Jianpi Qinghua Sanyu Decoction attenuates insulin-like growth factor 1-induced GES-1 cell proliferation by suppressing the AKT pathway based on network pharmacology analysis

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Article

Keywords: Jianpi Qinghua Sanyu Decoction, raised erosive gastritis, pharmacological analysis, phosphatidylinositol 3-kinase/protein kinase B
Abstract

Raised erosive gastritis (REG) is a gastric mucosal lesion with characteristic radiological and endoscopic features. Jianpi Qinghua Sanyu Decoction (JPQHSYD) may be effective against REG, but its therapeutic effects and underlying molecular mechanisms have yet to be investigated. A network pharmacology analysis was performed to identify its JPQHSYD active ingredients, potential targets, and pathways in REG treatment. Following database mining, our current study identified 245 compounds and 320 potential targets, as well as 94 common targets with REG. The construction of a traditional Chinese medicine (TCM) comprehensive network (Drug-Ingredients-Gene symbols-Disease network) identified ten potentially active compounds (including Quercetin) and 35 potential targets (including AKT1), as well as enriched signaling pathways (including cell proliferation and phosphatidylinositol 3-kinase and protein kinase B (PI3K/Akt)). In vitro studies revealed that JPQHSYD significantly inhibits cell growth and viability while also down-regulating p-AKT, Bcl-2, CDK4, and CyclinD1 expression and up-regulating Bax protein levels in insulin-like growth factor 1 (IGF-1)-stimulated GES-1 cells. This study provides novel insights into the mechanism of action of JPQHSYD in REG treatment, implying that suppressing the AKT pathway may be one of the essential underlying anti-REG mechanisms of JPQHSYD.

1 Introduction

In the national literature, raised erosive gastritis (REG), also known as gastric varioliform lesions or varioliform gastritis (VG), is a lesion of the gastric mucosa characterized by multiple small flat or tiny elevated lesions with or without central depressions or erosions involving the antrum. The presence of numerous small elevated lesions with an umbilical-like depression (“octopus’ sucker” gastritis) is the most notable endoscopic feature. Lesions are often multiple, linearly arranged on the longitudinal fold of the distal gastric antrum, and converge toward the pylorus\(^1\).

Until now, there have been few studies on the etiopathogenesis of REG. In 1994, REG was classified as a precursor to gastric cancer at the World Congress of Gastroenterology\(^2\). Some researchers observed “in situ” carcinomatous transformation in a patient with REG, and other groups have recently reported similar findings\(^3\). REG is currently linked to Helicobacter pylori (H. pylori) infection, hyperacidity, mechanical overload reflux, gastric atrophy, and background mucosa vulnerability\(^4\).

Numerous epidemiological studies have attempted to shed light on the factors influencing precancerous lesions, such as a history of aspirin use, excessive smoking and drinking, pickled food consumption, and tea consumption, among others. The mainstays of REG treatment are anti-Helicobacter pylori, acid suppressors, gastric mucosa protection, and endoscopic treatment, but high recurrence rates and endoscopic therapy-related side effects persist. Therefore, controlling this disease would have a major impact on the benefits of health care.

In China, traditional Chinese medicine (TCM) has been used to treat digestive diseases for thousands of years. A recent systematic review concluded that a Chinese classical formula, Banxia Xiexin decoction
(BXD), was more effective and safer for patients with chronic atrophic gastritis and cold-heat complex syndrome\(^5\). In addition to BXD, Jianpi Qinghua Sanyu Decoction (JPQHSYD) is another Chinese formula widely used in REG treatment based on TCM theories of clearing heat and resolving dampness. JPQHSYD consists of 14 herbs, including Codonopsis Radix, Radix Salviae, Scopariae, Atractylodes Macrocephala, Carapax Trionycis, Lablab Semen Album, Poria Cocos, Pinellia Ternata, Cortex Magnoliae Officinalis, Coptidis Rhizoma, Codonopsis Radix, Amomum Villosum, Citrus Reticulata, and Licorice. However, the underlying mechanisms remain unclear.

