Therapeutic efficacy evaluation and mechanism of action based on meta-analysis and network pharmacology of Li Chong Decoction (Bolus) for cancer treatment

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Abstract: Li Chong Decoction (Bolus) is a traditional Chinese medicine (TCM) prescription with notable antitumor efficacy. This study focused on evaluating the therapeutic efficacy and mechanism of action of Li Chong Decoction (Bolus) for cancer treatment, using meta-analysis and network pharmacology. Firstly, we adopted an evidence-based medicine research method to collect as many clinical research reports as possible on the treatment of tumors with Li Chong Decoction (Bolus). We then conducted a meta-analysis to confirm the efficacy and safety of Li Chong Decoction (Bolus) in treating tumors. Lastly, we used the network pharmacology method to predict the intervention mechanism of Li Chong Decoction (Bolus) in treating tumors. The meta-analysis showed that the total effective rate and safety of Li Chong Decoction (Bolus) were significantly higher than those of the control group ($P < 0.01$). From network pharmacology, we identified 111 compounds in Li Chong Decoction (Bolus) and 339 targets shared between Li Chong Decoction (Bolus) and cancer. The significant protein targets of Li Chong Decoction (Bolus) for cancer intervention were AKT1, TP53, TNF, IL6, JUN, VEGFA, MYC, ESR1, EGFR, and CASP3. GO analysis was performed, in which the entries with the highest number of enriched genes were protein binding, cytosol, and nucleus, with 291, 179, and 166 genes distributed, respectively. For KEGG analysis, 1894 channels were enriched, with pathways related to cancer among the top 20, such as pathways in cancer, prostate cancer, endocrine resistance, proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, bladder cancer, pancreatic cancer, IL-17 signaling pathway, C-type lectin receptor signaling pathway, and hepatocellular carcinoma. Molecular docking results showed that quercetin, luteolin, and kaempferol could dock well with AKT1, TNF, VEGFA, and EGFR, and luteolin-AKT1 and quercetin-AKT1 had the best binding degree. Li Chong Decoction (Bolus) maintained its efficacy and safety in cancer treatment. The effective TCM components of Li Chong Decoction (Bolus) might inhibit cancer by targeting multiple biological processes of hub genes mentioned above. Currently, research on the treatment of non-gynecological cancers with Li Chong Decoction (Bolus) mainly focuses on hepatocellular carcinoma.

Keywords: Li Chong Decoction (Bolus); Traditional Chinese medicine; Cancer; Meta-analysis; Network pharmacology; Data mining

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1. Introduction

Cancer is a significant threat to human public health security as it can potentially become the leading cause of mortality by 2060, according to the WHO[1,2]. Unfortunately, apart from a few types of cancers, most malignancies do not have effective therapies, resulting in heavy burdens for individuals, families, and societies. As a result, it is crucial to continue enhancing modern research on tumors and explore therapies in traditional medicine as well.

Recent studies have demonstrated that traditional Chinese Medicine (TCM) has promising clinical effects in treating cancer[3]. TCM has been found to participate in the metabolic pathways of tumor cells and can exert antitumor effects by inhibiting tumor cell regeneration, propagation, and metastasis while enhancing mitophagy and apoptosis[4,5]. Regarding immunity, TCM can modulate the immune system by using Chinese herbal medicine

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to fight pathogenic factors and strengthen the body's resistance[6]. Moreover, TCM can intervene in tumors through non-pharmacological modalities, such as acupuncture and moxibustion, manipulation, and breathing techniques. The modernization of TCM has been the subject of several studies, which has helped researchers abroad gain a better understanding of TCM. This, in turn, has contributed to the promotion and dissemination of TCM, making it a significant area of research[7].

Li Chong Decoction (Bolus) is a formula created by Mr. Xichun Zhang, a master of modern Chinese medicine, to treat abdominal masses[8]. It has been widely used in clinical settings, and recently, researchers have found that Li Chong Decoction (Bolus) can aid in the treatment of hysteromyoma through multiple mechanisms[9]. Related experimental studies have shown that Li Chong Decoction (Bolus) can regulate hypoxia-inducible factor-1α and VEGF to exert its anti-uterine effects[10]. Li Chong Decoction (Bolus) can also reduce the mRNA expressions of Bcl-2 and Bcl-2-related X protein[11]. Similarly, Li Chong Decoction (Bolus) can reduce the expression of IGF-1 mRNA[12]. These alterations are all beneficial for the management of uterine fibroids. Some researchers have also found that Li Chong Decoction (Bolus) can be used to treat other tumors. For instance, Xiaofeng Zhang has used Li Chong Decoction (Bolus) to treat colorectal cancer and achieved good results[13]. In addition, Wenhui Niu's experiments using Li Chong Decoction have shown that the decoction inhibits the viability, migration, and invasion of human hepatoma Hep G2 cells[14].

However, current research on Li Chong Decoction (Bolus) is mainly focused on uterine fibroids and ovarian cancer, and its therapeutic effect on other malignant tumors lacks credible research. In order to further explore the potential of Li Chong Decoction (Bolus) for the treatment of tumors, especially malignancies, we decided to conduct new research using meta-analysis and network pharmacology as methods.

Meta-analysis and network pharmacology are widely accepted research methods[15]. Meta-analysis, in particular, has become the cornerstone of modern evidence-based medicine[16]. Network pharmacology is a new research method that combines drug ingredients with drug and disease targets. It can predict the mechanism of action of TCM formulas through target binding, pathway enrichment, and molecular docking, making it a valuable approach[17–19].

In our present study, we first conducted a meta-analysis to gather as many published articles on the treatment of cancer with Li Chong Decoction (Bolus) as possible. This helped us confirm the effectiveness of Li Chong Decoction (Bolus) in treating cancer. We then applied network pharmacology to predict the potential mechanism by which Li Chong Decoction (Bolus) exerted therapeutic effects on cancer. This provided a research basis for the next steps, such as cellular and animal experiments, and also laid a foundation for further clinical use. Additionally, we hope that this study will offer new ideas for oncologists in clinical decision-making.

2. Methods

2.1. Meta-analysis

2.1.1. Literature search

We conducted a literature search in both English and Chinese between January 2000 and December 2022 on the treatment of tumors using Li Chong Decoction (Bolus). We searched publicly available articles using

computerized databases, including Web of Science, PubMed, Cochrane Library, CNKI, Wanfang, and Weipu. Our search terms were focused on the following subject headings: “Li Chong Decoction (Bolus)”, “neoplasm”, “effective rate”, and “clinical observation”.

2.1.2. Inclusion and exclusion criteria

The types of studies included in our search were clinical studies on the treatment of tumors using Li Chong Decoction (Bolus), which were published online. The inclusion criteria for literature were as follows: the exposure factor was the use of Li Chong Decoction, the outcomes were related to neoplastic diseases, and the study design was a randomized controlled trial (RCT). The literature needed to provide an association (odds ratio and 95% confidence interval) between Li Chong Decoction (Bolus) and disease cure rate. The exclusion criteria for literature were studies on the use of non-rational punch decoction, studies on disease risk caused by tumors, and studies with duplicate reports. Additionally, studies with unclear observational indexes and studies that could not provide reliable raw data for meta-analysis were excluded. Lastly, studies that were not sufficiently informative to be utilized were also excluded from the analysis.

2.1.3. Literature data extraction and quality assessment

The screening and data extraction process should be carried out independently by two researchers, and any discrepancies should be discussed and resolved among the involved professionals. If a consensus cannot be reached, a third person should be consulted for arbitration. The extracted data should include the first author’s name, age, publication year, subject population, number of studies, disease type, experimental grouping, and follow-up time. The quality of the literature should be evaluated based on the Cochrane Handbook’s quality criteria for RCTs, which were categorized as high-risk, low-risk, or unclear. Studies with a high risk of bias, such as an unjustified design protocol, subject and testing indication bias, and high-risk stigmata, should be excluded during the statistical analysis and data interpretation.

2.1.4. Statistical methods

In this study, we conducted a meta-analysis of the collected data using Review Manager 5.3 software. Dichotomous variables were expressed using odds ratios (OR) and 95% confidence intervals (CIs). We performed a heterogeneity test, and if the *P*-value was greater than 0.05 and I^2 was less than 50%, we analyzed the data using a fixed-effects model. However, if the *P*-value was less than 0.05 or I^2 was greater than 50%, we adopted a random-effects model for analysis.

