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Analysis of potential mechanisms of improvement of abnormal uterine bleeding by Erzhi Pill based on network pharmacology and molecular docking technique

Xiao Han [1], Pengyu Han [1], Yixin Li [3] ,Xiaomao Liu [2] *

1. The First Clinical Medical College, Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, China.

2. Department of Gynecology, Yulin Hospital of Traditional Chinese Medicine, Yulin, Shaanxi, China

3. Xi'an Hospital of Traditional Chinese Medicine, Xi'an, Shaanxi, China

* Corresponding author: Xiaomao Liu, 1367916380@qq.com

Abstract

OBJECTIVE: This study aimed to uncover potential targets and mechanisms for the treatment of abnormal uterine bleeding through a network pharmacological study of Erzhi Pills

METHODS: Network pharmacology was used to predict the active ingredients and potential targets for abnormal uterine bleeding of Erzhi Pill, and the binding affinity was explored using molecular docking techniques. The relevant associated signalling pathways were studied using GO and KEGG enrichment analysis.

RESULTS: Based on the network pharmacology results, two key active ingredients, Acetin and Kaempferol, were screened, and three potential therapeutic targets, TP53, TNF, and AKT1, were screened using STRING and PPI networks. The enrichment analysis showed that these potential therapeutic targets were mainly involved in signalling pathways related to metabolism and inflammation. The binding was observed using the molecular docking technique, and both active ingredients and potential targets showed good affinity.

CONCLUSION: This study contributes new research evidence on the potential mechanism of Erzhi Pill in the treatment of abnormal uterine bleeding. The results of both network pharmacology and molecular docking techniques suggest a potential therapeutic effect of Erzhi Pill on abnormal uterine bleeding. However, these results need to be further investigated to provide more profound clinical information.

Keywords: Erzhi Pills; abnormal uterine bleeding; network pharmacology; molecular docking.

Abnormal Uterine Bleeding (AUB) is a common condition in women that focuses on abnormal changes in the four elements of a woman's menstrual period: regularity, cyclicity, length of the period, and amount of bleeding, and where the bleeding comes from the uterine cavity. It occurs in about one-third of women and dramatically affects women's quality of life and physical and mental health. 1 According to the classification of the International Federation of Gynecology

and Obstetrics (FIGO), AUB is classified into acute and chronic conditions. Generally, the diagnosis of chronic AUB requires a comprehensive assessment based on bleeding over the past six months, whereas the presence of heavy vaginal bleeding characterizes acute AUB. 2 As research on AUB continues to advance, the pathogenesis is becoming more apparent, and endocrine abnormalities, uterine fibroids, coagulation disorders, malignant tumours, and ovulatory dysfunction all appear to be potential causative factors for AUB.3,4

Hormone therapy is currently the preferred option for AUB treatment, and other patients who meet the indications for surgery can also be treated with surgical methods. 5,6 Deoxypregnene ethinyl estradiol, dydrogesterone, and estradiol valerate are common therapeutic drugs in the clinic. 7 Although hormone therapy can be therapeutically effective, it can still lead to some side effects, such as acne, obesity, digestive reactions, etc., and they are easy to fluctuate after stopping the drug. With the continuous development of Chinese medicine, Chinese medicine treatment has become a new field and has gradually been accepted by everyone; AUB adopted traditional Chinese medicine for treatment and achieved better efficacy. 8 Erzhi Pill is a traditional Chinese medicine compound formula that mainly comprises two medicines: Ligustrum lucidum fruit and Eclipta prostrata. 9 Ligustrum lucidum fruit has the efficacy of nourishing the liver and kidney, cooling the blood and stopping bleeding. In contrast, Eclipta prostrata has the efficacy of brightening the eyes and nourishing the liver and kidneys. Both medicines can enter the liver and kidney meridian of the twelve meridians and have the clinical efficacy of nourishing the liver and kidney and nourishing the essence and blood. They can be used to treat haemorrhage due to physical weakness. Traditional Chinese medicine believes that bleeding caused by physical weakness should be stopped by tonifying the body, so the Erzhi Pill has great potential in treating abnormal uterine bleeding.

