ORIGINAL RESEARCH

The Mechanism of Jinkui Wenjing Tang in the Treatment of Anovulatory Dysfunctional Uterine Bleeding was Explored Through Network Pharmacology and Molecular Docking Technology

Sisi Tang, MD; Shuangyan Weng, BD; Zhi Chen, MD; Yu Huang, MD; Renzhi Hu, MD

ABSTRACT

Objectives • Jinkui Wenjing Tang (JKWJT), a Traditional Chinese Medicine prescription for gynecological menstrual adjustment, is also used to treat continuous uterine bleeding and abdominal pain. However, the mechanism of action, potential targets, and active ingredients of JKWJT in the treatment of anovulatory dysfunctional uterine bleeding (ADUB) remain unknown. Therefore, it is imperative to explore the molecular mechanism of JKWJT.

Methods • The chemical composition and target of JKWJT were obtained by using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), and the SwissTargetPrediction website. ADUB-related targets were collected through the GeneCards database. The protein-protein interaction network was constructed from the target protein. Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were performed. The binding of the compounds in JKWJT and the potential therapeutic target molecules was verified by molecular docking. Finally, immunohistochemistry, Western Blotting, and qPCR were used for target expression validation within ADUB patient tissues.

Results • The putative targets of JKWJT for the treatment of ADUB mainly involve ESR1, VEGFA, TNF, AR, OXTR, and PCNA. Functional enrichment analysis showed that the therapeutic effect of JKWJT on ADUB was correlated to response to estradiol, gland development, regulation of hormone levels as well as endocrine resistance, estrogen signaling pathway, HIF-1 signaling pathway, EGFR tyrosine kinase inhibitor resistance, MAPK signaling pathway, prolactin signaling pathway. Molecular docking showed that the target OXTR was expected to have a good binding affinity with 4 corresponding compounds (Girinimbin, icosa-11, 14, 17-trienoic acid methyl ester, Kanzonol F, and Obacunone). After treatment with JKWJT, OXTR relative protein expression and mRNA levels were significantly reduced in ADUB patients.

Conclusion • In this study, the basic pharmacological effects, and mechanisms of JKWJT in the treatment of ADUB were elucidated, thus providing a clinical basis for the treatment of ADUB by JKWJT. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

According to the different pathogenesis, dysfunctional uterine bleeding (DUB) is mainly divided into 2 types, namely, ovulation (ODUB) type and anovulatory (ADUB) type, of which the incidence of ADUB is about 80%.[1] ADUB primarily arises from the dysfunction of the hypothalamic-pituitary-ovarian (HPO) axis neurosecretion, resulting in abnormal uterine bleeding with non-organic lesions.[2,3] It is one of the most prevalent gynecological disorders, necessitating the exclusion of organic causes of uterine bleeding during diagnosis. The clinical manifestations of DUB are characterized by irregular menstrual cycle, unstable menstrual flow (excessive or inexhaustible vaginal bleeding), accompanied by cold pain in the lower abdomen, and even anemia in severe cases.[4,5]

Primary treatment strategies for DUB encompass symptomatic approaches involving hormones, antibiotics, endocrine regulation, and surgery.[4,6] With the advancement of clinical practice, at present, ADUB is mostly treated with drugs or surgery, among which, the treatment with drugs can not only inhibit the proliferation of the endometrium but also prevent the atrophy of the endometrium. However, long-term hormone usage can easily induce hormone-dependent tumors and cause liver damage,[7] while the long-term use of contraceptives will suppress ovulation,[8] affect fertility, and lead

to various side effects. Surgical interventions such as diagnostic curettage cannot be repeated, which has a significant impact on the patient both physically and psychologically, causing low compliance and acceptance, noticeable side effects, and a high recurrence rate.[9] Therefore, to enhance the efficacy of drug treatment, minimize drug-related side effects, and improve patient compliance and acceptance, it became imperative to explore new drug development and conduct clinical research on the underlying mechanisms.