2.2. Network pharmacology

2.2.1. Collection of active ingredients in Li Chong Decoction (Bolus)

In the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and Swiss Target Prediction database, all the ingredients of Li Chong Decoction (Bolus) were searched, including “raw astragalus”, “codonopsis pilosula”, “atractylodes macrocephala”, “zedoary”, “rhizome sparganii”, “Chinese angelica”, “leech”, “membranes of chicken gizzards”, “anemarrhena asphodeloides”, “peach kernel”, “trichosanthes root”, and “Chinese yam”. The compound screening conditions were set to include drug-like properties (DL) ≥ 0.18 and oral bioavailability (OB) ≥ 30. After removing duplicates and irrelevant compounds, an Excel table was imported to establish a library of easily absorbed compounds from the active components of Li Chong Decoction (Bolus). The library was further supplemented and improved by consulting the relevant literature.

2.2.2. Component target prediction of Li Chong Decoction and construction of "drug-component-target" network diagram

The study utilized the TCMSP database and SWISS Target prediction database to search for relevant target information on the active ingredients. Following screening and deduplication, human target and standard gene names were selected through the Uniprot database. A drug active ingredient target network diagram was created using Cycloscape 3.9.0 software.

2.2.3. Acquisition of disease-related targets

We used the keywords "cancer", "benign tumor", "malignant tumor", and "malignancy" to search for disease-related targets in the GeneCards database. This allowed us to establish a database of tumor-related targets.

2.2.4. Screening of drug-disease common targets and construction of interaction network diagram

We used the search words "cancer", "benign tumor", "malignant tumor", and "malignancy" to collect disease-related targets from the GeneCards database and establish a tumor-related targets database. Then, we used Venny 2.1 to intersect the predicted targets of "Li Chong Decoction (Bolus)" with the related targets of cancer to establish the gene target database of "Li Chong Decoction (Bolus)" against cancer. The common gene targets were introduced into STRING, and the species selected was Homo sapiens. We obtained the target interaction network and imported it into the Cytoscape 3.9.0 software for topology analysis and construction of the protein-protein interaction (PPI) network diagram. We adjusted the attributes of nodes in the network according to the degree value of the nodes.

2.2.5. Analysis of gene pathway and biological function

The anticancer target of "Li Chong Decoction (Bolus)" was uploaded to the DAVID platform for enrichment analysis of the KEGG pathway ($P < 0.05$) and annotation of GO biological function. The top 20 KEGG pathways and top 30 GO biological functions were screened out for visualization.

2.2.6. Molecular docking between core components and core targets

The AKT1, TNF, VEGFA, and EGFR proteins are currently the focus of both clinical and basic research, as evidenced by their high Degree values in the Cycloscape 3.9.0 software. These proteins were selected as docking receptors, while quercetin, luteolin, and kaempferol, the three most potent active ingredients, were selected as ligands for docking using AutoDock tools.

3. Results

3.1. Results of the meta-analysis

Figure 1 displays the results of five studies (involving 332 patients) that compared the efficacy of Li Chong Decoction (Bolus) treatment to that of a control group for the treatment of various diseases[20–24]. When $P < 0.05$ and $I^2 > 50\%$, a random effect model was utilized. The Li Chong Decoction (Bolus) treatment group showed a significantly higher total effective rate of patients compared to the control group [OR = 7.61, 95% CI (4.45, 13.02), $P < 0.001$] (Fig. 1A).

Furthermore, a subsequent safety analysis of three studies (220 patients)[24–26] indicated that, compared to the control group, the total effective rate of patients in the Li Chong Decoction (Bolus) treatment group was also significantly higher [OR = 0.36, 95% CI (0.18, 0.71), $P < 0.01$] (Fig. 1B). The efficacy and safety bias analyses are presented in Figure 1C and 1D, respectively.

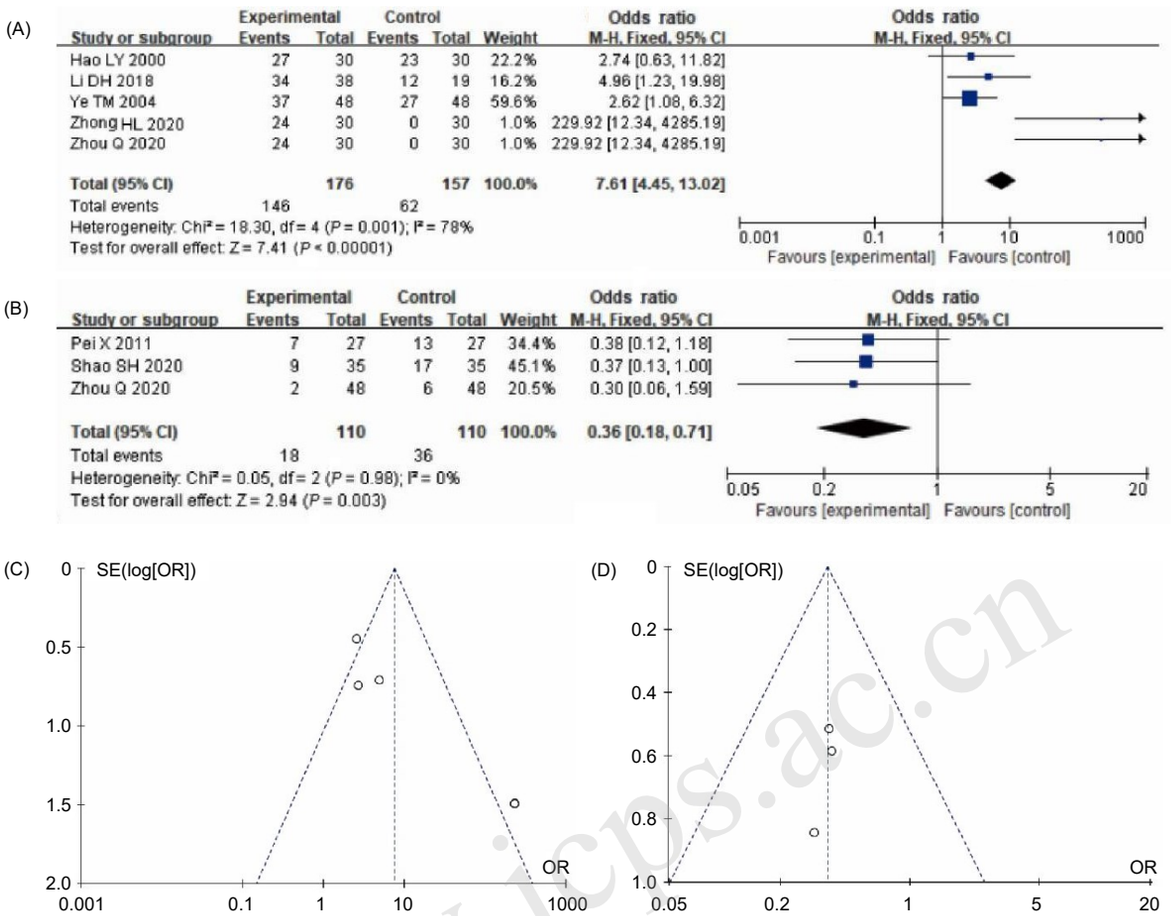

Figure 1. The results of the meta-analysis. (A) Comparison of total effective rate between Li Chong Decoction (Bolus) group and control group; (B) Comparison of safety between Li Chong Decoction (Bolus) group and control group; (C) Funnel chart of total effective rate in literature publication bias; (D) Funnel chart of safety in literature publication bias.

3.2. Results of network pharmacology

3.2.1. Screening results of active ingredients of Li Chong Decoction (bolus)

The study screened the potential active compounds of each medicinal herb by utilizing TCMSP and herb databases. A total of 18 compounds from Astragalus membranaceus, 19 compounds from Codonopsis pilosula, six compounds from Atractylodes macrocephala, two compounds from snakegourd root, 14 compounds from Chinese yam, 12 compounds from Anemarrhena asphodeloides, five compounds from curcuma zedoary, two compounds from Chinese angelica, 19 compounds from peach kernels, six compounds from membranes of chicken gizzards, and five compounds from leech were retrieved and included in the study. A detailed list of these compounds can be found in the attached document. Compounds with degree values > 20, OB% and DL values were also identified and are presented in the following section (Table 1).

