System Pharmacological Mechanism and Compatibility Laws of Jianghuang from Traditional Chinese Medicine In Blood-Regulating Formulae

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Research

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Abstract

**Background:** Jianghuang (JH) is a popular ingredient in blood-regulating traditional Chinese Medicine (TCM) that could be effective for the treatment of various diseases. We demonstrate the compatibility laws and system pharmacological mechanisms of the key formula containing JH by leveraging data mining of bioinformatics databases.

**Material/Methods:** The compatibility laws of blood-regulating formulae containing JH from the Chinese Traditional Medicine Formula Dictionary were analyzed using a generalized rule induction (GRI) algorithm implemented. The putative target gene and miRNA were retrieved via a combination of the Arrowsmith knowledge discovery tool and FunRich 3.1.3. System pharmacological mechanisms are traced by their protein-protein interaction (PPI) network, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted using Uniprot, the Human Protein Atlas (HPA), STRING 11.0, and KOBAS 3.0.

**Results:** We found that the JH-CX-DG formula (Ligusticum chuanxiong-Angelica sinensis) could represent a key formula containing JH in blood-regulating TCM formulae. The JH-CX-DG formula was observed to directly target AKT, TLR4, caspase-3, PI3K, mTOR, p38 MAPK, VEGF, iNOS, Nrf2, BDNF, NF-κB, Bcl-2, and Bax 13 targets and regulate targets through 13 miRNA. The PPI network and KEGG pathway enrichment analysis showed that the JH-CX-DG formula possess potential pharmacological effects including anti-inflammatory, improving microcirculation, and anti-tumor through the regulation of multiple pathways including PI3K/Akt, MAPK, Toll-like receptor, T cell receptor, EGFR, VEGFR, Apoptosis, HIF-1 (p < 0.05).

**Conclusion:** The JH-CX-DG formula can exert beneficial pharmacological effects through multi-target and multi-pathway interactions. It can be effectively administered for the treatment of inflammatory diseases, microcirculation disorders, cardiovascular disease, and cancer. We found a new effective drug formula through analyzing the compatibility law and systemic pharmacological mechanism of JH. Our study provides a theoretical basis and directions for subsequent research on the JH-CX-DG formula.

**Background**

Jianghuang (JH), from traditional Chinese medicine (TCM), is the dried rhizome of the Curcuma Longa, a Zingiberaceae plant. This compound is characterized by its unique aroma and bitter and spicy taste. It is known to exert its effects on the spleen and liver meridian. Among its functions are promoting blood circulation, which aids in removing blood stasis and regulating immunity to relieve symptoms of dysmenorrhea and pain. In China, formulae of JH were first recorded in WUBI Decoction from Taiping Huimin Heji Ju Fang. A TCM formula containing JH was used to treat several afflictions including wind-cold-dampness pattern (TCM syndromes), dysmenorrhea, pain, and bone injury [1]. JH is also used as a national herbal medicine or health food to treat skin diseases, infections, and mental diseases in countries such as Iran, India, and Thailand [2]. JH has attracted the attention of many scholars owing to its therapeutic effects on various diseases, with some studying the molecular pharmacological
mechanisms of JH. Hay et al. [3] analyzed the modern pharmacological mechanism of JH and found that help to improve metabolic disorders, chronic diseases, regulate bodily functions, and serve as an antioxidant. Deguchi et al. [4] demonstrated that curcumin is the main functional ingredient in JH. Noting that this compound is highly safe and can mediate multiple targets and multiple pathways to exert anti-inflammatory and anti-tumor effects.

