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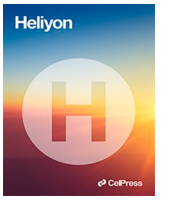

Research article

Integrating network pharmacology and experimental models to investigate the mechanisms of XCHD and YCSLS in preventing HUA progression via TLR4/MYD88/NF-κB signaling

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ABSTRACT

With the alterations in dietary structure and the augmentation of the human living standard, hyperuricemia (HUA) has emerged as a significant factor impacting contemporary human health. It has also been scientifically validated as an independent risk determinant for the progression of renal disease. Existing literature indicates that XCHD (Xiao Chai Hu Decoction) and YCSLS (Yinchen Siling San) possess a capability to ameliorate UA levels and fortify renal function, yet a comprehensive understanding of their mechanisms of action remains elusive. This investigation is designed to elucidate the therapeutic efficacy and mechanistic underpinnings of XCHD/YCSLS on the renal tissues of HUA-afflicted rats, with the objective of fortifying the evidence base to advocate its clinical application. Our preliminary findings substantiated that XCHD and YCSLS impede HUA progression through the inhibition of inflammatory and oxidative stress pathways. Further, we synthesized data from publicly accessible repositories to forecast interactions between XCHD, YCSLS, and their prospective targets in HUA, including the associated signaling pathways. This approach facilitated the identification of shared targets of XCHD/YCSLS, and HUA, and the subsequent correlation analysis of these targets employing KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) methodologies. The findings indicate that the TLR4/MYD88/NF-κB signaling constitutes one of the potential crucial conduits engaged in XCHD and YCSLS-induced HUA mitigation. In conclusion, the analysis of WB and IHC from HUA rat models corroborated that XCHD and YCSLS do indeed attenuate the expression of TLR4/MYD88/NF-κB, reinforcing the hypothesized pivotal role of the its signaling cascade in HUA. This warrants subsequent scholarly exploration.

1. Introduction

Hyperuricemia (HUA) is a widespread metabolic disease, with its prevalence escalating globally, posing a significant health challenge and contributing to the increasing burden on healthcare systems over recent decades [1–3].Statistical data indicate that approximately 120 million individuals in China suffer from HUA, constituting about 10 % of the nation's total population [4].

Abbreviations: Hyperuricemia, (HUA); XCHD, (Xiao Chai Hu Decoction); YCSLS, (Yinchen Siling San); TLR4, (Toll-like Receptors 4); UA, (Uric Acid); KEGG, (Kyoto Encyclopedia of Genes and Genomes); MyD88, (Myeloid Differentiation Factor 88); PBS, (Phosphate-Buffered Saline); GO, (Gene Ontology); NF-κB, (Nuclear Factor kappa-B).

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Hyperuricemia is defined as a metabolic aberration characterized by an elevated serum urate concentration, a manifestation that may arise due to overproduction and/or diminished excretion of urate in the gut and kidneys. This condition is indicative of systemic disorders in purine metabolism and a reduction in uric acid excretion, culminating in an elevation of serum uric acid, a salient clinical feature of metabolic syndrome. Elevated concentrations of UA may precipitate in tissues, engendering diverse pathological conditions and constituting a risk factor for renal impairment [5–7].

Clinically, HUA is predominantly treated with oral medications such as propylene sulfate, benzbromarone, allopurinol, etc [8]. However, propylene sulfate and benzbromarone may lead to UA salt crystal deposition within the urinary tract, thereby causing renal damage, while allopurinol may provoke hypersensitivity, gastrointestinal stimulation, and liver dysfunction [6,9]. Although these pharmaceutical agents are effective in reducing serum uric acid levels, their utilization is often accompanied by significant adverse effects. Consequently, there is an exigent need for the exploration of novel targetable medications and combination therapies to augment clinical benefits and enhance therapeutic outcomes in HUA patients.

Within the realm of Traditional Chinese Medicine (TCM), the herbal formulation XCHD has demonstrated satisfactory efficacy against HUA. Representing a seminal prescription in Chinese medicine, XCHD, first documented in the Treatise on Febrile Diseases, comprises seven traditional Chinese herbs [10,11]. Contemporary pharmacological research has elucidated that the constituents of XCHD exhibit properties including anti-inflammatory, antioxidant, anti-tumor, and immune regulatory effects, thus contributing to the prevention and treatment of HUA and associated kidney injury [12–15]. Likewise, YCSLS, composed of five traditional Chinese medicines, has been shown to modulate abnormal human water metabolism and demonstrate pharmacological attributes, like antioxidant, hepatoprotection, anti-inflammatory and immune regulation [16–19].