3.2.2. Collection of predicted targets and diseases of Li Chong Decoction (Bolus)

Active ingredients in Li Chong Decoction (Bolus) were gathered from the TCMSP and SWISS databases, resulting in 480 predicted ingredient targets after removing duplicates. A total of 5374 gene targets related to diseases were collected from the GeneCards database (Fig. 2). The analysis identified 339 common targets shared between Li Chong Decoction (Bolus) and the diseases.

Table 1. Compounds with degree values > 20 in Li Chong Decoction (bolus) and OB%, DL values.

Active ingredient	Source of compound	OB%	DL
Quercetin	Raw astragalus	46.43	0.28
Luteolin	Codonopsis pilosula	36.16	0.25
Kaempferol	Raw astragalus, Anemarrhena asphodeloides	41.88	0.24
Isorhamnetin	Raw astragalus	49.60	0.31
Formononetin	Raw astragalus, Rhizoma sparganii	69.67	0.21
7-Methoxy-2-methyl isoflavone	Codonopsis pilosula	42.56	0.20
7-*O*-Methylisomucronulatol	Raw astragalus	74.69	0.30
Anhydroicaritin	Anemarrhena, Asphodeloides	45.41	0.44
β-Sitosterol	Raw astragalus, Chinese angelica, Peach kernel	36.91	0.75
Cherianoine	Membranes of chicken gizzards		
β-Ursolic acid	Leech		
14-Acetyl-12-senecioyl-2E,8E,10E-atractylentriol	Atractylodes macrocephala	60.31	0.31
Schottenol	Snakegourd root	37.42	0.75
Mangiferolic acid	Anemarrhena asphodeloides	36.16	0.84
(3*R*)-3-(2-Hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	Raw astragalus	67.67	0.26
Stigmasterol	Codonopsis pilosula, Chinese yam, Anemarrhena asphodeloides, Rhizomasparganii, Chinese angelica	43.83	0.76
Taraxerol	Codonopsis pilosula	38.40	0.77
α-Amyrin	Atractylodes macrocephala	39.51	0.76
Spinasterol	Codonopsis pilosula, Snakegourd root	42.98	0.76
Calycosin	Raw astragalus	47.75	0.24
Glycitein	Codonopsis pilosula	50.48	0.24

Note: Cherianone, ranked 10th and beta-ursolic acid, ranked 11th, were detected in Swiss ADME. The OB% and DL values were inconsistent with those in TCMSP, so they were not filled in.

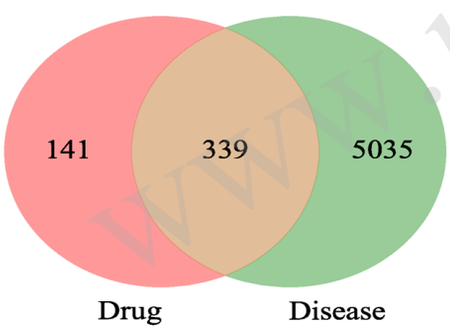

Figure 2. Wayne diagram of Li Chong Decoction (Bolus) in treating tumor.

3.2.3. Target network of drug components of Li Chong Decoction (Bolus) for tumor treatment

The 339 common targets shared between the active ingredients in Li Chong Decoction (Bolus) and diseases were visualized using Cytoscape 3.9.0 software (Fig. 3). The software allowed for the visualization of the "traditional Chinese medicine, active ingredients, and targets" relationship. Node size and order were adjusted based on the degree value, with larger nodes indicating higher Degrees. The target network consisted of 462 nodes and 1535 edges, and further details can be found in the attached document.

3.2.4. Construction of PPI network map of Li Chong Decoction (bolus) for tumor treatment

The predicted targets of Li Chong Decoction (Bolus) and tumor-related targets were compared and intersected using Venny, resulting in the identification of 339 prediction targets of Li Chong Decoction (Bolus) with clear antitumor effects. These results were imported into the STRING database for protein interaction analysis, and the analysis results were visualized using Cytoscape 3.9.0 to create a PPI network (Fig. 4). Non-interaction targets were removed, resulting in a network of 335 nodes and 15 118 mapping relationships. The size of targets in the network was adjusted based on their network degree, with higher degrees indicating more target points for drug molecules. The degrees of 60 proteins were found to be higher than 150, including AKT1, TP53, TNF, IL6, JUN, VEGFA, MYC, ESR1, EGFR, CASP3, and other targets,

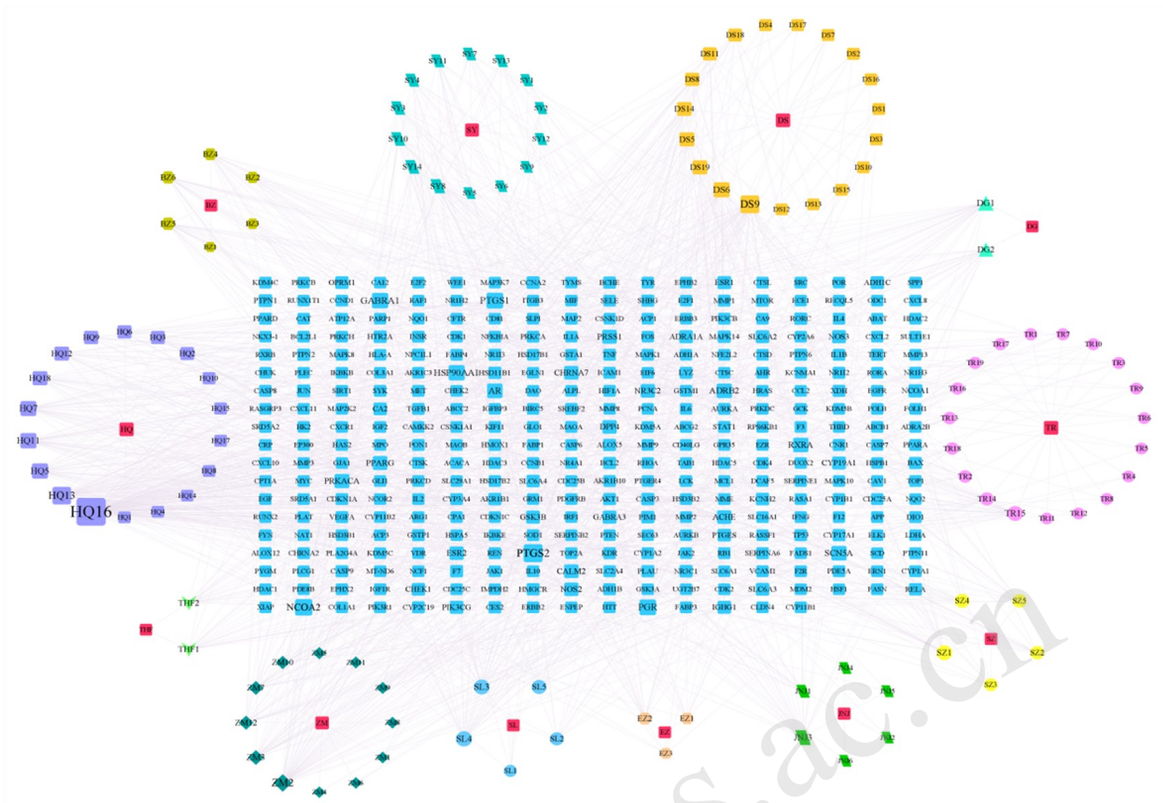

Figure 3. "Drug-component-target" network of Li Chong Decoction (Bolus) for cancer treatment.