Although these studies revealed several pharmacological mechanisms of JH, most of them focus on the JH in isolation. The research concerning the compatibility laws and system pharmacological mechanisms are scarce. We hypothesize that the compatibility laws of TCM must influence the abilities of JH to treat diseases. The TCM theory states that "TCM has a powerful personality and the formulae have a wonderful combination", and that the efficacy of TCM formulae are different, owing to the functions of "sovereign drug, minister drug, assistant drug, envoy drug" as recorded in Shennong's Classic of Materia Medica—the first TCM book. Therefore, the exploration of turmeric's pharmacological effects must be extended to an overall analysis of its compatibility laws. Recent advances on data mining provide an alternative method for studying the compatibility laws of TCM. Generalized rule induction (GRI) algorithms can extract compatibility laws in TCM formulae. This approach has been widely employed in the discovery of TCM compatibility laws and key formulae [5]. We used the GRI algorithm, Arrowsmith knowledge discovery tool, bioinformatics databases, and other means to study the compatibility laws and pharmacological mechanism of JH in blood-regulating formulae to provide theoretical foundation and devise technical clues.

Material And Methods

Data Source and Normalization

We built a database of all TCM formulae based on the Chinese Traditional Medicine Formula Dictionary—a dictionary containing over 60,000 TCM formulae [6]. The collected TCM formulae were screened and their names standardized according to: (1) The TCM formulae contained JH and was designed as blood-regulating formulae; the formulae was recognized by two professional TCM doctors. (2) Excluded TCM formulae with over 15 ingredients since it would be challenging to appreciate the effects of JH. (3) Surgical TCM formulae were excluded. (4) When two TCM formulae were composed of the same ingredients but with different name, only one was analyzed. (5) Standardized the names of TCM formulae according to Chinese Medicine Classification of People’s Republic of China Pharmacopoeia (2015 Edition) [7].

Data GRI Algorithm Analysis

The GRI algorithm toolbox and the network graphics in SPSS Clementine 12.0 were used to analyze and calculate the compatibility laws of JH in blood-regulating TCM formulae. The main parameters included support, confidence, and lift. Support of X refers to the ratio of a certain quantity X in respect to the total quantity. Confidence of X→Y is the probability that the TCM formulae containing X also contains Y. Lift of X→Y is the ratio of confidence of X→Y to support of Y.
Targets prediction of key formulae containing JH

We analyzed the system pharmacological mechanisms of key formulae containing JH via the Arrowsmith knowledge discovery tool. Briefly, we searched for “Curcuma Longa” or “Jianghuang” on the Arrowsmith database and set it to dataset A. Next, we searched for names of other TCM on the Arrowsmith database and set it to dataset C. Finally, we used Arrowsmith to associate dataset A to dataset C and set it as dataset B. We set the retrieval semantics of dataset B to “Genes & Molecular Sequences, and Gene & Protein Names” to discover targets of key formulae containing JH.

Analysis of systematic pharmacological mechanism

We constructed a PPI network by using targets of key formulae containing JH via STRING 11.0 to select hub targets. Then, we used FunRich3.1.3 to predict non-coding miRNA targeting key formulae containing JH to select potential miRNA targets. We also investigated features such as tissue location and subcellular location of target genes via Uniprot and the Human Protein Atlas. We used KOBAS 3.0 to determine KEGG pathway enrichment of key formulae containing JH for treating various diseases. For the STRING analysis we selected the parameters as follows: The Species was "Homo sapiens", the source of interaction data was "Text Mining, Experiments, Databases" and the threshold was set to "high confidence = 0.7". The KOBAS 3.0 parameters were: The Species is "Homo sapiens" while the source of pathways were "KEGG Pathway" and "KEGG Disease". Finally, we summarized systematic pharmacological mechanisms of key formulae containing JH by thorough analysis of the information collected.

