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Exploring the mechanism and experimental validation of Fuzi Lizhong Tang in treating gastric cancer based on network pharmacology and molecular docking

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Abstract. – OBJECTIVE: This study aimed to explore the mechanism of Fuzi Lizhong Tang (FZLZT) in treating gastric cancer using network pharmacology and molecular docking, and to validate the results through *in vitro* experiments.

MATERIALS AND METHODS: Active ingredients and target genes of FZLZT were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, while disease targets of gastric cancer were collected from GeneCards, OMIM, and DrugBank databases. The "herb-active ingredient-target gene" network was constructed using Cytoscape software, and core active ingredients were obtained through topological analysis. Protein-protein interaction analysis was performed using the STRING database, and core targets were obtained through topological analysis. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis were performed using the DAVID database. Molecular docking was conducted using AutoDock Vina software to verify the interaction between core ingredients and core targets. Cholecystokinin octapeptide (CCK-8) assay was used to determine the proliferation inhibition effect of FZLZT on AGS, BGC823, HGC-27, MGC-803, and SGC-7901 gastric cancer cell lines, and ANNEXIN V-FITC/PI double staining combined with flow cytometry was used to measure the cell apoptosis rate.

RESULTS: Network pharmacology analysis revealed 117 active ingredients and 261 target genes of FZLZT, and 211 overlapping targets with gastric cancer. Ten core active ingredients were identified through topological analysis, including quercetin, 7-methoxy-2-methyl isoflavone, kaempferol, luteolin, naringenin, isorhamnetin, quercetagetin, glycyrrhizic acid A, β-sitosterol, and medioresinol. GO and KEGG enrichment analysis showed that the mechanism of FZLZT in treating gastric cancer mainly involves cancer, inflammation, metabolism, and blood rheology-related pathways, and may act through 7 core targets (*CDKN1A, MYC, MAPK1, MAPK14, RB1, RELA,* and *STAT3*). Molecular docking results further confirmed the prediction of network pharmacology. *In vitro* experiments showed that FZLZT inhibited the proliferation of all five gastric cancer cell lines, with the strongest effect on SGC-7901 cells, and induced apoptosis in SGC-7901 cells.

CONCLUSIONS: FZLZT has a multi-component, multi-target, and multi-pathway characteristic in treating gastric cancer. Its active ingredients may regulate the expression of proteins such as *CDKN1A, MYC, MAPK1, MAPK14, RB1, RELA,* and *STAT3* to activate cancer-related signaling pathways to achieve its therapeutic effect.

Key Words:
Fuzi Lizhong Tang, Gastric cancer, Network pharmacology, Molecular docking, Mechanism of action.

Introduction

Gastric cancer is a common malignant tumor worldwide, originating from the epithelial cells of the gastric mucosa, and the incidence rate in China ranks first among digestive system tumors. It often has a hidden onset, with nonspecific symptoms, usually manifesting as digestive disorders such as nausea and vomiting, and a small percentage of patients may have black stools caused by upper gastrointestinal bleeding[1,2]. Diagnosis is usually in the middle and late stages, posing a serious threat to patients' health and lives. In recent years, traditional Chinese medicine (TCM) has shown good efficacy in treating gastric cancer and has gradually gained attention and recognition. Clinical studies[3] have shown that TCM has a positive role in preventing and treating gastric cancer, improving clinical symptoms, preventing disease progression, preventing recurrence and metastasis, and enhancing the efficacy and reducing the toxicity of chemotherapy.

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Fuzi Lizhong Tang (FZLZT) comes from Zhang Zhongjing's "Shanghan Lun", containing five Chinese herbal medicines: Fuzi, Ganjiang, Baizhu, licorice, and Ginseng/Dangshen. Ginseng was used in the original recipe, but modern preparations mostly use Dangshen. Its functions are warming and dispelling cold, strengthening qi and invigorating the spleen, mainly used for spleen and kidney yang deficiency or spleen and stomach cold deficiency[4]. Fuzi and Ganjiang in the formula are monarch drugs, which warm the center and dispel cold; Ginseng/Dangshen are ministerial drugs, which are sweet, warm, and tonify the spleen; Baizhu is an assistant drug, which invigorates the spleen and dries dampness; licorice is an envoy drug, which harmonizes the medicinal properties and relieves pain[5,6]. This study used network pharmacology combined with molecular docking technology to systematically analyze the active ingredients, targets, and signaling pathways of FZLZT, and studied its molecular mechanism of treating gastric cancer through *in vitro* anti-tumor experiments, providing scientific data and theoretical basis for clinical application.

Materials and Methods

Screening of Active Ingredients and Targets of Fuzi Lizhong Tang

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspe.com/tcmsp.php) was logged in, and Fuzi, Ganjiang, Baizhu, licorice, and Ginseng/Dangshen were used as keywords to search for active chemical components contained in FZLZT, and the active chemical components corresponding to the targets were collected using the targets in the TCMSP database, with the screening criteria being oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18.

Collection of Disease-Related Targets

GeneCards database (https://www.genecards.org/), OMIM database (https://www.omim.org/), and DrugBank database (https://go.drugbank.com/) were used to search for disease targets of gastric cancer with "gastric cancer" as the key search term. Venny 2.0 database (https//bioin-fogp.cnb.csic.es/tools/venny/index.html) was used to obtain the intersection of drug targets and gastric cancer targets as effective targets for FZLZT in treating gastric cancer.

Construction of the "Herbal Medicine-Active Ingredient-Target" Network

The relationship between the herbal medicine, active ingredients, and target genes of Fangji LiZhong decoction were used as nodes to build a network using Cytoscape 3.7.2 software for visualization.

Construction of Protein-Protein Interaction Network

The gastric cancer-related targets of Fangji LiZhong decoction were uploaded to the STRING database (https://string-db.org/) and limited to "Homo sapiens" species. The minimum interaction threshold was set to medium confidence (> 0.4), and other parameters were set to default to obtain protein-protein interaction (PPI) data. The PPI data were imported into Cytoscape 3.7.2 software (Cytoscape Consortium, San Diego, CA, USA) and CytoNCA plugin (Cytoscape Consortium, San Diego, CA, USA) was used to calculate the degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC). Targets with DC, BC, and CC greater than their respective medians were screened as core targets. The core targets were used to construct a PPI network.

GO Function and KEGG Pathway Enrichment Analysis

The gastric cancer-related targets of Fangji LiZhong decoction were input into the DAVID database (https://david.ncifcrf.gov/home.jsp) and analyzed with "Homo sapiens" selected as the species. The significance level was set at $p < 0.05$, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed. Enrichment information for biological processes (BP), cellular components (CC), molecular functions (MF), and KEGG key signaling pathways were obtained and visualized.