In recent years, network-based drug discovery has been regarded as one of the most promising approaches for cost-effective drug development, thanks to rapid advances in bioinformatics, systems biology, and polypharmacology. Network pharmacology uses a “network target” as a mathematical and computable representation of various connections between botanical formulas and diseases\(^6\). It has significant benefits in terms of locating alternative herbal drug targets, finding multi-target pharmaceuticals, and providing new perspectives on TCM research. Therefore, we used this system biology-based approach to describe the association of multiple components with multiple targets and pathways, as well as reveal potential mechanisms of JPQHSYD against REG at the system level, as a reference for subsequent pharmacological studies and clinical treatments of REG (Fig. 1). Finally, in vivo experiments were conducted to validate the key pathways of JPQHSYD against REG.

2 Materials And Methods

2.1 Collection of JPQHSYD compounds and target ingredients

All of the components in JPQHSYD were collected from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP; http://tcmsp.com/tcmsp.php\(^7\) and the Analysis Platform (TCMSP) and Bioinformatics Analysis Tool for Molecular mechanism (BATMAN; http://bionet.ncpsb.org.cn/batman-tcm/). The target was then transformed using the UniProt knowledge database (http://www.uniprot.org) and the SymMap database (http://www.symmap.org)\(^8\), with Homo sapiens as the selected species. The data were merged after redundant items were removed to obtain gene symbols.

2.2 Acquisition of REGWe obtained gene targets

REG gene targets were obtained from two different sources. The first source was the GeneCards database (https://www.genecards.org\(^9\). The keywords “raised erosive gastritis or gastric varioliform lesions or varioliform gastritis” were used to search this database. The second source was the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org)\(^10\).

2.3 Construction of a Drug-Ingredients-Gene symbols-Disease (D-I-G-D) network

First, a Venn diagram of the intersected gene symbols was created by intersecting the drug targets with disease-related genes. Then, a network of complex information was constructed based on interactions
between the drug (JPQHSYD), ingredients, gene symbols, and disease (REG). Following that, Cytoscape Version 3.7.2, a network analysis and editing software with a graphical display, was used to visually analyze the D-I-G-D network.

2.4 Protein-protein interaction (PPI) network construction and topological analysis

The PPI network was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://cn.string-db.org/), with the common drug-disease targets screened. The biological species was set to “Homo sapiens,” and the minimum interaction score was set at the highest confidence level (0.900). The PPI network was then topologically analyzed using Cytoscape 3.7.2 software. The core genes were sorted by degree values and genes with scores greater than the mean value. This means that the desired therapeutic target is screened by betweenness centrality, closeness centrality, and degree value.

2.5 Enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways

The intersected gene symbols were imported into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) database (https://david.ncifcrf.gov/tools.jsp) for GO and KEGG enrichment analysis, and the top 20 entries in the GO enrichment analysis and the top 20 pathways in the KEGG enrichment analysis were ranked according to the number of genes involved in the enriched pathways, using a p-value less than 0.01 as the screening condition. The results were displayed as a bubble chart.

2.6 Experimental validation

2.6.1 JPQHSYD aqueous extract preparation

JPQHSYD consisted of 15 g of Codonopsis Radix, 15 g of Radix Salviae, 9 g of Artemisiae Scopariae, 9 g of Atractylodes Macrocephala, 18 g of Carapax Trionycis, 15 g of Lablab Semen Album, 15 g of Poria Cocos, 9 g of Pinellia Ternata, 9 g of Cortex Magnoliae Officinalis, 3 g of Coptidis Rhizoma, 9 g of Currumae Rhizoma, 6 g of Amomum Villosum, 6 g of Citrus Reticulata, and 3 g of Licorice. JPQHSYD granules were obtained from Beijing Kang Rentang Pharmaceutical Co. Ltd., batch no. 22005431 of Codonopsis Radix, no. 22007711 of Radix Salviae, no. 22000541 of Artemisiae Scopariae, no. 22000821 of Atractylodes Macrocephala, no. 21025411 of Carapax Trionycis, no. 21033791 of Lablab Semen Album, no. 20022852 of Poria Cocos, no. 21039382 of Pinellia Ternata, no. 21017371 of Cortex Magnoliae Officinalis, no. 22006291 of Coptidis Rhizoma, no. 21013841 of Currumae Rhizoma, no. 21034141 of Amomum Villosum, no. 21019951 of Licorice, no. 22007871 of Citrus Reticulata. JPQHSYD granules were dissolved in complete phosphate-buffered saline and prepared into a solution with a concentration of 100 mg/ml, which was then stored at −20°C for subsequent use.