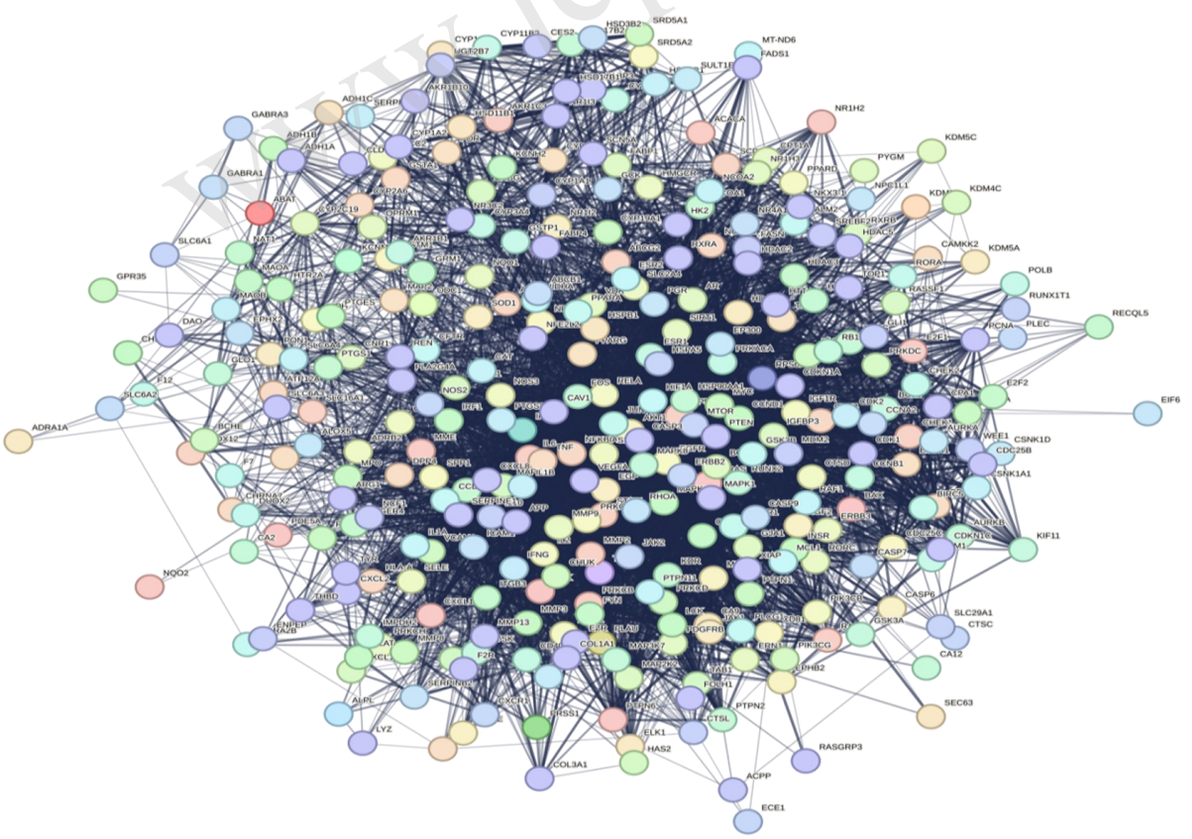

Figure 4. PPI network of intersecting therapy targets.

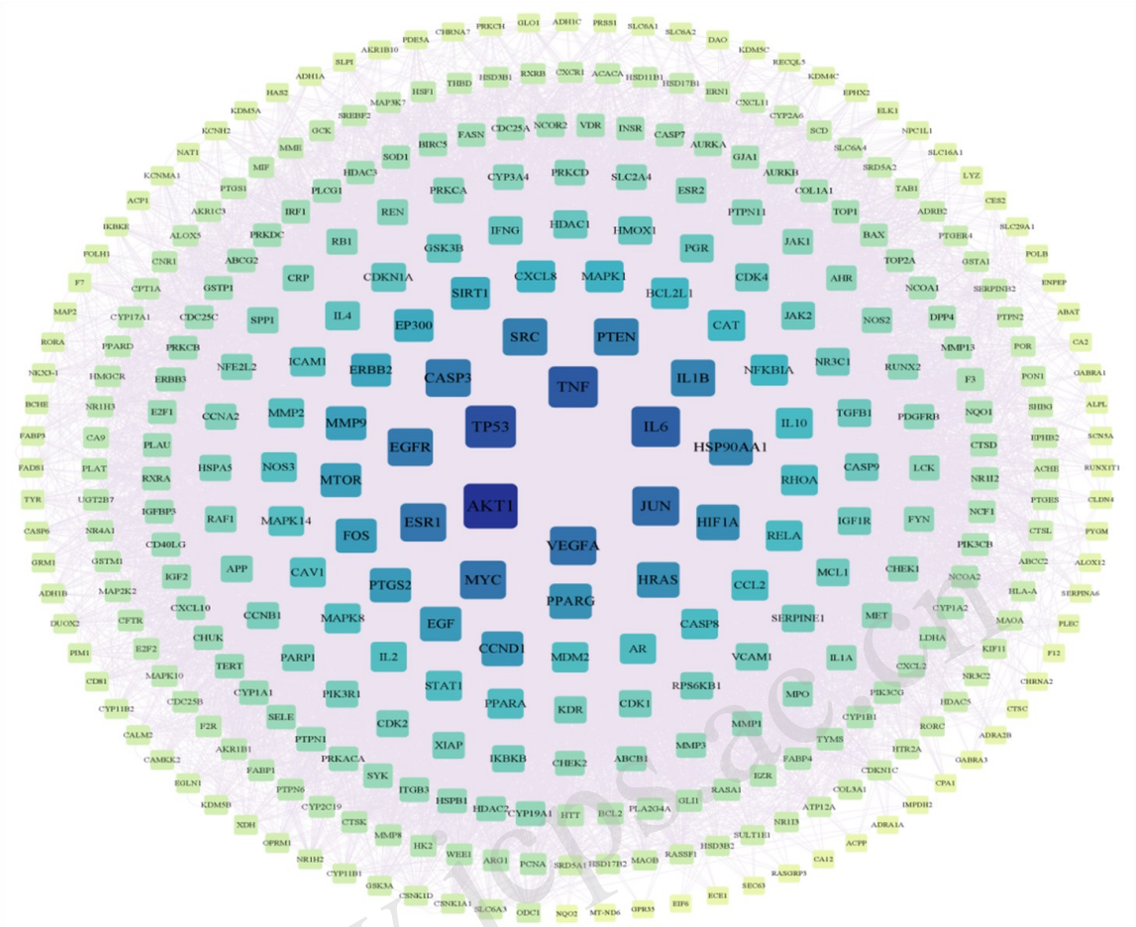

Figure 5. Visualization analysis of PPI network of intersection therapy targets.

indicating their high contribution and important role in the PPI network. Key targets were also visualized (Fig. 5).

3.2.5. Gene pathway and biological function analysis of anticancer target of Li Chong Decoction (Bolus)

An ontological analysis of the GO gene and an annotation analysis of the KEGG target enrichment pathway were conducted on 339 potential antitumor targets of Li Chong Decoction (Bolus) with a significance level of $P < 0.05$. In the GO analysis, the 339 intersection targets were examined, resulting in the identification of 1059 biological process (BP) entries, 131 cell component (CC) entries, and 261 molecular function (MF) entries. Among these, the categories with the largest number of genes were protein binding, cytosol, and nucleus, with 291, 179, and 166 genes, respectively (Fig. 6).

KEGG analysis: the above 339 intersection targets were analyzed by KEGG, and 1894 channels were enriched. Among them, the top five pathways, according to their significance, are pathways in cancer, lipid, and atmosphere, AGE-RAGE signaling pathway in diagnostic complexes, prostate cancer, and hepatitis B. It is necessary to further analyze the correlation with the target disease and then analyze the potential therapeutic targets in the above pathways, such as the EGFR signaling pathway (Fig. 7). Specific contents can be found in the discussion.

3.2.6. Molecular docking of compounds in Li Chong Decoction (Bolus) with cancer treatment targets

Quercetin, luteolin, and kaempferol are the core compounds in Li chong Decoction (Bolus) for cancer

treatment, and they demonstrate a strong binding affinity with AKT1, TNF, VEGFA, and EGFR (Fig. 8). 12 sets of ligand-receptor docking results were obtained using a threshold value, and among them, the best results were observed for luteolin-AKT1 and quercetin-AKT1 (Table 2).

