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Xiangsha Weiling Pills alleviate intestinal barrier damage via the TGFB1/IL6 in diarrhea with syndrome of spleen deficiency with dampness encumbrance

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

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Abstract

Introduction and Objective. Diarrhea with syndrome of spleen deficiency with dampness encumbrance (D-SSDDE) is a clinically debilitating condition, and the therapeutic mechanisms of Xiangsha Weiling Pills (XsWlP) against it remain unclear. The aim of the study is to explore the multidimensional regulatory mechanisms of XsWlP on D-SSDDE, and predict the key targets of its therapeutic effects using network pharmacology.

Materials and Method. In the D-SSDDE mouse model, physiological disorders were assessed by measuring loose stool rates and abdominal temperature, and using enzyme-linked immunosorbent assay (ELISA) to measure serum triglyceride (TG) levels. Gut barrier function was assessed by detecting goblet cell proportions using flow cytometry and quantifying tight junction protein (ZO-1, Claudin-1, MUC2) expression levels via reverse transcription quantitative polymerase chain reaction (RT-qPCR). The potential targets of XsWlP were predicted using network pharmacology. Additionally, we quantified interleukin 6 (IL6) and transforming growth factor beta 1 (TGFB1) expression levels in mouse intestinal tissues and serum via RT-qPCR and ELISA.

Results. XsWlP substantially reverses D-SSDDE-induced metabolic disruptions, including reduced loose stool rate, restored abdominal temperature, and normalized serum TG levels, while repairing gut barrier integrity by adjusting goblet cell ratio and inducing tight junction proteins. Molecular docking validates TGFB1 and IL6 binding affinity. Moreover, XsWlP markedly suppresses the aberrant expression of D-SSDDE-induced pro-inflammatory factors, such as IL-6 and TGF-β1.

Conclusions. The study demonstrates that XsWlP reduces intestinal barrier disruption in D-SSDDE via TGFB1/IL-6 signaling.

Key words

diarrhea, network pharmacology, Interleukin 6, spleen deficiency syndrome with dampness encumbrance, Xiangsha Weiling Pills, transforming growth factor beta 1

INTRODUCTION

Diarrhea, one of the most common diseases, is a public health issue with potentially fatal impacts. It also poses a major health threat to the global population, whether in subtropical or tropical developing countries [1]. The World Health Organization (WHO) defines diarrhea clinically as the passage of three or more loose or watery stools within 24 hours, with subclassifications based on duration: acute (<14 days) or chronic (≥14 days) [2]. In Traditional Chinese Medicine (TCM), diarrhea pathogenesis is intrinsically linked to the concept of 'dampness'. Prolonged exposure to cold and damp conditions, termed 'seasonal pathogens', can impair spleen and stomach function, leading to 'diarrhea with syndrome of spleen deficiency with dampness encumbrance (D-SSDDE)'. This condition presents with watery stools, hyperactive bowel sounds, and a desire for warmth. It is commonly seen in high-altitude-induced diarrhea, acute infantile diarrhea in cold environments, and is associated with such modern conditions as irritable bowel syndrome with diarrhea (IBS-D) and functional diarrhea [3, 4]. Thus, D-SSDDE is one of the six common TCM diarrhea patterns [5].

Various treatments are available for D-SSDDE, including TCM formulations, acupuncture, and massage. TCM, such as Shenling Baizhu San (containing *Panax ginseng*, *Poria cocos*, and *Atractylodes macrocephala*), can enhance spleen function, remove dampness, and alleviate IBS-D symptoms, making it suitable for patients with chronic diarrhea and fatigue [6]. Huoxiang Zhengqi San has shown potential in mouse models to regulate intestinal lactase activity and restore microbiota balance, highlighting its role in resolving gastrointestinal disorders [7]. Xiangsha Weiling Pills (XsWlP) comprise 11 Chinese herbs: Aucklandiae Radix (50g), Amomi Fructus (50 g), Atractylodis Rhizoma (stir-heating with bran, 150 g), Magnoliae Officinalis Cortex (processed with ginger, 150 g), Atractylodis Macrocephalae Rhizoma (stir-heating with bran, 150 g), Citri Reticulatae Pericarpium (150 g),

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Poria cocos (Schw.) Wolf (150 g), Alismatis Rhizoma (100 g), *Polyporus umbellatus* (Pers.) Fries (100 g), Cinnamomi Cortex (50 g), and Glycyrrhizae Radix et Rhizoma (60 g). In clinical practice, Chinese physicians use XsWlP in the treatment of D-SSDDE. Many of its herbal components are closely related to diarrhea treatment, for example, *Poria Cocos* (Schw.) Wolf., which reduces dampness-induced diarrhea and enhances spleen/stomach function [8]. Research shows *Poria Cocos* (Schw.) Wolf. can inhibit PARP-1 activation and reduce macrophage pyroptosis, protecting the gut barrier [9]. Atractylodis Macrocephalae Rhizoma, rhizome of *Atractylodes Macrocephala* Koidz., strengthens the spleen, dries dampness, and improves digestion [10]. Studies indicate it can activate the aryl hydrocarbon receptor via tryptophan metabolites produced by gut microbiota, thereby improving metabolic disorders of sugar and lipids [11]. Magnoliae Officinalis Cortex, the cortex of *Magnolia Officinalis* Rehd Et Wils., can dry dampness, resolve phlegm, and relieve abdominal distension [12]. Research shows it can improve intestinal pathological damage and barrier gene expression, reduce pro-inflammatory cytokines like interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α), and inhibit the PI3K/AKT/NF-κB pathway, thereby significantly alleviating intestinal injury [13]. The synergy of these components offers some theoretical support for D-SSDDE treatment.