2.6.2 Cell culture and treatment
GES-1 cells were obtained from the Chinese Academy of Sciences cell bank (Shanghai, China). They were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. For all experiment, cells were cultured at 37°C in 5% CO2.

After overnight culture, a subset of the cells was stimulated with different concentrations (0, 2.5, 5, 10, 20, 40, 80, 100, 150, and 200 ng/mL) of the AKT pathway activator insulin-like growth factor 1 (IGF-1, E10501061, Cyage). The remaining cells were given JPQHSYD at different concentrations (0, 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 ug/mL). The optimal doses were finally chosen for subsequent experiments.

2.6.3 Cell viability assay

The cells were stimulated for 24 hours with different concentrations of AKT pathway activator (IGF-1) and JPQHSYD and then inoculated in 96-well plates. Next, 10 μL of cell counting kit (CCK)-8 reagent was added to each well, and the cells were incubated for 2 hours in an incubator at 37°C. After incubation, the absorbance was measured at 450 nm on an enzyme standard meter, and the proliferation curves were plotted based on the absorbance values to estimate cell viability.

2.6.4 Cell growth morphology assessment and cell counting

The cells were stimulated with an AKT pathway activator (IGF-1) and three different concentrations of JPQHSYD (low, medium, and high) for 24 hours. The cell morphology and growth were observed and photographed using an inverted microscope. After observation, the cells were digested with a 0.25% trypsin digestion solution, and single-cell suspensions were made from a fresh culture medium. The cell suspensions were gently blown with a pipette, and a small volume of the cell suspension was mixed with an equal volume of Taipan blue, and 20 μL of the mixture were then added to a cell counting plate and counted using a Constar cell counter.

2.6.5 Western blotting assay

The cells were homogenized and lysed in RIPA buffer. Total proteins were extracted by centrifugation at 4°C for 20 minutes at 14000 rpm. Subsequently, total proteins were subjected to 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The membranes were blocked in 5% nonfat milk, and primary antibodies of AKT (4685S, CST), p-AKT (4040S, CST), Bcl2 (ABM0010, ABBKINE), Bax (5023S, CST), CDK4 (12790s, CST), Cyclin D1 (12790s, GENTEX), and β-actin (4970S, CST) were added and incubated at 4°C overnight. Horseradish peroxidase-goat anti-rabbit antibody (L3012, SAB) was added and incubated at room temperature for 60 minutes. Finally, the membranes were scanned with a chemiluminescence system. The β-actin protein expression was used as an internal control. The protein levels were analyzed with ImageJ software.

2.6.6 Statistical analysis
All data were expressed as a mean and standard deviation. The data were analyzed using GraphPad Prism 6.02 and the Statistical Package for the Social Sciences (SPSS) 21.0 software. The Student’s t-test was used to compare quantitative data between groups, and a p-value of less than 0.05 was considered significant.

3 Results

3.1 Identification of JPQHSYD potential targets for REG treatment

The active components of the JPQHSYD formula, comprising multiple herbs, including Codonopsis Radix, Radix Salviae, Herba Artemisiae Scopariae, Atractylodes macrocephala, Lablab Semen Album, Poria Cocos, Pinellia ternata, Cortex Magnoliae Officinalis, Coptidis Rhizoma, Curcumae Rhizoma, Amomum villosum, Licorice, and Citrus Reticulata, were identified based on TCMSP with oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. In addition, the effective components of Carapax Trionycis were screened in the BATMAN database with a setting score cutoff of 20 or more and a p-value of 0.05 or less. Furthermore, 245 JPQHSYD candidate ingredients were selected from databases (Supplementary).

Meanwhile, the targets of these active ingredients were also obtained, including 185 targets of Codonopsis Radix ingredients, 881 targets of Radix Salviae ingredients, 343 targets of Herba Artemisiae Scopariae ingredients, 23 targets of Atractylodes Macrocephala ingredients, 2 targets of Carapax Trionycis ingredients, 22 targets of Lablab Semen Album ingredients, 149 targets of Poria Cocos ingredients, 168 targets of Pinellia Ternata ingredients, 33 targets of Cortex Magnoliae Officinalis ingredients, 278 targets of Coptidis Rhizoma ingredients, 24 targets of Curcumae Rhizoma ingredients, 75 targets of Amomum Villosum ingredients, 1504 targets of Licorice ingredients, and 73 targets of Citrus Reticulata ingredients. Finally, there were 320 targets left after removing duplicates.