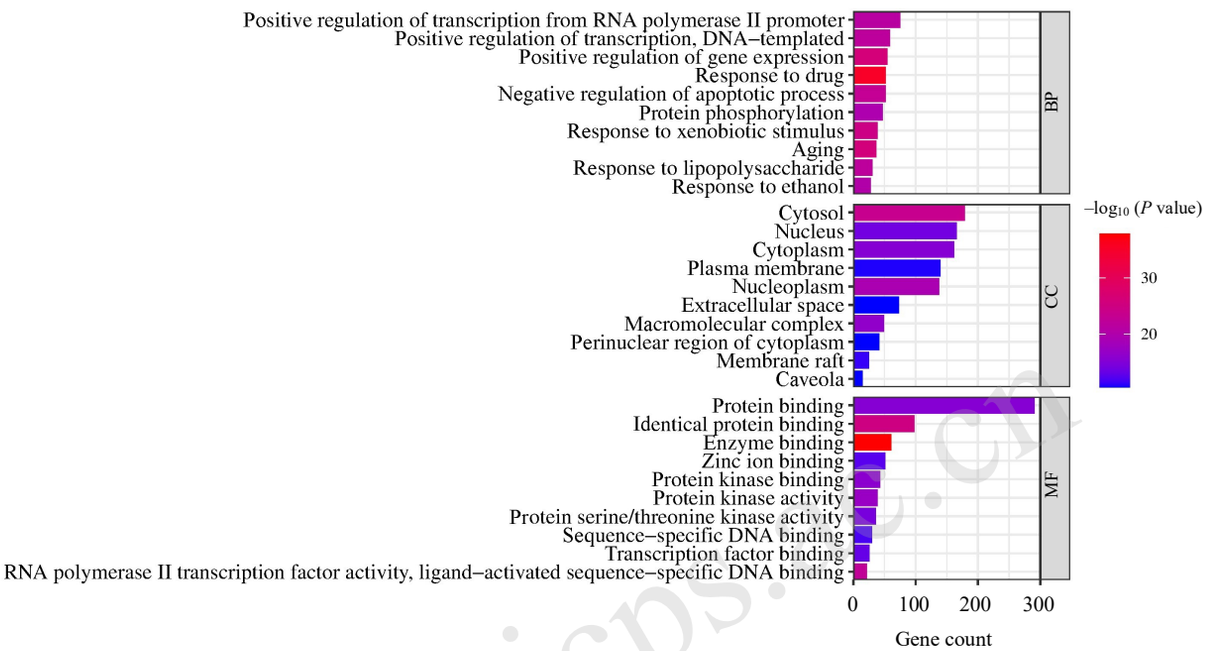

Figure 6. GO analysis of therapeutic targets of Li Chong Decoction (Bolus) for tumor intersection.

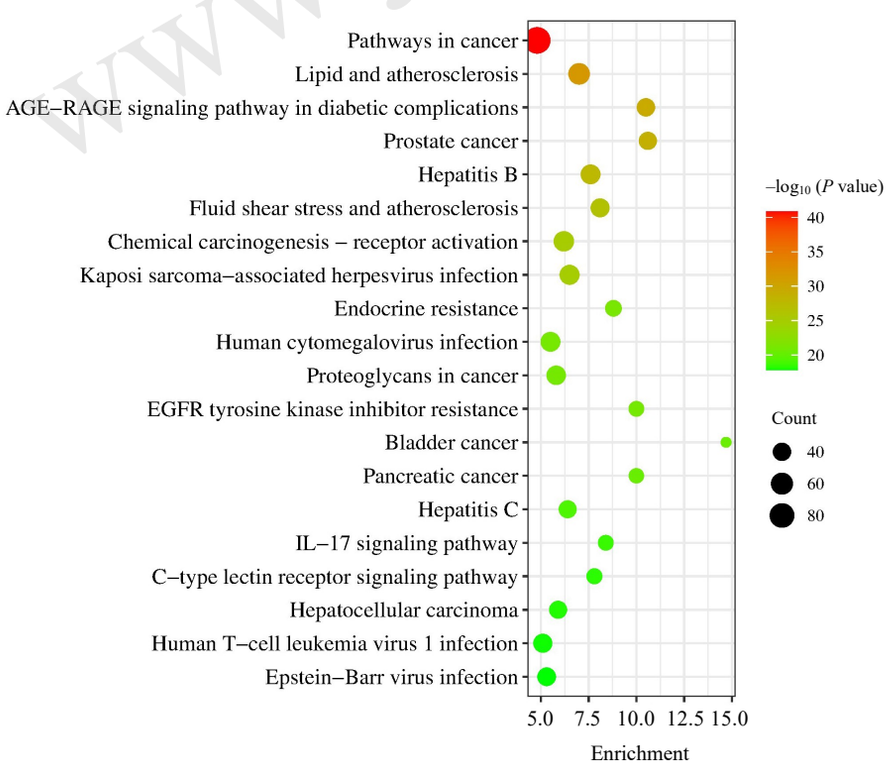

Figure 7. Enrichment analysis of KEGG pathways of tumor intersection therapy targets of Li Chong Decoction (Bolus).

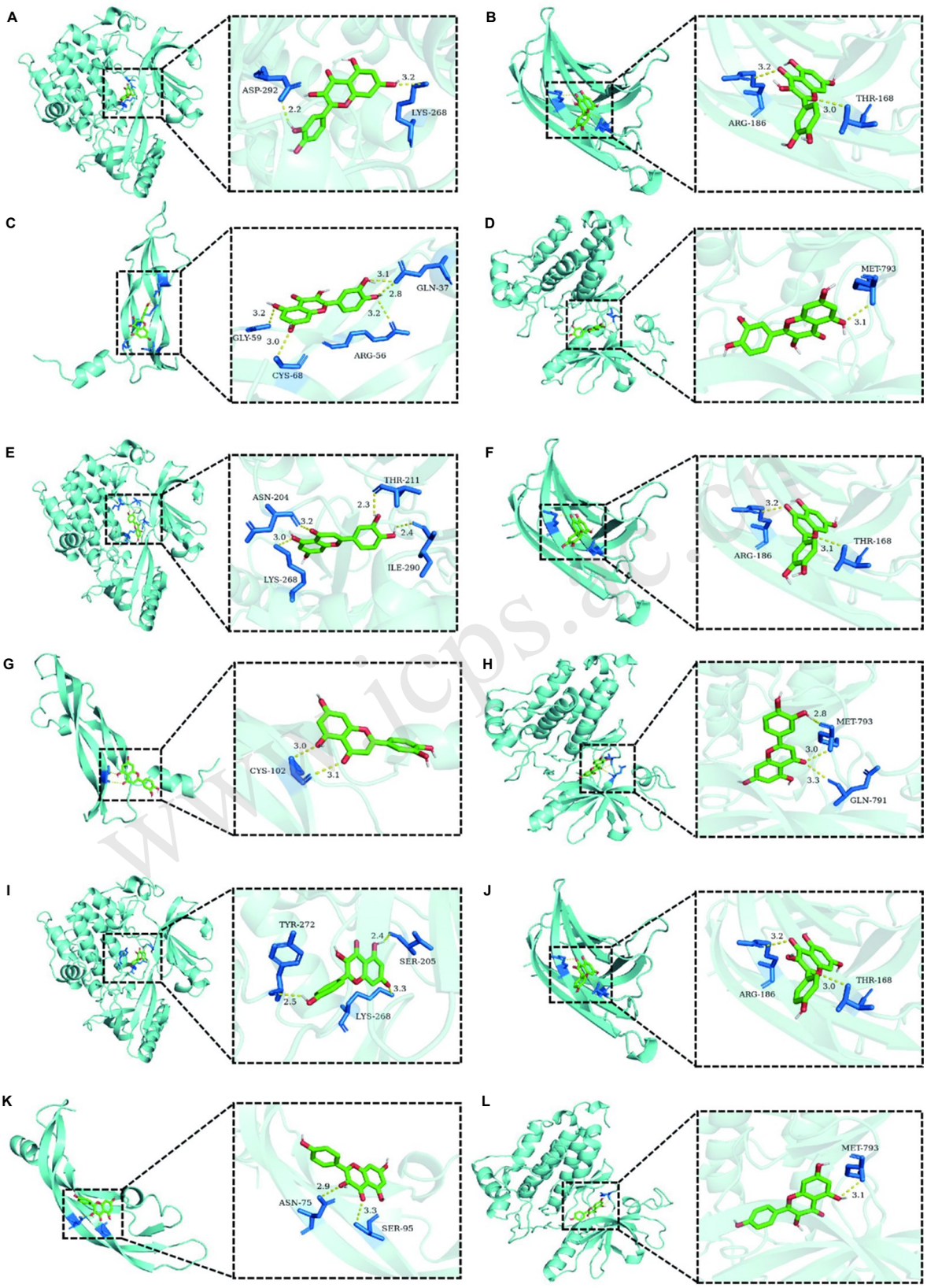

Figure 8. Docking diagram of core compounds and important target molecules of Li Chong Decoction (Bolus). (A–D) Docking diagram of quercetin and AKT1, TNF, VEGFA, and EGFR targets of Li Chong Decoction (Bolus); (E–H) Docking diagram of Lluteolin and AKT1, TNF, VEGFA, and EGFR targets of Li Chong Decoction (Bolus); (I–L) Docking diagram of kaempferol and AKT1, TNF, VEGFA and EGFR targets of Li Chong Decoction (Bolus).