Although there is no record of anovulatory dysfunctional uterine bleeding in traditional Chinese medicine (TCM), it is classified as "metrorrhagia" based on clinical manifestations, and its etiology is complicated, and the disease is prolonged and repeated, mostly as an emergency and severe disease.[10] TCM believes that the disease mechanism of "metrorrhagia" is the deficient cold of thoroughfare and conception channels and stasis stops internally. It is believed that the pathogenesis of "uterine bleeding" is mainly due to various pathogenic factors that lead to thoroughfare and conception channels' damage, which cannot restrict menstrual blood, and lead to the uterine dysfunction.[11] Jinkui Wenjing Tang (JKWJT) from Zhang Zhongjing, was used to treat uterine bleeding and abdominal pain, and on this basis, both the disease of insufficient qi and blood caused by the deficient cold of thoroughfare and conception channels, blood stasis, and internal obstruction are treated.[12] It is referred to as gynecological menstrual adjustment and is widely used in clinical practice, but the application and clinical research in treating uterine bleeding are lacking. Guo et al. used JKWJT to treat 80 cases of dysfunctional uterine bleeding, achieving a good therapeutic effect with a low recurrence rate.[13] Wuzhuyu, Shengjiang, and Guizhi in the formula can warm the meridian, disperse cold, warm the uterus, and promote blood stasis. Mudanpi, Danggui, Chuanxiong, and Baishao can nourish blood and promote blood stasis. Renshen and Gancao can tonify middle Jiao and Qi. Banxia and Maidong can moisten dryness and reduce adverse reactions. Various medicinal herbs can warm and nourish thoroughfare and conception channels, nourish the blood, promote blood stasis, and mainly support the right and dispel evil. JKWJT has twelve ingredients and can be used to treat any gynecological miscellaneous disease that belongs to the Xiayuan deficiency, is deficient cold of thoroughfare and conception channels, and involves blood stasis.

This study enrolled 5 patients diagnosed with ADUB at Chongqing Hospital of Traditional Chinese Medicine from October 2020 to September 2022 as research subjects. The study aimed to explore the mechanism of JKWJT combined with acupuncture in the treatment of ADUB. Network pharmacology was employed to investigate the potential active ingredients and candidate gene targets of JKWJT for ADUB treatment. Therefore, the objective of this study is to investigate the mechanism of JKWJT in the treatment of ADUB using network pharmacology and molecular docking technology, to provide a clinical basis for ADUB treatment and contribute to the advancement of TCM in this field.

MATERIALS AND METHODS

JKWJT preparation

The JKWJT decoction consists of twelve herbs, including Evodiae Fructus (Wuzhuyu, 18 g), Ginger Officinale Roscoe (Shengjiang, 12 g), Cinnamomi Ramulus (Guizhi, 12 g), Cortex Moutan (Mudanpi, 12 g), Angelicae (Danggui, 12 g), Chuanxiong Rhizoma (Chuanxiong, 12 g), Paeoniae Radix Alba (Baishao, 12 g), Panax Ginseng C. A. Mey. (Renshen, 12 g), Asini Corii Colla (Ejiao, 12 g), Licorice (Gancao, 12 g), Arum Ternatum Thunb. (Banxia, 12 g), and Camellia petelotii (Merr.) Sealy (Maidong, 12 g). JKWJT was prepared by adding 1000 mL of water and frying, then boiling for 30 min with low heat, filtering 150 mL of medicinal liquid into sealed packaging, and refrigerating for later use. 1 dose per day, divided into 3 times in the morning, noon, and evening, 30 min after meals, with continuous treatment for 3 months, and stop taking the drug during menstruation.

Specimen collection

The study collected 5 patients with ADUB who were treated in the gynecology clinic of Chongqing Traditional Chinese Medicine Hospital from October 2020 to September 2022, and the treatment lasted for 3 months through oral administration of JKWJT combined with acupuncture. Pre- and post-treatment endometrial tissue was collected for follow-up studies. This study was approved by the hospital ethics committee (2021ZY3940-1.0). Patients were informed of the study, including all endometrial tissue biopsies and they signed a consent form.