A total of 523 REG-related targets were obtained from the GeneCards and OMIM database. The intersection of 320 drug targets and 523 disease targets yielded 94 common targets (Fig. 2A), which were used as JPQHSYD prediction targets in REG treatment.

3.2 Network analysis and key components

The D-I-G-D network constructed using the Cytoscape 3.7.2 software obtained the JPQHSYD core components and ranked them based on degree values; the higher the value, the greater the importance (Fig. 2B). This contributes to a better understanding of the potential pharmacodynamic substances and targets of JPQHSYD in REG treatment. The top ten were chosen for subsequent analysis (Table 1). Among these bioactive components, Quercetin had the highest correlation with REG targets based on the degree value, followed by Luteolin, Kaempferol, Naringenin, Beta-sitosterol, and so on.

Table 1. Top active ingredients of JPQHSYD identified in the component-target network.
<table>
<thead>
<tr>
<th>MOL ID</th>
<th>Name</th>
<th>Degree</th>
<th>OB (%)</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOL000098</td>
<td>Quercetin</td>
<td>210</td>
<td>46.43</td>
<td>0.28</td>
</tr>
<tr>
<td>MOL000006</td>
<td>Luteolin</td>
<td>62</td>
<td>36.16</td>
<td>0.25</td>
</tr>
<tr>
<td>MOL000422</td>
<td>Kaempferol</td>
<td>25</td>
<td>41.88</td>
<td>0.24</td>
</tr>
<tr>
<td>MOL004328</td>
<td>Naringenin</td>
<td>21</td>
<td>59.29</td>
<td>0.21</td>
</tr>
<tr>
<td>MOL000358</td>
<td>Beta-sitosterol</td>
<td>20</td>
<td>36.91</td>
<td>0.75</td>
</tr>
<tr>
<td>MOL002773</td>
<td>Beta-carotene</td>
<td>18</td>
<td>37.18</td>
<td>0.58</td>
</tr>
<tr>
<td>MOL000354</td>
<td>Isorhamnetin</td>
<td>18</td>
<td>49.60</td>
<td>0.31</td>
</tr>
<tr>
<td>MOL003896</td>
<td>7-Methoxy-2-methyl isoflavone</td>
<td>18</td>
<td>42.56</td>
<td>0.20</td>
</tr>
<tr>
<td>MOL007154</td>
<td>Tanshinone iia</td>
<td>17</td>
<td>49.89</td>
<td>0.40</td>
</tr>
<tr>
<td>MOL005828</td>
<td>Nobiletin</td>
<td>15</td>
<td>61.67</td>
<td>0.52</td>
</tr>
</tbody>
</table>

### 3.3 PPI network analysis of the core genes

The 94 common targets were imported into the STRING database to construct the PPI network, which had 94 nodes and 422 edges, an average node degree of 8.98, and a PPI enrichment p-value of less than 1.0e-16 (Fig. 3A). The degree, PPI network topological eigenvalues, degree of node color, size of the reaction center, edge thickness, and color depth were then analyzed using Cytoscape Version 3.7.2 to yield a combined score (Fig. 3B). The average degree value of the PPI network was 9.48. Topological analysis of the PPI networks identified 35 genes with scores greater than the average as core targets. The top ten targets in terms of degree value were STAT3, JUN, AKT1, EP300, tumor necrosis factor (TNF), TP53, interleukin 6 (IL6), mitogen-activated protein kinase 14 (MAPK14), CTNNB1, and MAPK1 (Table 2).

