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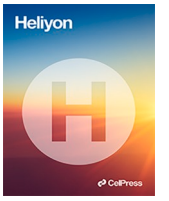

Mechanism of Yushenhuoxue prescription in treating endometriosis based on network pharmacology and the effect on the TNF pathway

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ABSTRACT

Endometriosis is a common disease in the field of gynaecology, exhibiting clinical manifestations such as dysmenorrhoea, pelvic masses, and infertility, affecting 2–10% of women of reproductive age worldwide. Currently, the acceptance rate of hormonal drugs in patients is low and certain side effects exist. In this study, based on network pharmacology, it was found that the Yushenhuoxue (YSHX) formula could potentially affect endometriosis through the TNF signalling pathway. Clinical studies indicated that YSHX demonstrated the ability to reduce the vas score of dysmenorrhoea, resulting in a significant down-regulation of serum ca125 and inflammatory factors (IL-6, IL-1β, TNF-α). In vivo studies showed that stem cell mice in the YSHX group exhibited significantly reduced lesion volumes than those in the model group. Serum levels of IL-1β and IL-6 were significantly decreased. Moreover, the phosphorylation levels of NF-κB p65 and the expression of TNF-α protein were significantly decreased. In vitro studies have shown that YSHX inhibits the proliferation, invasion, and migration of endometriotic cells. This study partially verified that YSHX contributed to the treatment of endometriosis by regulating the TNF signalling pathway and improving the inflammatory state of endometriosis.

1. Introduction

Endometriosis (EM) is a prevalent and challenging gynaecological disease, estimated to affect 2–10% of women of childbearing age [1]. Oestrogen-dependent illnesses are characterised by the development of endometrial glands and stroma outside the uterine cavity, along with the continuous expansion, infiltration, and bleeding of ectopic lesions, causing pain, masses, and infertility [2]. Superficial peritoneal implantation, ovarian EM, and deep-infiltrating EM are the three different forms of EM [3]. Although it is a benign disease, it

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exhibits malignant biological behaviours such as invasion, metastasis, and recurrence [4]. Presently, hormonal drugs are primarily used; however, certain side effects are present, and the acceptance of these drugs among patients is low [5].

Traditional Chinese medicine (TCM) has demonstrated effective in treating EM [6]. Yushenhuoxue Decoction (YSHX) is an empirical prescription for the treatment of EM developed by Professor Hu Guohua, a renowned TCM doctor in Shanghai. Its effectiveness in the clinical treatment of EM has been confirmed [7]. Its components are complex and have multi-component, multi-target, and multi-approach characteristics [8]. Network pharmacology integrates systems biology, bioinformatics, and pharmacology. Different from the traditional research mode of "single component, single target, and single approach", its construction mechanism is consistent with the "holism" in TCM [9,10]. In this study, network pharmacology was used to analyse the targets of YSHX in the treatment of EM and reveal the possible mechanisms. Experiments were conducted to further validate the analytical results. Fig. 1 illustrates the design of the experimental protocol.

2. Materials and methods

2.1. Screening of YSHX active chemical components and target of potential active ingredients

The active ingredients of the 10 herbs in YSHX were retrieved using the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, https://old.tcmsp-e.com/). The oral bioavailability (OB) ≥30% and drug-like properties (DL) ≥0.18 were used as screening criteria. The predicted gene targets of the active ingredients were obtained, and the eligible target information of the active ingredients was standardised using Uniport database.

2.2. Analysis of targets related to EM

GeneCards database (https://www.genecards.org/) and OMIM database (https://omim.org/) were searched for EM-related genes with the keyword “endometriosis”. Duplications were eliminated to extract disease-related target genes.

2.3. Intersection targets of YSHX in the treatment of EM

Targets related to YSHX and disease-related targets of EM were interposed, and a network of medicine-active ingredient-target was constructed using Cytoscape 3.7.

2.4. Protein-protein interaction (PPI) network and screening of core targets

The intersection target information of drugs and diseases was imported into the String database, the species was selected as “Homo sapiens”, and the confidence level >0.4 was used as the screening condition to download the PPI information of the intersection target, and Cytoscape software was used to visualise it.

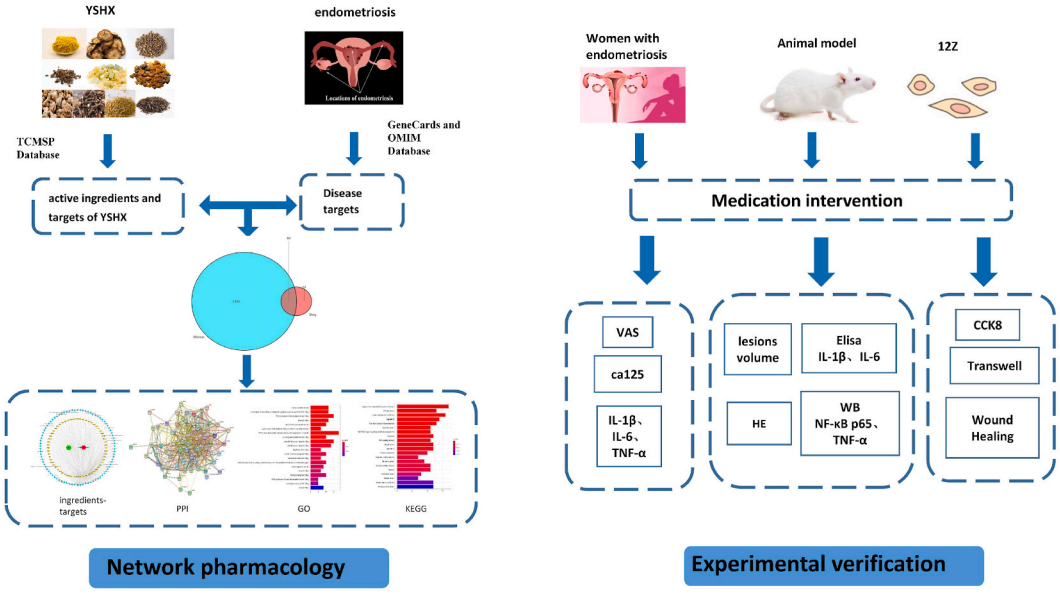

Fig. 1. The idea and process of this research.

2.5. Gene ontology (GO) function and Kyoto Encyclopaedia of genes and Genomes (KEGG) pathway analysis

GO and KEGG pathway enrichment analyses were performed using the intersection target PPI database and the species was limited to humans. Biological processes and signalling pathways with $P < 0.05$ were screened and visualised using R package (ggplot 2).