Table 2. The binding energy of the key components of the Li Chong Decoction (Bolus) and the target gene molecules.

Compound name	Binding energy (kcal/mol)			
	AKT1 (6S9W)	TNF (1XU1)	VEGFA (6V7K)	EGFR (3IKA)
Quercetin	–9.9	–6.1	–5.2	–7.7
Luteolin	–10.1	–6.2	–5.5	–7.8
Kaempferol	–9.8	–6.2	–5.2	–7.5

4. Discussion

Based on a review of the literature, the current focus of antitumor research on Li Chong Decoction (Bolus) is primarily on uterine fibroids and ovarian cancer. Although there are sporadic studies suggesting that Li Chong Decoction (Bolus) may have efficacy in treating other non-gynecological tumors, none of these investigations have yielded conclusive findings. Therefore, further research into the antitumor effects of Li Chong Decoction (Bolus) is necessary to fully realize its therapeutic potential. This study was initiated with the aim of providing a reliable direction for future research programs.

A total of 111 compounds were screened from TCMSP and Swiss AEMD databases for Li Chong Decoction (Bolus), with the top 10 ranked by degree value being quercetin, luteolin, kaempferol, isorhamnetin, formononetin, 7-methoxy-2-methyl isoflavone, 7-*O*-methylisoketal, anhydroicaritin, beta-sitosterol, and cherianoine. Many of these compounds have demonstrated antitumor biological activity. Quercetin, for example, has been recognized as an anticancer agent and has been found to intervene in the treatment of esophageal cancer[27], ovarian cancer[28], breast cancer[29], prostate cancer[30], colon cancer[31], and other cancers through a variety of biological pathways. Similarly, luteolin is believed to have the potential to treat breast cancer[32], esophageal cancer[33], lung cancer[34], rectal cancer[35], skin cancer[36], and other cancers. Using cellular assays, researchers have found that kaempferol may be involved in treating many types of cancer, including pancreatic[37], gastric[38], and prostate[39] cancers. Taken together, these findings suggest that Li Chong Decoction (Bolus) has a material basis for treating cancer, which provides a prerequisite for further research in this area.

From the PPI network map, we identified the top 10 core targets: AKT1, TP53, TNF, IL6, JUN, VEGFA, MYC, ESR1, EGFR, and CASP3. Preclinical medical research has demonstrated that many of these targets are strongly linked to tumors. For instance, Herberts et al. have shown that the use of an AKT1 antagonist can significantly reduce the level of prostaglandin-specific antigen, playing a crucial role in anti-prostate cancer[40]. Similarly, Deng et al. have found that inhibiting AKT1 activity can delay the progression of gastric cancer, and the mechanism may be related to decreased expression of the ENO1-AKT1 complex[41]. TP53 plays a critical role in gynecologic cancer, and studies have demonstrated that mutations in TP53 may contribute to the development of breast and ovarian cancer[42]. Mu et al. have found that TNF and VEGFA are associated with the neovascularization of prostate cancer. Inhibiting their expression can have an anti-prostate cancer effect, and the expression level is regulated by miRNA-130b[43]. EGFR, a classic antitumor target protein, has been found to be associated with various types of tumors, including esophageal cancer, ovarian cancer, prostate cancer, bladder cancer, etc. Inhibiting its expression can significantly improve the prognosis of tumor patients[44].

Using these five important antitumor target proteins as an example, we confirmed that Li Chong Decoction (Bolus) could intervene with the currently known important

and extensively studied tumor target proteins. This finding gave us greater confidence that Li Chong Decoction (Bolus) could be used in cancer treatment.

The KEGG pathway enrichment analysis revealed that several signaling pathways were associated with cancer, including pathways in cancer, prostate cancer, endocrine resistance[45], proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance[46], bladder cancer, Pancreatic cancer, IL-17 signaling pathway[47], C-type lectin receptor signaling pathway[48], and hepatocellular carcinoma. These pathways are closely related to the occurrence, development, and prognosis of cancer.

Further analysis of the KEGG enrichment pathways revealed that the signals of pancreatic cancer, bladder cancer, prostate cancer, and liver cancer were particularly evident, indicating that Li Chong Decoction (Bolus) had the potential to treat these types of cancer. Among the current research on the treatment of non-gynecological cancers with Li Chong Decoction (Bolus), most of the studies focus on hepatocellular carcinoma. For instance, Wang et al. have found that Li Chong Decoction (Bolus), either alone or in combination with 5-fluorouracil, can inhibit the growth of subcutaneously transplanted liver cancer in mice, suppress the expression of epithelial-mesenchymal transition (EMT)-related factors, decrease the number of lung metastases, and inhibit the migration, invasion, and adhesion ability of human liver cancer cells (HepG2). The mechanism may be related to the upregulation of E-cadherin expression and downregulation of N-cadherin, Snail, Vimentin, Twist expression, which are related to EMT[49,50].

Based on the results of molecular docking, the core compounds quercetin, luteolin, and kaempferol in Li Chong Decoction (Bolus) were found to be able to effectively bind to AKT1, TNF, VEGFA, and EGFR, providing further evidence that Li Chong Decoction (Bolus) contained the necessary chemical components for cancer treatment. Therefore, it is important to pay attention to Li Chong Decoction (Bolus) as it may be an essential prescription for cancer treatment.

Finally, we would like to introduce the theoretical basis of TCM for Li Chong Decoction (Bolus) in cancer treatment. According to TCM, cancer is characterized by a mixed pattern of deficiency and excess, which requires a treatment strategy that nourishes the vital qi while attacking the evil influence. The combination of astragalus, codonopsis pilosula, atractylodes macrocephala, and Chinese yam in Li Chong Decoction (Bolus) helps strengthen the spleen and replenish qi. Anemarrhena asphodeloides and trichosanthin can help counteract the drying effects of astragalus, codonopsis pilosula, and atractylodes macrocephala, thereby preventing any biases in the body's temperature. Leech, membranes of chicken gizzards, zedoary turmeric, and rhizoma sparganii can resolve blood stasis and soften hard lumps, while peach kernel and angelica can assist in activating blood circulation and removing blood stasis. When these herbs are combined, they work together to tonify healthy qi and combat the evil influence, providing a theoretical basis for the good clinical efficacy of Li Chong Decoction (Bolus). While TCM theory can be complex and challenging to understand, the practice of syndrome differentiation and treatment remains a fundamental principle for achieving optimal clinical outcomes in TCM.

5. Conclusion

Li Chong Decoction (Bolus) has been shown to maintain both efficacy and safety in the treatment of cancer. The effective TCM components of Li Chong Decoction (Bolus) may inhibit cancer by targeting multiple biological processes involving the hub genes mentioned above. Currently, research on the treatment of non-gynecological cancers with Li Chong Decoction (Bolus) primarily focuses on hepatocellular carcinoma.

References

[1] Zhang, X.Y.; Qiu, H.A.; Li, C.S.; Cai, P.P.; Qi, F.H. The positive role of traditional Chinese medicine as an adjunctive therapy for cancer. *BioSci. Trends.* **2021**, *15*, 283–298.

[2] Neha, D.; Shikha, S. Cancer chemotherapy with novel bioactive natural products. *J. Chin. Pharm. Sci*. **2022**, *31*, 589.

[3] Wang, Y.F.; Zheng, Y.; Ku, B.S.; Yao, H.Y.; Yao, G.Y.; Wan, Y.L. Anti-tumor activity of Hedyotis diffusa Willd. in mice. *J. Chin. Pharm. Sci*. **2013**, *22*, 272–276.