Inclusion and exclusion criteria

Inclusion criteria: Participants who met the diagnostic criteria outlined in the draft of the Clinical Diagnosis and Treatment Guidelines for Dysfunctional Uterine Bleeding.[14]

Exclusion criteria: (1) Individuals with severe pathological or reproductive tract lesions, complex atypical hyperplasia, malignant tumors, or incomplete medical records; (2) Participants who have taken medications causing bleeding within the past month or may influence the results of this study; (3) Individuals with coexisting coagulation disorders, mental abnormalities, or pregnancy.

Oxytocin receptor (OXTR) expression detection in endometrial tissue

Immunohistochemistry (IHC) was used to detect the expression localization and expression level of oxytocin receptor (OXTR) in endometrial tissue. After obtaining endometrial tissue under sterile conditions, it was fixed in a 4% formaldehyde solution, and all samples were immunohistochemically detected for the expression of OXTR in the endometrium. Western blotting (WB) was used to detect OXTR protein expression in endometrial tissue. Briefly, radioimmunoprecipitation assay (RIPA) cell lysate was added to endometrial tissue, total protein was extracted, supernatant was centrifuged, and quantitative detection was performed with bicinchoninic acid (BCA) kit (P0012,

Beyotime, Shanghai, China). 50 μg of the tested protein was mixed with 5 times the volume of loading buffer and then boiled in a water bath for 10 min, centrifuged, followed by electrophoresis, and then transferred to a polyvinylidene fluoride (PVDF) membrane. Skim milk powder was added and then placed in a shaker (room temperature) sealing for 2 h. OXTR primary antibody (1:500) was added after washing, and then incubated overnight at 4°C. Thereafter, goat anti-rabbit IgG secondary antibody (1:2000) was added, and incubated at room temperature for 2 h. Enhanced chemiluminescence (ECL) solution was added after washing, exposed in the dark room, scanned, and analyzed with a gel imaging system. In addition, quantitative reverse transcription polymerase chain reaction (qRT-PCR) detects the expression of OXTR mRNA in the endometrium. Total RNA from tissues was extracted using Trizol reagent, RNA was reverse-transcribed to cDNA by reverse transcription kit (R211, Vazyme, Nanjing, China), and qRT-PCR reaction was performed using HiScript II One Step qRT-PCR Probe Kit (Q222-01, Vazyme, Nanjing, China). The relative expression level of OXTR mRNA was calculated by the $2^{-\Delta\Delta CT}$ method using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference, and the primer sequences were as follows: OXTR-F CTGAACATCCCGAGGAACT, OXTR-R CTCTGAGCCACTGCAAATGA.

Search and integration of chemical components of JKWJT

The main chemical components of Wuzhuyu, Shengjiang, Guizhi, Mudanpi, Danggui, Chuanxiong, Baishao, Renshen, Ejiao, Gancao, Banxia were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php), respectively. Oral availability (OB) ≥30%, and drug-like properties (DL) ≥0.18 were initially screened for active ingredients to obtain active compounds. The small molecule targets were retrieved through the SwissTargetPrediction website, and Probability >0.1 was used as a screening principle for candidate target genes collection.

ADUB disease target acquisition

ADUB-related targets in the GeneCards (https://www.genecards.org/) database were queried and downloaded by taking "anovulatory dysfunctional uterine bleeding" as the search term. The intersection analysis of active ingredient targets and disease-related targets was performed to obtain intersection genes.

Construction of the JKWJT-ADUB Network

To scientifically explain the therapeutic effect of JKWJT on ADUB by network pharmacology, this study predicted the relationship between compounds and targets by constructing a visualization network through Cytoscape 3. 6.1 Software. The nodes in the network represent drug components, disease targets, and drug targets, and if a compound acts on a potential target, it is connected by edges. The protein-protein interaction (PPI) network was built by uploading the intersection targets to String11. 0 database (https://string-db.org/), the organism species was set to "homo sapiens", and the rest of the settings were maintained as default.

