RESEARCH ARTICLE

Network pharmacology and experimental validation to explore the molecular mechanisms of kidney and blood refreshing recipe for the treatment of intrauterine adhesions

Renzhi Hu[1] · Min Xia[1] · Shuangya Weng[1] · Zhi Chen[1] · Zhen Wang[1] · Xuemei Zou[1] · Yan Zhang[1] · Yiming Chen[1] · Sisi Tang[1]

Abstract

Kidney and blood refreshing recipe formula is usually used to effectively treat intrauterine adhesions (IUAs). However, little is known about the underlying mechanism of kidney and blood refreshing recipes. We investigated the pharmacological mechanism of Bushen Yixue recipe on IUA through pharmacological and experimental validation. By network pharmacological analysis, 183 active constituents of the kidney and blood refreshing recipe were screened out (retrieved from TCMSP and TCM-ID). According to Venn diagram analysis, there were 102 common targets of the kidney and blood refreshing recipe. PPI analysis of the above common targets showed that there were 102 nodes and 1678 edges. Subsequently, GO enrichment and KEGG pathway analyses were also performed, which resulted in 1582 biological processes, 111 molecular functions, 82 cell components, and 174 related pathways. The active component of the kidney and blood refreshing recipe was also determined by liquid mass spectrometry. Finally, a rat model of IUA was constructed and tested in vivo. After kidney and blood refreshing recipe treatment, HE staining, Masson staining and immunohistochemistry (TGF-β1, SDF-1, VEGF) were performed on the uterine tissues of rats. The results showed that fibrosis in the uterine tissue was inhibited. The kidney and blood refreshing recipe inhibits fibrosis by inhibiting the expression of genes typical of fibrosis.

Keywords Intrauterine adhesions · Kidney and blood refreshing recipe · Network pharmacology · PPI · Animal model

Background

Intrauterine adhesion (IUA), also known as Asherman's syndrome (AS), was first proposed by Joseph Asherman in 1948 (Asherman 1948). IUA is a complication caused by damage to the basal layer of the endometrium, which can be attributed to mechanical trauma, infection, and other factors (Song et al. 2021). Furthermore, IUA causes severe endometrial dysfunction, which can lead to partial or complete occlusion of the cervical canal and/or uterus (Khan and Goldberg 2018; March 2011), eventually leading to distortion or even disappearance of the uterine cavity (Zhu et al. 2019). It has been shown that IUA formation is often associated with uterine trauma and postoperative infections, including early miscarriage, cervical biopsy, polypectomy, or insertion of an intrauterine device (IUD) (Evans-Hoeker and Young 2014). The incidence of IUA is approximately 1.5%. Notably, the incidence of intrauterine insufficiency was increased to 39% by repeated curettage caused by abortion (Deans and Abbott 2010; Lee et al. 2021). Hysteroscopic adhesiolysis is the first choice for the treatment of IUA, but while early cure effects are good, the current situation is of poor prognosis (Hooker et al. 2014). At present, the pathogenesis of IUA is not well understood, so it is important to explore the mechanism.

Studies have shown that transforming growth factor-β1 (TGF-β1) is significantly related to the occurrence and development of IUA, which is an important mediator promoting fibrosis (Abudukeyoumu et al. 2020). The expression levels of TGF-β1 and Smad3 were increased in IUA animal models and human endometrial samples (Li et al. 2016). Furthermore, in patients, TGF-β1 expression was significantly higher in severe IUA than in the endometrium of mild and moderate IUA (Zhou et al. 2018). Stromal-derived

✉ Sisi Tang
  sisi_03162023@163.com

1 Department of Gynecology, Chongqing Traditional Chinese Medicine Hospital, No. 6, Panxi, 7th Branch Road, Jiangbei District, Chongqing 400021, China

factor (SDF)-1 is a dynamically altered chemokine secreted by damaged tissues (Zhou et al. 2018). In a rat model, the kidney and blood refreshing recipe affected the expression of rat endometrial vascular endothelial growth factor (VEGF) (Lv et al. 2020). Studies have shown that downregulation of TGF-β1, SDF-1 and VEGF can effectively slow IUA, reduce fibrotic tissue, and improve endometrial regeneration (Feng et al. 2023; Zhang et al. 2019).

In China, traditional Chinese medicine (TCM) has a history of thousands of years as the main treatment for diseases (Shi et al. 2014). Through the synergistic action between various drugs, TCM has shown obvious advantages over single-drug therapy, especially for diseases caused by multiple complex factors (Stone 2008; Tapmeier et al. 2008). Chinese herbal medicine has relatively low toxicity and is widely used to treat diseases (Xiao and Tao 2017). As a traditional Chinese medicine prescription, the kidney and blood refreshing recipe is widely used in the treatment of gynaecological diseases (Lyu et al. 2018). Studies have shown that the kidney and blood refreshing recipe formula can reduce endometrial development (Ma et al. 2015). In particular, the immune microenvironment in the endometrium is regulated (Gong et al. 2017). However, the mechanisms involved are not fully understood. Network pharmacology is a new discipline that can be used to study the mechanism of disease and drug action. It needs to be carried out in the background of the biological network (Fotis et al. 2018). Therefore, a new method, network pharmacology of TCM, is established by combining TCM with the method of network pharmacology, which can further reveal the correlation between drugs, genes and diseases and analyse the network regulation effect of TCM formula (Li and Zhang 2013; Liang et al. 2014).

In this study, based on the strategy of network pharmacology, the active ingredients of kidney and blood refreshing recipe were screened, and targets were predicted. In vivo experiments (construction of an intrauterine adhesion rat model) were conducted to explore the active ingredients, potential targets and molecular mechanisms of kidney and blood refreshing recipe against IUA to provide an experimental basis for the next application.

Materials and methods

Screening the active ingredients and targets of the kidney and blood refreshing recipe

In this study, all components of the kidney and blood refreshing recipe were obtained utilizing retrieval in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and Traditional Chinese Medicine Information Database (TCM-ID). The obtained components were input into the TCMSP platform for information matching. In this study, the criteria for screening active ingredients were as follows: oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18. Subsequently, the components of the kidney and blood refreshing recipe were sorted out through the literature, and the predicted results were supplemented. PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the molecular structure of the active ingredient SDF. Finally, the Swiss Target Prediction platform was used to predict its potential target.