Results

Results of Compatibility Laws Analysis

We found a total of 126 herbs of TCM among the 49 formulae included in the analysis. The results of the compatibility laws are shown in Fig. 1, Table 1, Table 2, and Fig. 2. We found 27 herbs of TCM associated with JH with a frequency of more than 4. The top three herbs of TCM according to their frequency of use are Danggui (from Angelica sinensis), Chuanxiong (from Ligusticum chuanxiong), and Chishao (from Radix Paeoniae Rubra). According to the association rules of GRI algorithm, the results showed that the correlation of "Jianghuang, Chuanxiong→Danggui" is the strongest (Confidence 93.33%, Support 31.91%, Lift 1.51). Meanwhile, Chuanxiong increased the frequency of "Jianghuang, Dangui" from 61.70% to 93.33%. From our analyses, we conclude that "Jianghuang, Chuanxiong → Danggui" (JH-CX-DG formula) is the most critical formulae containing JH.
Table 1
Frequency distribution of Jianghuang compatible drugs

| NO. | Herb Category          | Support (%) | Frequency |
|-----|------------------------|-------------|-----------|
| 1   | Jianghuang-Danggui     | 61.70       | 29        |
| 2   | Jianghuang-Chuanxiong  | 31.91       | 15        |
| 3   | Jianghuang-Chishao     | 29.79       | 14        |
| 4   | Jianghuang-Rougui      | 27.66       | 13        |
| 5   | Jianghuang-Yanhusuo    | 25.53       | 12        |
| 6   | Jianghuang-Puhuang     | 21.28       | 10        |
| 7   | Jianghuang-Honghua     | 19.15       | 9         |
| 8   | Jianghuang-E'zhu       | 17.02       | 8         |
| 9   | Jianghuang-Moyao       | 17.02       | 8         |
| 10  | Jianghuang-Muxiang     | 17.02       | 8         |
| 11  | Jianghuang-Wulingzhi   | 17.02       | 8         |
| 12  | Jianghuang-Sanleng     | 14.89       | 7         |
| 13  | Jianghuang-Shudi       | 14.89       | 7         |
| 14  | Jianghuang-Gancao      | 12.77       | 6         |
| 15  | Jianghuang-Mudanpi     | 12.77       | 6         |
| 16  | Jianghuang-Niuxi       | 12.77       | 6         |
| 17  | Jianghuang-Qingpi      | 12.77       | 6         |
| 18  | Jianghuang-Taoren      | 12.77       | 6         |
| 19  | Jianghuang-Baishao     | 10.64       | 5         |
| 20  | Jianghuang-Qianghuo    | 10.64       | 5         |
| 21  | Jianghuang-Shengdi     | 10.64       | 5         |
| 22  | Jianghuang-Baizhu      | 8.51        | 4         |
| 23  | Jianghuang-Chenpi      | 8.51        | 4         |
| 24  | Jianghuang-Dahuang     | 8.51        | 4         |
| 25  | Jianghuang-Heye        | 8.51        | 4         |
| 26  | Jianghuang-Ruxiang     | 8.51        | 4         |
| NO. | Herb Category         | Support (%) | Frequency |
|-----|-----------------------|-------------|-----------|
| 27  | Jianghuang-Yuanhua    | 8.51        | 4         |
| NO. | Herb Category                        | Confidence (%) | Support (%) | Lift  |
|-----|--------------------------------------|----------------|-------------|-------|
| 1   | Jianghuang, Chuanxiong→Daungui       | 93.33          | 31.91       | 1.51  |
| 2   | Jianghuang, Chishao→Danggui          | 92.86          | 29.79       | 1.50  |
| 3   | Jianghuang, Puhuang→Chishao          | 70.00          | 21.28       | 2.35  |
| 4   | Jianghuang, Yanhusuo→Rougui          | 58.33          | 25.53       | 2.11  |
| 5   | Jianghuang, Mudanpi→Chuanxiong       | 100.00         | 12.77       | 3.13  |
| 6   | Jianghuang, Moyao→Yanhusuo          | 62.50          | 17.02       | 2.45  |
| 7   | Jianghuang, Puhuang→Rougui           | 50.00          | 21.28       | 1.81  |
| 8   | Jianghuang, Ruxiang→Moyao           | 100.00         | 8.51        | 5.87  |
| 9   | Jianghuang, Moyao→Ruxiang           | 50.00          | 17.02       | 5.88  |
| 10  | Jianghuang, Niuxi→Shudi             | 66.67          | 12.77       | 4.48  |
| 11  | Jianghuang, Shudi→Niuxi             | 57.14          | 14.89       | 4.48  |
| 12  | Jianghuang, Mudanpi→E’zhu           | 66.67          | 12.77       | 3.92  |
| 13  | Jianghuang, Muxiang→Sanling         | 50.00          | 17.02       | 3.36  |
| 14  | Jianghuang, E’zhu→Honghua           | 50.00          | 17.02       | 2.61  |
| 15  | Jianghuang, Moyao→Rougui            | 50.00          | 17.02       | 1.81  |
| NO. | Herb Category         | Confidence (%) | Support (%) | Lift  |
|-----|-----------------------|----------------|-------------|-------|
| 16  | Jianghuang, Dahuang→Heye | 75.00          | 8.51        | 8.81  |
| 17  | Jianghuang, Heye→Dahuang | 75.00          | 8.51        | 8.81  |
| 18  | Jianghuang, Shengdi→Baizhu | 60.00          | 10.64       | 7.05  |
| 19  | Jianghuang, Heye→Chishao | 75.00          | 8.51        | 2.52  |
| 20  | Jianghuang, Taoren→Wulingzhi | 50.00          | 12.77       | 2.94  |
| 21  | Jianghuang, Qingpi→Wulingzhi | 50.00          | 12.77       | 2.94  |
| 22  | Jianghuang, Ruxiang→Gancao | 50.00          | 8.51        | 3.92  |