Bioinformatics

The intersecting target genes of JKWJT and ADUB were independently subjected to rigorous bioinformatic scrutiny utilizing the DAVID database (https://david.nciferf.gov/ Version 6.8) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/). Notably, the analysis was confined to the human species. A stringent significance threshold of $P < .05$ was implemented. The investigation encompassed both Gene Ontology (GO) and KEGG metabolic pathway enrichment analyses. The enrichment results with significant differences were screened in ascending order according to the *P*-value size, and the top 30 GO terms and the top 30 KEGG signal pathways with the most significant enrichment results were selected and shown in the figure.

Molecular docking

Firstly, the 2D structure of the core component of the drug (mol2 format file was obtained through the TCMSP database, and Chem: https://pubchem.ncbi.nlm.nih.gov/ database), was obtained and Chem 3D was used to convert it into a 3D structure. Secondly, the 3D structure of the core target protein was obtained (downloaded the pdb format file of the macromolecular target protein from the PDB database: https://www.rcsb.org/). Water molecules and small molecule ligands were separated by PyMOL, and molecular docking preparation was performed using Auto Dock 4.2.6 for receptor hydrogenation and charge calculation. Molecular docking between the receptor protein and the small molecule was then performed by AutoDock Vina 1.1.2 to score the binding energy. PyMOL was used to visualize the interaction between receptor proteins and small molecules.

Statistical processing

SPSS 23.0 software was used for data analysis, and the measurement data were represented by mean ± standard deviation ($\bar{x} \pm s$), and the *t* test was used for comparison between the two groups, and $P < .05$ indicated that the difference was statistically significant.

RESULTS

Potential targets of JKWJT in treating ADUB

The twelve chemical components of JKWJT were screened by OB ≥30% and DL ≥0.18 to obtain 209 active ingredients, and a total of 1033 targets remained after duplication was deleted. Using "anovulatory dysfunctional uterine bleeding" as the search term, 500 ADUB-related target genes were obtained through the GeneCards database (Figure 1A). The 1033 targets corresponding to the chemical composition of drugs in JKWJT and 500 ADUB-related targets were analyzed. 32 potential JKWJT targets for ADUB treatment were obtained, such as ESR1, VEGFA, TNF, AR,

Figure 1. The Intersecting Genes of Targets of JKWJT Active Ingredients and ADUB-Related Targets. (A) The Intersecting Gene Venn Diagram. (B) PPI Network Diagram of Targets of JKWJT Treating ADUB. (C) GO and (D) KEGG Enrichment Analysis Bubble Plots.

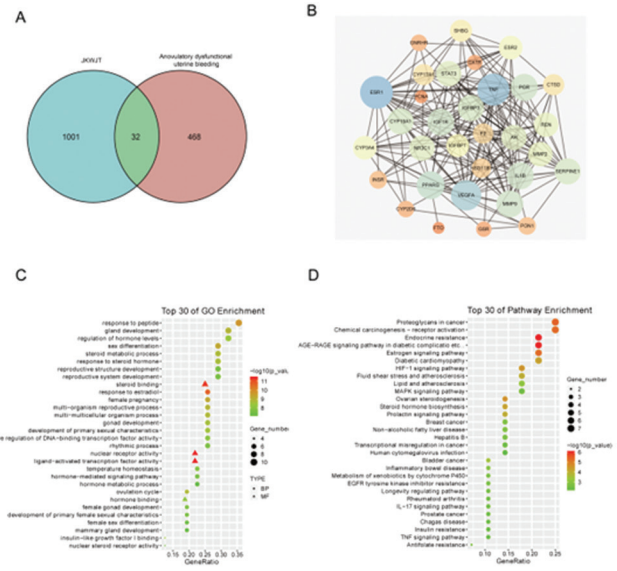
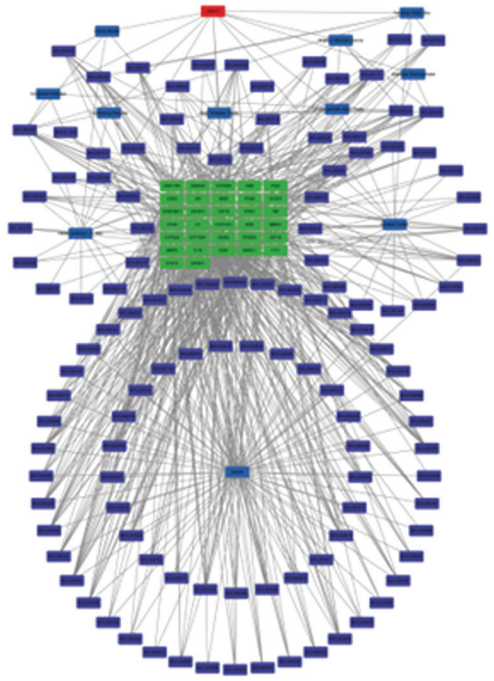