Search targets related to intrauterine adhesions

"Intrauterine adhesion" was used in the keywords in the Gene Cards database (https://www.genecards.org/) and OMIM database (https://omim.org/) for retrieval and filtering (Amberger et al. 2015). The obtained targets were summarized, and duplicate values were removed. The UniProt database was used to standardize the names of disease target proteins, and the insomnia target database was established.

Construction of the target network

Integration of the kidney and blood refreshing recipe and common targets as key genes was performed using Venny2.1 (https://bioinfogp.cnb.csic.es/tools/venny/), and the entries were targets of drug targets and disease. After integrating the data, a Venn diagram was drawn online to obtain the targets of drug and disease interactions. IUA active ingredients and key targets were imported into Cytoscape3.7.2 software. The "drug—active ingredient—key target" regulatory network was constructed according to the corresponding relationship and attributes.

Construction of the PPI network

The STRING database (https://string-db.org/) was used to analyse key targets (Szklarczyk et al. 2017) and construct protein−protein interaction (PPI) networks with biological species as "Homosapiens".

GO enrichment and KEGG pathway analyses

Key target genes were imported into the Metascape platform (https://metascape.org/) for Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. GO enrichment can be used to study the mechanism of action of molecular function (MF), biological process (BP) and cellular component (CC). KEGG enrichment analysis was used to study signalling pathways that were associated with treating IUAs. These results are saved through the microscopic letter (http://www.bioinformatics.com.cn/) to visualize the results analysis.

Liquid mass spectrometry

The boiled kidney and blood refreshing recipe was sent to a company (Shanghai Fuda Detection Technology Group Co., Ltd.) for liquid mass spectrometry analysis. Thermo QE plus liquid chromatograph and Compound Discover data processing software were used to analyse the chemical components of the samples.

Analysis of animal models

Sixty SPF-grade female 8-week-old rats (weight 160–180 g) were purchased from the Laboratory Animal Center of Chongqing Medical University. Two animals in each cage were fed and allowed to drink freely at a room temperature of 25 ± 1 °C, relative humidity of $55 \pm 5\%$ and light time of 12 h/d. After 7 days, they were randomly divided into the following 6 groups: sham operation group, negative control group, positive control group, low dose group, medium dose group and high dose group. Animal experiments pass the experimental animal ethics review (2021120001B) and provide humanitarian care in strict accordance with the 3R principle.

A daily vaginal smear was performed to observe the rat's oestrous. The rats with oestrous cycles were subjected to open abdominal modelling until all rats were completed. A cotton thread was soaked in lipopolysaccharide saline (6 mg/L) at 4 °C for 24 h. The rats were anaesthetized with chloral hydrate (10%) at a dose of 4 mg/kg. After confirming full anaesthesia, the uterus was separated by laparotomy under sterile conditions, and the uterus incision and abdominal incision were sutured after corresponding treatment according to the group. The sham operation group only underwent opening of the abdomen without any treatment of the uterus. For other groups, endometrial curettes were used to scrape the endometrium at the upper two-thirds of the uterus until the inner wall was rough and felt granulated. Then, lipopolysaccharide cotton thread was placed in the uterine cavity, and the cotton thread was removed 7 days later. After modelling, each rat was injected intraperitoneally continuously with gentamicin for 3 d, and weekly weight changes were recorded.

Immunohistochemistry, HE and masson staining

According to the experimental design requirements, rat uteri were collected in groups. The tissues were sent to Wuhan Xavier Biotechnology Co., Ltd. for immunohistochemical, HE and Masson staining.

Statistical analysis

The data were expressed as broken line changes (mean ± standard deviation), with each broken line change representing at least three independent experiments, repeated three times. Statistically significant differences were determined by Student's t test or multivariate analysis of variance.

Results

Screening of the kidney and blood refreshing recipe active ingredients

The compounds of the kidney and blood refreshing recipe were retrieved from TCMSP and TCM-ID. OB $\geq 30\%$ and DL ≥ 0.18 were set according to pharmacokinetic parameters, and 187 active components were screened out. After removing duplicate values, 183 components were obtained, involving 25 kinds. Among them, RS1 had the largest number of targets, which was 27. MY7, MY6, MY16, DS12 and CX1 had the fewest targets, with 15 targets. The main active ingredients are shown in Table 1.

Therapeutic target prediction

By querying the TCMSP database, 187 compound targets were found. Then, after correction by the UniProt database, 1004 targets were obtained. A total of 505 disease targets were obtained by searching "intrauterine adhesion" in the Gene Cards and OMIM databases and screening repeats. Then, the above data were imported into the Venny2.1 online platform for analysis. Finally, the data were presented in the form of a Venn diagram, and the results showed that there were 1088 targets for Bushen Huoxuette prescription and 505 targets for intrauterine adhesion disease. Notably, the two share 102 targets (Fig. 1).

PPI network construction and analysis

To create protein−protein interaction (PPI) networks, common targets were visualized. Therefore, the common gene information involved in Fig. 1 (which belongs to the common information of the kidney and blood refreshing recipe and IUA) was submitted to the STRING11.0 platform to obtain the PPI network of kidney and blood refreshing recipe—IUA target (Fig. 2). In the PPI network diagram, 102 nodes and 1678 edges were involved. Their average degree value was 32.9. The larger the node in the figure, the higher the degree value, and the more likely it is to be the core target.