This study employs network pharmacology to investigate the therapeutic targets of XsWlP in alleviating diarrhea and to explore its effects in the D-SSDDE model. By analyzing the mechanisms of XsWlP's multiple components, targets, and pathways, the study highlights its significant regulatory effects on the key targets *TGFB1* and *IL6*.

MATERIALS AND METHOD

Mouse grouping and treatment. The experiments were carried out under the guidelines of ARRIVE and approved by the Institutional Animal Care and Use Committee of Xiuzheng Pharmaceutical Group in China (Approval ID: 202016). Thirty healthy mice were acclimatized for 3 days prior to randomization via a random number table into 3 groups (n=10/group): (1) Control, (2) D-SSDDE model, and (3) D-SSDDE+XsWlP co-treatment. From 08:00 – 12:00 midday, daily, mice in the D-SSDDE model and D-SSDDE+XsWlP groups were housed in an artificial climate chamber (temperature: 4 ± 0.5 °C; humidity: 90 ± 2%) for 4 hours/day over 7 consecutive days. During non-exposure periods, these mice were maintained under standard room conditions (24 ± 1 °C; relative humidity: 50 ± 3%). Control remained under standard conditions throughout the 7-day modeling phase.

Post-modeling, the D-SSDDE+XsWlP group received XsWlP aqueous solution via oral gavage for 3 days, with dosage calculated based on clinical equivalence. Control and D-SSDDE model groups were administered equivalent volumes of sterile water. Primary (loose stool rate) and secondary (abdominal temperature) symptoms were recorded post-treatment.

Upon completion of interventions, mice were anaesthetized for whole-blood and colon collection. Blood samples were incubated at room temperature for 2 hours, followed by centrifugation at 4 °C (3,000 rpm, centrifugal radius: 5 cm) for 15 minutes to isolate serum for downstream analyses. The colon tissues were extracted and freed of adherent fat and faeces.

Enzyme-linked immunosorbent assay (ELISA). Serum biochemical parameters were quantified using ELISA. Triglyceride (TG) levels were measured with a Mouse Triglyceride ELISA Kit (Yanqi, Shanghai). IL-6 concentrations were determined using a Mouse IL-6 ELISA Kit (Elabscience, Wuhan). Transforming Growth Factor-β1 (TGF-β1) levels were analyzed with a Mouse TGF-β1 ELISA Kit (BOSTER, Wuhan).

Flow cytometry for goblet cells in the mouse colon. Colon tissues were collected from mice, rinsed with ice-cold saline, minced into approximately 1 mm^3 pieces, and digested in a solution containing collagenase and DNase I at 37 °C for 20 minutes to prepare a single-cell suspension. The digested tissue was then filtered through a 70 μm cell strainer and centrifuged with saline to remove the digest and debris, yielding a relatively pure single-cell suspension. Subsequently, the cells were blocked with TruStain FcX™ anti-mouse CD16/32 antibody (1 μg per 10^6 cells, BioLegend 101319) to prevent non-specific labelling. After that, the cells were labelled with Alexa Fluor 647-conjugated anti-CD24 antibody (1:200, BioLegend 101818) and Atto 488-conjugated Ulex europaeus agglutinin (UEA-1) (5μg/ml, Sigma 19337). Dead cells were excluded using DAPI staining, and goblet cells ($UEA\text{-}1^+$ $CD24^-$) were identified via flow cytometry (Beckman Coulter Gallio's Flow Cytometer). Finally, the data were analyzed using Kaluza analysis 2.1 software to determine the proportion of goblet cells.

Network pharmacology. Bioactive component screening and target identification: phytochemical components of XsWlP were systematically screened from the TCMSP database using the following inclusion criteria: oral bioavailability (OB) ≥30%, drug-likeness (DL) ≥0.18, and Caco-2 permeability ≥-0.4. Potential targets associated with the identified bioactive components were subsequently retrieved from TCMSP through compound-specific queries.

Gene selection methodology: SSDDE-associated genes were identified by querying using the 'Syndrome of Spleen Deficiency with Dampness Encumbrance' in the ETCM 2.0 database, which provided a curated list of putative therapeutic targets linked to this TCM syndrome phenotype. For diarrhea-related genes, systematic data mining was conducted across 5 biomedical repositories: TCMSP, HERB, Therapeutic Target Database (TTD), GeneCards, and OMIM, using 'diarrhea' as the primary search term to collate disease-associated genetic targets.

Identification of XsWlP therapeutic targets in D-SSDDE-associated diarrhea: Potential therapeutic targets of XsWlP were derived through tripartite Venn analysis, intersecting three distinct gene sets: (1) pharmacological targets of XsWlP, (2) SSDDE syndrome-related genes from ETCM 2.0, and (3) diarrhea-associated genes curated from multi-database mining (TCMSP, HERB, TTD, GeneCards, OMIM). The consensus genes identified through this combinatorial screening represent possible targets through which XsWlP modulates D-SSDDE.

Pill-phytochemical-target-disease network. A retrospective analysis was conducted to trace the bioactive phytochemicals and corresponding herbs of XsWlP that interact with D-SSDDE-related targets. To systematically visualize these interactions, a multi-layered network was constructed, integrating 4 dimensions: (1) the pill XsWlP,

(2) phytochemical compounds, (3) molecular targets, and (4) disease associations. This network was generated using Cytoscape 3.9.1, with edge weights reflecting interaction probabilities derived from STITCH and HIT databases.

Gene annotation analysis. Potential targets of XsWlP acting on D-SSDDE were analyzed for protein-protein interactions (PPI) using the STRING database. The PPI network was visualized in Cytoscape (version 3.9.1) to identify hub targets. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using R packages to elucidate biological pathways and molecular functions.