In this study, network pharmacology and molecular docking methods were used to investigate the Erzhi Pill's potential mechanism for treating abnormal uterine bleeding. It is worth mentioning that network pharmacology is a more integrated approach that includes bioinformatics, which is widely used in the field of medicine, and its central role is to analyze the information from multiple databases comprehensively and then build an easy-to-understand framework for the interactions between the active ingredients of the drug and the disease target proteins; 10,11 The molecular docking technique is based on the realization of the microscopic level, and its main gazes focuses on the atomic level to further elucidate the underlying mechanisms by performing binding simulations between the active ingredients of diastatomimetic pills and the disease targets of AUB. 12 The combined use of the two approaches explores the molecular basis of Erzhi Pills in the treatment of AUB at a deeper level.

This study provides new perspectives and methods for the molecular mechanism of AUB treatment by Erzhimaru and likewise provides new clues for clinical diagnosis and treatment.

1. Information and methods

1.1 Acquisition of Active Components and Targets of Erzhi Pill Chinese Medicine

The TCMSP database (https://www.tcmsp-e.com) was used to retrieve the active ingredients of the traditional Chinese medicines (Eclipta prostrata lucidum and Ligustrum lucidum fruit) contained in Erzhi Pills, and the active ingredient components were screened according to oral bioavailability (OB) ≥30% and drug-like properties (DL) ≥0.18. The SMILES numbers of the known chemical constituents were retrieved through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the active ingredient targets were predicted using SwissTargetPrediction (http://swisstargetprediction.ch/). And select the target of Probability＞ 0. The UniProt database was used to obtain the standard coding gene names corresponding to the target proteins, and the gene names corresponding to the obtained targets were unified and corrected to establish a library of the active ingredients and targets of Erzhimaru traditional Chinese medicine.

1.2 Disease target and intersection target acquisition

The Gencards (https://www.genecards.org/) and OMIM (https://www.omim.org/) databases were searched with the keywords "AUB" and "Abnormal uterine bleeding", and the target library of abnormal uterine bleeding was established by combining and removing duplicates. Using Venn diagrams, the intersecting targets of the active ingredient targets of Erzhi Pill and the AUB-related gene targets, which will be used as potential targets of Erzhi Pill to improve AUB, were further investigated.13

1.3 Constructing "drug-target-disease" network diagrams

The intersection target points of Di-Chi Pill and AUB were imported into Cytoscape 3.10.2 software to construct a "drug-target-disease" visualized network graph, where nodes represent active ingredients of the drug and edges represent the interrelationships between the chemical components, targets and diseases. The network was analyzed using the Network Analyze function to determine the top active ingredients based on the degree value of the nodes.

1.4 Construction of PPI network diagram and selection of core genes

The interactions between targets were predicted using the STRING platform (https://cn.string-db.org/). The intersecting target genes were imported into the STRING platform, the "multiple proteins" analysis mode was selected, and the genus was limited to "Homo sapiens". "Homo sapiens", the minimum interaction score should be more than 0.7 to obtain the interaction relationship between target proteins. Blue lines connected the unvalidated target proteins, and purple lines connected the experimentally validated target proteins. The files were imported into Cytoscape software for PPI network diagram construction. The CentiScaPe2.2 plug-in in Cytoscape 3.10.2 software was used for computational analysis. 14 All nodes were sorted and organized according to Betweenness, Closeness, and Degree as follows:

1.4.1 CentiScape Analysis

In constructing the visualized PPI network, the analysis was carried out using the CentiScape 2.2 plug-in, which helps calculate the three centrality metrics of Betweenness, Closeness, and Degree.

1.4.2 Sorting and standardization

The centrality of the nodes is calculated during the calculation process, and these computed values give the nodes a rank, with the higher calculated values ranked relatively high. To make the analysis more accurate and standardized, it is also necessary to standardize the resulting values, which are generally narrowed down to the range of 0~1. After standardization, reordering will be done, and finally, the standardized rank of Betweenness, Closeness and Degree values will be used to reflect the importance. Nodes important in the study will use darker colours and larger nodes to indicate their significance. Finally, based on the Betweenness and Degree values of the nodes, the key target points will be visualized by drawing bubble plots using the ggplot2 package in the R 4.4.2 software.

1.5 GO and KEGG enrichment analysis

Enrichment analysis was performed using the clusterProfiler package in R 4.4.2 software (version), which links to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Ge-nomes (KEGG). The enrichment results obtained were visualized using the ggplot2 package to clarify the biological role of di to pills in AUB.