Molecular Docking Verification of Core Ingredients and Core Targets

The 2D structures of active ingredients were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The compounds were energy-minimized using Chem3D 14.0 software (PerkinElmer Inc. Waltham, Massachusetts, USA) and 3D structures were exported. The 3D structures of core targets were searched and downloaded from the PDB database (https://www.rcsb.org/). Using PyMOL software (Schrödinger, Inc., NY, USA), water molecules, solvent molecules, and ligands were removed from the core targets, and AutoDockTools 1.5.7

software (The Scripps Research Institute, La Jolla, CA, USA) was used for hydrogen and charge calculations. AutoDock Vina software (The Scripps Research Institute, La Jolla, CA, USA) was used for molecular docking to analyze the binding energy, and PyMOL software was used to visualize the docking results.

Cells and Drugs

Human gastric cancer cell lines AGS, BGC823, HGC-27, MGC-803, and SGC-7901 were purchased from Nanjing KeyGen Biotech Co., Ltd., and cultured in RPMI 1640 medium containing penicillin-streptomycin and 10% fetal bovine serum at 37°C in a 5% CO_2 incubator. The formula of Fuzi Lizhong decoction included Fuzi (18 g), Ganjiang (9 g), Baizhu (36 g), Gancao (18 g), and Dangshen (30 g), which was prepared as a decoction by the Department of Traditional Chinese Medicine at the First Affiliated Hospital of Nanyang Medical College and then concentrated to obtain a dry extract with a yield of 62 g, which was dissolved in saline to prepare a solution with a concentration of 2.0 mg/mL. Cisplatin (lot No. 4261022, HPLC ≥ 98.5%) was purchased from Beijing Solabiolife Science and Technology Co., Ltd. and prepared as a solution with a concentration of 20 μM in saline, which was stored at -20°C until use.

Reagents and Instruments

RPMI 1640 medium (lot No. 2378806), 0.25% trypsin- ethylenediamine tetraacetic acid (EDTA) (lot No. 1869505), and fetal bovine serum (lot No. 2036226) were purchased from Thermo Fisher Scientific (Wilmington, MA, USA); penicillin-streptomycin (100×, lot No. 20200317) and the ANNEXIN V-FITC/PI apoptosis detection kit (lot No. 20220312) were purchased from Beijing Solabiolife Science and Technology Co., Ltd. (Beijing, China); the cholecystokinin octapeptide (CCK-8) cell viability assay kit (lot No. TM566) was purchased from Dojindo Laboratories (Shiwa City, Iwate Prefecture, Japan).

The following instruments were used: a FA2204B electronic balance (Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China), an Olympus CKX41 inverted phase contrast microscope (Shinjuku-ku, Tokyo, Japan), an MCO-18AIC CO_2 incubator (Panasonic Healthcare Co., Ltd., Matsunokijima, Higashiyodogawa-ku, Osaka, Japan), a SpectraMax M5 microplate reader (Molecular Devices, LLC, San Jose, CA, USA), and a FACSCalibur flow cytometer [Becton, Dickinson and Company (BD), Becton-Dickinson, Franklin Lakes, NJ, USA].

CCK-8 Assay for Cell Proliferation

Logarithmic-phase human gastric cancer cells were harvested, digested with trypsin, and resuspended to obtain a cell suspension with a density of 3×104 cells/well, which was seeded into a 96-well plate. After 24 h of culture, different concentrations of Fuzi Lizhong decoction (250.00, 125.00, 62.50, 31.25, 15.63, 7.81, 3.91, and 1.95 μg/mL) were added and incubated for 48 h. The blank control group was used for zero calibration, and each group had at least 3 replicate wells. The experiment was repeated 3 times. At the end of the experiment, 10 μl of CCK-8 solution was added to each well, and the plate was incubated for another 3 h. The absorbance (A) at 450 nm of each well was measured using a microplate reader, and the cell viability was calculated as follows: Cell viability (%) = (OD value of experimental group - OD value of blank control)/(OD value of control group - OD value of blank control) × 100%.

Measurement of Cell Apoptosis Rate Using ANNEXIN V-FITC/PI Double Staining and Flow Cytometry

Human gastric cancer cells SGC-7901 in the logarithmic growth phase were treated with trypsin digestion, resuspended in cell suspension, and seeded in 6-well plates. After 24 hours of cell adherence, different concentrations of Fuzi Lizhong decoction (65 and 130 μg/mL) or cisplatin (3 μM) were added and incubated for 48 hours. Cells were collected after trypsin digestion and subjected to the ANNEXIN V-FITC/PI staining protocol strictly according to the kit instructions. The apoptosis rate was determined by flow cytometry.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). All data were presented as mean ± standard deviation. One-way analysis of variance was used for comparison between multiple groups, and t was used for comparison between any two groups. $p < 0.05$ was considered statistically significant.

Results

Active Ingredients and Target Proteins of Fuzi Lizhong Tang

A total of 117 active ingredients were collected from the TCMSP database by screening OB and DL values. Among them, licorice had 92 active ingredients, *Codonopsis pilosula* had

21, *Aconitum carmichaelii* had 19, *Atractylodes macrocephala* had 7, and dried Ginger had 5 (Table I). After removing duplicates, a total of 261 target proteins were obtained.

Collection of Gastric Cancer-Related Targets for Fuzi Lizhong Tang

After merging and removing duplicates from GeneCards, OMIM, and DrugBank databases, a total of 11,373 gastric cancer-related targets were obtained. The intersection of the active ingredient targets of Fuzi Lizhong Tang and gastric cancer targets was taken using the online analysis tool Venny 2.1.0, and a Venn diagram was drawn, which showed that there were 211 common target proteins (Figure 1).

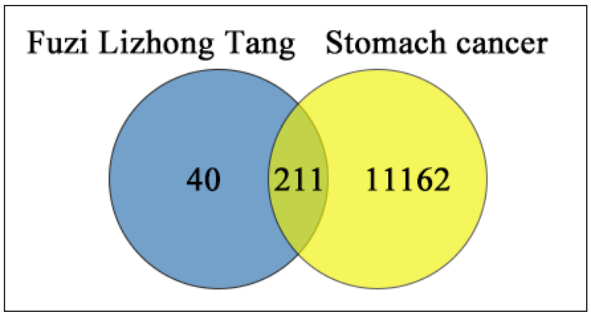

Figure 1. Venn diagram showing the common targets of the active ingredients in Fuzi Lizhong Tang and gastric cancer-related targets.