Figure 2. JKWJT-ADUB Target Network Diagram. Green Nodes Represent Target Proteins, Purple Nodes Represent Compound Components, and Red Nodes Represent JKWJT.



OXTR, and PCNA. As shown in Figure 1B of the PPI network, where the size of the node is proportional to the degree of connection. When more edges are connected to the node (the larger the degree corresponding from red to blue), the larger the node becomes.

GO and KEGG enrichment analysis of JKWJT in treating ADUB

According to the potential targets of JKWJT for the treatment of ADUB obtained in Figure 1A, GO and KEGG enrichment analysis was performed on these genes. The top 30 GO terms were displayed using $P < .05$ as the screening criterion, sorted by P-value, and a total of 4 molecular function (MF) entries and 26 biological process (BP) entries were found to be enriched (Figure 1C). In addition, it was found that BPs are mainly involved in response to estradiol, gland development, and regulation of hormone levels; while MFs mainly involve steroid binding, nuclear receptor binding, ligand-activated transcription factor activity, and hormone binding. KEGG pathway enrichment analysis was performed on 32 target genes of JKWJT for ADUB, and a total of 161 pathways were obtained. Taking $P < .05$ as the filter condition, 61 signal pathways are found to be enriched. 30 signaling pathways were screened according to the P-value (Figure 1D), including endocrine resistance, estrogen signaling pathway, hypoxia-inducible factor (HIF-1) signaling pathway, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance, mitogen-activated protein kinase (MAPK) signaling pathway, ovarian steroidogenesis, steroid hormone biosynthesis, and prolactin signaling pathway which are associated with the endometrium.

Network diagram of intersection target in the active ingredient therapy of JKWJT for treating ADUB

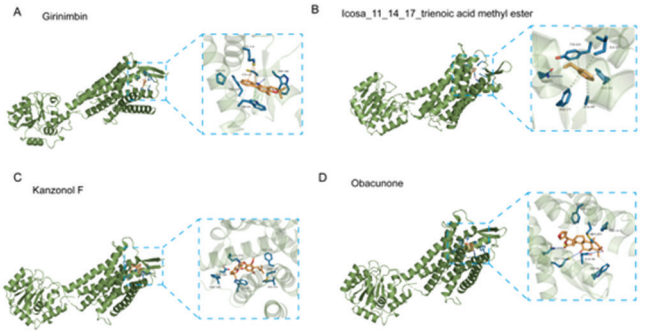
A drug-component-target-disease direct target network was constructed using Cytoscape software representing the interaction relationship of JKWJT-ADUB targets (Figure 2). The red rectangle represents JKWJT, the purple rectangle represents the composition of TCM, and the green rectangle represents the intersection of the target gene of JKWJT and the potential target of ADUB.