However, the therapeutic effect of XCHD and YCSLS, as well as their underlying anti-HUA mechanisms, remain subjects of ongoing investigation. Network pharmacology, a methodological paradigm integrating systems biology with network approaches, has emerged as an influential instrument for screening unknown targets for drugs [20]. It assists in constructing speculative 'drug-target-disease' models utilizing high-throughput screening and bioinformatics techniques [21].Integrating network pharmacology with in vivo models offers substantial value in discerning a possible cross-targets between XCHD/YCSLS and HUA, thereby elucidating the molecular mechanisms underpinning XCHD and YCSLS in HUA treatment. We employ HUA rat models to validate the anti-HUA roles of XCHD and YCSLS, and utilize network pharmacology to uncover the mechanism of XCHD and YCSLS in HUA. Data from network pharmacology suggest that NF-κB is a noteworthy target associated with both XCHD/YCSLS and HUA. Subsequent in vivo studies

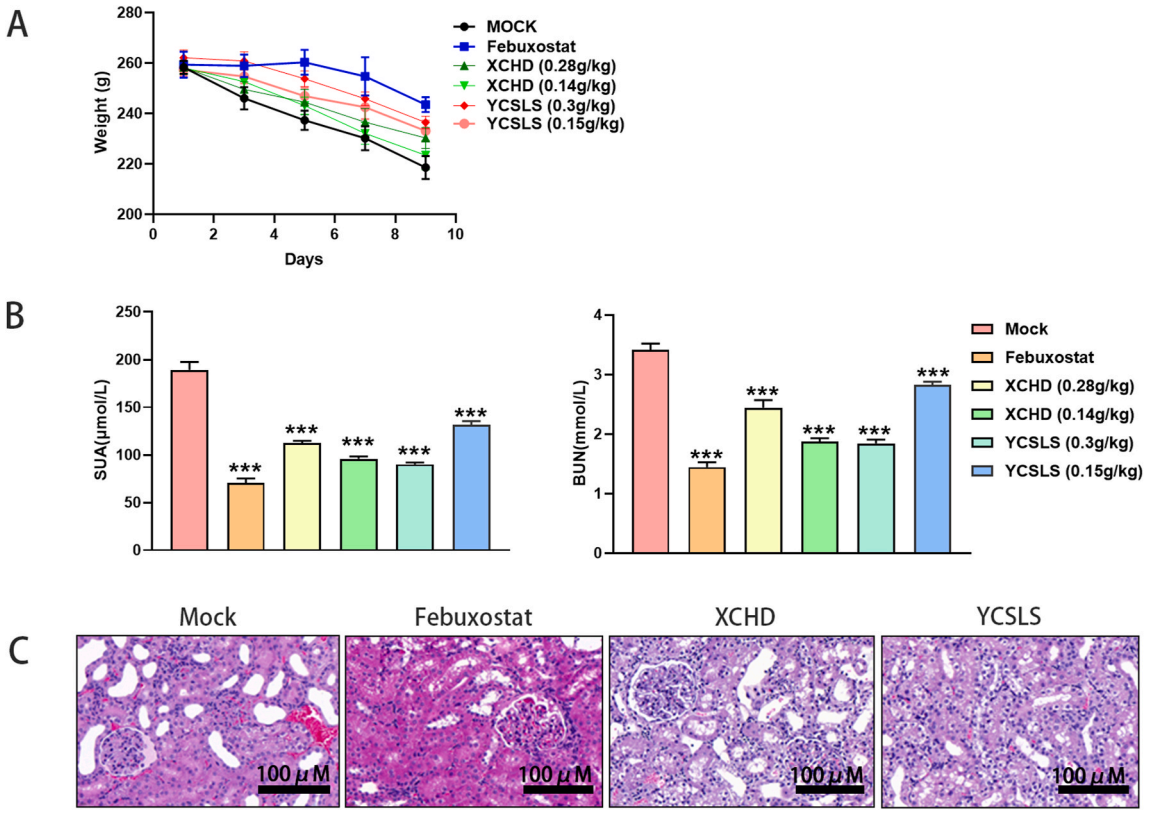

Fig. 1. XCHD and YCSLS restrained HUA progress. (A) The body weights of HUA rat were assessed each two days while being treated with PBS, Febuxostat, XCHD (0.14 g/kg, 0.28 g/kg), and YCSLS (0.15 g/kg, 0.3 g/kg) (n = 5). (B) The expression level of SUA and BUN in serum from HUA rat model (n = 5). (C) Representative H&E-stained sections of kidney from HUA rat model scale bar: 100 μm. Data were mean ± SD of three independent experiments (n = 5), ***P < 0.001 vs. Mock group. Mock: PBS, XCHD: Xiao Chai Hu Decoction, YCSLS: Yinchen Siling San.

reveal that the TLR4/MYD88/NF-κB signaling pathway may be a pivotal pathway leading XCHD/YCSLS-induced HUA inhibition via the induction of inflammation and oxidative stress.