Construction of PPI Network and Analysis of Core Target Genes

The 211 potential target genes of Fuzi Lizhong Tang for the treatment of gastric cancer were uploaded to the STRING database to construct a protein interaction network diagram (Figure 2), which consisted of 156 nodes and 566 edges. The DC, BC, and CC values of network nodes were calculated using the CytoNCA plugin in Cytoscape 3.7.2 software. The target genes with DC, BC,

Table I. Screening list of active ingredients in Fuzi Lizhong Tang (only showing the top 5 active ingredients ranked by OB value for each herb).

Traditional Chinese Medicine	MOLID	Molecular Name	OB (%)	DL
Licorice	MOL002311	Glycyrol	90.78	0.67
	MOL004990	7,2',4'-trihydroxy-5-methoxy-3-arylcoumarin	83.71	0.27
	MOL004904	Licopyranocoumarin	80.36	0.65
	MOL004891	Shinpterocarpin	80.3	0.73
	MOL005017	Phaseol	78.77	0.58
Odonopsis Pilosula	MOL002140	Perlolyrine	65.95	0.27
	MOL005321	Frutinone A	65.9	0.34
	MOL008400	Glycitein	50.48	0.24
	MOL008397	Daturilin	50.37	0.77
	MOL008407	(8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-1,2,4,7,8,9,11, 12,14,15,16,17-dodecahydrocyclopenta phenanthren-3-one	45.4	0.76
Fuzi	MOL002421	Ignavine	84.08	0.25
	MOL002419	(R)-Norcoclaurine	82.54	0.21
	MOL002398	Karanjin	69.56	0.34
	MOL002388	Delphin_qt	57.76	0.28
	MOL002395	Deoxyandrographolide	56.3	0.31
Atractylodes macrocephala	MOL000022	14-acetyl-12-senecioyl-2E,8Z,10E-atractylentriol	63.37	0.3
	MOL000020	12-senecioyl-2E,8E,10E-atractylentriol	62.4	0.22
	MOL000021	14-acetyl-12-senecioyl-2E,8E,10E-atractylentriol	60.31	0.31
	MOL000049	3β-acetoxyatractylone	54.07	0.22
	MOL000028	α-Amyrin	39.51	0.76
Dried Ginger	MOL002514	Sexangularetin	62.86	0.3
	MOL002501	[(1S)-3-[(E)-but-2-enyl]-2-methyl-4-oxo-1-cyclopent-2-enyl] (1R,3R)-3-[(E)-3-methoxy-2-methyl-3-oxoprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate	62.52	0.31
	MOL002464	1-Monolinolein	37.18	0.3
	MOL000358	Beta-sitosterol	36.91	0.75
	MOL000359	Sitosterol	36.91	0.75

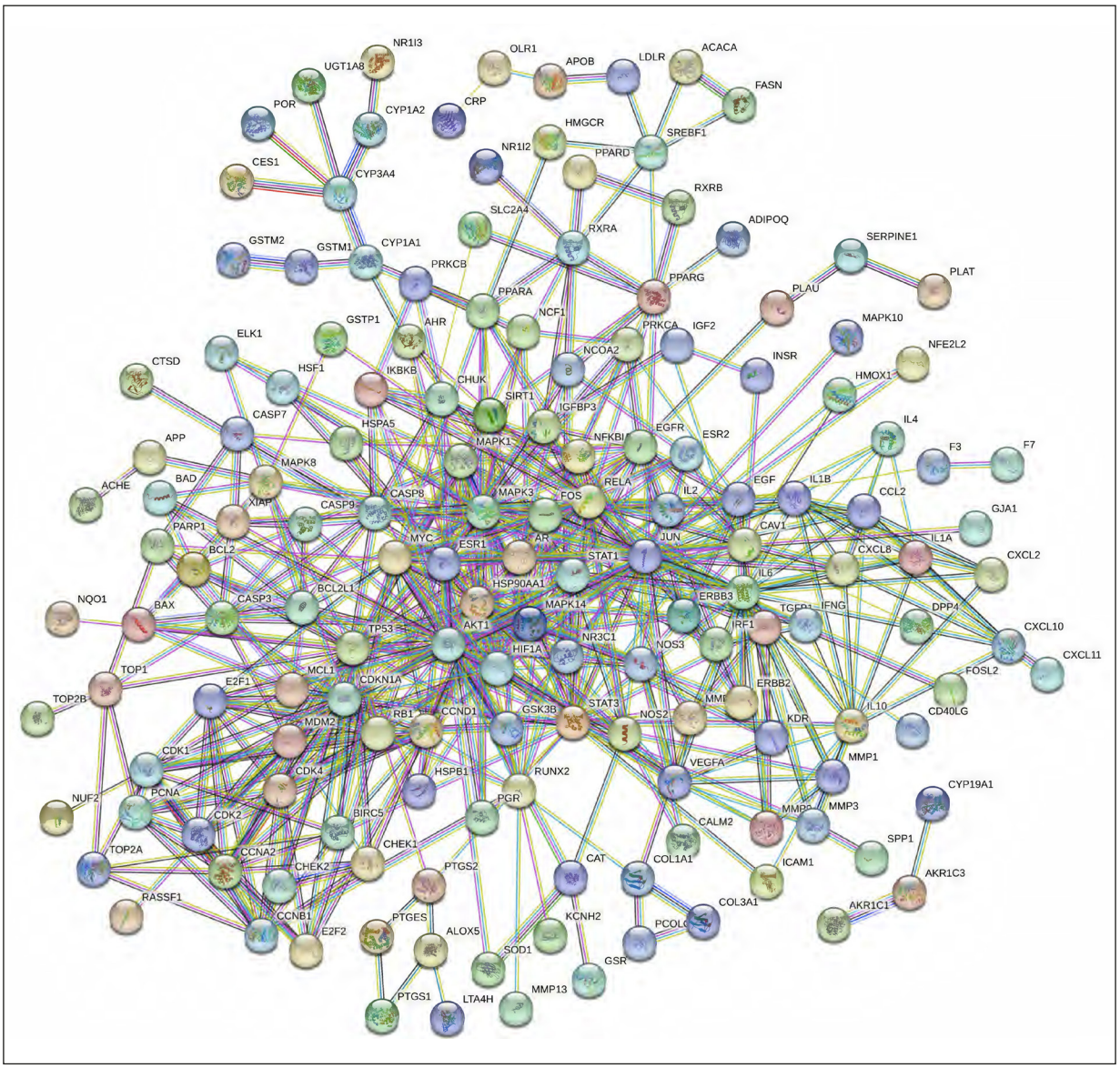

Figure 2. PPI network diagram of target genes of Fuzi Lizhong Tang for the treatment of gastric cancer.

and CC values greater than the median values of 5.0, 66.08, and 0.17, respectively, were first selected. Then, the target genes with DC, BC, and CC values greater than the median values of 11.0, 302.0, and 0.17, respectively, were further screened. Finally, seven core target genes were identified, including *CDKN1A, MYC, MAPK1, MAPK14, RB1, RELA,* and *STAT3* (Figure 3).