3. Clinical studies

3.1. Recruitment of clinical patients

Patients with EM were recruited from the Shanghai Hospital of Traditional Chinese Medicine. This study was approved by the hospital's ethics committee (No:2020SHL-KYYS-102) and registered at the China Clinical Research Trial Registration Center (ChiCTR2000036994). All patients who met the inclusion criteria voluntarily participated in the clinical trials and signed an informed consent form.

3.2. Diagnostic criteria

(Refer to the 2021 Guidelines for Diagnosis and Treatment of EM (the third edition)) [11] Conforming to the pathological diagnosis criteria of EM: The lesion site and scope can be explored laparoscopically, and lesion tissue can be obtained for histopathological diagnosis.

3.3. Inclusion criteria

(1) Conforming to the pathological diagnostic criteria of EM; (2) non-lactating patients aged 25–45 years; (3) regular menstrual cycle (21–35 days of menstrual cycle)

3.4. Exclusion criteria

(1) Patients with gynaecological diseases such as gynaecological tumours, vaginitis, cervical cancer, pelvic tumours, and pelvic abscesses; (2) pregnant women within half a year; (3) patients allergic to the test drug; (4) patients with complications from serious medical diseases; and (5) patients taking drugs similar to this experiment, before enrolment.

3.5. Discharge criteria

(1)Serious adverse reactions occurred during medication; (2) medications were not taken as required during the trial; (3) patients dropped out of the study without completing the trial; and (4) combined use of other drugs.

3.6. Treatment method

YSHX is composed of Spatholobus spatholobi 30 g (batch number:21074281), Puhuang 18 g (batch number:21071251), Yanhusuo 9 g (batch number:21071291), Bupleurum Chinense 9 g (batch number:21071491), Frankincense 3 g (batch number:20110871), Myrrh 3 g (batch number:20070771), Sanqi powder 2 g (batch number:21082091), Clematis root 18 g (batch number:21074341), Liu Jinu 9 g (batch number:20112151), Fenugreek 18 g (batch number:20110653), From Jiangyin Tianjiang Pharmaceutical Co., Ltd. Each patient took 150 mLYSHX half an hour after breakfast and dinner every day. All patients with EM were administered drugs for three months and were followed up.

3.7. Observation indicators and test methods

(1) VAS score: visual analogue scale (VAS) was used to evaluate dysmenorrhoea, with 0 end (0 point) representing "no pain" and 10 cm end (10 point) representing "unbearable pain". Patients marked their pain levels on a scale according to their feelings, and the length from 0 points to the marked point (cm reading) was the pain level. 0–0.4 cm was defined as having no pain, 0.5–4.4 cm as mild pain, 4.5–7.4 cm as moderate pain, and 7.5–10 cm as severe pain, respectively. (2) Serum CA125 And Serum Inflammatory Cytokines: Abdominal measurements were performed three days after menstruation, before and after treatment; all tests were performed in the clinical Laboratory of Shanghai Traditional Chinese Medicine Hospital.

4. In vivo experimental verification

4.1. Establishment of mice EM model and animal administration

SPF C57BL/6 female mice aged 6–7 weeks were provided by Shanghai Jisco (licence number: SCXK (Shanghai) 2018-0004). All mice were standardised and reared at the Laboratory Animal Centre of the Shanghai Traditional Chinese Medicine Hospital. Constant temperature: 20–24 °C, constant humidity: 40–70%, 12h light/12h dark alternate, free to feed animals and feed water.

Mice with a normal oestrus cycle were selected and categorized as donor and recipient mice at a ratio of 1:2, which were further divided into model, YSHX, sham operation, and normal groups, with 10 mice in each group. The mice were subcutaneously injected with β-oestradiol solution (2 μg•0.2 mL-1•20 g-1, Sigma, batch number: # WXBD3761V) on the 1st, 3rd, and 6th days before modelling, and the EM mouse model was established on the 7th day. (1)Endometrial retrieval from donor mice: Sutai 50 (France Vik Co., Ltd., batch No. BN 8ADTA) were sacrificed after anaesthesia. The abdominal cavity was opened to find "Y the uterus, the mesometrium and adipose tissue around the uterus were removed, and the uterus was placed in a DMEM culture dish for rinsing. The uterus was then cut longitudinally into fragments with size of about 1 mm^3. The uterus of one donor mouse was used for the two recipient rat mice. (2)Endometrial transplantation: The recipient mice were anaesthetised and placed on fixed plates. After abdominal disinfection, a longitudinal incision (approximately 0.7 cm) was made approximately 1.5 cm above the urethral orifice. Seven endometrial fragments were implanted along the periphery of the abdominal incision, and the inner and outer skin layers of the mice were sutured using 4-0 suture needles. After suturing, the incision was sterilised using iodarone. In the sham operation group, the uterus was replaced with fat. Penicillin sodium 80000 U was administered to each mouse for three consecutive days to reduce the risk of surgical infection. Oestrogen solution (2 μg•0.2mL-1•20g-1) was injected subcutaneously at 3, 6, and 9 days after the end of modelling. On 14th, 21st, and 28th days, the mice were dissected to establish the EM model, and three mice were sacrificed each day. The abdominal cavity was opened and the lesions were observed.

Animal administration: On 14th day after the establishment of the EM model, the mice were intragastrically administered at the same time every day for 21 days. In a previous study, the optimal dose of YSHX for EM model mice was in the high-dose group, which was the equivalent dose for humans and mice. The specific dosage of each group was as follows: (1) YSHX group: according to the body surface area ratio equivalent dose conversion, given 0.3 ml YSHX at a concentration of 3.2606 g/ml; (2) model, sham, and normal group: mice were gavaged with 0.3 ml normal saline every day; after 21 days of gavage, mice were sacrificed by cervical vertebra removal on day 22, ectopic lesions were removed, and the size was recorded. Mouse serum was obtained by eyeball blood sampling (Fig. 2).

4.2. HE staining

The sections of ectopic lesions were de-paraffinised, hydrated, successively placed in xylene for 15 min, absolute ethanol for 5 min, 75% alcohol for 2 min, and then cleaned with distilled water for 3 min. The slices were dried, stained with haematoxylin for 10 min, differentiated with 1% hydrochloric acid alcohol, blued with 0.6% ammonia water, stained with eosin for 3 min, dehydrated with absolute ethanol for 5 min, placed in xylene until transparent, and sealed with neutral gum. The results were observed, and images were acquired using an optical microscope.