2. Results

2.1. XCHD/YCSLS can reduce the symptoms of disease in rats caused by high uric acid kidney damage

The HUA model was adopted to investigate the therapeutic effect of XCHD/YCSLS on uric acid-induced kidney damage. Male SD rats were subjected to potassium oxyzincate (200 mg/kg, subcutaneously) and hypoxanthine (500 mg/kg, by gavage) to induce hyperuricemia, followed by treatment with varied dosages of XCHD/YCSLS. Post a nine-day drug treatment, sacrifice the animals, and harvest blood and kidney. Compared to the Mock (PBS), XCHD and YCSLS decreased Serum UA and Blood Urea Nitrogen (BUN) levels (Fig. 1B), and mitigated weight loss associated with hyperuricemia (Fig. 1A). Hematoxylin and Eosin (H&E) staining of the kidney corroborated that XCHD and YCSLS attenuated kidney damage consequent to hyperuricemia (Fig. 1C), affirming that XCHD and YCSLS could alleviate symptoms of disease in rats induced by elevated uric acid kidney impairment.

2.2. Effects of XCHD and YCSLS on serum inflammatory cytokines and ICAM-1 in rats with HUA kidney damage

Notably, IL-1β, TNF-α were discernibly decreased in the XCHD/YCSLC groups (Fig. 2A). Immunohistochemical (IHC) results further validated that XCHD and YCSLS substantially reduced IL-6 levels in kidney tissues (Fig. 2C). Concurrently, the ICAM-1 levels in XCHD/YCSLS-treated rats were considerably lower than those in PBS-treated rats (Fig. 2B). Collectively, these findings imply that XCHD and YCSLS treatment could notably ameliorate HUA-induced inflammation, potentially through inhibiting the secretion of inflammatory factors.