1.6 Molecular docking

The study also performed molecular docking using the top active ingredient and the key target to clarify further the binding of the key target and the two-to-pill active ingredient. Molecular docking simulations were performed separately using AutodockVina software (version 1.2.2) for top active ingredients and key targets. The molecular structures of the chemical components were obtained from the PubChem database, while the data of the key gene proteins TP53 (PDB No. 3D06), TNF (PDB No. 2E7A), and AKT1 (PDB No. 7MYX) were downloaded from the PDB (http://www.rcsb.org/). In the molecular docking simulation process, all the protein and molecular files were first converted to PDBQT format, hydrogen atoms were added after removing water molecules, and a mesh framework was set up that could ensure the free movement of the protein and cover it, with the centre coordinates and pocket sizes to ensure that the whole protein could be covered. Finally, the docking results were visualized using pymol software (version 4.5.0).

2. Results

2.1 Acquisition of main active ingredients and potential targets of Erzhi Pills

Using TCMSP database search, OB≥ 30% and DL≥ 0.18 were used as the screening conditions and predicted by SwissTargetPrediction, 10 active ingredients of Ligustrum virginianum and nine

active ingredients of Eclipta prostrata japonica were obtained, which are shown in Table 1. Further, the active ingredient-related targets were collected, and 285 potential targets were obtained by integrating and de-emphasizing them.

Table1: Main active ingredients of Erzhi Pill

herbal remedy	chemical composition	OB value	DL value	SMILES
Ligustrum lucidum fruit	beta-sitosterol	36.91	0.75	CC[C@H](CC[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C)C(C) C(C)C
	kaempferol	41.88	0.24	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O) O)O
	Lucidumoside D	48.87	0.71	CC=C1[C@@H](O[C@@H]2O[C@H](CO)[C@@H](O)[C@H](O)[C@H]2O)OC=C(C(=O)OC)[C@@H]1CC(=O)OCCc1ccc(OC)c(OC)c1
	(20S)-24-ene-3β, 20-diol-3-acetate	40.23	0.82	CC(=O)O[C@H]1CC[C@@]2(C)[C@H](CC[C@](CC[C@]3(C)[C@@H]2CC[C@H]2[C@@H]([C@@](C)(O)CCC=C(C)C)CC[C@@]23C)C1(C)C
	eriodictyol	71.79	0.24	C1[C@H](OC2=CC(=CC(=C2C1=O)O)O)O)C3=CC(=C(C=C3)O)O
	syringaresinol diglucoside_qt	83.12	0.8	COc1cc(O)c([C@H]2OC[C@@@H]3[C@H](c4c(O)cc(OC)c(O)c4OC)OC[C@H]23)c(OC)c1O
	Olitoriside	65.45	0.23	C[C@H]1O[C@@H](O[C@H]2CC[C@@]3(C=O)[C@H]4CC[C@]5(C)[C@@H](C6=CC(=O)OC6)CC[C@]5(O)[C@@H]4CC[C@]3(O)C2)C[C@@H](O)[C@@H]1O[C@@H]1O[C@@H](CO)[C@@H](O)[C@H](O)[C@H]1O
	Olitoriside_qt	103.23	0.78	C[C@]12CC[C@@H]3[C@@H](CC[C@]4(O)C[C@H](O)CC[C@@]34C=O)[C@@]1(O)CC[C@H]2C1=CC(=O)OC1
	luteolin	36.16	0.25	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O) O)O
	quercetin	46.43	0.28	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O)O
Eclipta	Linarin	39.84	0.71	C[C@H]1[C@@H]([C@H]([C@H]([C@H](O1)O

prostrata				C[C@@H]2[C@H]([C@H]([C@H]([C@@H](O2)OC3=CC(=C4C(=C3)OC(=CC4=O)C5=CC=C(C=C5)OC(OC)O)O)O)O)O)O
	acacetin	34.97	0.24	COC1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O
	butin	69.94	0.21	C1[C@@H](OC2=C(C1=O)C=CC(=C2)O)C3=CC(=C(C=C3)O)O
	1,3,8,9-tetrahydroxybenzofurano[3,2-c]chromen-6-one	33.94	0.43	C1=C(C=C2C(=C1O)C3=C(C4=CC(=C(C=C4O3)O)O)C(=O)O2)O
	3'-O-Methyloro bol	57.41	0.27	COC1=C(C=CC(=C1)C2=COC3=CC(=CC(=C3C2=O)O)O)O
	Pratensein	39.06	0.28	COC1=C(C=C(C=C1)C2=COC3=CC(=CC(=C3C2=O)O)O)O
	wedelolactone	49.6	0.48	COC1=CC(=C2C(=C1)OC(=O)C3=C2OC4=CC(=C(C=C43)O)O)O
	luteolin	36.16	0.25	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O) O)O
	quercetin	46.43	0.28	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O)O