Construction of "Herb-Active Ingredient-Target Gene" Network

A "Herb-Active Ingredient-Target Gene" network model was constructed using Cytoscape 3.7.2 software. The deep blue V-shaped

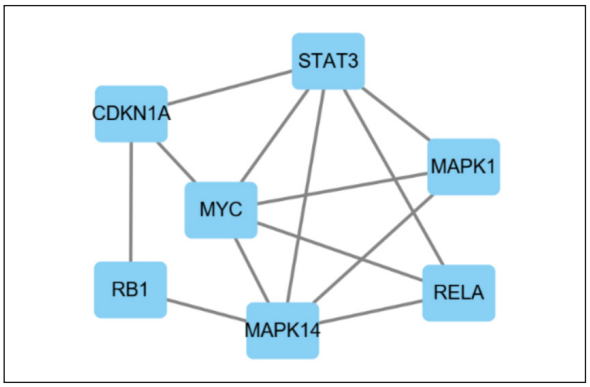

Figure 3. Interactions between core target genes for the treatment.

nodes represented herbs, the red rounded rectangle nodes represented active ingredients, and the light blue circular nodes represented target genes. A total of five herbs, 117 chemical components, and 235 target genes were involved in the network (Figure 4). The results showed that the same compound could act through different molecular targets, and different compounds could also act on the same target. This network reflects the characteristics of Fuzi Lizhong Tang in treating gastric cancer with multiple components and multiple targets. Using a screening condition of DC $\geq$ 30, ten core active ingredients were identified, including quercetin, 7-methoxy-2-methyl isoflavone, kaempferol, luteolin, naringenin, formononetin, isorhamnetin, licochalcone A, beta-sitosterol, and medicarpin.

GO Biological Function and KEGG Pathway Enrichment Analysis

The results from the DAVID database showed a total of 1,155 GO terms, including 858 biological process (BP) terms, which mainly involved positive regulation of gene expression (GO: 0010628), positive regulation of transcription from RNA polymerase II promoter (GO: 0045944), response to estradiol (GO: 0032355), response to xenobiotic stimulus (GO: 0009410), and positive regulation of transcription, DNA-templated (GO: 0045893).

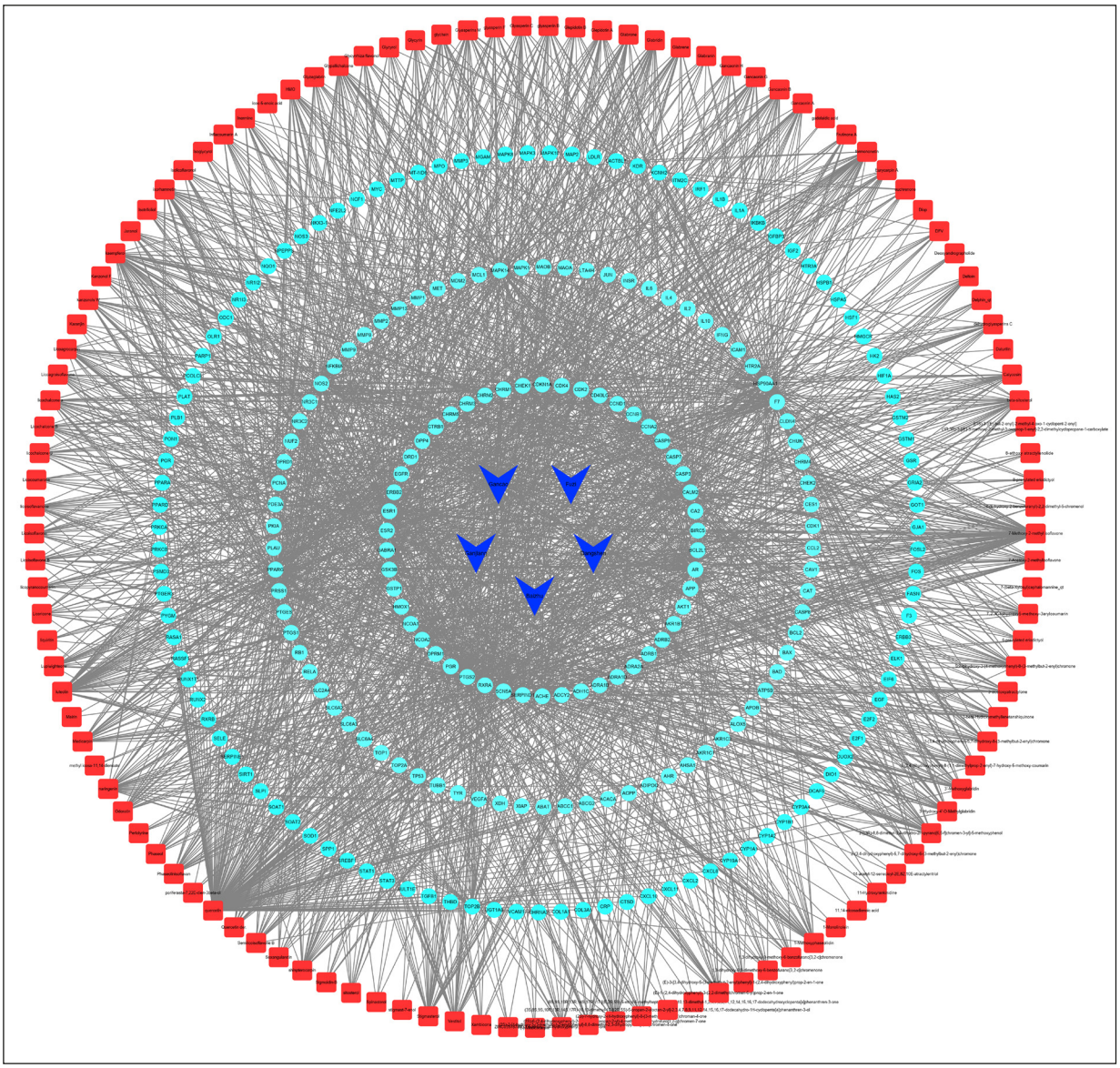

Figure 4. Network diagram of "Chinese herbs-active components-targets" for Fuzi Lizhong Tang.