4.3. ELISA assay

ELISA was used to detect serum levels of IL-1β and IL-6 in each group of mice (Shanghai Senjo Biological Co., Ltd.). Dual-wavelength detection was performed using a microplate reader (Thermo Fisher Scientific), and the OD value of each well was measured at 450 nm.

4.4. Western-blot TEST

The tissue was cut into small pieces and placed into a 1.5 ml centrifuge tube, and the appropriate cell tissue lysate, phosphatase inhibitor, and protease inhibitor (equal volume), and three sterilised steel beads were placed. The homogenate was homogenised twice using a homogeniser at 60 Hz for 2 min, allowed to fully lyse the tissue, and placed on ice for 30 min. At the end of standing, all centrifuge tubes were centrifuged at 4 °C at 12000r/min for 10 min. At the end of centrifugation, all tubes were placed on ice and the supernatant was aspirated. The protein concentration was determined using the BCA method. Add 5 × buffer to mix, and boil all protein samples in boiling water for 5 min. Protein samples were separated by electrophoresis on 12% SDS-PAGE, polyacrylamide gel

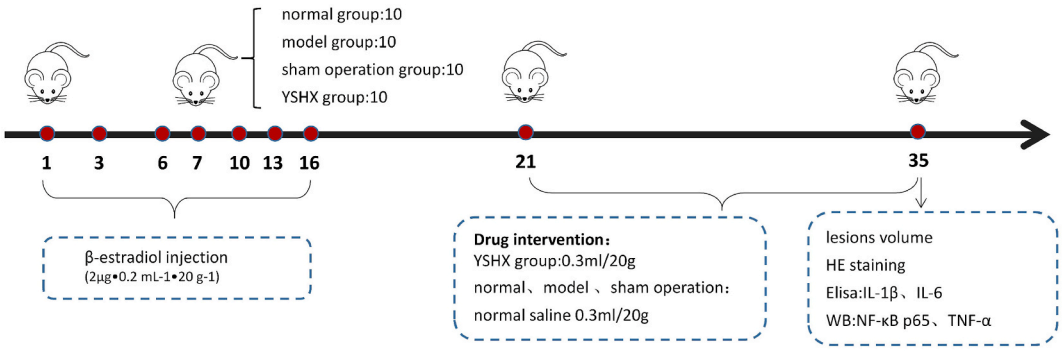

Fig. 2. Experimental procedures of the in vivo EM mouse model.

electrophoresis, and then transferred to PVDF membranes. Blocking solution containing 5% skim milk powder was added and blocked by shaking back and forth on a shaker for 1.5 h. TBST was added to wash the PVDF membrane three times, and then dilution containing primary antibodies against NF-κB, p65, and TNF-α was added and incubated overnight at 4 °C with shaking on a shaker. The PVDF membrane was washed three times with TBST, and the secondary antibodies HRP peroxidase-labelled sheep anti-rabbit (1:500) and sheep anti-mouse (1:10000) were diluted with TBST for 2 h. An appropriate amount of ELC working solution was dropped and transferred to a gel imaging analyser for exposure development.

5. In vitro experimental verification

5.1. Preparation of medicated serum

Prepare YSHX as before. Fifteen C57BL/6 mice were administered YSHX twice daily for seven consecutive times at a rate five times the equivalent dose for adults. Eyeball blood was taken 1–2h after the last administration, serum was centrifuged at 3000 rmp and inactivated at 56 °C for 30 min. The serum was filtered by a 0.22 μm filter and frozen at −20 °C for use.

5.2. Cell proliferation assay

After the 12Z (12Z cells derived from epithelial cells of peritoneal EM) cell count, 96-well plates were inoculated at 8 × 103 cells per well. The total culture medium was quantified at 200 μl and incubated in incubators for 24h, 48h, and 72h. Add 10 per hole μ L CCK-8 solution and incubation for 1h, the absorbance was detected at a wavelength of 450 nm by an automatic enzyme marker.

5.3. Cell invasion assay

A 750 μL complete medium was added to the 24-well plate, and 300 μL medium containing YSHX serum (1:300) (containing 2.5 × 104 cells) was added to the Transwell chamber. The medium was incubated for 12–16h, and the cells were fixed with para-formaldehyde after discarding the old solution. After 100% formaldehyde was added to increase cell permeability, the cells were stained with 0.5% crystal violet for 15 min. Cells that did not penetrate the filtration membrane were gently wiped with cotton swabs and observed under an optical microscope.

5.4. Cell wound healing assay

Three uniform horizontal lines were drawn on the back of the six-hole plate with marker pen. When the cell density was about 90% 24h after transfection, 3–4 scratches were made with 10 μL gun tip perpendicular to the crossed line. YSHX serum was added for culture at 0h, 24h, and 48h after scratching, and appropriate photographs were taken with the same field of vision.

5.5. Statistical analysis

Prism 9.1.1 and SPSS 25.0, were used for statistical analyses and drawing. Data are presented as mean ± standard deviation. For multiple-group comparisons, the data met the criteria of normal distribution and homogeneity of variance, and one-way analysis of variance was used. If the data were unsatisfactory, a rank-sum test was used. P was set at $p < 0.05$. significant.

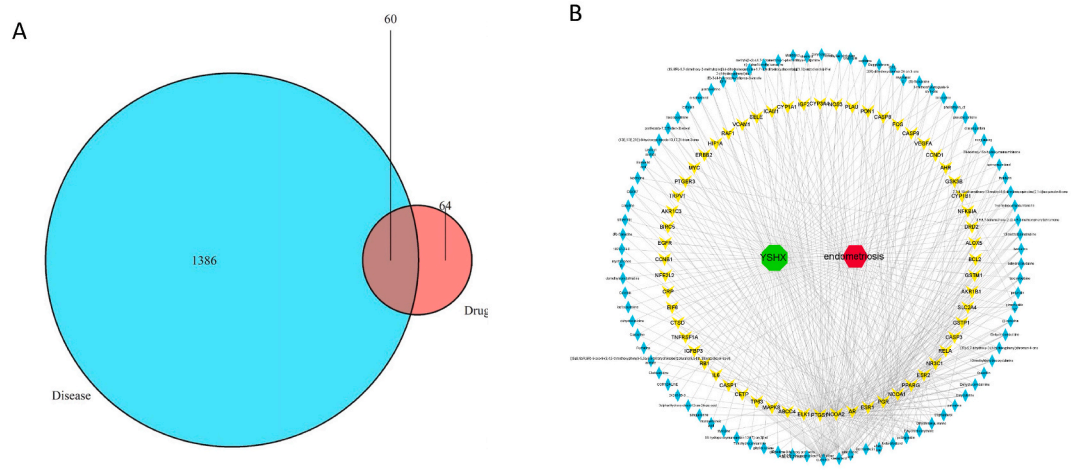

Fig. 3. (A)Venna diagram (blue is the disease targets; red is the drug targets; in the middle are common targets); (B)The compond-target network for YSHX on Endometriosis.