[4] Wang, S.; Fu, J.L.; Hao, H.F.; Jiao, Y.N.; Li, P.P.; Han, S.Y. Metabolic reprogramming by traditional Chinese medicine and its role in effective cancer therapy. *Pharmacol. Res*. **2021**, *170*, 105728.

[5] Wang, S.M.; Long, S.Q.; Deng, Z.Y.; Wu, W.Y. Positive role of Chinese herbal medicine in cancer immune regulation. *Am. J. Chin. Med*. **2020**, *48*, 1577–1592.

[6] Xiang, Y.N.; Guo, Z.M.; Zhu, P.F.; Chen, J.; Huang, Y.Y. Traditional Chinese medicine as a cancer treatment: modern perspectives of ancient but advanced science. *Cancer Med*. **2019**, *8*, 1958–1975.

[7] Tao, W.W.; Jiang, H.; Tao, X.M.; Jiang, P.; Sha, L.Y.; Sun, X.C. Effects of acupuncture, tuina, Tai Chi, Qigong, and traditional Chinese medicine five-element music therapy on symptom management and quality of life for cancer patients: a meta-analysis. *J. Pain Symptom Manag*. **2016**, *51*, 728–747.

[8] Zhao, Y.M.; Feng, Y.W.; Zhang, L.; Yu, C.H. Research progress in the treatment of uterine leiomyoma with Lichong Decoction. *Chin. J. Exp. Tradit. Med. Form.* **2021**, *27*, 228–234.

[9] Wang, Y.S.; Li, D.H.; Xu, X.; Qian, R.Y.; Zhang, Y.L.; Huang, Y.H.; Geng, J.G.; Zou, X.L.; Han, H.J.; Zhang, W.F. Lichong Decoction reduces Matrix Metalloproteinases-2 expression but increases Tissue Inhibitors of Matrix Metalloproteinases-2 expression in a rat model of uterine leiomyoma. *J. Tradit. Chin. Med*. **2016**, *36*, 479–485.

[10] Wang, W.; Zhang, W.; Li, D.; Qian, R.; Zhu, L.; Liu, Y.; Chen, C. Lichong Decoction inhibits micro-angiogenesis by reducing the expressions of hypoxia inducible factor-1α and vascular endothelial growth factor in hysteromyoma mouse model. *J. Tradit. Chin. Med*. **2020**, *40,* 928–937.

[11] Li, D.H.; Xu, X.; Qian, R.Y.; Geng, J.G.; Zhang, Y.; Xie, X.L.; Wang, Y.S.; Zou, X.L. Effect of Lichong Decoction on expression of Bcl-2 and Bcl-2-associated X protein mRNAs in hysteromyoma model rat. *J. Tradit. Chin. Med*. **2013**, *33*, 238–242.

[12] Li, D.H.; Zhang, Y.L.; Han, H.J.; Geng, J.G.; Xie, X.L.; Zheng, J.B.; Wang, Y.S.; Zou, X.L. Effect of Lichong Decoction on expression of IGF-I and proliferating cell nuclear antigen mRNA in rat model of uterine leiomyoma. *J. Tradit. Chin. Med*. **2012**, *32*, 636–640.

[13] Zhao, S.G.; Zhang, X.F. Zhang Xiaofeng's experience of use Li Chong Tang's in treating colorectal cancer. *Guangming Tradit. Chin. Med.* **2022**, *37*, 2718–2721.

[14] Niu, W.H. Research on the mechanism of Jiawei Lichong Decoction in treating hepatocellular carcinoma based on bioinformatics analysis. *Henan Univ. Tradit. Chin. Med.* **2020**.

[15] Yi, P.J.; Zhang, Z.Y.; Huang, S.Q.; Huang, J.H.; Peng, W.J.; Yang, J.J. Integrated meta-analysis, network pharmacology, and molecular docking to investigate the efficacy and potential pharmacological mechanism of Kai-Xin-San on Alzheimer's disease. *Pharm. Biol.* **2020**, *58*, 932–943.

[16] Foroutan, F.; Guyatt, G.; Alba, A.C.; Ross, H. Meta-analysis: mistake or milestone in medicine? *Heart.* **2018**, *104*, 1559–1561.

[17] Nogales, C.; Mamdouh, Z.M.; List, M.; Kiel, C.; Casas, A.I.; Schmidt, H.H.H.W. Network pharmacology: curing causal mechanisms instead of treating symptoms. *Trends Pharmacol. Sci*. **2022**, *43*, 136–150.

[18] Li, X.; Wei, S.Z.; Niu, S.Q.; Ma, X.; Li, H.T.; Jing, M.Y.; Zhao, Y.L. Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of Huanglian Jiedu Decoction against sepsis. *Comput. Biol. Med.* **2022**, *144*, 105389.

[19] Wang, Z.Y.; Wang, X.; Zhang, D.Y.; Hu, Y.J.; Li, S. Traditional Chinese medicine network pharmacology: development in new era under guidance of network pharmacology evaluation method guidance. *China J. Chin. Mater. Med.* **2022**, *47*, 7–17.

[20] Hao, L.Y.; Yang, J.Q. Treatment of 30 cases of hysteromyoma with modified Lichong decoction. *Jilin J. Tradit. Chin. Med.* **2000**, *06*, 32.

[21] Li, D.H.; Zhang, W.F.; Liu, X.M.; Zhu, L.J.; Chen, C.; Liu, Y. Observation on the therapeutic effect of "Lizhong Decoction", a traditional Chinese medicine for strengthening the body and removing blood stasis, on hysteromyoma. *Liaoning J. Tradit. Chin. Med.* **2018**, *45*, 1653–1656.

[22] Ye, T.M. Clinical observation on the treatment of 38 cases of women with oligoabdominal syndrome with Jiawei Lichong decoction. *J. Guangzhou Med. Coll.* **2004**, *02*, 91–92.

[23] Zhong, H.L. Clinical observation on the treatment of hysteromyoma of qi deficiency and blood stasis with Lichong decoction plus acupoint application. *Jiangxi Univ. Tradit. Chin. Med.* **2020**.

[24] Zhou, Q.; Zhou, F.; Zhang, X.H. Clinical study on the treatment of advanced ovarian cancer with Lichong decoction plus or minus TC regimen. *New Chin. Med.* **2020**, *52*, 39–43.

[25] Pei, X.; Du, Y.Q.; Liu, K.J. Clinical Study on the Treatment of Advanced Ovarian Cancer with Lichong Decoction Plus and Minus Formula Combined with Chemotherapy. *Liaoning J. Tradit. Chin. Med.* **2011**, *38*, 920–922.

[26] Shao S.Q. Analysis of the clinical efficacy of Lichong decoction plus minus formula combined with chemotherapy in the treatment of advanced ovarian cancer. *Heilongjiang Tradit. Chin. Med.* **2020**, *49*, 30–31.

[27] Davoodvandi, A.; Shabani Varkani, M.; Clark, C.C.T.; Jafarnejad, S. Quercetin as an anticancer agent: focus on esophageal cancer. *J. Food Biochem.* **2020**, *44*, e13374.

[28] Khan, K.; Javed, Z.; Sadia, H.; Sharifi-Rad, J.; Cho, W.C.; Luparello, C. Quercetin and microRNA interplay in apoptosis regulation in ovarian cancer. *Curr. Pharm. Des.* **2021**, *27*, 2328–2336.

[29] Khorsandi, L.; Orazizadeh, M.; Niazvand, F.; Abbaspour, M.R.; Mansouri, E.; Khodadadi, A. Quercetin induces apoptosis and necroptosis in MCF-7 breast cancer cells. *Bratislava Med. J.* **2017**, *118*, 123–128.

[30] Ghafouri-Fard, S.; Shabestari, F.A.; Vaezi, S.; Abak, A.; Shoorei, H.; Karimi, A.; Taheri, M.; Basiri, A. Emerging impact of quercetin in the treatment of prostate cancer. *Biomed. Pharmacother.* **2021**, *138*, 111548.

[31] Özsoy, S.; Becer, E.; Kabadayı, H.; Vatansever, H.S.; Yücecan, S. Quercetin-Mediated Apoptosis and Cellular Senescence in Human Colon Cancer. *Anticancer Agents Med. Chem.* **2020**, *20*, 1387–1396.