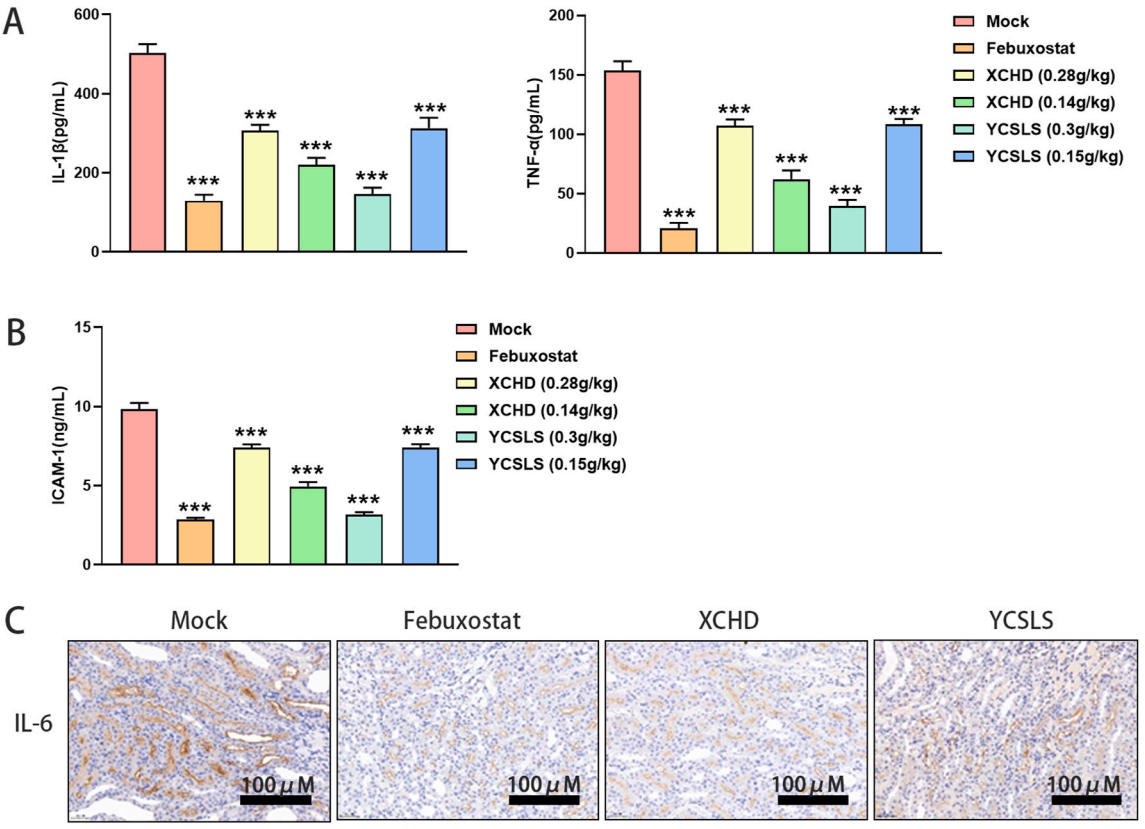

Fig. 2. XCHD and YCSLS inhibit inflammatory in HUA rat. (A,B) The expression IL-1β, TNF-α, ICAM-1 in serum from HUA rat model. (C) Expression of IL-6 in kidney was detected by immunochemistry assay, scale bar: 100 μm. The expression of IL-6 were reduced in the XCHD and YCSLS treatment groups. Data were mean ± SD of three independent experiment (n = 5), ***P < 0.001 vs. Mock group. Mock: PBS, XCHD: Xiao Chai Hu Decoction, YCSLS: Yinchen Siling San.

2.3. Effects of XCHD/YCSLS on oxidative stress of HUA rats

Physiologically, equilibrium prevails between oxidative and antioxidant systems, with antioxidant enzymes mitigating peroxide-related damage [22,23]. Specifically, SOD dismutates superoxide anions to hydrogen peroxide and oxygen, while CAT and GSH-PX promote hydrogen peroxide into oxygen and water, thereby synergistically safeguarding cells from peroxidative harm [24,25]. Malondialdehyde (MDA), a lipid peroxidation product, can crosslink with proteins, nucleic acids, and N-compounds, perturbing their physiological function. MDA, SOD, and GSH-PX are widely regarded as oxidative stress biomarkers [26,27].

Oxidative stress can lead to elevated uric acid, culminating in further renal injury [28,29]. In this study, the expression levels of MDA, SOD, and GSH-PX were assessed, and the findings revealed that XCHD and YCSLS significantly augmented the expression of SOD and GSH-PX while suppressing MDA production in HUA rats (Fig. 3A), and quantitative RT-PCR showed that XCHD and YCSLS can enhanced their expression, reduced MDA production in mRNA level (Fig. 3B), these results robustly substantiate that XCHD and YCSLS could counteract oxidative stress in HUA rats, through modulating the activities of SOD or GSH-PX and suppressing the activity of MDA.