There were 105 cellular component (CC) terms, including extracellular space (GO: 0005615), macromolecular complex (GO: 0032991), nucleoplasm (GO:0005654), membrane raft (GO: 0045121), and chromatin (GO: 0000785). There were 192 molecular function (MF) terms, including enzyme binding (GO: 0019899), identical protein binding (GO: 0042802), RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding (GO: 0004879), protein binding (GO: 0005515), and protein homodimerization activity (GO: 0042803) (Figure 5).

The KEGG pathway analysis revealed a total of 177 enriched pathways, mainly focusing on cancer-related pathways (hsa05200: pathways in cancer), lipid and atherosclerosis (hsa05417: lipid and atherosclerosis), advanced glycation end products (AGE)/receptor for advanced glycation end products (RAGE) signaling pathway in diabetic complications (hsa04933: AGE-RAGE signaling pathway in diabetic complications), fluid shear stress and atherosclerosis (hsa05418: fluid shear stress and atherosclerosis), chemical carcinogenesis - receptor activation (hsa05207: chemical carcinogenesis - receptor activation), and interleukin (IL)-17 signaling pathway (hsa04657: IL-17 signaling pathway) (Figure 6).

Molecular Docking Validation

The 7 core targets and 10 active ingredients were subjected to molecular docking analysis. A lower binding energy indicates a more stable ligand-receptor interaction and a binding energy less than 0 indicates spontaneous binding. Generally, a binding energy lower than -5 kcal/mol is considered a good score. The results from Figure 7 and Figure 8 show that the main active ingredients in Fuzi Lizhong Tang had a binding energy lower than -5 kcal/mol with the core targets, indicating a good binding interaction. The lower the binding energy, the more stable the conformation. The results demonstrated that the molecular docking analysis was consistent with the network pharmacology screening results. The PyMOL

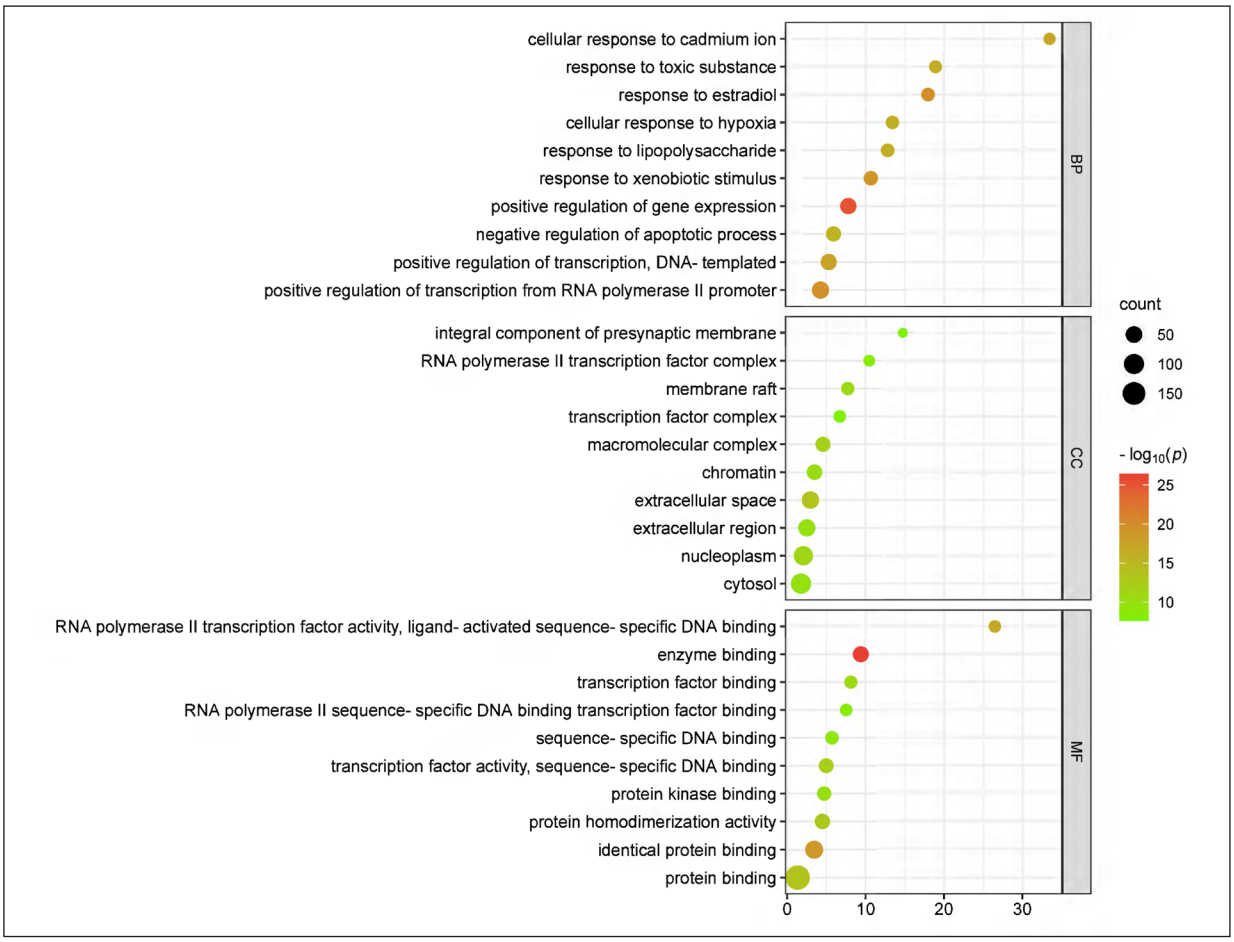

Figure 5. Top ten enriched GO terms related to the therapeutic effect of Fuzi Lizhong Tang on gastric cancer.