2.2 Disease target acquisition and intersecting target organization and analysis

A total of 2089 disease targets were collected through GeneCards, OMIM and other databases, and 1045 disease targets were identified after screening. Two sets of intersections were taken using Venn plots, and 70 intersecting targets were obtained, which may be potential targets for Erzhi Pills treatment of AUB, see Figure 1

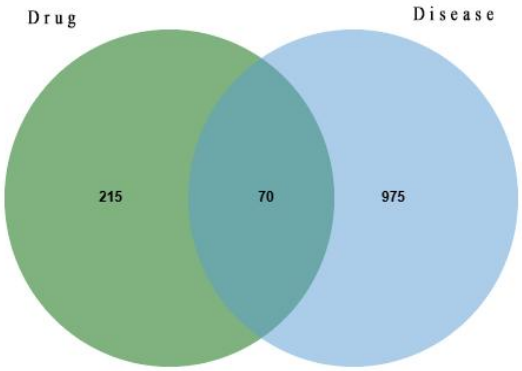

Figure 1: Intersecting Targets of Di- to Pills and AUB

2.3 Selection of key drug active ingredients

To visualize the key active ingredients of Erzhi Pill for AUB, a drug-target-disease co-expression network was constructed by Cytoscape 3.10.2 software. The active ingredients of Erzhi Pill and the intersecting targets of AUB were imported into the software, and a drug-target-disease co-expression network graph with 89 nodes and 169 edges was obtained. In the network graph, diamond-shaped nodes represent targets; circles represent active ingredients. The network graph was analyzed using the Network Analyze function, and different colours were used to render the network graph according to the order of Degree values, with darker colours representing high Degree values and vice versa for low Degree values. The results of the analysis showed that two active ingredients, Acacetin (MOL001689; Degree: 25) and Kaempferol (MOL000422; Degree: 24), were identified as the key drug active ingredients, which may play a critical role in the treatment of AUB. (Figure 2)

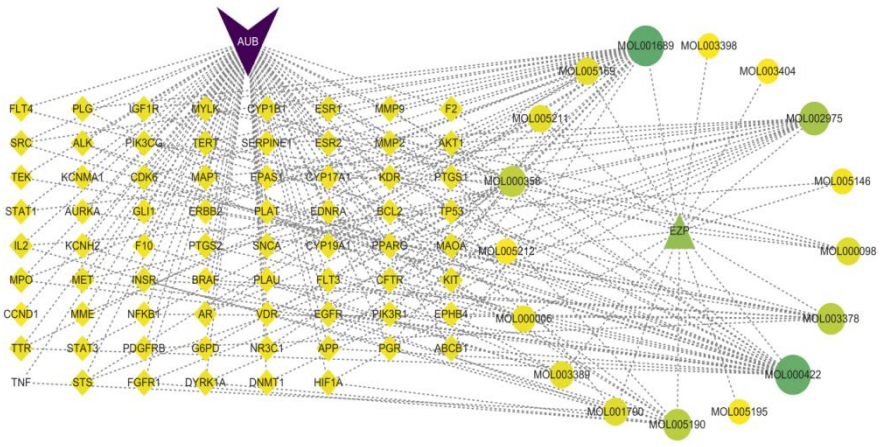

Figure 2: Drug-Target-Disease Network Diagram

Note: Node size is determined based on Degree value; in active ingredients, the larger the node, the darker the colour, proves that the node is more important