#### Table 2. The ranking of core targets, betweenness centrality, closeness centrality, and degree values.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Betweenness centrality</th>
<th>Closeness centrality</th>
<th>Degree</th>
</tr>
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<tbody>
<tr>
<td>STAT3</td>
<td>0.13</td>
<td>0.56</td>
<td>33</td>
</tr>
<tr>
<td>JUN</td>
<td>0.13</td>
<td>0.55</td>
<td>33</td>
</tr>
<tr>
<td>AKT1</td>
<td>0.10</td>
<td>0.51</td>
<td>28</td>
</tr>
<tr>
<td>EP300</td>
<td>0.08</td>
<td>0.51</td>
<td>28</td>
</tr>
<tr>
<td>TNF</td>
<td>0.08</td>
<td>0.50</td>
<td>26</td>
</tr>
<tr>
<td>TP53</td>
<td>0.07</td>
<td>0.48</td>
<td>25</td>
</tr>
<tr>
<td>IL6</td>
<td>0.05</td>
<td>0.49</td>
<td>24</td>
</tr>
<tr>
<td>MAPK14</td>
<td>0.04</td>
<td>0.51</td>
<td>23</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>0.05</td>
<td>0.49</td>
<td>22</td>
</tr>
<tr>
<td>MAPK1</td>
<td>0.09</td>
<td>0.51</td>
<td>22</td>
</tr>
</tbody>
</table>

STAT3, signal transducer and activator of transcription 3; AKT1, protein kinase B; TNF, tumor necrosis factor; TP53, tumor protein P53; IL6, interleukin 6; MAPK14, mitogen-activated protein kinase 14; CTNNB1, beta-Catenin; MAPK1, mitogen-activated protein kinase 1.

3.4 GO and KEGG enrichment analyses of common target genes

The 94 common targets were analyzed using GO and KEGG pathway enrichment, which identified the potential molecular mechanisms for JPQHSYD in REG. GO enrichment analysis yielded cellular component (CC), molecular function (MF), and biological process (BP) results. CC analysis revealed a higher proportion of protein in the cytoplasm, nucleus, and extracellular space. The MFs were mainly related to “protein binding,” “identical protein binding,” and “enzyme binding.” The main BPs were associated with “cell proliferation,” “cell cycle regulation,” and “inflammatory response.” Furthermore, GO analysis revealed the top 20 enriched conditions in the CC, MF, and BP categories (Figs. 4A–C).

To investigate the signaling pathways and functions of these target genes, a KEGG pathway functional enrichment analysis was performed (Fig. 4D). The signaling pathways were identified by screening their statistical significance ($p < 0.01$), and the resulting target genes were found to primarily interact with the IL17, TNF, MAPK, phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), hypoxia-inducible factor-1, FoxO, and T-cell receptor signaling pathways. Thus, these signaling pathways appear to be closely related to the potential impact of JPQHSYD on REG.

3.5 Experimental validation using in vitro CCK-8 assay

First, the effects of various IGF-1 doses on GES-1 cell viability were determined using the CCK-8 assay (Fig. 5A). An IGF-1 concentration of 2.5 ng/mL resulted in high cell viability. Therefore, a concentration of 2.5 ng/mL was selected for subsequent experiments. The effect of JPQHSYD on GES-1 cell viability was
investigated. GES-1 cells were incubated with JPQHSYD at concentrations of 0, 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/mL for 24 hours. The results revealed that JPQHSYD treatment at 200 µg/mL for 24 hours significantly reduced GES-1 cell viability, while lower concentrations had no significant effect (Fig. 5B). Therefore, the concentrations of 25, 50, and 100 µg/mL were selected for subsequent experiments. After treatment with 50 and 100 µg/mL of JPQHSYD for 24 hours, the number of GES-1 cells was significantly lower than that in the control group (p < 0.05; Figs. 5C–D). IGF-1 stimulation was shown to promote GES-1 cell proliferation, while medium and high JPQHSYD concentrations were able to inhibit IGF-1-induced cell proliferation.