Molecular docking analysis

The PPI network diagram and the results of the GO and KEGG pathway enrichment analysis suggest that OXTR may play a key role in the treatment of ADUB using JKWJT, by participating in the regulation of endometrium-related responses to estradiol, female pregnancy, gland development, and other biological processes. To further verify the regulatory effect of JKWJT on OXTR, according to the drug-component-target-disease direct target network diagram, the top components connected to OXTR were searched, and the binding effect of components and OXTR was analyzed by molecular docking. A total of four components, Girinimbin (Panax Ginseng C. A. Mey.), icosa-11, 14, 17-trienoic acid methyl ester (Evodiae Fructus), Kanzonol F (licorice), and Obacunone (Evodiae Fructus) were obtained, and the molecular docking analysis with OXTR was carried out. The results are shown in Figure 3. The minimum binding energy of Girinimbin to the OXTR target is less than -8.6 kcal/mol, forming six hydrophobic interactions with OXTR (PRO95, TRP99, PHE105, and TRP188), one hydrogen bond (CYS187),

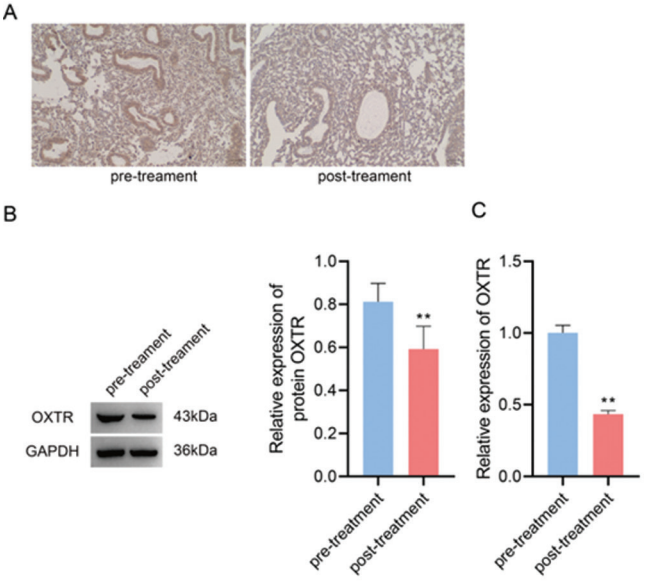
and three Pi-stacking (TRP99) (Figure 3A). The minimum binding energy of icosa-11, 14, 17-trienoic acid methyl ester to OXTR is less than -8.1 kcal/mol, forming five hydrophobic interactions with OXTR (PHE175, ALA189, PHE191, TYR200, and ILE201) and one hydrogen bond (GLN171) (Figure 3B). The minimum binding energy of Kanzonol F to OXTR is less than -8.8 kcal/mol, forming nine hydrophobic interactions with OXTR (TRP99, PHE105, LYS116, ASP186, PHE311, ILE312, and MET315) and one hydrogen bond interaction (LYS116) (Figure 3C). The minimum binding energy of Obacunone to the OXTR target is less than -9.6 kcal/mol, forming five hydrophobic interactions (TRP99, PHE291, PHE311, and MET315) and three hydrogen bond interactions (GLN96, LYS116, and GLN171) with OXTR, indicating that the above molecules have good binding activity to OXTR gene targets, and Obacunone has the best binding activity to OXTR (Figure 3D).

Figure 3. PyMOL Plotted Interactions Between Small Molecules Binding to OXTR Receptors. Proteins are Represented in Cartoon Form, with Blue Sticks Representing Protein Interaction Residues and yellow Sticks Representing Small Molecules.



After JKWJT treatment, the expression of OXTR in endometrial tissue in ADUB patients decreased. IHC, WB, and qPCR were used to detect OXTR protein and mRNA expression levels in endometrial tissues, respectively. As shown in Figure 4A, OXTR is mainly expressed in the nucleus of tissues. The relative protein expression of OXTR decreases after JKWJT treatment (Figure 4B) and the transcription level of the OXTR gene is significantly reduced (Figure 4C). These results suggest that JKWJT may be able to treat ADUB by modulating OXTR.

Figure 4. OXTR Expression in Endometrial Tissue in ADUB Patients. (A) IHC to Verify OXTR Expression in Endometrial Tissue. (B) WB Validates OXTR Expression Within Endometrial Tissue. (C) qPCR Verifies OXTR Gene Expression Within Endometrial Tissue.