2.4 PPI Network Topology Analysis

The 70 intersecting targets obtained were imported into the STRING platform, and the confidence interval was set to 0.7. It was found that all 70 targets were interconnected targets with no isolated nodes. (Figure 3a) Subsequently, TSV files were generated and imported into the Centiscape2.2 plugin in Cytoscape 3.10.2 software to visualize the intersecting targets. Key genes were selected to visualize and analyze the core targets with 70 nodes and 896 edges. In the network graph, a higher degree of centrality Degree value indicates higher importance. The betweenness centrality value represents how often a node appears in the shortest path between other node pairs, and the higher the frequency of appearance, the more critical it is. Proximity centrality Closeness reflects the inverse of the average shortest path length from a node to all other nodes. (Figure 3b) To analyze the importance of the target points further, we reflect on them using the bubble graph. It can be found that among the intersecting targets, Tumor Protein p53 (TP53), Tumor Necrosis Factor (TNF), V-akt Murine Thymoma Viral Oncogene Homolog 1 (AKT1), SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase (SRC) four targets were more critical. Based on the Degree value of these four targets, TP53 (Degree: 57), TNF (Degree: 58), and AKT1 (Degree: 56) were selected as the key genes to be used in the next step of the study. (Figure 3c) It is worth mentioning that these three key genes have been indicated in dark red colour in the PPI plot.

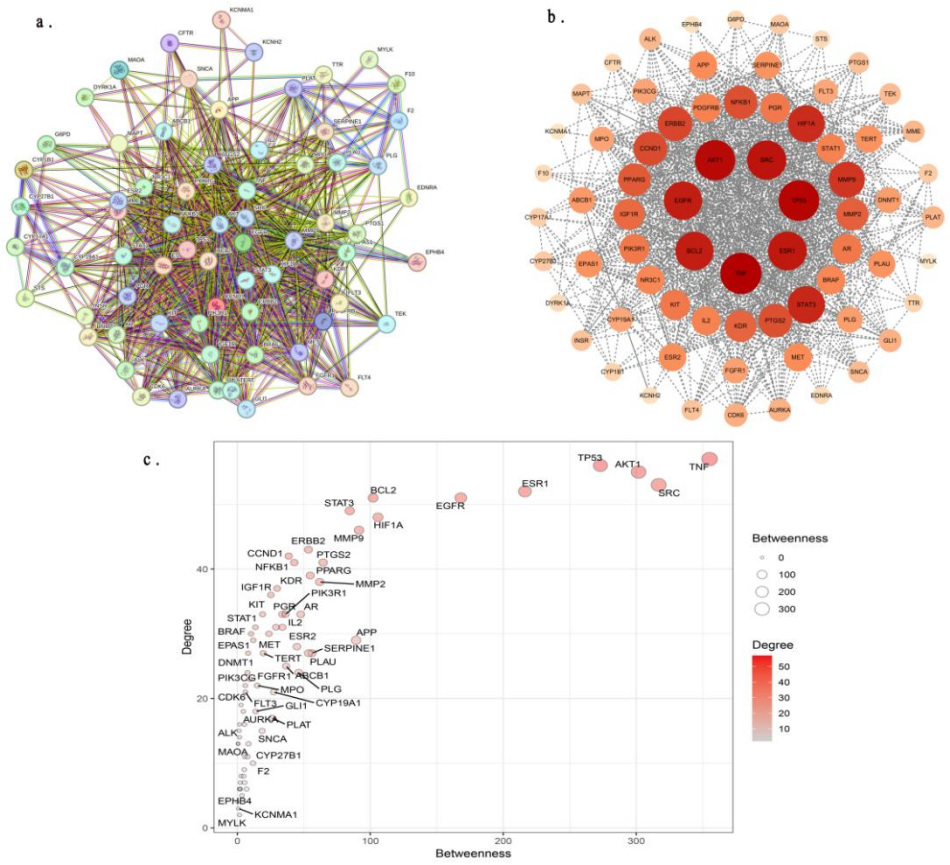

Figure 3: PPI network diagram construction for core targets. (a) Construction of protein interaction network using STRING. (b) PPI network of core targets. (C) Bubble diagram of core targets.

Note: In the PPI network diagram node size and color shades are determined by the Degree value, the higher the Degree the more important the node is considered to be.