Table 1 Main active components of the kidney and blood refreshing recipe

Sequence	Name	Component	Number of targets	Source
1	ZHC7	dehydrocorybulbine	18	human placenta
2	ZHC4	4-pregnene-17α,20β,21-triol-3,11-dione	18	human placenta
3	ZHC1	(+)-corybulbine	16	human placenta
4	ZGC5	liquiritigenin	17	baked licorice
5	XJ1	(2 s)-5-methoxy-6-methyl-flavan-7-ol	17	Blood Scorpion
6	TSZ3	isorhamnetin	18	chinese dodder seed
7	SZY3	Hydroxygenkwanin	19	fruit of medicinal cornel
8	RS1	GomisinB	27	ginseng
9	MY8	myrrhanoneA	16	myrrh
10	MY7	myrrhanolC	15	myrrh
11	MY6	(7S,8R,9S,10R,13S,14S,17Z)-17-ethylidene-7-hydroxy-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15-decahydrocyclopenta[a]phenanthrene-3,16-dione	15	myrrh
12	MY2	pelargonidin	18	myrrh
13	MY19	Stigmasterol	19	myrrh
14	MY16	[(5aS,8aR,9R)-8-oxo-9-(3,4,5-trimethoxyphenyl)-5,5a,6,9-tetrahydroisobenzofurano[6,5-f][1,3]benzodioxol-8a-yl]acetate	15	myrrh
15	MY11	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	20	myrrh
16	HJT2	octanoicacid	18	integripetal rhodiola herb
17	FL7	(2R)-2-[(5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,4,10,13,14-pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoicacid	16	tuckahoe
18	F1	quercetin	18	Eucommia/Myrrh/Dodder
19	DS20	tanshinaldehyde	15	root of red rooted salvia
20	DS18	przewaquinonec	18	root of red rooted salvia
21	DS15	formyltanshinone	21	root of red rooted salvia
22	DS12	(E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylicacid	15	root of red rooted salvia
23	CX1	Myricanone	15	chuanxiong rhizome
24	C1	kaempferol	18	Eucommia/Angelica/Myrrh
25	BZ1	14-acetyl-12-senecioyl-2E,8Z,35E-atractylentriol	16	largehead atractylodes rhizome

Fig. 1 Kidney and blood refreshing recipe components IUA target Venn diagram

Kidney and Blood Refreshing Recipe

986   102   403
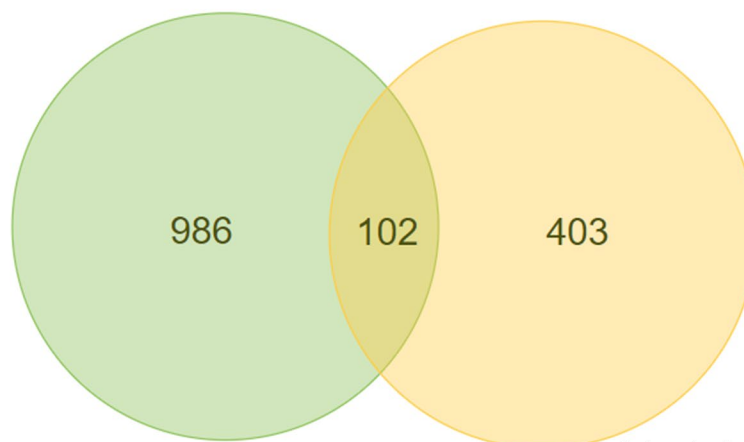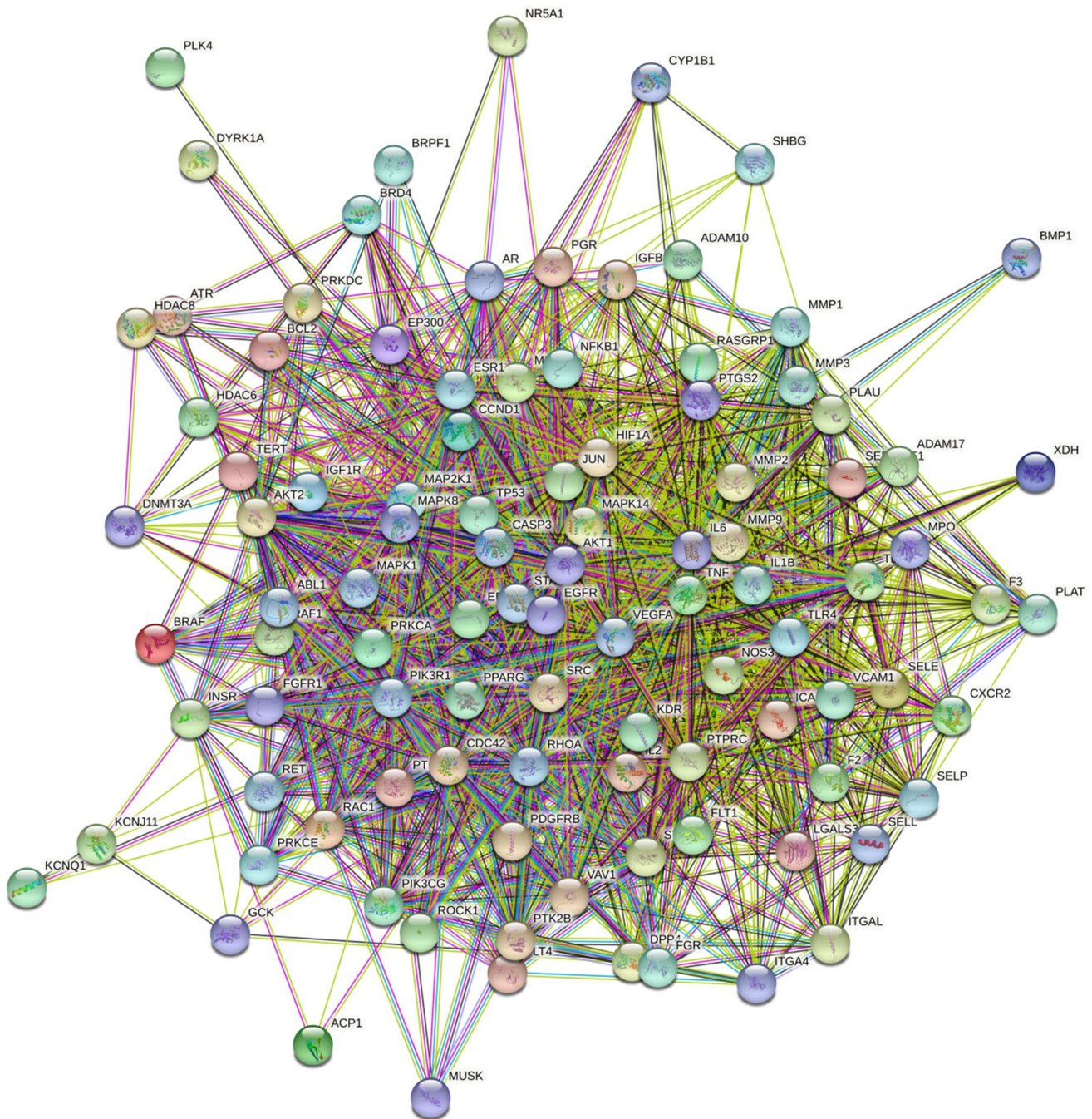
intrauterine adhesion