6. Results

6.1. Network pharmacology analysis

6.1.1. Common targets of YSHX and EM screening

The potential targets of YSHX in EM were compared after deduplication, and 60 intersection targets were identified (Fig. 3A). Among them, quercetin, kaempferol, arachidonic acid, isorhamnetin, stigmasterol, beta-sitosterol, and ellagic acid had the largest number of EM targets, corresponding to 40, 26, 11, 11, 9, 8, and 7 targets, respectively, which may be the key components of YSHX in the treatment of EM(Fig. 3B).

6.1.2. PPI network analysis

PPI network analysis was performed on the 60 intersection targets using the STRING database, of which 59 genes had protein-protein interactions, and 555 edges represented protein-protein interactions (Fig. 4A). The top 10 key genes were IL6, VEGFA, EGFR, MAPK8, CASP3, ESR1, MYC, FOS, CCND1, and AR (Fig. 4B).

6.1.3. GO function and KEGG pathway analysis

GO function analysis of the 60 intersection targets showed that 82 biological functions were affected ($P < 0.05$) (Fig. 5A). It mainly affects nuclear receptors and transcription factor activity. Direct ligand-regulated sequence-specific DNA binding, RNA polymerase II transcription factor binding, steroid binding, steroid hormone receptor activity, and cysteine-type endopeptidase activity are involved in the apoptotic process These genes were significantly enriched in 118 pathways ($P < 0.05$), among which the TNF signalling pathway was the most significant (hsa 04668) (Fig. 5B).

6.2. Clinical studies

6.2.1. Basic data of patients

Sixty patients with EM were enrolled based on inclusion and exclusion criteria, a total of 60 EM patients were enrolled. The mean age was 31.43 ± 5.93 years (range, 23–42 years). The disease duration was 0.3–10 years, with an average of (4.81 ± 6.32) years.

6.2.2. Pain VAS score

The EM patients' before treatment VAS score was 4.65 ± 1.08; after 3 months of YSHX treatment, VAS score was 3.35 ± 0.23, which was significantly lower than that before treatment ($P < 0.05$) (Table 1).

6.2.3. Serum Ca125 values

Before treatment the EM patients' serum Ca125 score was 83.17 ± 3.53; after three months of YSHX treatment, Ca125 score was 36.56 ± 1.98, which was significantly lower than those before treatment ($P < 0.05$) (Table 2).

6.2.4. Serum Inflammatory Cytokines

EM patients Serum Inflammatory Cytokines in high level, IL-6 (40.72 ± 11.92 pg/ml), IL-1β (22.20 ± 7.52 pg/ml), TNF-α (12.89 ± 0.49 pg/ml), after 3 months YSHX treatment, Serum Inflammatory Cytokines at a normal level, IL-6 (5.02 ± 2.69 pg/ml), IL-1β(5.46 ± 0.85 pg/ml), TNF-α (5.34 ± 0.15 pg/ml) were significantly lower than those before treatment ($P < 0.05$) **(Table 3)**.

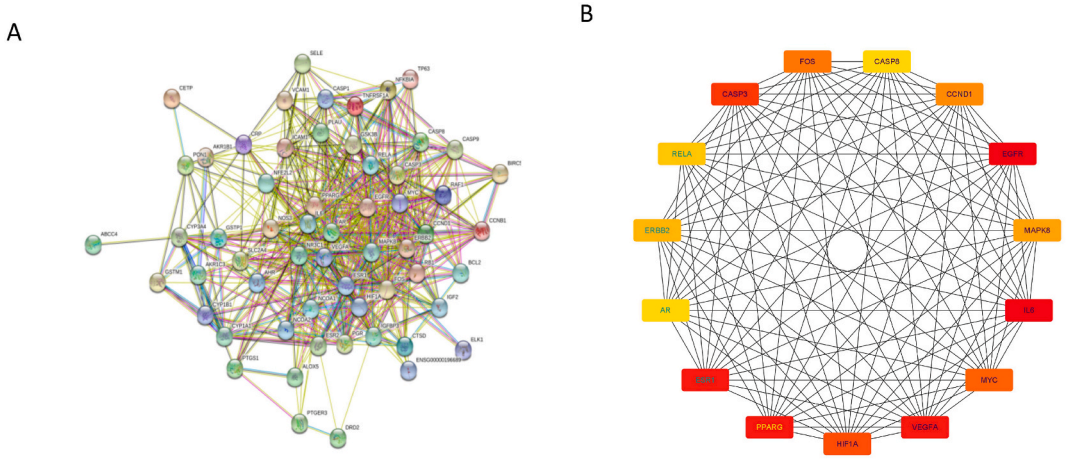

Fig. 4. Result of core target screening.(A)PPI network (B)Core target diagram (the darker the node, the more important the target.

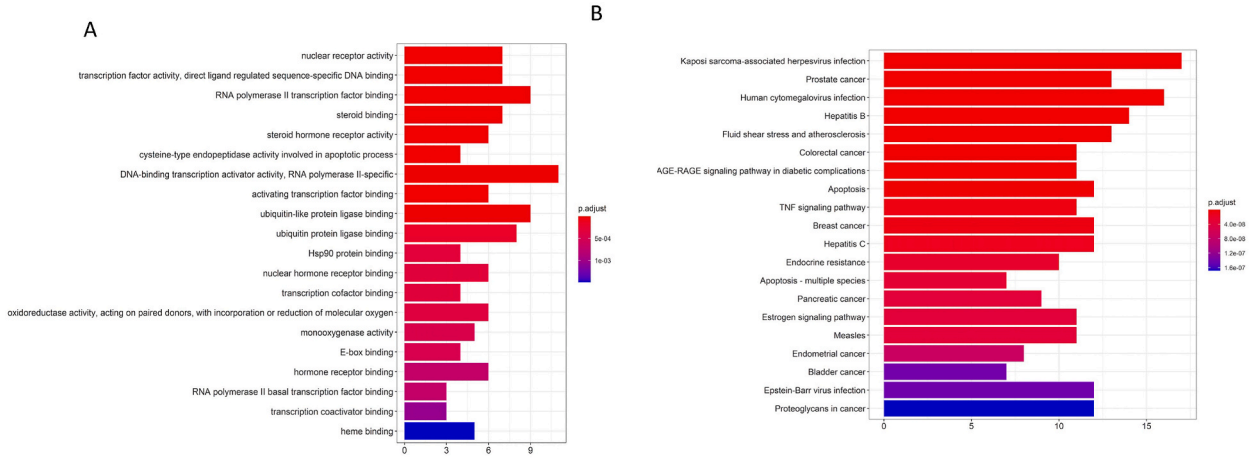

Fig. 5. Analysis of the enrichment histogram of GO(A) and KEGG pathway(B).

