed all the experiments and drafted the manuscript. SZ and QZ conceived the idea, designed the experimental plan, and revised the manuscript. LF, XH, and YW helped in the collection and validation of the data, provided the supervision of the results, and assisted in revising the manuscript.

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**Ethics declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Figures
Figure 1

Property analysis of the compounds related to genus Lobelia and targets associated with neuroprotection mechanism. A) IUPHAR classification of the overlapped genes (Set II) shown in terms of percentage; B) chemical diversity of the collected compounds belonging to the genus Lobelia. ECFP4 fingerprint was used. The numbering of the cluster is shown in descending order of the cluster size; C) chemical structure of the representative compound in each cluster.
Figure 2

Gene Ontology (GO) term and signaling pathway enrichment analysis of the overlapped genes (Set II) related to the neuroprotective mechanism of the genus Lobelia. (A) GO analysis of the terms biological process, molecular function, and cellular component. (B) KEGG/REACTOME pathways enrichment of the overlapped genes. (C) Key candidate pathways-targets interaction network.
Figure 3

The compound-target network of the active ingredients of the genus Lobelia and the targets related to the neuroprotective mechanism.
Figure 4

The PPI network of the neuroprotection associated candidate genes of the genus Lobelia.
Figure 5

The binding pose of the selected ligands. A) Lobelanidine docked into the binding cavity of the protein MAOB; B) 2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone docked into the binding cavity of the protein MAOB; C) Hydroxygenkwanin docked into the binding cavity of the protein PRKCB; D) Quercetin docked into the binding cavity of the protein PRKCB. The ligand is shown in cyan color and sticks representation. The main
The binding pose of the selected ligands. A) Luteolin docked into the binding cavity of the protein ANDR; B) Chryseriol docked into the binding cavity of the protein ANDR; C) 2-[(2R,6S)-6-[(2R)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanol docked into the binding cavity of the protein AChE; D) Lobelanidine docked into the binding cavity of the protein AChE. The ligand is shown in cyan color and sticks representation. The active site residues are shown as orange sticks. The main atoms involved hydrogen bonds are indicated as yellow dashes. The key residues participating in hydrogen bonds and hydrophobic interactions are labeled.

Figure 6
hydrogen bonds are indicated as yellow dashes. The key residues participating in hydrogen bonds and hydrophobic interactions are labeled.

Figure 7

The binding pose of the selected ligands. A) Acacetin docked into the binding cavity of the protein AChE; B) Luteolin docked into the binding cavity of the protein ERβ; C) Quercetin docked into the binding cavity of the protein ERβ. The ligand is shown in cyan color and sticks representation. The active site residues
are shown as orange sticks. The main atoms involved hydrogen bonds are indicated as yellow dashes. The key residues participating in hydrogen bonds and hydrophobic interactions are labeled.

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