Fig. 2 Protein−protein interaction network diagram of the kidney and blood refreshing recipe in IUA treatment

GO Enrichment and KEGG pathway analysis

To further explore the mechanism of the kidney and blood refreshing recipe on intrauterine adhesion, GO and KEGG enrichment analyses were performed on 102 intersecting genes (as shown in Fig. 1) on the Metascape platform. The results showed 1,582 biological processes, 82 cell components and 111 molecular functions. A microscopic letter (http://www.bioinformatics.com.cn/) online platform

was used for analysis. The top 20 GO enrichment data were displayed in the form of a bubble map. As shown in Fig. 3A, GO enrichment analysis (Biological Process) mainly involves (top 20) positive regulation of cell migration, protein phosphorylation, regulation of cell adhesion, response to hormone, tube morphogenesis and response to cytokine. In terms of molecular function, GO enrichment analysis (Fig. 3B) mainly involved the top 20 terms: side of membrane, receptor complex, membrane raft, focal adhesion
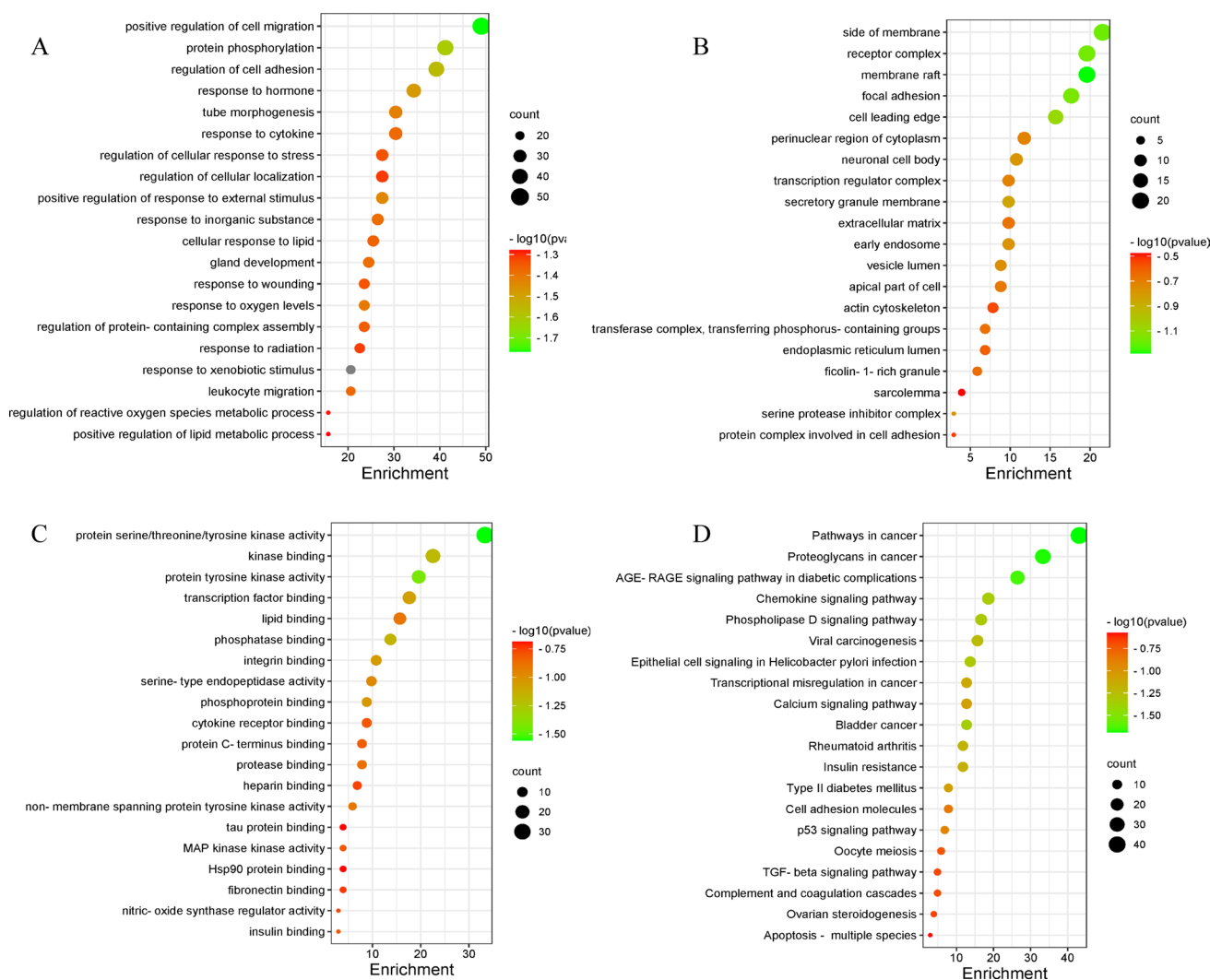
Fig. 3 GO enrichment and KEGG enrichment analyses. **A** GO-BP analysis **B** GO-CC analysis **C** GO-MF analysis **D** KEGG analysis

and cell leading edge. As shown in Fig. 3C, GO enrichment analysis (cell components) mainly revealed the following (top 20): protein serine/threonine/tyrosine kinase activity, kinase binding, protein tyrosine kinase activity, transcription factor binding and lipid binding. To explore the signal pathway mechanism of the kidney and blood refreshing recipe in the treatment of IUAs, we performed a KEGG enrichment analysis, and 174 related pathways were obtained. The top 20 signalling pathways (Fig. 3D) included pathways in cancer, proteoglycans in cancer, the GE-RAGE signalling pathway, the P53 signalling pathway and the TGF-β1 signalling pathway.