**Targets of the JH-CX-DG formula**

The targets of key formula containing JH (JH-CX-DG formula) were predicted via Arrowsmith knowledge discovery tool. We found that the number of intersections of the Jianghuang targets dataset and the Danggui targets dataset is 227, where 33 were targets with a score over 0.95. We found that the number of intersections of the Jianghuang targets dataset and the Chuanxiong targets dataset is 175, with 22 targets with a score over 0.95. Finally, the results shown in Fig. 3 and Table 3 show that we determined 13 common targets with a score over 0.95 of the JH-CX-DG formula, with these 13 targets containing 27 related family genes.
Table 3
Targets of the JH-CX-DG formula (score ≥ 0.95)

| No. | Target | Full name of target | Related family target genes |
|-----|--------|---------------------|-----------------------------|
| 1   | AKT    | Protein kinase B    | AKT1, AKT2, AKT3            |
| 2   | TLR4   | Toll like receptor 4| TLR4                        |
| 3   | caspase-3 | caspase-3     | CASP3                       |
| 4   | PI3K   | Phosphatidylinositol 3 kinase | PIK3CA, PIK3CB, PIK3CG, PIK3CD |
| 5   | mTOR   | Mechanistic target of rapamycin kinase | mTOR |
| 6   | p38 MAPK | p38 Mitogen-activated protein kinases | MAPK11, MAPK12, MAPK13, MAPK14 |
| 7   | VEGF   | vascular endothelial growth factor | VEGFA, VEGFB, VEGFC |
| 8   | iNOS   | inducible nitric oxide synthase | NOS2                        |
| 9   | Nrf2   | Nuclear factor E2 related factor 2 | NFE2L2                      |
| 10  | BDNF   | Brain derived neurotrophic factor | BDNF                        |
| 11  | NF-κB  | Nuclear factor-κB    | NFκB1, NFκB2, RELα, RELA, RELB |
| 12  | Bcl-2  | B-cell lymphoma-2    | BCL2                        |
| 13  | Bax    | Bcl-2 Associated X Protein | BAX                        |

PPI network and location of targets

Figure 4 PPI network
Table 4
Node parameters in the PPI network.