3.7 JPQHSYD inhibited cell proliferation via PI3K/Akt pathway \textit{in vitro}

To validate the underlying signaling pathway for JPQHSYD action on GES-1 cells predicted by network pharmacology, the expression changes in key genes in the PI3K/Akt signaling pathway were detected using the network pharmacology enrichment index (Figs. 6A–B). Compared to the control group, IFG-1 significantly upregulated p-AKT, Bcl2, CDK4, and CyclinD1 protein expression (p < 0.05), while AKT and Bax protein expression were not significantly different, and Bax/Bcl was downregulated (p < 0.05). Compared to the IGF-1 group, JPQHSYD treatment at 50 and 100 µg/mL significantly inhibited p-AKT, Bcl2, CDK4, and CyclinD1 protein expression (p < 0.05), with no significant difference in AKT or Bax protein expression and upregulation of Bax/Bcl2 (p < 0.05). In contrast, JPQHSYD at 25 µg/mL had no significant effect. These findings suggest that IGF-1 may promote the expression of its downstream proteins Bcl2, CDK4, and CyclinD1 by activating the AKT signaling pathway. JPQHSYD promoted apoptosis by inhibiting the AKT signaling pathway and suppressing AKT phosphorylation, which in turn inhibited its downstream Bcl2 expression. In addition, it suppressed cell cycle transition and proliferation by inhibiting CDK4 and CyclinD1 expression. Overall, we found that JPQHSYD could inhibit cell proliferation by inhibiting the PI3K/Akt pathway.

4 Discussion

Our current study aimed to explore the potential underlying anti-REG mechanism of JPQHSYD using network pharmacology analysis. Our findings identified 320 JPQHSYD target genes, 523 REG-associated genes, and 94 overlapping JPQHSYD-REG gene symbols that were significantly enriched in several GO processes, including cell proliferation, cell cycle regulation, inflammatory response, and apoptotic process regulation, among others. Furthermore, analysis of the network’s topological variables revealed that STAT3, JUN, and AKT1 genes were highly ranked throughout the network. KEGG analysis revealed that JPQHSYD’s therapeutic effects on REG involved the PI3K/AKT signaling pathway, the MAPK signaling pathway, and others. In vitro experiments revealed that JPQHSYD treatments reduced cell density, number, and viability. Furthermore, JPQHSYD inhibited IGF-1-induced GES-1 cell proliferation by suppressing the AKT pathway.

Networked pharmacology has recently emerged as an interdisciplinary field encompassing computational biology, conventional pharmacology, structural biology, and multi-omic strategies. The overlapping 94 JPQHSYD- and REG-related genes were entered into the STRING database to construct the
PPI network. Analysis of the network's topological variables revealed that STAT3, JUN, and AKT1 genes were highly ranked throughout the network. STAT3 is a key signaling protein that a multitude of growth factors and cytokines use to elicit diverse biological outcomes, including cellular growth, differentiation, and survival\(^\text{13}\). JUN acts on the AP-1 (c-JUN) signaling pathway and plays an important role in inflammatory cell proliferation and differentiation, and its expression is associated with the secretion of cytokines and inflammatory mediators\(^\text{14-15}\). AKT1 is a serine/threonine protein kinase that is involved in the PI3K pathway. When AKT1 is activated, it can activate or inhibit a series of downstream effectors that regulate a variety of biological processes, such as cell metabolism, proliferation, differentiation, apoptosis, and migration\(^\text{16-17}\). The anticancer gene TP53 controls a variety of cellular processes, including apoptosis, cell cycle, and cell migration and invasion\(^\text{18}\). EP300 is a histone acetyltransferase that plays an important role in cell proliferation, transformation, and differentiation. The lack of EP300 in cells increases cancer cell activity and invasiveness and induces epithelial-mesenchymal transformation\(^\text{19}\). Based on the aforementioned study findings, it is clear that JPQHSYD has a significant therapeutic and preventive role in EGR caused by multiple targets.

The PI3K/Akt signaling pathway regulates many intracellular signaling pathways and is essential for cell survival, proliferation, and anti-apoptosis. AKT is a PI3K downstream effector molecule that, when activated, promotes cell proliferation and growth. When PI3K is activated, it activates the downstream signaling molecule AKT. The activated AKT, p-AKT, can cause cells to proliferate continuously, promote angiogenesis, and regulate downstream signaling molecules such as Bcl-2, Bax, CyclinD1, mTOR, and others to regulate cell cycle changes and promote cell proliferation and survival\(^\text{20-21}\). At present, the expression of the p-AKT protein in warty gastritis has received little attention both at home and abroad. Various studies have found that p-AKT is highly activated in breast cancer, liver cancer, lung cancer, and other cancers, and that high p-AKT is associated with cancer progression. Therefore, the PI3K/AKT signaling pathway may play an important regulatory role in the abnormal proliferation of REG gastric mucosal cells.