Construction of the network graph (kidney and blood refreshing recipe -IUA-pathway)

To further reveal the relationship between signalling pathways and candidate targets and compounds, the "pathway-target-compounds" network was constructed by Cytoscape 3.7.2 software, and correlation analysis was carried out. As shown in Fig. 4, 20 pathway tiles (inverted triangles), 25 components (circular shapes), and 71 targets (diamond shapes) were analysed and networked. Twenty key genes were predicted according to the degree value, such as MMP9, EGFR, KDR, MET, ESR1, and IGF1R, as shown in Table 2.

The active constituents and compounds of the kidney and blood refreshing recipe were identified by liquid mass spectrometry

The composition of the kidney and blood refreshing recipe was determined by Thermo QE plus liquid chromatography. Compound discovery data processing was used to analyse the chemical constituents of the samples. According to the detected compounds, the samples were
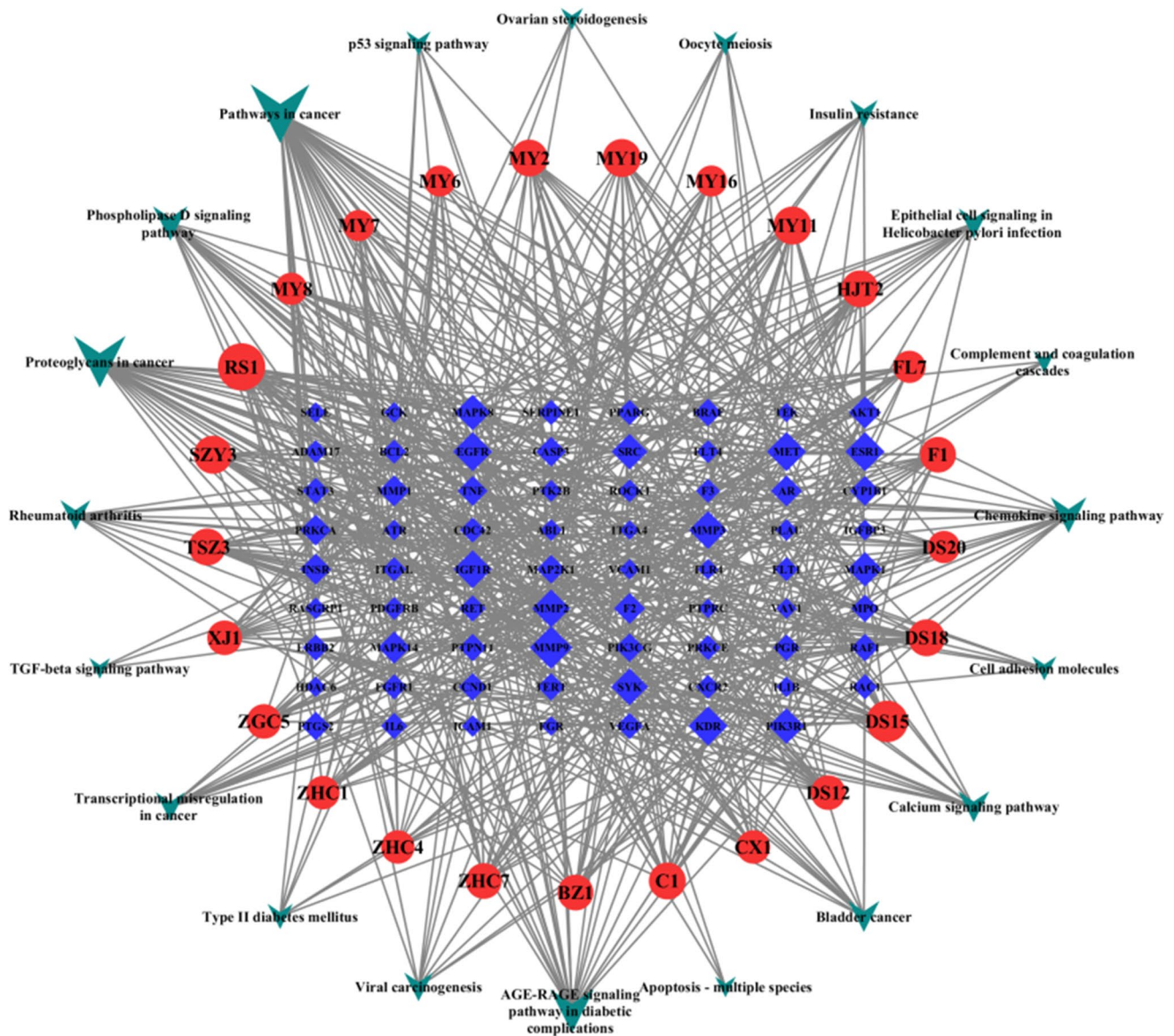
Fig. 4 Drug active ingredient-target-pathway diagram

compared with the traditional Chinese medicine components in the database, and it was concluded that the samples might be composed of traditional Chinese medicine components. The results involved 25 TCM ingredients. The names of TCM ingredients with the top 10 contents are shown in Table 3. Only olibanum, roots of red-rooted salvia, roots of herbaceous peony and liquorice accounted for more than 7 percent. Their percentages were 24.474%, 10.567%, 7.618% and 7.198%, respectively. A total of 156 compounds were identified. The relative contents (%) of the top 10 compounds are shown in Table 4. Among them, the content of acetyl-11-keto-β-boswellic acid was the highest, reaching 20.843%.