| Gene   | Type                  | Degree | Closeness       | Betweenness    | Main Subcellular location |
|--------|-----------------------|--------|-----------------|----------------|---------------------------|
| AKT1   | Protein kinases       | 20     | $81.25 \times 10^{-2}$ | $40.25 \times 10^{-2}$ | Plasma membrane          |
| PIK3CA | Enzymes               | 12     | $61.90 \times 10^{-2}$ | $8.08 \times 10^{-2}$ | Plasma membrane          |
| M Tor  | Protein kinases       | 10     | $59.09 \times 10^{-2}$ | $3.93 \times 10^{-2}$ | Cytoplasm                |
| RELA   | Transcription factors | 10     | $60.47 \times 10^{-2}$ | $12.02 \times 10^{-2}$ | Nucleus                  |
| AKT2   | Protein kinases       | 10     | $56.52 \times 10^{-2}$ | $3.31 \times 10^{-2}$ | Plasma membrane          |
| AKT3   | Protein kinases       | 10     | $54.17 \times 10^{-2}$ | $1.75 \times 10^{-2}$ | Plasma membrane          |
| NF KB1 | Transcription factors | 9      | $59.09 \times 10^{-2}$ | $10.35 \times 10^{-2}$ | Nucleus                  |
| CASP3  | Enzymes               | 8      | $55.32 \times 10^{-2}$ | $3.83 \times 10^{-2}$ | Cytoplasm                |
| MAPK14 | Protein kinases       | 8      | $57.78 \times 10^{-2}$ | $12.91 \times 10^{-2}$ | Cytoplasm                |
| VEGFA  | Growth factors        | 8      | $53.06 \times 10^{-2}$ | $6.33 \times 10^{-2}$ | Extracellular             |
| PIK3CB | Enzymes               | 7      | $50.98 \times 10^{-2}$ | $0.05 \times 10^{-2}$ | Plasma membrane          |
| PIK3CD | Enzymes               | 7      | $50.98 \times 10^{-2}$ | $0.05 \times 10^{-2}$ | Plasma membrane          |
| PIK3CG | Enzymes               | 6      | $50.00 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Plasma membrane          |
| BCL2   | Apoptosis regulator   | 5      | $49.06 \times 10^{-2}$ | $0.32 \times 10^{-2}$ | Mitochondrion            |
| NOS2   | Enzymes               | 5      | $52.00 \times 10^{-2}$ | $0.68 \times 10^{-2}$ | Cytoplasm                |
| TLR4   | Pattern recognition receptors | 5 | $53.06 \times 10^{-2}$ | $1.00 \times 10^{-2}$ | Plasma membrane          |
| NF KB2 | Transcription factors | 4      | $40.00 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Nucleus                  |
| Gene    | Type                  | Degree | Closeness     | Betweenness  | Main Subcellular location |
|---------|-----------------------|--------|---------------|--------------|---------------------------|
| RELB    | Transcription factors | 4      | $40.00 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Nucleus                   |
| REL     | Transcription factors | 4      | $40.00 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Nucleus                   |
| MAPK11  | Protein kinases       | 4      | $50.00 \times 10^{-2}$ | $3.24 \times 10^{-2}$ | Cytoplasm                 |
| VEGFC   | Growth factors        | 4      | $49.06 \times 10^{-2}$ | $2.36 \times 10^{-2}$ | Extracellular             |
| BAX     | Apoptosis regulator   | 3      | $47.27 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Mitochondrion             |
| MAPK12  | Protein kinases       | 3      | $38.24 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Cytoplasm                 |
| MAPK13  | Protein kinases       | 3      | $38.24 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Cytoplasm                 |
| BDNF    | Growth factors        | 3      | $48.15 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Extracellular             |
| VEGFB   | Growth factors        | 2      | $35.62 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Extracellular             |
| NFE2L2  | Transcription factors | 2      | $46.43 \times 10^{-2}$ | $0.00 \times 10^{-2}$ | Nucleus                   |