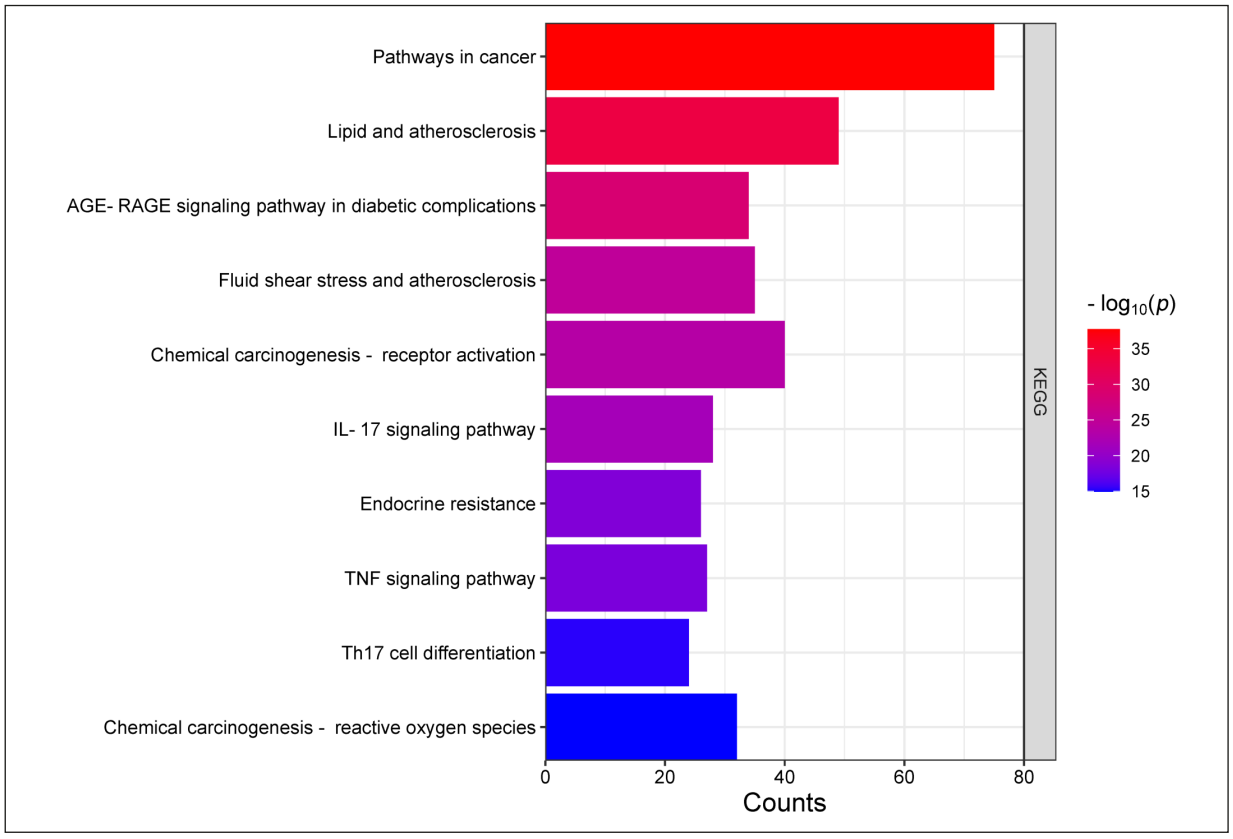

Figure 6. Top ten enriched KEGG pathways related to the therapeutic effect of Fuzi Lizhong Tang on gastric cancer.

software was used to visualize the best conformations of the docked ligands and targets, showing that the active ingredients of Fuzi Lizhong Tang were tightly bound to the target proteins through hydrogen bonds and hydrophobic interactions.

Effects of Fuzi Lizhong Tang on the Proliferation of Various Human Gastric Cancer Cell Lines

The study used the CCK-8 assay to evaluate the proliferation rate of multiple human gastric cancer cell lines (AGS, BGC823, HGC-27, MGC-803, and SGC-7901) treated with Fuzi Lizhong Tang. The results showed that Fuzi Lizhong Tang had an inhibitory effect on the proliferation of all five cell lines, with an IC_{50} value of (64.12 ± 16.89) μg/mL for SGC-7901, which exhibited the strongest growth inhibitory effect (Table II).

Effects of Fuzi Lizhong Tang on Apoptosis of Human Gastric Cancer Cells

Based on the IC_{50} values obtained from the CCK-8 assay for the five human gastric cancer cell lines, SGC-7901, with the lowest IC_{50} value, was selected for the ANNEXIN V-FITC/PI double staining assay and flow cytometry analysis of apoptosis. The results showed that compared to the control group with an apoptotic rate of (2.39 ± 0.91)%, the treatment with Fuzi Lizhong Tang at doses of 65 and 130 μg/mL resulted in apoptotic rates of (21.22 ± 3.38)% and (33.68 ± 2.61)%, respectively. The apoptotic rate induced by cisplatin (3 μM) was (35.41 ± 3.17)% (Figure 9).

Table II. IC_{50} values of Fuzi Lizhong Tang on multiple human gastric cancer cell lines.

Gastric cancer cells	Fuzi Lizhong Tang (μg/mL)
AGS	227.69±42.93
BGC823	655.04±48.81
HGC-27	388.59±86.94
MGC-803	190.70±18.38
SGC-7901	64.12±16.89

Discussion

The incidence of gastric cancer has been increasing globally, making it a significant health