The kidney and blood refreshing recipe significantly inhibited uterine fibrosis in rats

The rat uteruses were treated in six different ways according to the experimental design. The results of HE staining showed significant changes between treatments (Fig. 5A). In the sham operation group, the endometrium was intact, the structure of epithelial cells was intact, and the endometrium stroma showed arranged and tufted glands without inflammatory cell infiltration. Pathological phenomena of different degrees were found in the other 5 experimental treatment groups, such as endometrial tissue necrosis, reduction and absence of glands. Among them, the distilled purified water

Table 2 Kidney and blood refreshing -core target of IUA

Name	Target name	Degree
MMP9	Matrix metalloproteinase-9	22
EGFR	Epidermal growth factor receptor	19
KDR	Vascular endothelial growth factor receptor 2	19
MET	beiHepatocyte growth factor receptor	19
ESR1	Estrogen receptor	19
IGF1R	Insulin-like growth factor 1 receptor	18
MMP2	72 kDa type IV collagenase	18
MMP3	Matrix metalloproteinase-3	18
SYK	yrosine-protein kinase	17
MAPK8	Mitogen-activated protein kinase 8/9/10	16
SRC	Proto-oncogene tyrosine-protein kinase	16
PIK3R1	Phosphoinositide-3-kinase regulatory subunit alpha/beta/delta	16
MAPK1	Mitogen-activated protein kinase 1	15
MAPK14	Mitogen-activated protein kinase 14	13
AKT1	RAC-alpha serine/threonine-protein kinase	13
AR	Androgen receptor	12
F2	Prothrombin	12
INSR	Insulin receptor	12
MMP1	Matrix metalloproteinase-1	12
PRKCA	Protein kinase C alpha type	12

group and low-dose intervention group were the most significant. Notably, the high-dose intervention group was the mildest of the five groups.

Masson staining was also performed to compare fibrosis between groups. The results showed (Fig. 5B) that there were many blue collagen fibres in the distilled water group, the low dose group and the positive control group, and the number of collagen fibres in the positive control group and the medium and high dose groups were significantly reduced. Standardized scores were used for gland number and fibrosis. The degree of intimal fibrosis is proportional

to the score, with higher scores indicating greater severity. The results showed (Table 5) that the low-dose group had the highest score, and the high-dose group had the lowest score, indicating that the Bushen Huoxue prescription had a significant dose–effect relationship. The positive control group had a lower fibrosis score, but the glandular number score was significantly higher than that of the high-dose group. Thus, the total score was also significantly higher in the high-dose group.

The kidney and blood refreshing recipe regulated the expression of VEGF, SDF-1 and TGF-β1

Immunohistochemistry of VEGF, SDF-1 and TGF-β1 was performed on the uterine tissues of rats in each group, and the results are shown in Fig. 6 and Table 6. Compared with the sham operation group, the oestrogen expression levels of VEGF and TGF-β1 were significantly increased in the distilled water group, while the positive expression levels of SDF-1 were significantly decreased. Additionally, in the high-dose intervention group, the expression level of VEGF was significantly downregulated ($p < 0.05$), and the positive expression level of SDF-1 was effectively upregulated ($p < 0.05$). Compared with medium- and low-dose interventions, it had a significant dose–effect relationship. It is worth noting that the positive expression of TGF-β1 was significantly downregulated in the low-, middle- and high-dose intervention groups, indicating that TGF-β1 had an obvious regulatory effect ($p < 0.05$).

Discussion

This study used network pharmacology and animal model experiments to determine the mechanism by which Bushen Huoxue Fang affects intrauterine adhesions. The results indicated that the active ingredients and network targets of the

Table 3 Matching results of TCM components

Sequence	Medicine name	Total peak area	Relative content (%)
1	olibanum	23,472,480,551.92	24.474
2	root of red rooted salvia	10,134,508,239.33	10.567
3	root of herbaceous peony	7,306,280,196.41	7.618
4	liquorice	6,902,854,999.20	7.198
5	largehead atractylodes rhizome	5,280,839,148.02	5.506
6	chinese ligusticum rhizome	4,679,419,292.71	4.879
7	common aucklandia root	4,023,869,547.75	4.196
8	human placenta	2,673,177,929.91	2.787
9	fruit of medicinal cornel	2,374,625,507.33	2.476
10	chinese dodder seed	2,104,244,042.45	2.194

Table 4 Information on the identified compounds

Sequence	Names	Relative content (%)
1	Acetyl-11-keto-β-boswellic acid	20.483
2	Citric acid	8.699
3	Paeoniflorin	6.255
4	18 β-Glycyrrhetintic Acid	4.722
5	Atractylenolide I	4.494
6	Cryptotanshinone	4.451
7	Ligustilide	4.102
8	Dehydrocostus lactone	3.429
9	Tanshinone IIA	2.987
10	Lindenenol	2.668

kidney-tonifying and blood-activating formula accurately match, reflecting its good value in the multicomponent and multitargeted treatment of IUA and overall treatment of diseases with traditional Chinese medicine.

In order to reveal the related pathways of Bushen Huoxue Recipe in intrauterine adhesion, we obtained and screened the top 20 key pathways through KEGG analysis, mainly including MAPK pathway and TGF-β1 pathway. Research has shown that MMP2/9, the PI3K-AKT pathway, the MAPK pathway, and the TGF-β1 pathway are closely related to endometrial fibrosis (Abudukeyoumu et al. 2020; Gualdoni et al. 2022; Guo et al. 2022; Li et al. 2021; Liu et al. 2020; Yao et al. 2019; Zhang et al. 2022; Zhou et al. 2022). However, intrauterine adhesions are often characterized by abnormal expression of the endometrium, leading to fibrosis, and endometrial fibrosis is a common cause of uterine infertility (Zhou et al. 2022). We also verified the components of Bushen Huoxue Formula using liquid chromatography−mass spectrometry, and the results were consistent with the drug components screened in the database.

To explore the molecular mechanism by which Bushen Huoxue Fang can treat intrauterine adhesions, three representative targets (TGF1, SDF-1 and VEGF) were screened based on network pharmacology and the existing research results. TGF-β1, SDF-1 and VEGF were detected by HE staining, Masson staining, and immunohistochemistry. Existing evidence indicates that the expression of TGF-β1 and VEGF was inhibited and the expression of SDF-1 was enhanced, which can reduce fibrosis (Li et al. 2019; Yao et al. 2019; Zhang et al. 2019).

The key to healing endometrial trauma is angiogenesis, and fibrosis is a pathological process characterized by tissue scarring that can lead to the destruction of organ structure and functional decline. Endometrial fibrosis is the common pathological manifestation of IUA, and fibrin is the main contributor to the formation of tissue bridges between the walls of the uterine cavity (Evans-Hoeker and Young 2014). TGF-β1 is a key mediator of scar formation in tissue fibrosis (Liao et al. 2003), and some evidence indicates that TGF-β1 is closely associated with the occurrence and development of IUA and is an early risk factor for disease recurrence (Abudukeyoumu et al. 2020). Research shows that inhibiting TGF-β1 and overexpressing Smad2 and Smad3 can block the process of fibrosis and improve the adhesion state.