2.5 GO and KEGG enrichment analysis

GO and KEGG enrichment analysis of the intersecting targets revealed that protein phosphorylation, gland development, phosphatidylinositol 3-kinase/protein kinase B signalling, epithelial cell proliferation, and oxidative stress response were significantly enriched in terms of bioprocesses (BP); whereas in terms of cellular structures (CC), membrane valves and membrane microdomains might be the process of AUB treatment by di- to-pill In terms of molecular function (MF), it was mainly enriched in the activation of various protein kinases, hormone binding, transcriptional coactivator binding, estrogen response element binding, growth factor binding, etc. KEGG enrichment analysis showed that these targets were enriched in the MAPK signalling pathway, PI3K-Akt signalling pathway, endocrine-associated signalling pathway, and multiple cancer signalling pathways. According to the results of GO and KEGG enrichment analyses, the treatment of AUB in di to pills mainly involves metabolic and endocrine pathways, which reduce oxidative stress by regulating metabolism and endocrinology, promote gland development and hormone secretion, and control protein phosphorylation to affect cell proliferation and apoptosis. (Figure 4)

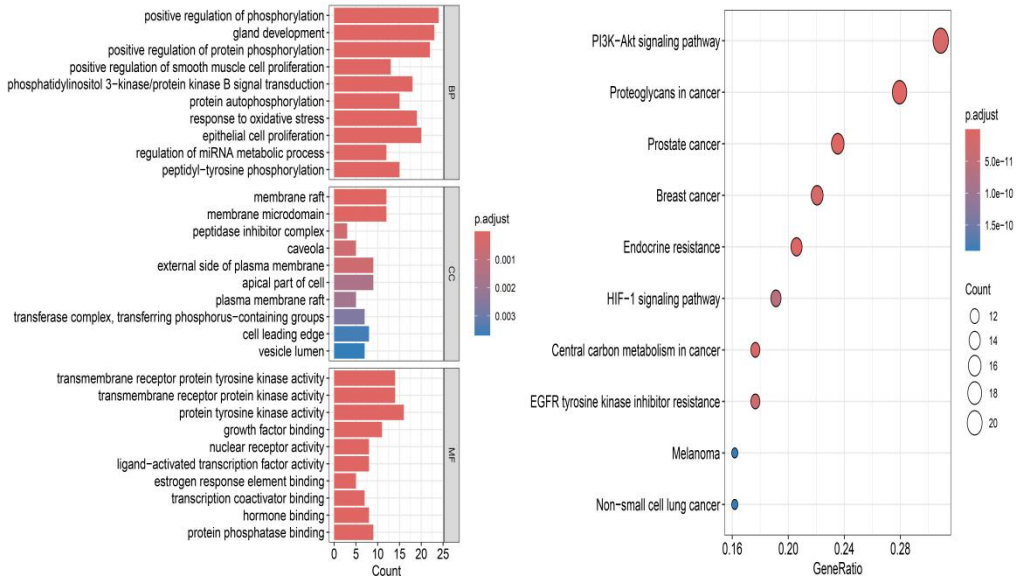

Figure 4: GO and KEGG enrichment analysis results

2.6 Molecular docking results

In order to delve into the therapeutic potential of Erzhi Pill for AUB, the study was carried out

using the molecular docking technique for in-depth analysis and validation of interactions between the active ingredients of Erzhi Pill and the key targets. (Figure 5a-f) Based on the network pharmacology results, Acacetin and Kaempferol have been selected as the main active ingredients to be docked to the key targets. In general, the molecular binding energy can visualize the binding affinity; when the binding energy is <0 kcal/mol, the active ingredient and the target have the possibility of binding; <-4.25 kcal/mol, the active ingredient has a better affinity with the target; <-7 kcal/mol can indicate that there is a relatively strong affinity between the two. Acacetin and Kaempferol The binding energy with TP53 and TNF is <-7 kcal/mol, indicating a strong interaction between these two active ingredients and the two key targets. This can reflect that Erzhimaru mainly relies on regulating cell proliferation and apoptosis and reducing inflammatory factor production to achieve therapeutic effects in treating AUB. (Figure 5g)

Specific analysis of the binding situation reveals that one residue, ASN-268, is labelled when Acacetin binds to TP53, forming a potential hydrogen bond with the ligand. In contrast, three disabilities are labelled when they bind to TNF, ASN-112, ARG-98, and ARG-103, forming potential hydrogen bonds with the ligand. Three residues are similarly labelled when binding to AKT1, ARG-25, ASN-53, and ARG-23, forming a potential hydrogen bond with the ligand. residues are labeled as ARG-25, ASN-53, and ARG-23, which similarly form potential hydrogen bonds with the ligand. (Figure 5a-c) Kaempferol formed four residues upon binding to TP53, LYS-139, GLN-136, THR-123, LEU-114; five residues were generated upon binding to TNF, ARG-98, GLN-102, ARG-98, SER-99, ASN-112; and five residues were generated upon binding to AKT1, ARG-99, SER-99, ASN-112. AKT1, two residues were generated upon binding, ASN-53 and LYS-14. Similarly, each of these residues formed potential hydrogen bonds with the ligand. (Figure 5d-f) It is worth noting that the hydrophobic portion of the active ingredient may form van der Waals forces with the surrounding hydrophobic side chains during docking. This phenomenon enhances the binding, and although this effect is relatively minor, it still plays a crucial role.