**miRNA target prediction**

We hypothesized that the 13 potential targets (27 genes) found could be inhibited by unknown miRNAs regulating the JH-CX-DG formula. Therefore, by using FunRich3.1.3, we predicted miRNA interactions and constructed a miRNAs-Genes network using 27 genes. The result shown in Table 5 and Fig. 6 reveal that these 27 genes can interact with 140 miRNAs. Degree, closeness, and betweenness of the hsa-miR-124-3p, hsa-miR-29a-3p, and other 11 miRNAs were ranked to be within the top 10% of the parameters. Therefore, we infer that these are targets regulated by the JH-CX-DG formula.
Table 5

| miRNA          | Degree | Closeness    | Betweenness |
|----------------|--------|--------------|-------------|
| hsa-miR−124−3p | 5      | 30.39 × 10^−2 | 23.04 × 10^−2 |
| hsa-miR−29a−3p | 5      | 29.81 × 10^−2 | 8.09 × 10^−2  |
| hsa-miR−29b−3p | 5      | 29.81 × 10^−2 | 8.09 × 10^−2  |
| hsa-miR−29c−3p | 5      | 29.81 × 10^−2 | 8.09 × 10^−2  |
| hsa-miR−15a−5p | 4      | 28.39 × 10^−2 | 2.90 × 10^−2  |
| hsa-miR−15b−5p | 4      | 28.39 × 10^−2 | 2.90 × 10^−2  |
| hsa-miR−16−5p  | 4      | 28.39 × 10^−2 | 2.90 × 10^−2  |
| hsa-miR−195−5p | 4      | 28.39 × 10^−2 | 2.90 × 10^−2  |
| hsa-miR−424−5p | 4      | 28.39 × 10^−2 | 2.90 × 10^−2  |
| hsa-miR−497−5p | 4      | 28.39 × 10^−2 | 2.90 × 10^−2  |
| hsa-miR−6838−5p| 4      | 28.39 × 10^−2 | 2.90 × 10^−2  |

**Enrichment analysis of the hub genes of the JH-CX-DG formula**

The KOBAS 3.0 database was analyzed for Gene pathway enrichment analysis of hub genes in the JH-CX-DG formula. We found a total 153 pathways (P < 0.05). Figure 7, shows the first 40 pathways. From these, 8 pathways are related to cancer, 12 pathways are related to infection and inflammation, 8 pathways are cell signal transduction pathways, 5 pathways are related to neuroendocrine diseases, 5 pathways are involved in general biological processes, and 2 pathways are related to other diseases. We depict the main pathways of the first 40 pathways into Fig. 7. From our results, we predict that the JH-CX-DG formula have potential pharmacological effects with anti-tumor and anti-tumor angiogenesis properties, anti-inflammatory, improving microcirculation, and protecting brain nerves via multiple pathways including PI3K/Akt, MAPK, Toll-like receptor, T cell receptor, EGFR, VEGFR, Apoptosis, HIF-1.

**Discussion**

Blood-regulating formulae based on TCM theory has been observed to exert beneficial effects such as regulating blood circulation, removing blood stasis, cooling blood, and analgesic. It is believed that blood-regulating formulae have the effects of treating trauma, inflammation, gynecological diseases, cardiovascular diseases, and cancer. We prove that the JH-CX-DG formula (Ligusticum chuanxiong-
Angelica sinensis) is a key formula containing JH in blood-regulating TCM formulae. Furthermore, JH, CX, and DG belong to blood-regulating formulae based on TCM. Our results by using the Arrowsmith knowledge discovery tool revealed that the JH-CX-DG formula can target the AKT, TLR4, caspase-3, PI3K, mTOR, p38 MAPK, VEGF, iNOS, Nrf2, BDNF, NF-κB, Bcl-2, Bax 13 drug targets. We also predicted that the JH-CX-DG formula can regulate the expression of 13 miRNAs including hsa-miR-124-3p and hsa-miR-29a-3p. Zhao et al. [8] demonstrated that curcumin (a compound from JH) can cause apoptosis in SKOV3 cells by upregulating miR-124 by employing a cell Counting Kit-8 (CCK-8) and a colony formation assay. Zhang et al. [9] found that curcumin inhibited TGFβ1-induced cell proliferation and collagen synthesis in NIH-3T3 cells via upregulating miR-29a by a conventional molecular biology experiment. Curcumin has been observed to possess an anti-proliferation effect on leukemic cells by up-regulation of miR-15a and miR-16. It can also inhibit cell proliferation of laryngeal cancer cells by preventing Bcl-2 and PI3K/Akt signaling pathway via upregulation of miRNA-15a [10, 11]. Moreover, Liu et al. [12] found that curcumin has a protective effect on Alzheimer's disease via upregulated miR-15b-5p by RT-qPCR. These studies indicate that JH can directly regulate the expression of various miRNA. Our results were shown to be consistent with the discussed studies.