Molecular docking. Molecular docking was performed on the CB-DOCK2 platform (https://cadd.labshare.cn/cb-dock2/php/blinddock.php) using the Auto Blinding Docking module. Docking was performed based on the detected candidate pockets using AutoDock Vina.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from mouse tissue samples using the RNA/Protein Isolation Kit (Beyotime Biotechnology, R0018S) following the manufacturer's instructions. The PrimeScript™ RT reagent Kit (Takara, RR037Q) was employed for reverse transcription under the following conditions: 5 minutes at 25 °C, 20 minutes at 46 °C, 1 minute at 95 °C, and held at 4 °C. For qPCR, 1 μL of the resulting cDNA was amplified using Power SYBR™ Green PCR Master Mix (Thermo Fisher Scientific, 4367659) on a QuantStudio Dx Real-Time PCR System (Thermo Fisher Scientific). The thermal cycling conditions were as follows: 2 minutes at 50 °C, 2 minutes at 95 °C for activation, followed by 40 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Relative gene expression levels of ZO-1, Claudin 1, MUC2, *IL6*, and TGFB1 were normalized to the internal control GAPDH. The primer sequences used in this study are listed in Table 1. Fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis. Quantitative data are presented as mean ± SD (n ≥ 3 biological replicates). Intergroup comparisons were analyzed through one-way ANOVA with two-tailed Student's t-test using GraphPad Prism 9.3.1; $p < 0.05$ was considered statistically significant.

Table 1. Primer sequences used for RT-qPCR

Gene	5′–3′	Primer sequence
ZO-1	Forward	CCAGCAACTTTCAGACCACC
	Reverse	TTGTGTACGGCTTTGGTGTG
Claudin 1	Forward	GGAGTCAGTGTTTCAGCCTATGGT
	Reverse	GAAGGGTTCATGCCTCTCATCT
MUC2	Forward	ATGCCCACCTCCTCAAAGAC
	Reverse	GTAGTTTCCGTTGGAACAGTGAA
IL-6	Forward	CCCCAATTTCCAATGCTCTCC
	Reverse	CGCACTAGGTTTGCCGAGTA
TGF-β1	Forward	CCCTGGATACCAACTATTGCTTC
	Reverse	AGTAGACGATGGGCAGTGGCT
GAPDH	Forward	CCTCGTCCCGTAGACAAAATG
	Reverse	TGAGGTCAATGAAGGGGTCGT

RESULTS

XsWlP exerts a beneficial effect on D-SSDDE-induced physiological disorders. In this study, the impact of XsWlP on D-SSDDE-induced physiological disorders was evaluated. Results showed that D-SSDDE treatment significantly increased the rate of loose stools (from 6.37±2.45% to 12.91±3.06%), an effect that was markedly reversed with the combined application of XsWlP (resulting in 7.30±1.76% of loose stool rate) ($p < 0.001$). The differences between the normal and D-SSDDE+XsWlP groups were not significant (6.37±2.45% versus 7.30±1.76%) (Fig. 1A). Similarly, D-SSDDE administration caused a significant decrease (from 36.10±0.24 °C to 35.66±0.22 °C) in abdominal temperature, which was nearly restored to normal following the addition of XsWlP (36.38±0.36 °C; $p < 0.01$) (Fig. 1B). Furthermore, D-SSDDE treatment led to a significant decrease (from 2.47±0.16 mmol/L to 1.33±0.29 mmol/L) in TG levels, whereas the administration of XsWlP resulted in a substantial increase in these levels, although still below normal values (1.83±0.25 mmol/L versus 1.33±0.29 mmol/L; $p < 0.001$) (Fig. 1C). Overall, these findings indicate that XsWlP exerts a significant ameliorative effect on physiological disorders induced by D-SSDDE.

XsWlP restores intestinal barrier function. The results of molecular analysis revealed that XsWlP intervention effectively reversed D-SSDDE-induced intestinal barrier dysfunction through dual regulatory mechanisms. Compared to the normal group, the proportion of goblet cells decreased significantly in the D-SSDDE group (12.60±0.60% versus 8.14±0.96%), while XsWlP treatment restored it nearly to baseline levels (12.25±0.78%; $p < 0.001$) (Fig. 2A). Experimental analyses demonstrated that D-SSDDE markedly inhibited the relative expression of key barrier genes, including ZO-1 (from 1.00±0.11 to 0.58±0.08), Claudin 1 (from 1.00±0.10 to 0.50±0.09), MUC2 (from 1.00±0.11 to 0.53±0.13), while the addition of XsWlP significantly restored these mRNA expressions nearly to normal (0.94±0.06, 0.91±0.08, and 0.93±0.08, respectively; $p < 0.001$) (Fig. 2B-D). In summary, XsWlP was demonstrated to rebalance goblet cell homeostasis and reinforce tight junction integrity, thereby maintaining gut barrier function under D-SSDDE-related pathophysiology.

Network pharmacology of XsWlP. Based on the inclusion criteria of oral bioavailability (OB) ≥30%, drug-likeness (DL) ≥0.18, and Caco-2 permeability ≥-0.4, a total of 143 phytochemical components were collected for XsWlP from TCMSP database, which targeted 224 genes (supplementary Fig. 1). Among these target genes, twenty genes were shared with those related to diarrhea and syndrome of spleen deficiency with dampness encumbrance (SSDDE) (supplementary Fig. 2). By tracing the herbs and phytochemicals, we constructed a "Pill-herb-phytochemical-target-disease" network, which showcased the 9 herbal components of XsWlP, encompassing 88 chemical entities, and targeting 20 genes associated with D-SSDDE (Fig. 3).