[32] Wu, H.T.; Lin, J.; Liu, Y.E.; Chen, H.F.; Hsu, K.W.; Lin, S.H.; Peng, K.Y.; Lin, K.J.; Hsieh, C.C.; Chen, D.R. Luteolin suppresses androgen receptor-positive triple-negative breast cancer cell proliferation and metastasis by epigenetic regulation of MMP9 expression *via* the AKT/mTOR signaling pathway. *Phytomedicine.* **2021**, *81*, 153437.

[33] Zhao, J.; Li, L.; Wang, Z.; Li, L.; He, M.; Han, S.; Dong, Y.; Liu, X.; Zhao, W.; Ke, Y.; Wang, C. Luteolin attenuates cancer cell stemness in PTX-resistant oesophageal cancer cells through mediating SOX2 protein stability. *Pharmacol. Res.* **2021**, *174*, 105939.

[34] Zhang, M.; Wang, R.; Tian, J.; Song, M.Q.; Zhao, R.; Liu, K.D.; Zhu, F.; Shim, J.H.; Dong, Z.G.; Lee, M.H. Targeting LIMK1 with luteolin inhibits the growth of lung cancer *in vitro* and *in vivo*. *J. Cell Mol. Med.* **2021**, *25*, 5560–5571.

[35] Pandurangan, A.K.; Esa, N.M. Luteolin, a bioflavonoid inhibits colorectal cancer through modulation of multiple signaling pathways: a review. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 5501–5508.

[36] Juszczak, A.M.; Wöelfle, U.; Končić, M.Z.; Tomczyk, M. Skin cancer, including related pathways and therapy and the role of luteolin derivatives as potential therapeutics. *Med. Res. Rev.* **2022**, *42*, 1423–1462.

[37] Wang, F.; Wang, L.; Qu, C.; Chen, L.; Geng, Y.; Cheng, C.; Yu, S.; Wang, D.; Yang, L.; Meng, Z.; Chen, Z. Kaempferol induces ROS-dependent apoptosis in pancreatic cancer cells *via* TGM2-mediated Akt/mTOR signaling. *BMC Cancer*. **2021**, *21*, 396.

[38] Kim, T.W.; Lee, S.Y.; Kim, M.; Cheon, C.; Ko, S.G. Kaempferol induces autophagic cell death *via* IRE1-JNK-CHOP pathway and inhibition of G9a in gastric cancer cells. *Cell Death Dis.* **2018**, *9*, 875.

[39] Zhang, Y.M.; Chen, J.Q.; Fang, W.X.; Liang, K.Y.; Li, X.N.; Zhang, F.; Pang, Y.Z.; Fang, G.; Wang, X.N. Kaempferol suppresses androgen-dependent and androgen-independent prostate cancer by regulating Ki67 expression. *Mol. Biol. Rep.* **2022**, *49*, 4607–4617.

[40] Herberts, C.; Murtha, A.J.; Fu, S.; Wang, G.; Schönlau, E.; Xue, H.; Lin, D.; Gleave, A.; Yip, S.; Angeles, A.; Hotte, S.; Tran, B.; North, S.; Taavitsainen, S.; Beja, K.; Vandekerkhove, G.; Ritch, E.; Warner, E.; Saad, F.; Iqbal, N.; Wyatt, A.W. Activating *AKT1* and *PIK3CA* mutations in metastatic castration-resistant prostate cancer. *Eur. Urol.* **2020**, *78*, 834–844.

[41] Deng, T.Y.; Shen, P.; Li, A.M.; Zhang, Z.Y.; Yang, H.L.; Deng, X.J.; Peng, X.M.; Hu, Z.; Tang, Z.B.; Liu, J.H.; Hou, R.T.; Liu, Z.; Fang, W.Y. CCDC65 as a new potential tumor suppressor induced by metformin inhibits activation of AKT1 *via* ubiquitination of ENO1 in gastric cancer. *Theranostics.* **2021**, *11*, 8112–8128.

[42] Silwal-Pandit, L.; Langerød, A.; Børresen-Dale, A.L. *TP53* Mutations in breast and ovarian cancer. *Cold Spring Harb. Perspect. Med.* **2017**, *7*, a026252.

[43] Mu, H.Q.; He, Y.H.; Wang, S.B.; Yang, S.; Wang, Y.J.; Nan, C.J.; Bao, Y.F.; Xie, Q.P.; Chen, Y.H. miR-130b/TNF-α/NF-κB/VEGFA loop inhibits prostate cancer angiogenesis. *Clin. Transl. Oncol.* **2020**, *22*, 111–121.

[44] Nicholson, R.I.; Gee, J.M.W.; Harper, M.E. EGFR and cancer prognosis. *Eur. J. Cancer.* **2001**, *37*, 9–15.

[45] Saatci, O.; Huynh-Dam, K.T.; Sahin, O. Endocrine resistance in breast cancer: from molecular mechanisms to therapeutic strategies. *J. Mol. Med.* **2021**, *99*, 1691–1710.

[46] Cheng, W.L.; Feng, P.H.; Lee, K.Y.; Chen, K.Y.; Sun, W.L.; Van Hiep, N.; Luo, C.S.; Wu, S.M. The role of EREG/EGFR pathway in tumor progression. *Int. J. Mol. Sci.* **2021**, *22*, 12828.

[47] Brevi, A.; Cogrossi, L.L.; Grazia, G.; Masciovecchio, D.; Impellizzieri, D.; Lacanfora, L.; Grioni, M.; Bellone, M. Much more than IL-17A: cytokines of the IL-17 family between microbiota and cancer. *Front. Immunol.* **2020**, *11*, 565470.

[48] Li, M.H.; Zhang, R.F.; Li, J.; Li, J.N. The role of C-type lectin receptor signaling in the intestinal microbiota-inflammation-cancer axis. *Front. Immunol.* **2022**, *13*, 894445.

[49] Wang, H.; Wu, J.; Chen, M.; Liu, S.L.; Xu, L.Z. Effect of Modified Lichong Decoction Combined with 5-fluorouracil on Epithelial Interstitial Transformation of Transplanted Tumor Cells in H22 Bearing Mice. *Chin. J. Exp. Tradit. Med. Form.* **2019**, *25*, 82–89.

[50] Wang, H.; Xu, L.Z.; Wang, J.; Sun, Q.M.; Chen, M.; Liu, S.L. Effect of Modified Lichong Tang Combined with 5-fluorouracil on Epithelial Interstitial Transformation of Human HepG2 Liver Cancer Cells. *Chin. J. Exp. Tradit. Med. Form.* **2019**, *25*, 14–21.

基于meta分析和网络药理学 理冲汤(丸)治疗癌症的疗效评价及作用机制研究

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摘要: 理冲汤(丸)是一种具有显著抗肿瘤功效的中医处方。本研究基于meta分析和网络药理学的方法, 对理冲汤治疗癌症的疗效评价和作用机制进行了研究。首先, 采用循证医学的研究方法, 收集尽可能多的理冲汤(丸)治疗肿瘤的临床研究报告, 然后进行荟萃分析, 以确认理冲汤(丸)治疗肿瘤的疗效和安全性; 接下来, 利用网络药理学的方法预测了理冲汤(丸)对肿瘤的干预机制。Meta分析显示, 理冲汤(丸)的总有效率和安全性显著高于对照组, 具有统计学意义($P < 0.01$)。理冲汤(丸)共筛选出化合物111个; 与癌症的交集靶点共339个; 由PPI网络图可知, 其核心靶点是: AKT1、TP53、TNF、IL6、JUN、VEGFA、MYC、ESR1、EGFR、CASP3; 进行GO分析, 富集基因数量最多的是protein binding, cytosol, nucleus, 分别分布了291, 179, 166个基因; KEGG分析共富集通路1894条。分子对接结果显示, 槲皮素、木犀草素、山奈酚能够很好地与AKT1、TNF、VEGFA、EGFR对接, 木犀草素-AKT1和槲皮素-AKT1结合程度最佳。发现, 理冲汤(丸)在癌症治疗中保持其疗效性和安全性; 理冲汤(丸)的有效中药成分可能通过上述多个靶点和通路, 发挥出多种生物活性来抑制癌症; 目前理冲汤(丸)治疗非妇科癌症的类型主要集中在肝细胞癌上。

关键词: 理冲汤(丸); 中医; 癌症; Meta分析; 网络药理学; 数据挖掘