Our PPI and the location of targets analysis via pathways enrichment predicted that the JH-CX-DG formula have potential pharmacological effects including anti-inflammatory, improving microcirculation, protecting brain nerves, anti-tumor and anti-tumor angiogenesis via the regulation of the PI3K/Akt, MAPK, Toll-like receptor, T cell receptor, EGFR, VEGFR, Apoptosis, HIF-1 pathways. Liu et al. [13] indicated JH could lead to cell apoptosis in ovarian cancer cell lines SK-OV-3 by inhibiting the AKT/mTOR pathway by using an MTT assay. Ashrafizadeh et al. [13] found that JH possess various effects including antioxidant, antibacterial, antineoplastic and anti-inflammatory via targeting the Nrf2 signaling pathway. There are reports that show Chuanxiong (Ligusticum chuanxiong) and Danggui (Angelica sinensis) also can exert anti-tumor effects. Shen J et al. [14] used RT-qPCR and western blot analysis to show that CX significantly decreased the Akt expression and activity, increased the activity of caspase-3, and was also shown to have anti-tumor effects in several types of cancer. DG can induce gastric cancer cell death through inhibiting mTOR protein and can significantly prolong the survival rate of cancer patients [15]. DG also affected a variety of apoptotic factors, Bcl-2 Associated X Protein (Bax), Bcl-2 and caspase-3 expression and promoted apoptosis in human breast cancer cells, therefore it has proved to be a promising therapeutic agent for breast cancer treatment [16]. Additionally, the JH-CX-DG formula may have an anti-tumor angiogenesis effect. An ELISA assay revealed that JH suppressed VEGF secretion from tumor cells and inhibited cancer angiogenesis [17]. Shen et al. [18] found that DG could prevent cancer cell proliferation and cancer angiogenesis by inhibiting the PI3K/AKT/mTOR signaling pathway and downregulating the expression of HIF-1α and VEGF. These studies indicate that the JH-CX-DG formula can exert anti-tumor effects. Our results demonstrate a strong agreement with the aforementioned studies.

We inferred that the JH-CX-DG formula could also display anti-inflammatory, protecting brain nerves effects, and improving microcirculation effects. Zhu et al. [19] used immunohistochemistry, ELISA, and Western blot, to demonstrate that JH have the potential to alleviate acute inflammatory injury in
experimental traumatic brain injury by inhibiting the TLR4/MyD88/NF-κB signaling pathway. Data demonstrated that DG can upregulate TLR4 protein, TLR4, and miRNA expression, suggesting this could represent an effective therapy for anti-inflammatory and anti-nociceptive affictions [20]. Experiments have demonstrated that CX diminished the expression of inflammatory cytokines and adhesion molecules on Vascular Endothelial Cells by Inhibiting p38 MAPK and NF-κB Signaling Pathways. These studies confirm our hypotheses regarding the anti-inflammatory effect of the components of JH-CX-DG formula. Wu et al. [21] demonstrated that JH can exert beneficial effects on ischemic cerebral injury by upregulating the caspase3 and Bcl-2 expression, downregulating the Bax expression, and promoting PI3K/AKT pathway activation. Hurley et al. [22] showed that JH also resulted in a dose-dependent increase in hippocampal BDNF and protected the brain nerves. Cheng et al. [23] found that DG pretreatment protected patients from cerebral infarct and improved neurological damage by downregulating the caspase3 and upregulated p-p38 MAPK, hypoxia-inducible factor (HIF-1), vascular endothelial growth factor-A (VEGF-A). Chen et al. [24] found that a Danggui Sini decoction could be effectively used to protect microvascular endothelial cells of islet microvasculature via PI3K/Akt/iNOS pathway. CX can also significantly enhance BCL-2, VEGF and reduced caspase-3 and BAX and protects against cerebral ischemia by anti-oxidation, apoptosis inhibition, and angiogenesis [25]. Our results are in agreement with the above studies, demonstrating the pharmacological effects of protecting brain nerves and improving of the JH-CX-DG formula.