The uterine cavity is rich in local blood vessels. After uterine injury, regeneration and repair of the endometrium are closely related to local microvessels and the blood supply. VEGF promotes the permeability of venules and veins, the proliferation of vascular endothelial cells, the accumulation of cytoplasmic calcium, and the induction of angiogenesis (McLaren 2000). When the endometrium is damaged or infected, local endometrial ischemia can stimulate the expression of VEGF (Merighi et al. 2006). After binding to specific receptors, VEGF increases vascular permeability, increases fibrinogen exudation in blood vessels, further stimulates and induces fibroblast differentiation and proliferation, and promotes the secretion of large amounts of extracellular matrix, thus affecting endometrial regeneration and repair and leading to IUA. Therefore, inhibiting the expression of VEGF can effectively reduce the excessive deposition of ECM and degrade collagen fibers.

SDF-1, which is also known as CXCL12, promotes angiogenesis by binding to specific receptors and plays an important role in intimal proliferation and embryo implantation. When the uterus is damaged, amount of secretion is significantly reduced, and during the process of self-repair, the secretion gradually increases (Thevenot et al. 2010; Tsuzuki et al. 2012). Immunohistochemical analysis also revealed a significant decrease in the expression of SDF-1 in rats in the control group (distilled water group). After treatment with low, medium, or high doses of the kidney-tonifying and blood-activating formula, dose-dependent recovery was observed, especially in the high-dose group, indicating that this treatment could effectively promote regeneration and repair of the endometrium.

At present, the main treatments for fibrosis include signaling pathway regulation, anti-inflammatory drugs, enzyme inhibitors, kinase inhibitors, metabolic pathway regulation, and stem cell interstitial therapy (Ito et al. 2010), all of which have limited clinical efficacy and a high recurrence rate. Our goal has been to actively explore drugs or treatment methods that have greater utility and can effectively reduce the risk of recurrence. However, long-term exploration and development of traditional Chinese medicine has effectively verified its advantages in treating chronic refractory recurrent diseases. The Bushen Huoxue Formula has advantages

Fig. 5 Uterine HE staining **A** and Masson staining **B** of rats in each experimental group. The experiment was repeated three times
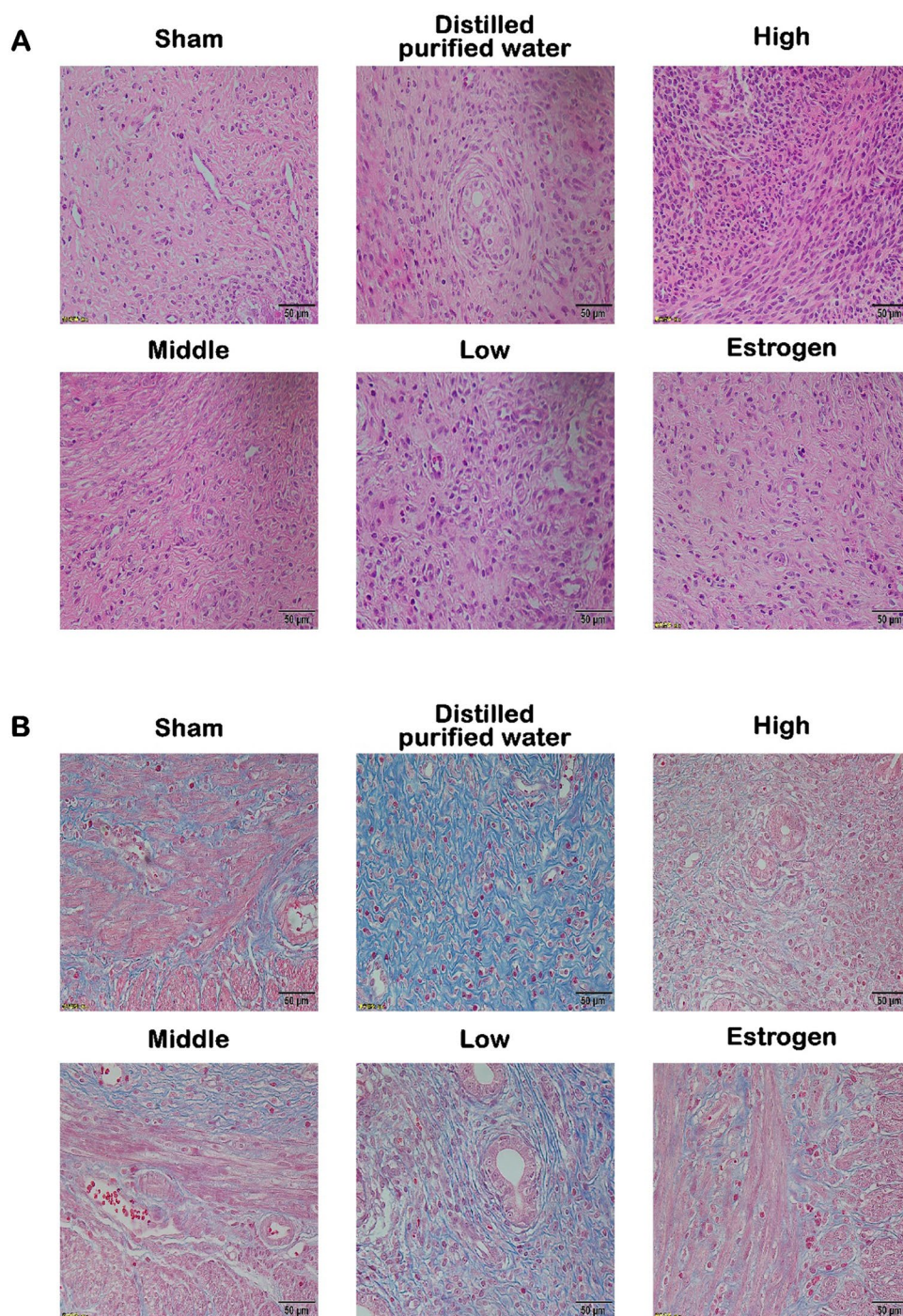

Table 5 Number of glands and fibrosis score in each experimental group

Group	Gland number score	Fibrotest	Aggregate score
Sham	2.77	1.80	4.57
Distilled purified water	2.77	6.20	8.97
High	1.85	3.55	5.40
Middle	3.49	4.93	8.41
Low	4.63	6.40	11.03
Estrogen	3.75	4.88	8.63

in treating intrauterine adhesions. This study also has several limitations. The accuracy and limitations of database data still need further improvements. However, the information obtained in the present study, including the main active compounds, core targets, and pathways affected by Bushen Huoxue Formula in treating intrauterine adhesions, may provide new insights for the prevention and treatment of intrauterine adhesions and provide support for clinical applications.