Table 1
The visual analogue scale (VAS) scores of EM patients ($\bar{x} \pm s$) .

	case	Vas	t	*P*
Before treatment	60	4.65 ± 1.08	7.93	< 0.001
After treatment	60	3.35 ± 0.23***		

Notes: ***$P < 0.001$, vs.Before treatment.

Table 2
The Serum Ca125 values of EM patients ($\bar{x} \pm s$) .

	case	Ca125	t	*P*
Before treatment	60	83.17 ± 3.53	12.62	< 0.001
After treatment	60	36.56 ± 1.98***		

Notes: ***$P < 0.001$, vs.Before treatment.

7. In vivo experimental verification

7.1. The weight of mouse and the size of ectopic lesion

After modelling, the body weights of mice in each group were recorded. No significant differences were found between the body weights of the mice in each group at the time points of 1, 7, 14, 21, 28, and 35 days ($P > 0.05$) (Fig. 6A). Based on the assessment of the longest diameter of ectopic lesions in mice, those in the YSHX group showed statistically significant reduction compared to those in the model group ($P < 0.001$) (Fig. 6B).

7.2. YSHX inhibits EM by regulating inflammatory cytokines

The HE staining results showed that the endometrial glands in the uterine sections of the normal and sham groups were neatly arranged and short and columnar, with a large number of glands and a complete glandular cavity. The sections of ectopic lesions in the model group showed obvious endometrial glands and endometrial stroma. The glands were columnar but irregular; the endometrial stromal cells were dense and tightly packed; inflammatory cells were observed in the glandular cavity; and the tissue had scattered

Table 3
The Serum Inflammatory Cytokines of EM patients ($\bar{x} \pm s$) .

		IL-6 (pg/ml)	IL-1β (pg/ml)	TNF-α (pg/ml)
Before treatment	60	40.72 ± 11.92	22.20 ± 7.52	12.89 ± 0.49
After treatment	60	5.02 ± 2.69***	5.46 ± 0.85***	5.34 ± 0.15***
t		16.26	12.69	14.21
P		< 0.001	< 0.001	< 0.001

Notes: ***$P < 0.001$, vs.Before treatment.

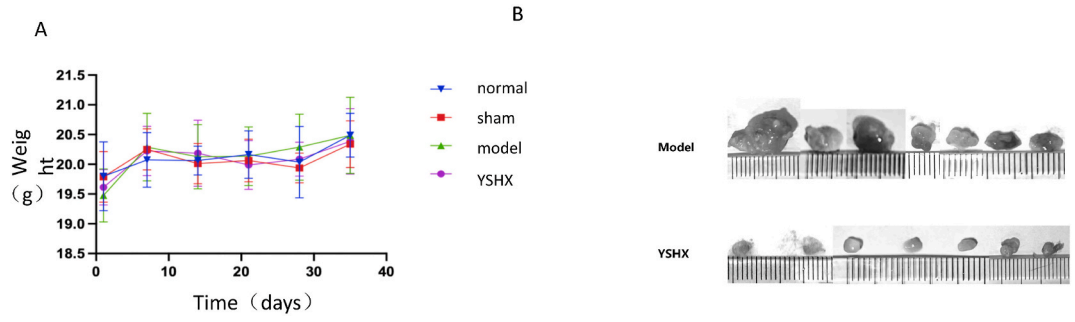

Fig. 6. (A)The weight of mice in each group; The ectopic lesion of each group.

bleeding points. Compared to the model group, the pathological sections of ectopic lesions in the YSHX group showed obvious atrophy of the endometrial glands, a reduction in the number of glands, a loose arrangement of glands, and a reduction in the number of stromal cells. (Fig. 7).

7.3. YSHX inhibits inflammatory cytokines in EM through the TNF signalling pathway

For validation, we selected the TNF signalling pathway related to inflammatory factors from the enrichment analysis of the KEGG signalling pathway. ELISA results indicated that the serum IL-1β and IL-6 levels in the sham group had no significant differences compared to those in the normal group ($P > 0.05$); however, the serum IL-1β and IL-6 levels in the model group were significantly higher ($P < 0.01$). Serum levels of IL-1β and IL-6 in the YSHX group were significantly lower than those in the model group ($P < 0.01$) (Fig. 8A–B). According to the Western blot, the sham operation group had no significant differences compared with the normal group ($P > 0.05$), and the phosphorylation of NF-κB p65 and TNF-α proteins in the model group showed a significant upward trend ($P < 0.05$). Compared to the model group, the phosphorylation of NF-κB p65 and the expression of TNF-α protein in the YSHX group showed a downward trend ($P < 0.05$). (Fig. 8C–F).

8. In vitro experimental verification

8.1. YSHX inhibits the proliferation, migration and invasion of EM cells

Based on CCK8 cell proliferation experiments, YSHX was found to inhibit cell proliferation in a concentration-dependent manner (low:50 μmol/L; Medium:100 μmol/L; High 200 μmol/L) (Fig. 9 A). The Transwell assay showed that YSHX inhibited the invasion of 12z cells (Fig. 9 B). The results of the wound healing experiment suggested that YSHX significantly reduced the migratory ability of 12z cells (Fig. 9C–D).