**Conclusions**

We demonstrated that the JH-CX-DG formula is a key formula containing JH in blood-regulating TCM formulae. The JH-CX-DG formula was shown to possess with multi-targets and multi-pathways properties and could prove effective in the treatment of inflammation, microcirculation disorders, cardiovascular disease, and cancer. We found a new drug formula and analyzed the compatibility laws and systemic pharmacological mechanisms of JH. Our study provides a theoretical foundation and directions for future research on the JH-CX-DG formula.

**Abbreviations**

JH
Jianghuang (from Curcuma Longa); CX:Chuanxiong (from Ligusticum chuanxiong); DG:Jianghuang (from Angelica sinensis); TCM:Traditional Chinese Medicine; GRI:Generalized rule induction (GRI) algorithms; KOBAS:KEGG Orthology Based Annotation System; PPI:Protein-protein Interaction

**Declarations**

1. Ethics approval and consent to participate*Not applicable*

2. Consent for publication*Not applicable*
3. Availability of data and materials

All data are available in the manuscript and they are showed in figures, tables and supplement file.

4. Competing interests

The authors declare that they have no conflict of interest.

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6. Authors’ contributions

LZQ: Study Design, Data Collection, Statistical Analysis, Data Interpretation, Manuscript Preparation, Literature Search, Funds Collection

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7. Acknowledgements ‘Not applicable’

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26. Include figure and or table legend.

Figures
Figure 1

Jianghuang Compatibility Network with Frequency $\geq 4$. 
Figure 1

Jianghuang Compatibility Network

Frequency ≥ 4.
Figure 2
Compatibility laws scatter plot.
Figure 2

This figure shows a scatter plot with axes labeled 'support' on the x-axis and 'confidence' on the y-axis. The data points are color-coded and numbered, indicating their corresponding values. The 'lift' scale is represented on the right side of the plot, ranging from 3 to 7.

Points are labeled with numbers ranging from 1 to 22, each representing a different data entry or category.
Compatibility laws scatter plot.

Figure 3

Venn diagram of number of targets (score $\geq 0.95$).

Figure 3

Venn diagram of number of targets (score $\geq 0.95$).
Figure 4

PPI network
Figure 4

PPI network
Figure 5

Tissue organ location
Figure 5

Tissue organ location
Figure 6
miRNAs-Targets Network
Figure 7
KEGG pathway enrichment analysis.
"Jianghuang - Danggui - Chuanxiong" regulate this process

Figure 8

Anticancer pathways.
Figure 8

Anticancer pathways.

"Jianghuang - Danggui - Chuanxiong" regulate this process

Survival, Differentiation, Growth, Proliferation
Figure 9

Pathways of immune response regulation.

"Jianghuang - Danggui - Chuanxiong" regulate this process.

Leukocyte and Inflammatory factor are activated.

Increased vascular permeability.
Figure 9

Pathways of immune response regulation.
Figure 10

Pathways of Angiogenesis regulation.

"Jianghuang - Danggui - Chuanxiong" regulate this process.

1. Angiogenesis 2. Vasculartone 3. Migration
Figure 10

Pathways of Angiogenesis regulation.