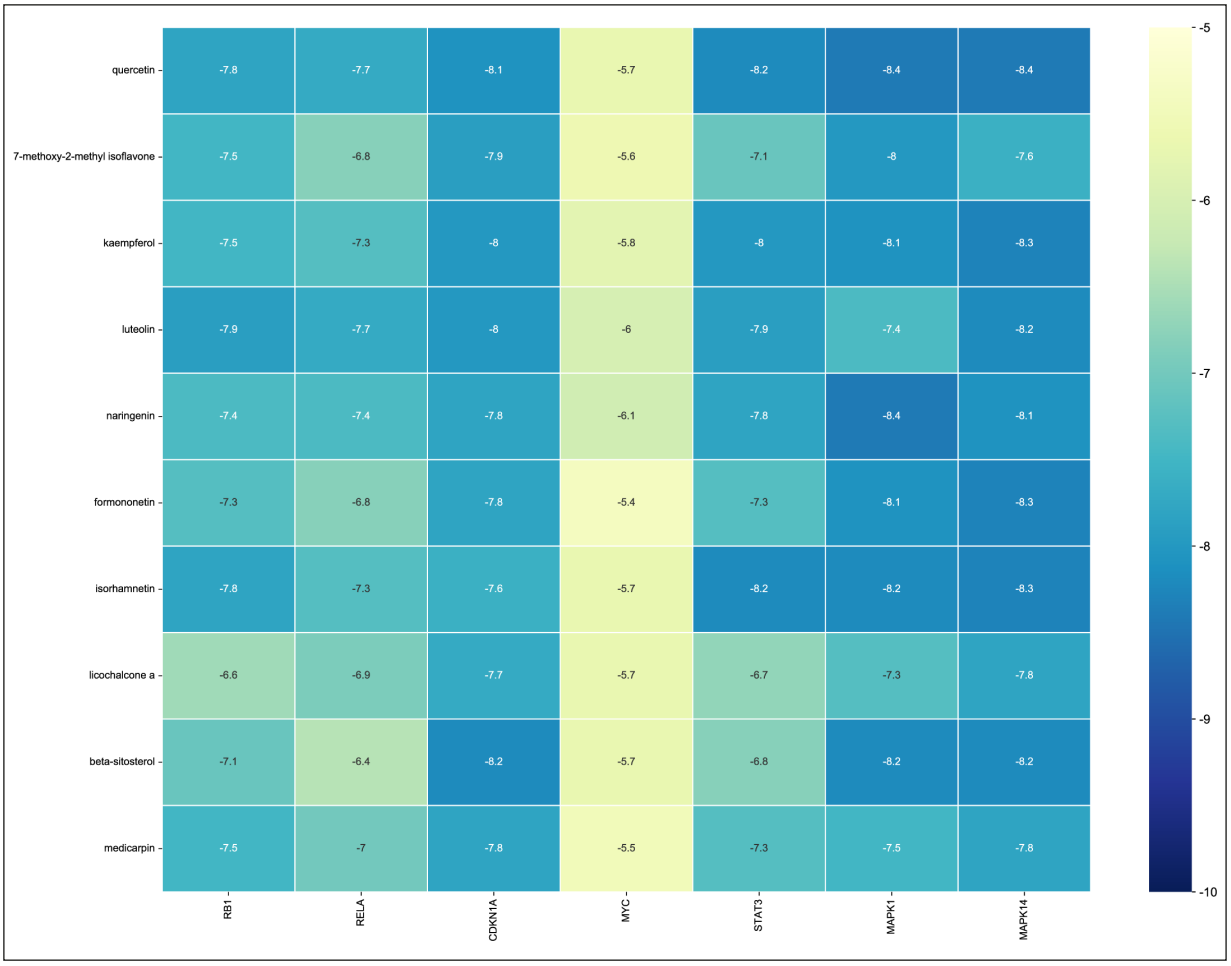

Figure 7. Heat map analysis of the binding energy between the core components and core targets of Fuzi Lizhong Tang.

concern with high mortality rates among tumor diseases. Current treatment options mainly involve surgery, radiotherapy, and chemotherapy[7-9]. However, to improve clinical outcomes, there is a growing trend towards adopting comprehensive diagnosis and treatment methods that include combined biological therapy and traditional Chinese medicine. Traditional Chinese medicine has gained attention for its potential in preventing and treating gastric cancer, giving it a unique advantage in the treatment of gastric cancer in China[10-13]. In this study, the researchers investigated

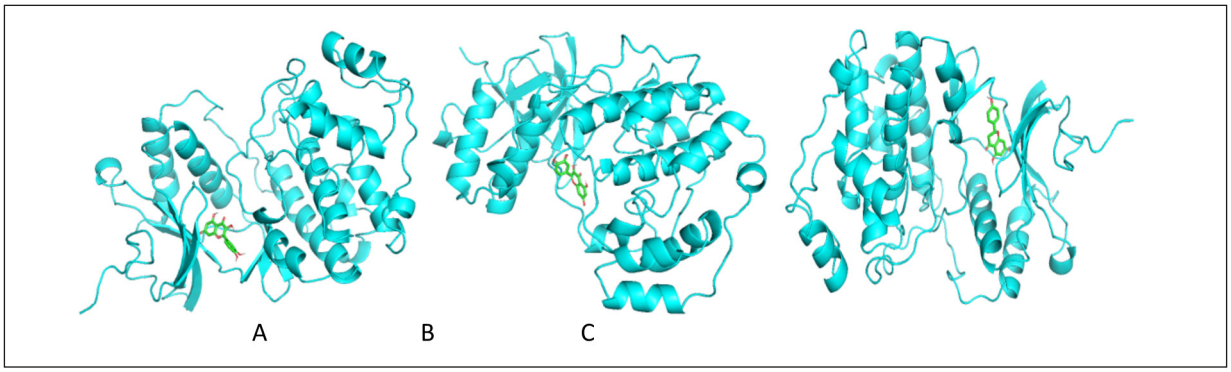

Figure 8. Heat map of binding energies between core components and core targets of Fuzi Lizhong Tang **A**, quercetin and MAPK1. **B**, quercetin and MAPK14. **C**, naringenin and MAPK1. Binding energies for all are -8.4 kcal/mol.