Prediction for key targets of XsWlP. Subsequently, KEGG pathway analysis (Fig. 4A) revealed notable enrichment of key targets, including IL6 and TGFB1, in pathways related to 'inflammatory bowel disease (IBD)'. The PPI

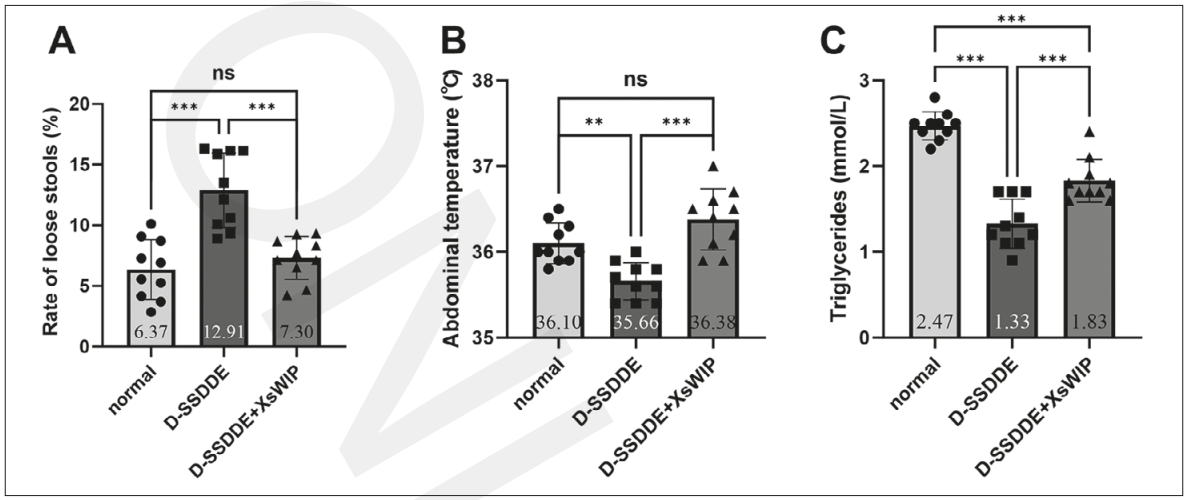


Figure 1. Effects of different treatments on mouse diarrhea rate, abdominal temperature, and triglyceride levels**.** (A) Effects of different treatments on the rate of loose stools. (B) Effects of different treatments on mouse abdominal temperature. (C) Effects of different treatments on mouse serum TG levels. n-10 mice per group; $^{**}p < 0.01$; $^{***}p < 0.001$; ns-not significant