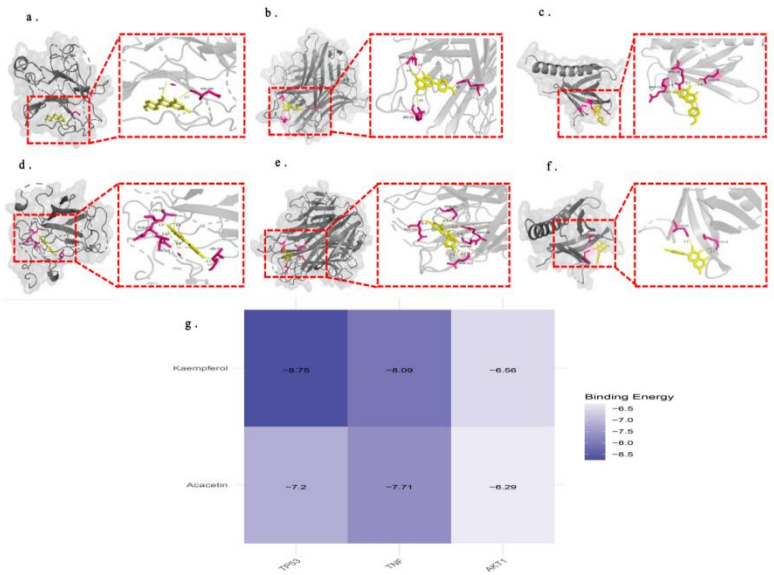

Figure 5: Molecular docking between the main active ingredient and TOP targets. (a-c) Visualization of molecular docking of Acacetin with TP53, TNF, AKT1. (d-f) Visualization of molecular docking of Kaempferol with TP53, TNF and AKT1. (g) Thermogram of molecular docking binding energy

3. Discussion

AUB originates from the uterine cavity and is closely related to the endometrium, and any factor that affects the normal secretion of the endometrium may cause irregular vaginal bleeding. Furthermore, factors such as polycystic ovary syndrome, insulin resistance, obesity, and thyroid-related endocrine disorders can affect ovulation function, resulting in menstrual disorders and seriously affecting women's reproductive health. 15,16 Western medical treatment is mainly based on hormone therapy. Although Western medicine treatment can be effective in the short term, the side effects will still bring certain physical and mental burdens to women, which is an unavoidable problem. Traditional Chinese medicine (TCM) has a history of thousands of years in China, and Erzhi Pill, as a classic compound formula, is commonly used in modern times for the treatment of a series of gynaecological diseases such as AUB and its specific therapeutic mechanism is not yet precise, despite the good clinical response. 17 In this study, Erzhi Pill was effectively analyzed using network pharmacology and molecular docking techniques, and intermolecular affinity analysis was performed. Cyberpharmacology was used because it is a comprehensive interdisciplinary approach that can help screen out key compounds and targets in Erzhi Pills and build an easy-to-understand framework for how compounds affect the organism. At the same time, molecular docking technology provides an in-depth view of intermolecular interactions by exploring and visualizing them at the atomic level. In this study, 19 active ingredients and 285 targets were identified in Erzhi Pill, and two key active ingredients, Acacetin and Kaempferol, were further screened by prediction. A total of 70 targets were obtained from the intersection of the two groups of targets, and STRING and PPI were utilized for constructing protein interactions network maps, and TP53, TNF, and AKT1 were used as TOP targets for in-depth study.