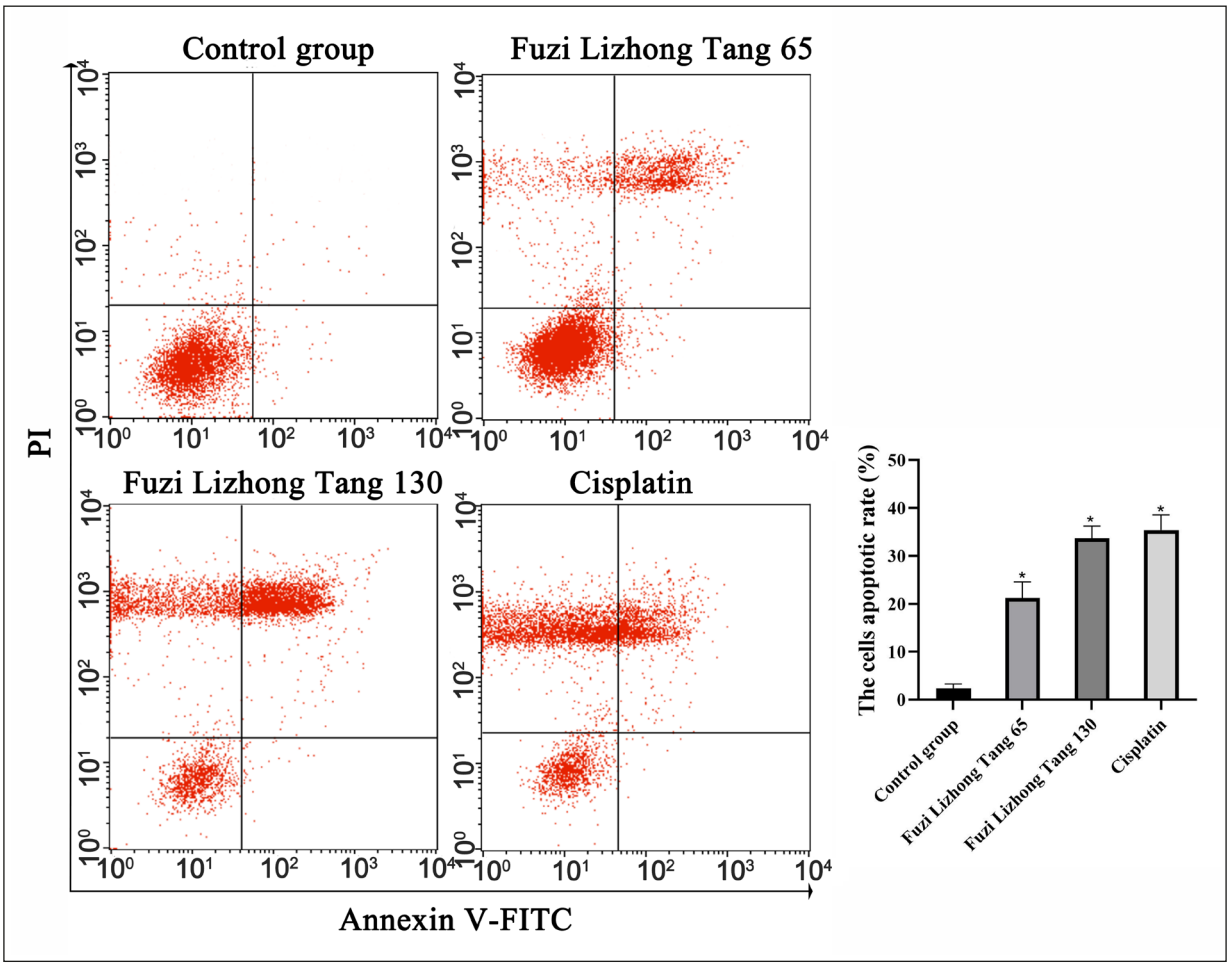

Figure 9. Effects of Fuzi Lizhong Tang on apoptosis in human gastric cancer cells SGC-7901 using ANNEXIN V-FITC/PI double staining and flow cytometry. N = 3/group, *compared to the control group, $p < 0.01$.

the therapeutic potential of Fuzi Lizhong Decoction, a traditional Chinese medicine with a history of use in various conditions, including cancer. Modern pharmacological research[14-16] has shown that this decoction possesses multiple biological effects, including anti-inflammatory, analgesic, immune-enhancing, anti-arrhythmia, blood sugar, blood lipid-lowering, and anti-tumor effects. A clinical study[17] reported that combining Fuzi Lizhong Tang. Decoction with conventional chemotherapy resulted in a higher short-term effective rate and reduced adverse reactions in postoperative gastric cancer patients compared to conventional treatment alone.

To elucidate the underlying mechanisms of Fuzi Lizhong Tang in treating gastric cancer, we employed network pharmacology and molecular docking techniques. Through "Traditional Chinese Medicine-Active Ingredient-Target" network analysis, we identified several active ingredients with anti-tumor effects, including quercetin, kaempferol, luteolin, naringenin, and others[18]. These active ingredients have been previously reported for their inhibitory effects on various cancers, including gastric cancer. For instance, quercetin was found[19] to inhibit epithelial-mesenchymal transition and promote apoptosis in gastric cancer cells. Kaempferol was shown[20] to induce apoptosis and autophagic death of gastric cancer cells. Luteolin inhibited gastric cancer cell proliferation and angiogenesis mimicry formation[21]. Naringenin reduced gastric cancer cell proliferation, adhesion, invasion, and migration[22]. These findings support the potential of Fuzi Lizhong Tang as an anti-gastric cancer therapy. Some studies[23-26] investigated the anti-cancer effects of specific active ingredients identified in our study, such as quercetin, kaempferol, luteolin, and naringenin. These studies consistently demonstrated the potential of traditional Chinese medicine in treating gastric cancer

and highlighted the importance of targeting key signaling pathways, similar to our research.

Furthermore, topological analysis of the protein-protein interaction (PPI) networks revealed seven core targets crucial in the treatment of gastric cancer with Fuzi Lizhong Tang (FZLZT). These targets are *CDKN1A, MAPK1, MAPK14, MYC, RB1, RELA,* and *STAT3*, and they play significant roles in regulating cell cycle, proliferation, apoptosis, and immune responses in various cancers, including gastric cancer[27].

The researchers performed GO and KEGG enrichment analysis, which indicated that Fuzheng Lizhong decoction mainly targets signaling pathways related to cancer, inflammation, metabolism, and hemorheology in the treatment of gastric cancer. The biological functions of the core anti-cancer targets, as identified through network pharmacology, are primarily concentrated in the Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3), P38MAPK, extracellular signal-regulated kinase (ERK), and nuclear factor-kappaB (NF-κB) signaling pathways, crucial targets for cancer treatment[28]. Experimental validation *in vitro* confirmed that Fuzheng Lizhong decoction (FZL) effectively inhibited the proliferation of human gastric cancer cells and induced apoptosis, consistent with the predicted anti-cancer effects through network pharmacology and molecular docking[29]. Comparative analysis with existing research on Traditional Chinese medicine and anti-gastric cancer therapies revealed similarities and differences in active ingredients and target pathways. For instance, quercetin, kaempferol, luteolin, and naringenin have been identified in other studies[30] for their anti-cancer properties in various cancer types, including gastric cancer. However, specific interactions with target proteins may vary, suggesting that Fuzi Lizhong Tang could offer a unique combination of active ingredients and pathways for treating gastric cancer.

Conclusions

In conclusion, this study provides valuable insights into the potential therapeutic effects of Fuzi Lizhong Tang Decoction in treating gastric cancer, highlighting the active ingredients, core targets, and molecular mechanisms involved. The integration of network pharmacology and molecular docking offers a powerful approach to identifying potential anti-cancer compounds and their targets. However, further *in vivo* experiments and clinical trials are essential to validate the findings and establish the efficacy and safety of Fuzi Lizhong Tang in clinical applications.

Ethics Approval

The protocol was approved by the ethics committee of First Affiliated Hospital of Nanyang Medical College. Ethical No.: 9278115.

Informed Consent

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Conflict of Interest

The authors declare that they have no competing interests.

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None.

Authors' Contributions

Fangyi Zhang designed the research study and performed the research. Suicheng Guo conducted experiments and analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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