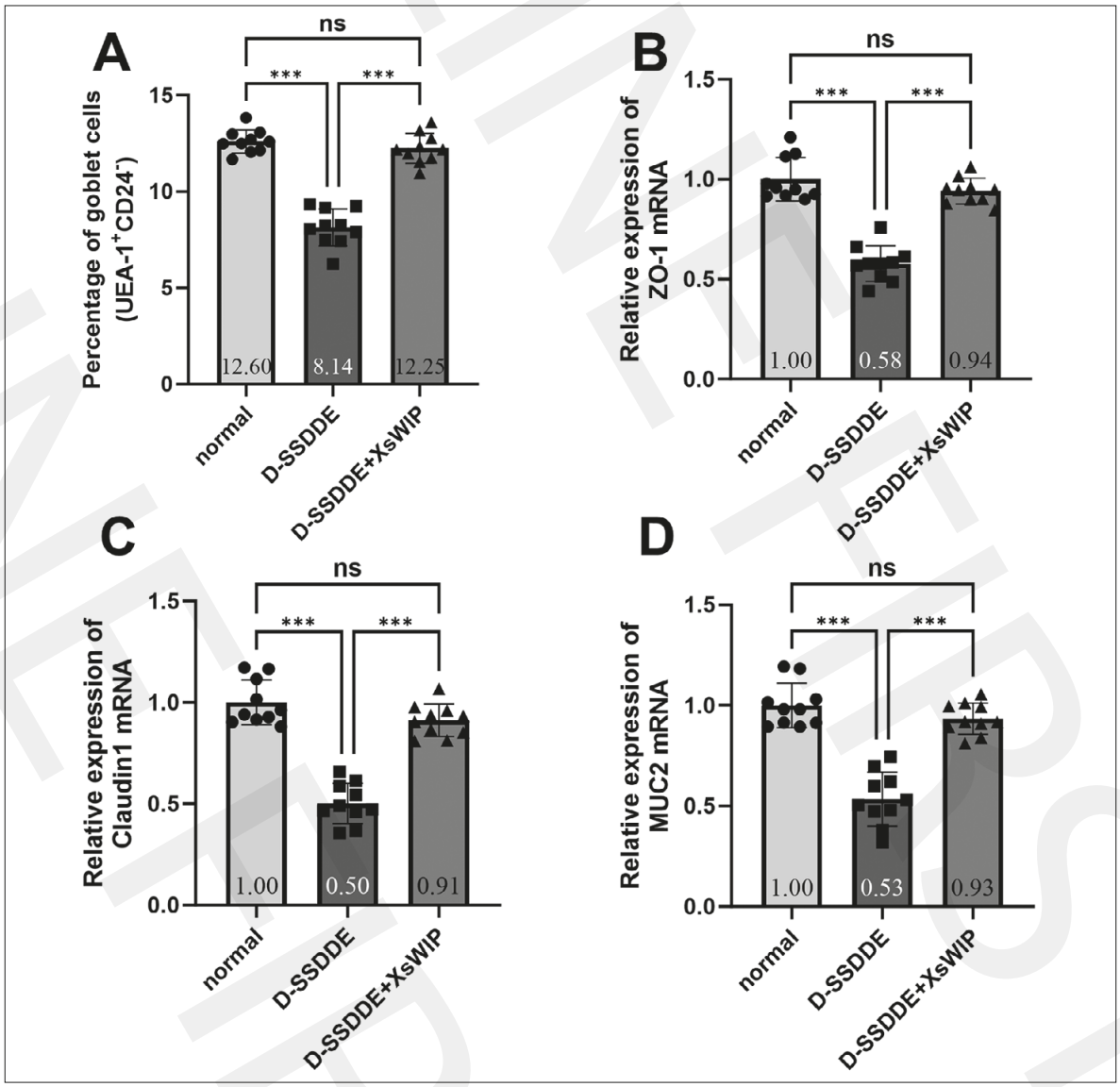


Figure 2. Effects of different treatments on the proportion of goblet cells and tight junction factors in mouse colon tissues. (A) Effects of different treatments on the proportion of goblet cells. (B) Effects of different treatment groups on the expression level of ZO-1 mRNA. (C) Effects of different treatment groups on the expression level of Claudin 1 mRNA. **(D)** Effects of different treatment groups on the expression level of MUC2 mRNA. n-10 mice per group; $^{***}p < 0.001$; ns-not significant

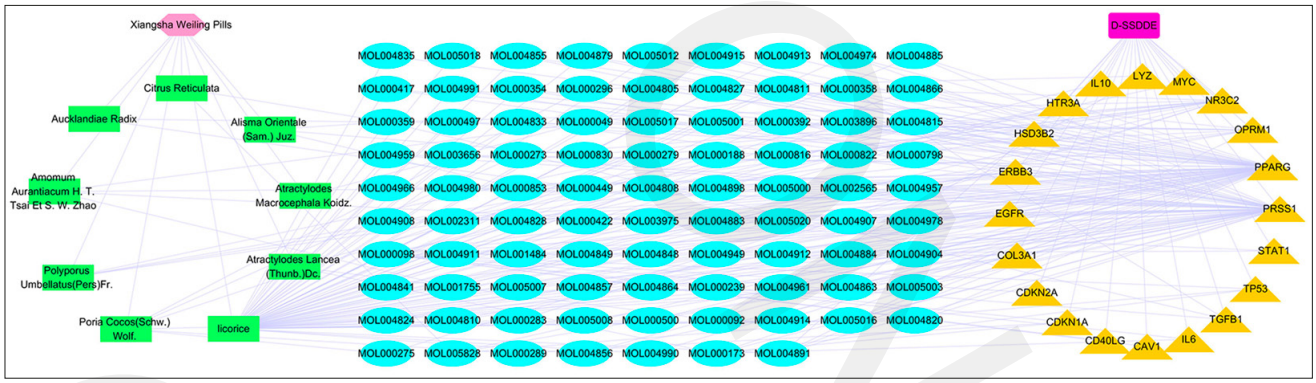


Figure 3. Construction of the 'Pill-phytochemical-target-disease' network

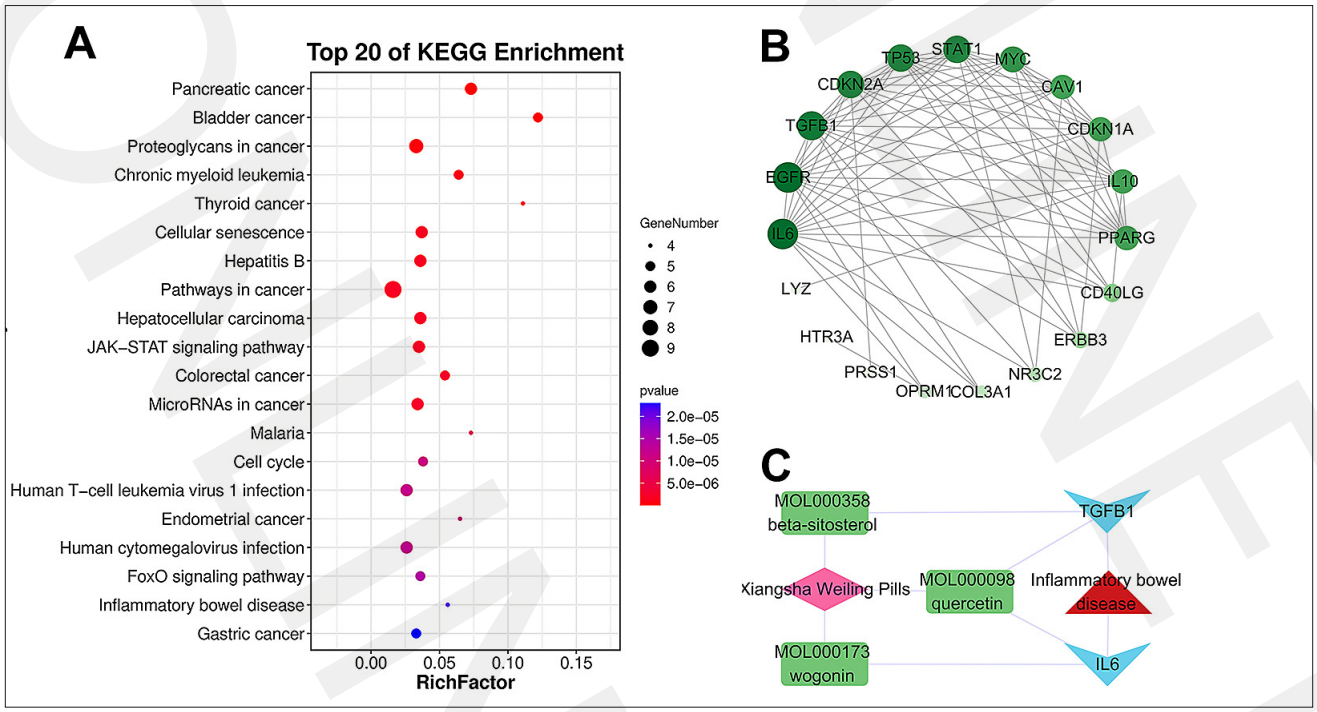
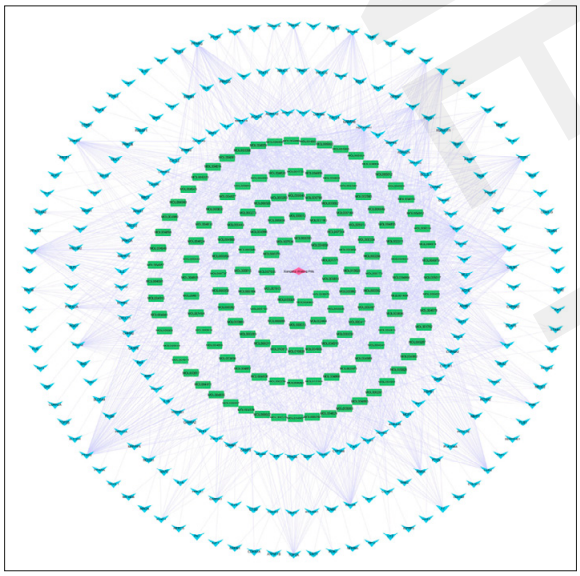
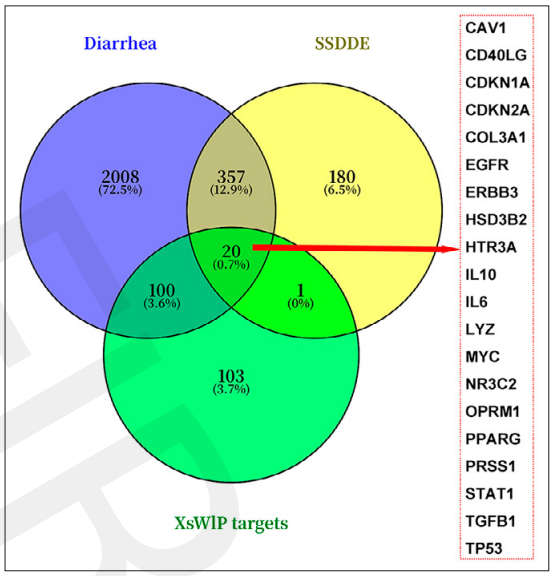