9. Discussion

EM is an oestrogen-dependent disease characterised by the appearance of functional endometrioid tissues outside the uterus that

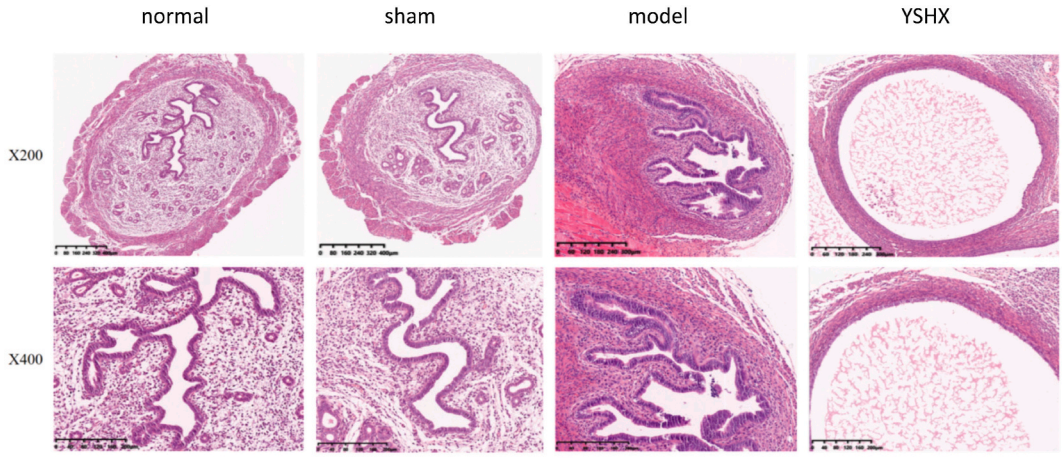

Fig. 7. HE stained pathological sections of the tissues taken from each group of mice (X200,X400).

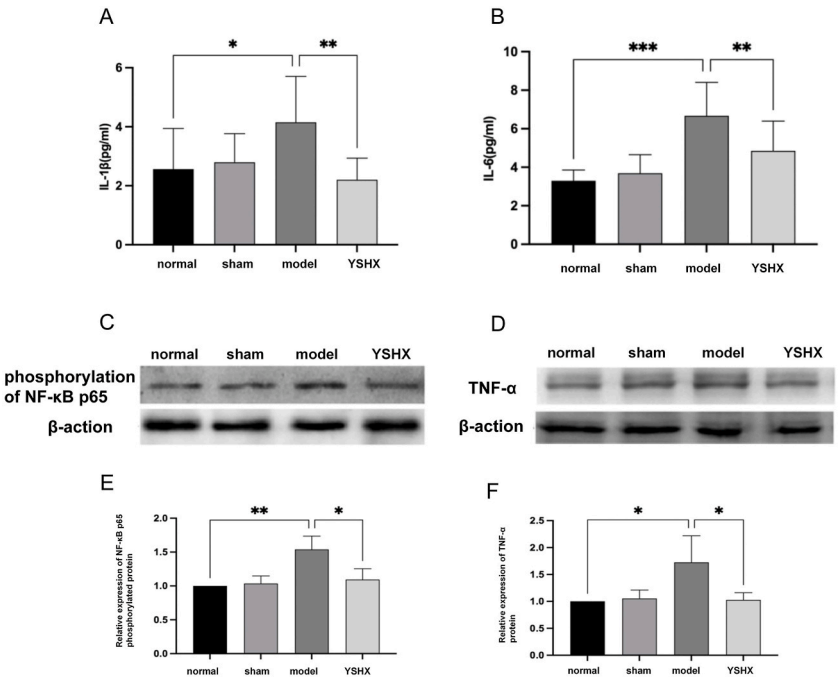

Fig. 8. (A–B) The expressions of serum inflammatory cytokines IL-1β and IL -6 detected by Elisa (C–F)The proteins expression of NF-κB p65 and TNF-α in endometrial tissues detected by WB, Notes: $*P < 0.05$,$**P < 0.01$,$**P < 0.001$vs Control.

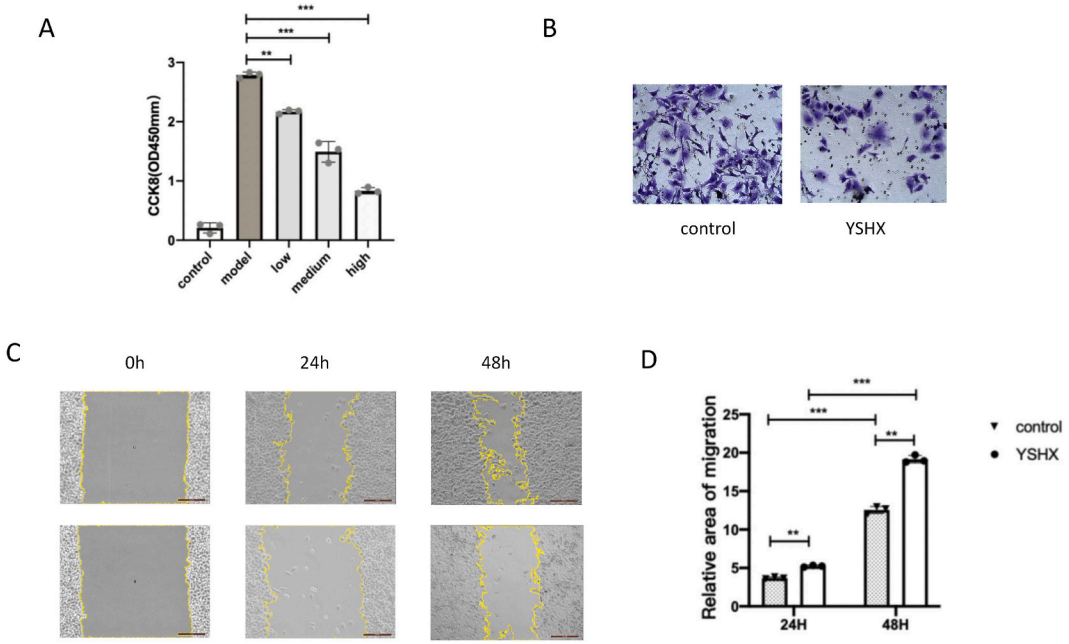

Fig. 9. (A)Effect of YSHX on12z proliferation detected by CCK8; (B)The effect of YSHX on 12z invasion detected by tanswell; (C-D)Effect of YSHX on12z migration detected by wound healing.

grow and infiltrate, causing repeated bleeding, abdominal pain, pelvic masses, infertility, and other symptoms. The incidence rate in women of childbearing age is approximately 10%–15%, with a yearly increasing trend. This rate rises to over 40–50% among women experiencing infertility and chronic pelvic pain [12,13]. It affects >176 million women globally. Currently, there are two clinical treatments: drugs and surgery. For ovarian EM, surgical treatment can be considered based on meeting surgical indications. However, surgery may not be suitable for all types of EM, and the postoperative recurrence rate remains high. Drug treatment includes

non-steroidal anti-inflammatory drugs (NSAID), progesterone, compound oral contraceptives (COC), gonadotropin-releasing hormone agonists (GnRHa), and TCM. However, hormonal drugs are often less accepted due to their tendency to cause vaginal bleeding, perimenopausal symptoms, and other side effects. TCM is effective for the treatment of EM, has received increasing attention, and is widely used in clinical practice. Several systematic reviews have demonstrated the efficacy and safety of TCM for treating EM over extended periods [14,15].