The current study used western blotting to validate the PI3K/AKT signaling pathway predicted by KEGG. Hence, the pharmacological mechanism of JPQHSYD in REG treatment can be better understood. Notably, our results revealed that JPQHSYD significantly reduced p-AKT and Bcl-2 protein expression levels while increasing the Bax/Bcl-2 ratio when compared to the IGF-1 group. Furthermore, it suppressed cell cycle transition by inhibiting CDK4 and CyclinD1 expression, thereby inhibiting cell proliferation. Bax and Bcl-2, as AKT downstream molecules, are linked to apoptosis. The present study found that JPQHSYD could inhibit Bcl-2 expression by regulating the PI3K/AKT signaling pathway, promoting REG cell apoptosis. It has been shown that CDK4, a key regulator of cellular G1/S phase transition, inhibits tumor cell proliferation by inactivating the phosphorylation of tumor cell proteins when it forms a complex with cytosolic CyclinD1\(^\text{22}\). These findings confirmed JPQHSYD’s pro-apoptotic and anti-proliferative effects on REG-aberrantly proliferating cells.

5 Conclusion
In the present study, the PPI network-based pharmacological analysis was combined with biological validation to investigate the mechanism of action of JPQHSYD against REG at the systemic level. We found that JPQHSYD’s anti-REG effect involved multiple targets in the PI3K/AKT signaling pathway. Future research on other pathways or mechanisms predicted by this study is recommended.

**Declarations**

**Author Contributions**

Q.H.L and P.L.Z conceived and designed the study. X.Y.L and X.R.Z conceived and designed the experimental validation *in vitro*. W.Y.F, W.R.W, J.Y.H,YC,and L.M.L responsible for data acquisition and extraction.Q.H.L drafted the paper, which was revised by A.L.S. XK supervised the study. All authors participated in drafting of the manuscript and revising it before final submission. These authors: Q.H.L and P.L.Z have contributed equally to this work and share first authorship.

**Data Availability Statement**

The data used to support the findings of our study are included within the article, or within the Supplementary Material.

**Acknowledgements**

Not applicable.

**Funding**

This work was supported by the National Natural Science Foundation of China (Nos.81973621).

**Conflict of interest**

The authors declare that they have no conflicts of interest.

**References**


Figures

**Figure 1**

An illustration of the network pharmacology analysis. JPQHSYD, Jianpi Qinghua Sanyu Decoction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction.
Figure 2

(A) 94 overlapping gene symbols between the disease and the drug. (B) The D-I-G-D network. The purple node represents JPQHSYD, and the yellow node represents REG. The 245 blue nodes represent the active ingredients in JPQHSYD. The 94 green nodes represent the overlapping gene symbols between the disease and the drug. The edges denote that nodes can interact with one another.
Figure 3

(A) The PPI relational network. (B) The topological analysis network. PPI, protein-protein interaction.

Figure 4

(A) Statistics of Cellular Component Enrichment

(B) Statistics of Molecular Function Enrichment

(C) Statistics of Biological Process Enrichment

(D) Statistics of KEGG Pathway Enrichment
Figure 4

(A) The top 20 significantly enriched cellular components; (B) the top 20 significantly enriched molecular functions; (C) the top 20 significantly enriched biological processes; and (D) KEGG pathway enrichment analysis of 74 overlapping genes of the top 20 significantly enriched pathways.

Image not available with this version

Figure 5

(A) GES-1 cell stimulation with different solubility levels of IGF-1, *p < 0.05 vs. the 0 ng/mL; and (B) various JPQHSYD levels for GES-1 cell stimulation, *p < 0.05 vs. the 0 µg/mL. (C) Effect of different JPQHSYD concentrations on IGF-1-induced GES-1 cell growth. (D) JPQHSYD treatment inhibited IGF-1-induced GES-1 cell activation. *p < 0.05 vs. the control group. #p < 0.05 vs. the IGF-1 group.
Figure 6

Effect of JPQHSYD on PI3K/AKT pathway-related protein expression in IGF-1-stimulated GES-1 cells. (A) Western blots showing p-AKT, AKT, Bcl2, and Bax expression following JPQHSYD treatment. (B) Western blots showing CDK4 and CyclinD1 expression following JPQHSYD treatment. *p < 0.05 vs. the control group. #p < 0.05 vs. the IGF-1 group.
Supplementary Files

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