Figure 4. Prediction and analysis of key targets of XsWIP in treating D-SSDDE. (A) KEGG pathway analysis of key targets. (B) PPI network of key targets. (C) Phytochemicals targeting key genes



Supplementary Figure 1. Collection of phytochemical components of XsWIP from the TCMSP database



Supplementary Figure 2. A Venn diagram illustrating the shared target genes among XsWIP, diarrhea, and SSDDE

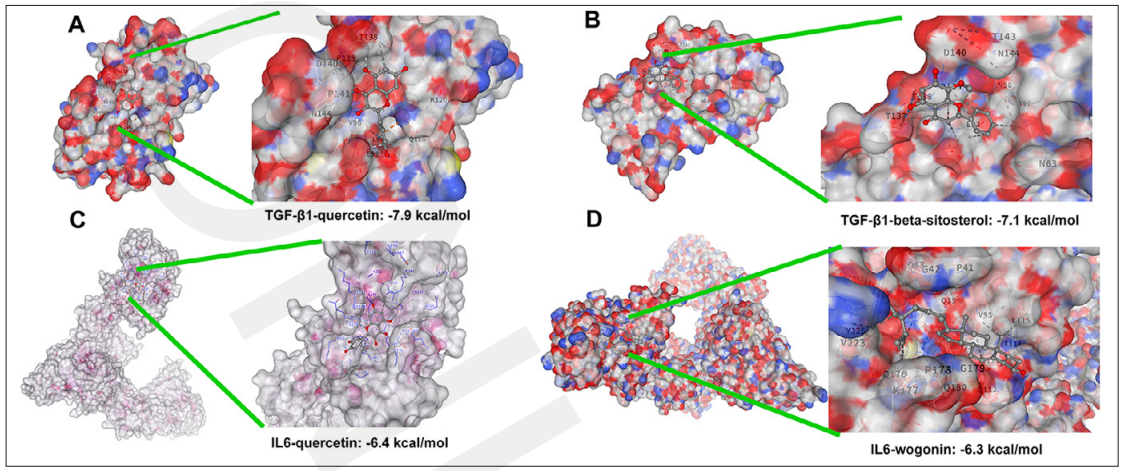


Figure 5. Molecular docking models of the active components of XsWIP with TGF-β1 and IL-6. (A) Molecular docking model of quercetin with TGF-β1. (B) Molecular docking model of beta-sitosterol with TGF-β1. (C) Molecular docking model of quercetin with IL-6. (D) Molecular docking model of wogonin with IL-6