It is worth mentioning that hormone levels are inextricably linked to inflammation and metabolism. Studies have shown that hormone secretion in the human body can be disrupted by a large number of inflammatory factors. 18 Through the study of network pharmacology, it can be found that Acetin and Kaempferol are the main active ingredients of Erzhi Pill. Both belong to progesterone compounds and have a wide range of pharmacological effects. Acacetin is capable of anti-inflammatory, antioxidant, obesity inhibition, and nerve protection.19 A modern pharmacological study has shown that Acacetin can inhibit inflammation by blocking the activation of MAPK/NF-κB and NLRP3 inflammatory vesicles. 20 Interestingly, it was found that low-dose intake of Acacetin can activate estrogen receptors, thus reducing estrogen levels and inhibiting excessive cell proliferation. 21 Kaempferol has anti-inflammatory, metabolism-regulating,

and anti-cancer properties. 22 Some studies have shown that Kaempferol can regulate metabolic function and improve inflammation by regulating multiple signalling pathways such as NF-κB, Nrf2, AMPK, PI3K/AKT, etc. 23 However, there are fewer studies on Kaempferol to reduce abnormal vaginal bleeding, but based on its anti-inflammatory properties, Kaempferol may play a protective role in endometrial health. 24 TP53 is a key gene that regulates the cell cycle. Studies have shown that DNA damage or abnormalities may occur during cell mitosis, and these abnormalities will be repaired at two cell cycle checkpoints. TP53 can prolong the cell cycle to complete DNA repair and avoid abnormal cellular appreciation. 25 The main role of TNF is pro-inflammatory, which can activate the NF-κB signaling pathway to promote large cell proliferation, and it has been found that in some cases TNF can also inhibit excessive cell proliferation; AKT1 is mainly used to achieve cell proliferation by activating the PI3K-AKT signaling pathway and then inhibiting the expression of anti-proliferative and pro-apoptotic genes by decreasing the expression of FOXO proteins.26,27

Analysis by GO and KEGG enrichment showed that these targets were mainly enriched in gland development, protein phosphorylation, and epithelial cell proliferation, involving membrane valves and membrane microdomains in the cell, affecting hormone binding and protein kinase activation, and correlating with multiple pathways associated with endocrinology and metabolism, suggesting that ErzhiPills may, through regulating hormone levels in women's bodies phosphorylating or dephosphorylating a variety of proteins and so on. Improving endometrial conditions. It is well known that the occurrence of AUB is associated with a variety of endometrial pathologic changes that are significantly affected by female hormones. It has been suggested that estrogen and progesterone instability may be an essential cause of pathological changes in the endometrium. 28 Progesterone can activate the AKT1 signaling pathway to reduce the overproliferation of endometrial cells; whereas estrogen promotes proliferation by increasing the expression level of AKT1 and phosphorylating related cell proliferation factors. 29,30 It is noteworthy that the treatment of AUB with diastole pills is affected by a combination of mechanisms, and the enrichment analysis results are the potential main mechanism rather than the only one.

The results of molecular docking showed that Acacetin and Kaempferol had good affinity for TP53 and TNF, proving that the molecules had strong interactions. Notably, the binding energies of Kaempferol with TP53 and TNF were -8.75 kcal/mol and -8.09 kcal/mol, which proved that Kaempferol had a better affinity with these two targets. Combined with the biological roles of Kaempferol and TP53, it can be hypothesized that Kaempferol can regulate the cell cycle and control cell proliferation and apoptosis through the activation of TP53; combined with the role of TNF, it can be hypothesized that Kaempferol can play an anti-inflammatory role through the inhibition of TNF-related signalling pathway. The binding energies of Acacetin TP53 and TNF were -8.75kcal/mol and -8.09kcal/mol, respectively. Acacetin has a good affinity for TP53 and TNF with binding energies of -7.2kcal/mol and -7.71kcal/mol, respectively. Acacetin can up-regulate TP53 to affect cell proliferation and block the activation of TNF-related signalling pathways to reduce inflammation.

In summary, the treatment of AUB with Erzhimaru is mainly achieved by affecting the cell cycle

and inflammation-related target proteins, modulating the cell growth cycle, and attenuating the inflammatory effects. The study aims to provide an effective therapeutic option for the clinical management of AUB. Unfortunately, the study did not further analyze the expression of target proteins under the intervention of Erzhiman to understand the relationship between the two and clarify the therapeutic mechanism at a deeper level.

Author's contribution

Xiaoxiao Han: manuscript writing, data organization, visualization; Pengyu Han: visualization; Yixin Li: data organization; Shimao Liu: paper review, financial support

conflict of interest

No conflict of interest in this study

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