DISCUSSION

Endocrine disorders are common clinical manifestations of gynecological diseases such as ADUB.[15] Endocrine disorders can affect the hypothalamus, ovaries, anterior pituitary, and other tissues, affect reproductive function, and impact women's physiology, psychology, and other health problems. Coupled with the continuous increase in the incidence of the disease in recent years, if endocrine abnormality continues, it will influence the ovulatory function, leading to an increase in the incidence of infertility.[16] There are continuous clinical studies on ADUB and endocrine disorders, but there is no breakthrough in the treatment of this disease, and although the clinical treatment options can control bleeding, they have no ideal value for improving endocrine status. Conventional treatment mainly involves the administration of estrogen. Although it can stop bleeding through the endometrium, it can cause adverse reactions such as nausea and vomiting during treatment, and increase the amount of menstrual blood after stopping the drug.[17,18]

The pathogenesis of ADUB has not been fully elucidated, and in recent years, the research on ADUB has not only focused on the disorder of the reproductive endocrine axis but has also shifted the focus to the level of molecular biology.[19] Studies suggest that under neuroendocrine dysregulation, the local microenvironment of the uterus changes, including growth factors, vasoactive substances, sex hormone receptors, and matrix metalloenzymes.[20-22] Wuzhuyu, Shengjiang, and Guizhi in JKWJT can warm channels for dispelling cold and warm the uterus, remove blood stasis; Mudanpi, Danggui, Chuanxiong, and Baishao can nourish the blood; Gancao replenish qi; Banxia and Maidong can moisturize. All medicines warm up and flush the thoroughfare and conception channels, nourish blood and disperse the stasis, support the right, and dispel the evil. In this study, network pharmacology combined with the molecular docking method was used to explore the mechanism of action of JKWJT in the treatment of ADUB.

Through PPI analysis, 32 potential targets including ESR1, VEGFA, TNF, AR, OXTR, PCNA, etc. of JKWJT for the treatment of ADUB were obtained. As early as 1999, Hyder and Stancel believed that estrogen regulates vascular endothelial growth factor (VEGF) mRNA expression in human endometrial adenocarcinoma cells and rat uterus via

estrogen receptors (ER). VEGF expression is associated with changes in angiogenesis and vascular reactivity in placental tissue,[23] and thus estrogen can regulate angiogenesis and vascular permeability. Therefore, estrogen plays a key role in physiological health conditions such as DUB and endometriosis that are caused by physiological dysfunction of the female reproductive tract.[23] Studies at the cellular level have investigated the effects of estrogen on ADUB, and their findings indicate that estrogen can increase the expression of VEGF, thereby activating the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway to induce matrix metalloproteinase 2/9 (MMP2/9) expression.[24]

Interestingly, Lv et al's study indicates that the Chinese herbal medicine XBR has hemostatic, coagulative, and anti-inflammatory effects in SD rats, promoting the repair of rat endometrium by altering the mRNA expression level of matrix metalloproteinase 1 (MMP1).[25] Furthermore, the results of a prospective study also indicate that the expression frequency of MMP2/9 in the uterine endometrial matrix and the expression frequency of VEGF in uterine glands of ADUB-afflicted women are significantly higher than those in the control group, thus potentially playing a significant role in the pathogenesis of ADUB.[26] As an ER target, ESR1 can effectively improve the menstrual cycle of patients, promote endometrial repair, and reduce uterine bleeding. Case-control experiments have shown that ER and progesterone receptor (PR) levels are significantly elevated in patients with DUB.[27] Studies have shown that selective ESR modulator-clomiphene can be used for the initial treatment of DUB, suppress uterine bleeding, and promote ovulation cycle recovery.[28] As one of the inflammatory mediators, tumor necrosis factor (TNF) can not only fight tumors, but also induce apoptosis of egg cells, damage endothelial cells to affect ovarian blood supply, and then affect the secretion of hormones, leading to menstrual disorders and uterine bleeding. Thus, inhibiting the activation of TNF inflammation can effectively reduce the amount of bleeding, inhibit the inflammatory response, and promote the repair of the endometrium. The proliferating cell nuclear antigen (PCNA) has recently received attention in the study of gynecological endocrine diseases. PCNA is a nuclear protein specifically expressed by proliferating cells, with a molecular weight of 36 kDa. In the endometrium of women of normal childbearing age, the expression of PCNA changes periodically. Studies have shown that the expression of PCNA in the endometrial gland epithelium of patients with ADUB is stronger than that in the normal endometrial proliferative period, and the inhibition of apoptosis of endometrial glandular epithelial cells in patients with ADUB is enhanced compared with the normal proliferative period, and the overexpression of PCNA compensates for endometrial hyperplasia, indicating that PCNA has the bidirectional regulatory effect of inhibiting apoptosis and cell proliferation.[29,30] Yao et al. employed network pharmacology and molecular docking to investigate the identification of effective components in Huanghuai for the treatment of dysfunctional uterine bleeding. The results suggest that TNF may serve as a potential molecular basis for the Huanghuai's treatment of DUB.[31]