Clinical studies have confirmed the effectiveness of YSHX in EM treatment, suggesting its ability to enhance endometrial adhesion function by downregulating serum CA125 levels and whole blood viscosity. This effectively controls disease progression, reduces the recurrence rates, and enhances patients' quality of life [16–18]. To elucidate the molecular mechanism of YSHX in the treatment of EM on the network pharmacology, 61 active ingredients, including quercetin, kaempferol, arachidonic acid, isorhamnetin, sitosterol, -sitosterol, and ellagic acid, which act on EM through key target genes, such as IL6, VEGFA, EGFR, CASP3, ESR1, MYC, and CCND1, were evaluated. It mainly affects nuclear receptor activity, transcription factor activity, direct ligand-regulated sequence-specific DNA binding, RNA polymerase II transcription factor binding, steroid binding, steroid hormone receptor activity, and cysteine-type endopeptidase activity, which are involved in apoptosis and other biological processes. The KEGG enrichment pathway was mainly involved in apoptosis, the TNF signalling pathway, and the oestrogen signalling pathway. EM are localised pelvic diseases and systemic chronic inflammatory diseases. The occurrence of EM is closely related to inflammatory factors, and the inflammatory environment is conducive to the adhesion, invasion, and angiogenesis of endometrial tissues outside the uterine cavity [19–21]. Therefore, this experiment was verified based on TNF signalling pathway.

EM can also cause secondary pain. Previous study showed that 89.6% of EM patients were accompanied by dysmenorrhoea, and 77.2% had chronic pelvic pain, which seriously affected their quality of life [22,23]. In our study, 62 EM patients were recruited and treated with YSHX for three months. Before and after treatment, patients completed the dysmenorrhoea visual analogue scale (VAS), and serum tumour markers and inflammatory factors were measured. Our studies have shown that YSHX can effectively relieve pain in patients with EM without irregular vaginal bleeding, hot flashes, night sweats, or other symptoms caused by hormonal drugs. The mean VAS score was 4.65 ± 1.08 before treatment and decreased to 3.35 ± 0.23 after three months of YSHX treatment. The serum tumour marker ca125 also decreased significantly, from 83.17 ± 3.53 36.56 ± 1.98. And all the serum inflammatory factors (IL-6, IL-1β,TNF-α)were higher than normal before treatment , after YSHX treatment , they were at normal level. Endometriotic cells are similar to tumour cells in that they have obvious invasion, metastasis, proliferation, recurrence, and other malignant biological behaviour [24,25].

According to the pathological characteristics and pathogenesis of EM, reducing the level of inflammation in the body may be an entry point for treating daughter EM. The TNF-α/NF-κB signalling pathway is a combination of the signalling molecule TNF-α with the TNFR1 receptor on the cell membrane, resulting in the formation of trimer TNFR1, and then recruiting downstream signalling proteins to activate the NF-κB signalling pathway, thus promoting the formation of an inflammatory response [26]. TNF-α, a ligand belonging to the TNF superfamily, plays a promoting role in mediating inflammation and immune response and is involved in the chronic inflammatory response of EM [27]. TNF-α is an important risk factor for tumorigenesis, tumour progression, invasion, and metastasis. IL-6 is an important mediator of the inflammatory response in the body [28]. It plays an immunomodulatory role, induces the differentiation of T and B lymphocytes, enhances the function of monocytes and natural killer cells, and promotes the development of EM [29]. Serum levels of IL-1β stimulate the expression of genes associated with inflammation and EM and play an important role in immune regulation and inflammation. The increased activity of NF-κB P65 binding DNA is involved in the pathogenesis and proinflammatory state of EM [30].

The research explored the potential mechanisms of YSHX on EM by integrating network pharmacology and experimental verification. Clinical studies have shown that YSHX treatment in patients with EM can improve dysmenorrhoea score, decrease tumour markers ca125 and inflammatory cytokines (IL-6, IL-1β, TNF-α). In vivo validation was performed using an EM model, and the number of glandular and interstitial cells decreased. Serum levels of IL-1β and IL-6 decreased. The expression of NF-κB P65 phosphorylation and TNF-α protein in endometrial tissues was up-regulated. In vitro experiments have shown that YSHX can inhibit the proliferation, invasion, and migration of endometriotic cells. Combined with network pharmacology and experimental validation, it may reduce the expression of inflammatory factors and promote cell survival and proliferation through the TNF signalling pathway to treat EM.

10. Conclusion

Combined with network pharmacology and experimental validation, YSHX may reduce the expression of inflammatory factors and promote cell survival and proliferation through the TNF signalling pathway to treat EM.

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Availability of data and materials

The datasets included in the article are available from the corresponding author upon reasonable request.

Author contribution statement

Jing Chen, Guohua Hu: Conceived and designed the experiments.
Jiami Huang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Xu Zhang: Performed the experiments; Wrote the paper.
Jiayun Wang: Analyzed and interpreted the data.
Yanan Zhang, Cancan Gu: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data associated with this study has been deposited under the accession number ChiCTR2000036994.

Additional information

No additional information is available for this paper.

Ethics approval and consent to participate

All the experimental protocols were approved by the Research Ethics Committee (No. No:2020SHL-KYYS-102). Animal experiments were performed in accordance with the guidelines and regulations of the Centre for Laboratory Animal Care in Shanghai Traditional Chinese Medicine Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
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References