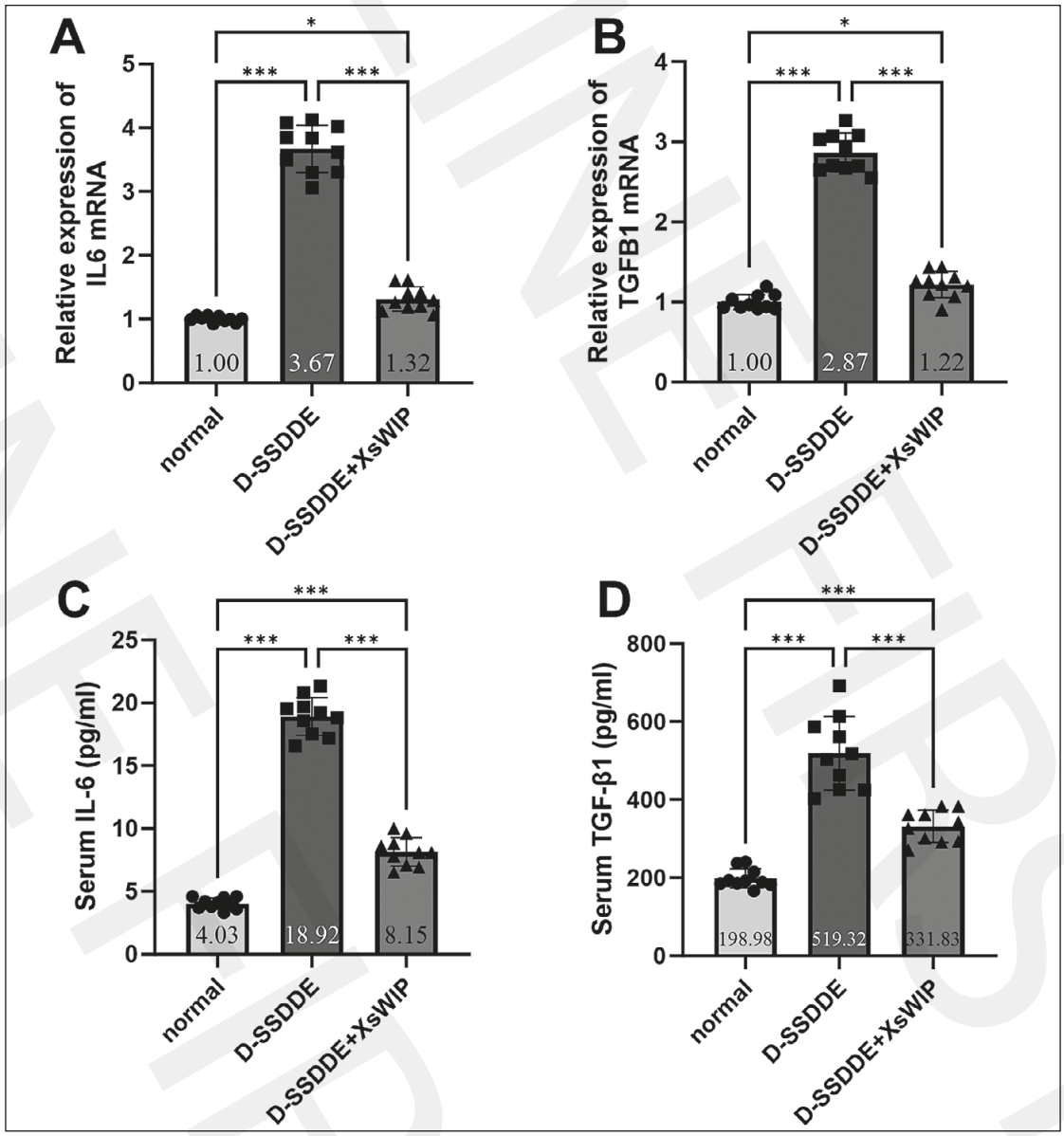


Figure 6. XsWIP moderated IL-6 and TGF-β1 expression levels in mice. (A) IL6 mRNA relative expression levels in mouse colon tissues across treatment groups. (B) TGFB1 mRNA relative expression levels in mouse colon tissues across treatment groups. (C) Serum IL-6 concentrations in response to different treatments. (D) Serum TGF-β1 concentrations in response to different treatments.
n-10 mice per group; *$p < 0.05$; ***$p < 0.001$.

network for these 20 genes according to the degree values showed that IL6 and TGFB1 ranked in the top 5 (Fig. 4B). By tracing the phytochemicals, quercetin and beta-sitosterol were found to target TGFB1, while quercetin and wogonin targeted IL6 (Fig. 4C). Collectively, these results suggested that TGFB1 and IL6 may be critical targets for XsWlP in treating D-SSDDE.

Molecular docking results of the core active components of XsWlP with TGF-β1 and IL6. The Molecular docking analysis revealed that the active components of XsWlP (quercetin, beta-sitosterol, and wogonin) bind to the key targets TGF-β1 and IL6. Quercetin demonstrated binding affinity to both TGF-β1 (binding energy: -7.9 kcal/mol) (Fig. 5A) and IL6 (binding energy: -6.4 kcal/mol) (Fig. 5C). Beta-sitosterol exhibited binding to TGF-β1 (binding energy: -7.1 kcal/mol) (Fig. 5B), and wogonin showed binding to IL6 (binding energy: -6.3 kcal/mol) (Fig. 5D). These results suggest that these compounds may modulate the activity of TGF-β1 and IL6, thereby exerting a potential therapeutic effect on D-SSDDE.

XsWlP exerts inhibitory effects on D-SSDDE-induced expression of inflammatory cytokines IL-6 and TGF-β1. Experimental analyses demonstrated that XsWlP regulates IL-6 and TGF-β1 expression in D-SSDDE models (Fig. 6A-D). Relative to untreated controls, D-SSDDE administration significantly elevated both IL6 (from 1.00±0.05 to 3.67±0.37; $p < 0.001$) (Fig. 6A) and TGFB1 (from 1.00±0.10 to 2.87±0.24; $p < 0.001$) (Fig. 6B) mRNA levels, with corresponding increases observed in serum IL-6 (from 4.03±0.45 pg/ml to 18.92±0.52 pg/ml; $p < 0.001$) (Fig. 6C) and TGF-β1 (from 198.98±24.41 pg/ml to 519.32±0.52 pg/ml; $p < 0.001$) (Fig. 6D) concentrations. These data collectively indicate D-SSDDE-induced activation of inflammatory factors. In contrast, XsWlP co-treatment substantially attenuated these effects, reducing IL-6 and TGF-β1 mRNA expression levels (1.32±0.19 and 1.22±0.66, respectively) and serum concentrations (8.15±1.14 and 331.83±40.88, respectively; $p < 0.05$) (Fig. 6A-D). These results confirm XsWlP's capacity to counteract inflammatory activation via targeted IL6/TGFB1.