The enrichment results of the GO and KEGG metabolic pathways showed that the treatment of ADUB by JKWJT may be related to BP such as response to estradiol, gland development, regulation of hormone levels, as well as endocrine resistance, estrogen signaling pathway, HIF-1 signaling pathway, EGFR tyrosine kinase inhibitor resistance, MAPK signaling pathway, prolactin signaling pathway, etc. which are endometrium-related pathways. The results of PPI, GO, and KEGG enrichment analysis suggest that OXTR may be a key regulator of JKWJT in the treatment of ADUB. Our GO enrichment shows that OXTR is involved in response to estradiol, gland development, regulation of hormone levels, etc. However, there are currently no research reports directly linking the mechanism of OXTR and ADUB. Oxytocin works by binding to OXTR, a nonapeptide neuroendocrine hormone released into the blood by the posterior pituitary gland, which has basic functions such as promoting childbirth, breastfeeding, and preventing postpartum hemorrhage. Oxytocin also has an anti-inflammatory effect and can inhibit oxidative stress-induced apoptosis.[32-34] The expression of OXTR in women with endometriosis is significantly increased, which is closely related to abnormal uterine contractile activity, thus affecting fertility and dysmenorrhea severity.[35] Recent research indicates a significant correlation between increased OXTR methylation level and elevated relative risk of postpartum hemorrhage in delivering patients. The evidence suggests an association between OXTR epigenetics and the dosage of oxytocin used during childbirth.[36] The interaction of small molecules (Girinimbin, icosa-11, 14, 17-trienoic acid methyl ester, Kanzonol F, Obacunone) and OXTR was analyzed by molecular docking experiments, and the results showed that Obacunone and OXTR had the most stable binding effect (-9.6 kcal/mol), forming three hydrogen bond interactions with OXTR (GLN96, LYS116, and GLN171) and multiple hydrophobic effects. IHC, WB, and qPCR experiments showed that the relative protein expression of OXTR was reduced and the transcription level of the OXTR gene was significantly decreased after JKWJT treatment, suggesting that JKWJT may treat ADUB by regulating OXTR.

JKWJT treatment of ADUB possibly works by targeting OXTR, modulating the endocrine resistance, estrogen signaling pathway, and prolactin signaling pathway; and involving in BP such as response to estradiol, gland development, regulation of hormone levels, etc. In the treatment of patients with ADUB, the effect of reducing the level of OXTR in endometrial tissue is significant. In this study, network pharmacology and molecular docking methods were employed to identify the potential active ingredients and OXTR targets in JKWJT for treating ADUB. However, the experimental validation of these findings, both *in vitro* and *in vivo*, has not been conducted extensively. Only the expression changes of OXTR after JKWJT treatment in ADUB patients were preliminarily examined. The cellular-

level *in vitro* experiments elucidating the mechanism of JKWJT's efficacy will be further investigated in subsequent experiments. Therefore, we encourage further exploration of relevant content and conduct biological experiments to gain a deeper understanding of the therapeutic mechanisms of JKWJT in treating ADUB.

AUTHOR DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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Not applicable.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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