Fig. 6 Immunohistochemistry of TGF-β, SDF-1 and VEGF in each experimental group. The experiment was repeated three times
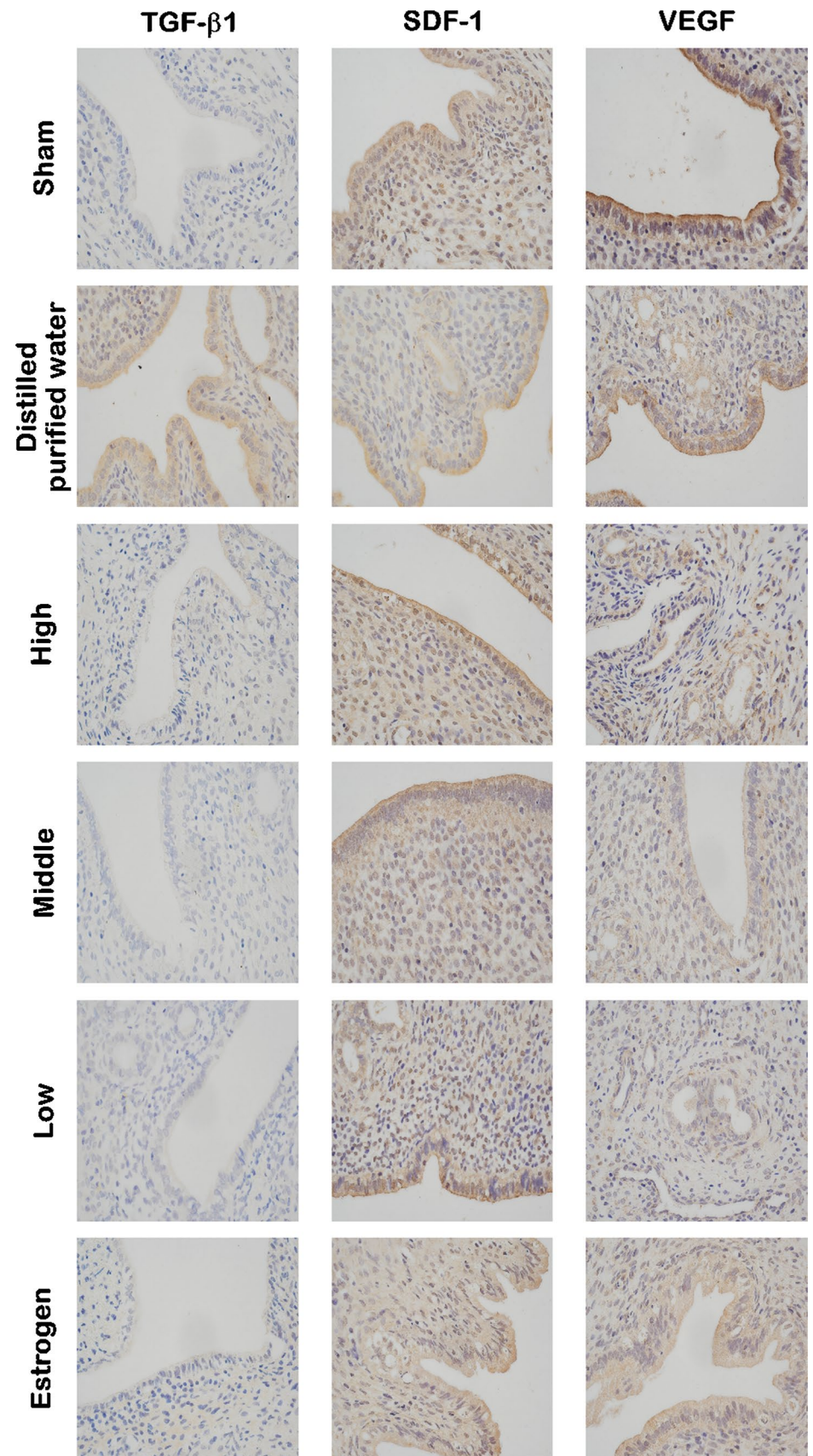
Table 6 Positive expression in each experimental group (%)

Group	VEGF	SDF-1	TGF-β1
Sham	34.55 ± 9.72	55.71 ± 7.83	3.50 ± 1.57
Distilled puri-fied water	52.27 ± 0.20	13.70 ± 3.36	58.95 ± 9.84
High	35.46 ± 18.41	59.31 ± 3.90	2.70 ± 1.61
Middle	43.644 ± 2.99	47.78 ± 5.67	2.78 ± 0.87
Low	48.81 ± 10.82	45.89 ± 6.03	3.43 ± 1.24
Estrogen	42.93 ± 1.54	48.91 ± 3.41	5.93 ± 3.82

Declarations

Ethical statement Not applicable, except that the animal study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Experimental animal Welfare Ethics Committee of Chongqing Hospital of Traditional Chinese Medicine (2022-DWSY-HRZ).

Consent for publication Not applicable.

Conflict of interest Renzhi Hu has no conflict of interest. Min Xia has no conflict of interest. Shuangya Weng has no conflict of interest. Zhi Chen has no conflict of interest. Zhen Wang has no conflict of interest. Xuemei Zou has no conflict of interest. Yan Zhang has no conflict of interest. Yiming Chen has no conflict of interest. Sisi Tang has no conflict of interest.

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