DISCUSSION

With the accelerated pace of modern life and increased levels of stress, irregular dietary habits, and excessive consumption of cold/raw foods have become prevalent, leading to a rising incidence of D-SSDDE. In this context, the present study employed a network pharmacology approach to systematically investigate the multi-dimensional regulatory effects of XsWlP on physiological function, intestinal barrier integrity, and inflammatory responses in the D-SSDDE.

Cold stimulation mediates the pathological progression of D-SSDDE through multidimensional mechanisms. Primarily, cold exposure directly suppresses the activity of key lipid-metabolizing enzymes, inducing dyslipidemia (elevated TG) [14] and impaired bile acid synthesis [15, 16], thereby weakening intestinal lipid digestion and absorption, and ultimately leading to diarrhea. The experimental results of the current study demonstrate that XsWlP significantly ameliorated D-SSDDE-related phenotypic alterations, including reduced diarrhea frequency, restored abdominal temperature, and modulated serum TG levels, indicating its potential efficacy in alleviating metabolic disturbances and energy imbalance associated with D-SSDDE. Furthermore, low-temperature conditions compromise intestinal mucosal barrier integrity [17]. The study also revealed that XsWlP restored gut barrier integrity by regulating goblet cell differentiation homeostasis and upregulating tight junction protein expression, thereby attenuating barrier damage.

Additionally, cold-dampness disrupts intestinal micro-environmental homeostasis by perturbing amino-acid metabolism (notably glutamate and tryptophan) and by synergistically up-regulating pro-inflammatory cytokines IL-6, IL-7, and IL-8, collectively contributing to intestinal barrier dysfunction [18]. Research indicates that IL-6 expression is upregulated in the inflamed mucosa and plasma of IBD patients, where it modulates tight junction protein expression through multiple signalling pathways, including MAPK/ERK, PI3K/AKT, and JNK, thereby increasing permeability to small molecules and compromising intestinal barrier integrity [19]. Meanwhile, TGF-β induces disruption of tight junction structures, leading to impaired intercellular connectivity in the epithelial layer [20]. These metabolic disturbances and inflammatory factors provide a theoretical foundation for targeted therapeutic strategies.

The network pharmacology investigation delineated the multi-component and multi-target therapeutic profile of XsWlP against D-SSDDE. Through the construction of an integrated 'formula-compounds-targets-disease' network, 88 bioactive constituents were identified as potentially modulating 20 key targets via synergistic mechanisms. Protein-protein interaction analysis highlighted IL6 and TGFB1 as central nodes, indicating that XsWlP may mediate its effects through a TGFB1/IL6-centric axis. This premise was substantiated by the identification of specific components-quercetin, β-sitosterol, and wogonin-as key regulators of these targets, with molecular docking validating their strong binding affinities to TGF-β1 and IL6. Pathway enrichment further revealed significant involvement of these targets in inflammatory bowel disease-related pathways, supporting a mechanism centred on anti-inflammatory activity and lipid metabolism regulation. These findings systematically elucidate the pharmacological basis of XsWlP in D-SSDDE treatment. The findings of the current study on the intestinal barrier-restoring effects of XsWlP are consistent with previous reports concerning its constituent herbs Citri Reticulatae Pericarpium and *Poria cocos* (Schw.) Wolf. Specifically, polyphenolic compounds from Citri Reticulatae Pericarpium upregulate tight junction proteins (ZO-1, occludin) to reinforce the intestinal barrier [21, 22]. Polysaccharides derived from *Poria cocos* contribute to barrier integrity by modulating tight junction proteins and gut microbiota, in addition to exerting antioxidant and anti-inflammatory activities [23]. While existing studies have largely focused on individual compounds or single herbs, the current study extends this evidence by demonstrating that the complete XsWlP formula produces integrated therapeutic outcomes in the complex context of D-SSDDE, underscoring the distinctive value of the classical formulation in the coordinated regulation of intestinal barrier function and inflammatory responses.

Limitations of the study. While this study integrated experimental validation and network pharmacology to reveal the potential of XsWlP in alleviating intestinal barrier injury in D-SSDDE via the TGFB1/IL6 pathway, several limitations should be acknowledged. First, although molecular docking supported the interactions between predicted bioactive components and key targets, further experimental confirmation using *in vitro* cellular assays or gene knockout approaches is necessary to establish direct regulatory relationships. Second, the study primarily focused on the TGFB1/IL6 signalling axis, leaving the contributions of other components in XsWlP and their potential involvement in additional pathways less explored. Moreover, while the animal model recapitulated certain pathological features of D-SSDDE, differences in pathophysiology between mice and humans warrant further validation in clinical settings.

CONCLUSIONS

In summary, XsWlP ameliorates D-SSDDE-induced physiological disturbances and alleviates intestinal barrier injury. Integrated network pharmacology and experimental validation demonstrate that XsWlP modulates TGF-β1 and IL6, thereby suppressing pro-inflammatory factor expression in the D-SSDDE model.

Future investigations will focus on the following directions: systematically validating the direct interactions between critical bioactive compounds and their targets using mechanistic cellular models; applying metabolomic and metagenomic approaches to elucidate the role of XsWlP in host-microbiome metabolic crosstalk. And translating these foundational findings into clinical evaluations to better assess their therapeutic efficacy.

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