[1] C. Mehedintu, M.N. Plotogea, S. Ionescu, et al., Endometriosis still a challenge, J Med Life 7 (3) (2014 Sep 15) 349–357. Epub 2014 Sep. 25.
[2] C. Chapron, L. Marcellin, B. Borghese, et al., Rethinking mechanisms, diagnosis and management of endometriosis, Nat. Rev. Endocrinol. 15 (11) (2019 Nov) 666–682.
[3] P. Vercellini, P. Viganò, E. Somigliana, et al., Endometriosis: pathogenesis and treatment, Nat. Rev. Endocrinol. 10 (5) (2014 May) 261–275.
[4] T. Falcone, R. Flyckt, Clinical management of endometriosis, Obstet. Gynecol. 131 (3) (2018 Mar) 557–571.
[5] A. Czyzyk, A. Podfigurna, A. Szeliga, et al., Update on endometriosis pathogenesis, Minerva Ginecol. 69 (5) (2017 Oct) 447–461.
[6] P. Dong, L. Ling, L. Hu, Systematic review and meta-analysis of traditional Chinese medicine compound in treating infertility caused by endometriosis, Ann. Palliat. Med. 10 (12) (2021 Dec) 12631–12642.
[7] J. Huang, Y. Wan, G. Hu, et al., Clinical effect of Tongjingning decoction on endometriosis of Qi stagnation and blood stasis type [J], J Guangzhou Univ Chinese Med 38 (11) (2021) 2364–2369.
[8] X. Zhu, L. Zhu, Application progress in the field of network pharmacology in Chinese medicine [J], J traditional Chinese medicine management journal (13) (2021) 98–102.
[9] Y. Zhu, J. Yu, K. Zhang, et al., Network pharmacology analysis to explore the pharmacological mechanism of effective Chinese medicines in treating metastatic colorectal cancer using meta-analysis approach, Am. J. Chin. Med. 49 (8) (2021) 1839–1870.
[10] C. Nogales, Z.M. Mamdouh, M. List, et al., Network pharmacology: curing causal mechanisms instead of treating symptoms, Trends Pharmacol. Sci. 43 (2) (2022 Feb) 136–150.
[11] Chinese Society of Obstetricians and Gynecologists, Chinese medical doctor association, Chinese society of obstetrics and gynecology, Chinese medical association endometriosis collaboration group. Guideline for diagnosis and treatment of endometriosis (third edition)[J], Chin. J. Obstet. Gynecol. 56 (12) (2021) 812–824.
[12] R.M. Hodgson, H.L. Lee, R. Wang, et al., Interventions for endometriosis-related infertility: a systematic review and network meta-analysis, Fertil. Steril. 113 (2) (2020 Feb) 374–382.e2.
[13] T. Tanbo, P. Fedorcsak, Endometriosis-associated infertility: aspects of pathophysiological mechanisms and treatment options, Acta Obstet. Gynecol. Scand. 96 (6) (2017 Jun) 659–667.
[14] P. Dong, L. Ling, L. Hu, Systematic review and meta-analysis of traditional Chinese medicine compound in treating infertility caused by endometriosis, Ann. Palliat. Med. 10 (12) (2021 Dec) 12631–12642.
[15] X. Wang, Y. Shi, L. Xu, et al., Traditional Chinese medicine prescription Guizhi Fuling Pills in the treatment of endometriosis, Int. J. Med. Sci. 18 (11) (2021 Apr 16) 2401–2408.
[16] J. Chen, N. Ye, H. Guo, et al., Clinical observation of Tongjingning in the treatment of endometriosis and dysmenorrhea of Qi stagnation and blood stasis type [J], Shanghai J. Tradit. Chin. Med. 51 (S1) (2017) 127–129+132.
[17] N. Ye, M. Zhang, J. Chen, Effect of Tongjingning on the expression of tumor suppressor gene PTEN in eutopic and ectopic endometrium of endometriosis rats [J], Shi Zhen Chinese Medicine and Chinese Medicine 28 (4) (2017) 850–854 (in Chinese).

[18] N. Ye, M. Zhang, H. Yang, et al., Effect of Tongjingning on the expression of ICAM-1, MMP-9 and VEGF in eutopic and ectopic endometrium of rats with endometriosis[J], J.Shanghai Univ.Traditional Chinese Medicine 32 (2) (2018) 75–81 (in Chinese).
[19] H.S. Taylor, A.M. Kotlyar, V.A. Flores, Endometriosis is a chronic systemic disease: clinical challenges and novel innovations[J], Lancet (2021), 10276, 397.
[20] H.S. Taylor, M. Alderman Iii, T.M. D'hooghe, et al., Effect of simvastatin on baboon endometriosis[J], Biol. Reprod. 97 (1) (2017) 32–38.
[21] Samimi Mansooreh, Pourhanifeh Mohammad Hossein, Mehdizadehkashi Abolfazl, et al., The role of inflammation,oxidative stress,angiogenesis, and apoptosis in the pathophysiology of endometriosis:Basic science and new insights based on gene expression[J], J. Cell. Physiol. 234 (2) (2019) 19384–19392.
[22] Ben-Meir Lee Cohen, Soriano David, Zajicek Michal, et al., The Association between Gastrointestinal Symptoms and Transvaginal Ultrasound Findings in Women Referred for Endometriosis Evaluation: A Prospective Pilot Study.[J], Ultraschall Med, 2020 undefined: undefined.
[23] H.M. Eid, P.S. Haddad, The antidiabetic potential of quercetin: underlying mechanisms, Curr. Med. Chem. 24 (4) (2017) 355–364.
[24] M. Reyes-Farias, C. Carrasco-Pozo, The anti-cancer effect of quercetin: molecular implications in cancer metabolism, Int. J. Mol. Sci. 20 (13) (2019 Jun 28) 3177.
[25] M. Imran, B. Salehi, J. Sharifi-Rad, et al., Kaempferol: a key emphasis to its anticancer potential, Molecules 24 (12) (2019 Jun 19) 2277.
[26] M. Li, W. Zhang, L. Yang, et al., The mechanism of xiaoyao san in the treatment of ovarian cancer by network pharmacology and the effect of stigmasterol on the PI3K/akt pathway, Dis. Markers (2021 Jun 29), 4304507, 2021.
[27] H. Bae, S. Park, J. Ham, et al., ER-mitochondria calcium flux by β-sitosterol promotes cell death in ovarian cancer, Antioxidants 10 (10) (2021 Oct 8) 1583.
[28] T. Chen, X. Zhang, G. Zhu, et al., Quercetin inhibits TNF-α induced HUVECs apoptosis and inflammation via downregulating NF-kB and AP-1 signaling pathway in vitro, Medicine (Baltim.) 99 (38) (2020 Sep 18), e22241.
[29] O.J. Sul, S.W. Ra, Quercetin prevents LPS-induced oxidative stress and inflammation by modulating NOX2/ROS/NF-kB in lung epithelial cells, Molecules 26 (22) (2021 Nov 17) 6949.
[30] Y. Liu, C. Yu, K. Ji, et al., Quercetin reduces TNF-α-induced mesangial cell proliferation and inhibits PTX3 production: involvement of NF-κB signaling pathway, Phytother Res. 33 (9) (2019 Sep) 2401–2408.