2.4. Identify the common targets of XCHD/YCSLS and HUA

Several investigations pertaining to HUA have illuminated the multifaceted roles of XCHD and YCSLC in inflammatory responses and oxidative stress, yet the precise targets and underlying mechanisms remain predominantly elusive. In an endeavor to discern the potential targets of XCHD and YCSLS within the context of HUA, we leveraged publicly accessible databases, employing network pharmacology to identify the shared targets of XCHD/YCSLS and HUA. A total of 1140 human targets specific to HUA were gleaned from GeneCards, OMIM, and DisGeNET. Simultaneously, 1026 and 668 drug targets each for XCHD and YCSLS were collected from the Swiss target prediction database (Fig. 4A and D). Subsequent intersection analysis revealed 185 common targets of XCHD and HUA, and 135 common targets of YCSLS and HUA (Fig. 4B and E), encompassing GAPDH, IL-6, TNF, IL-1β, SOD1, PPARG, BCL2, and others, suggesting a positive role of XCHD and YCSLS in HUA treatment. These targets were constructed a PPI network through STRING, requiring a minimum interaction score of 0.4. An examination of the centrality of target genes, including GAPDH, TNF, BCL2, NF-κB, identified these as key genes instrumental in the pathogenesis of HUA (Fig. 4C and F).

2.5. GO and KEGG analysis of XCHD/YCSLS and HUA intersection core targets

KEGG and GO analyses were undertaken on the probable targets of HUA in conjunction with XCHD/YCSLS, using the Metascape database. Fig. 5A delineates the enriched Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MF) ($p \leq 0.01$) associated with target proteins of HUA and XCHD, predominantly related to defense response regulation, cellular activation, xenobiotic stimulus response, catecholamine binding, kinase binding, oxidoreductase activity, etc. Fig. 5C illustrates the target proteins of HUA and YCSLS, chiefly linked to chemical stress response, MAPK cascade regulation, oxygen level response, gland development, kinase activity, phosphatase binding, oxidoreductase activity, among others. In addition, KEGG analysis revealed that targets allied with XCHD and HUA were primarily concentrated in 198 signaling pathways. YCSLS and HUA were predominantly enriched in 177 signaling pathways, including pathways similar to those found with XCHD and HUA, with the top 20 illustrated in Fig. 5B and D.

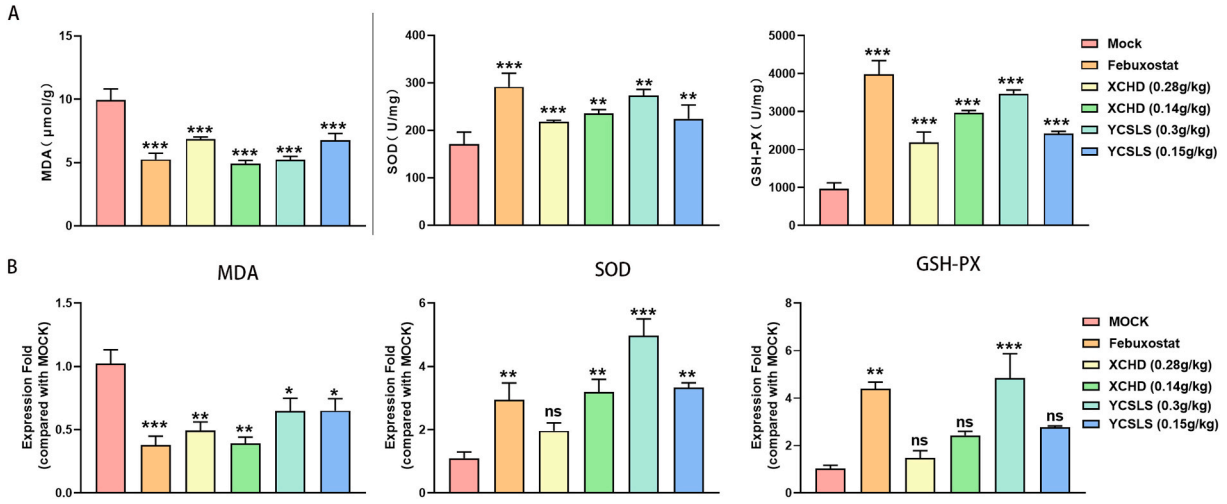

Fig. 3. XCHD and YCSLS inhibit Oxidative Stress in HUA rat. (A) The expression level of MDA, SOD, GSH-PX in serum from HUA rat model were detected. (B) Relative mRNA expression of MDA, SOD, GSH-PX measured by qRT-PCR in serum from HUA rat model after XCHD and YCSLS treated. Data were mean ± SD of three independent experiments (n = 5), *P < 0.01, **P < 0.05, ***P < 0.001 vs. Mock group. Mock: PBS, XCHD: Xiao Chai Hu Decoction, YCSLS: Yinchen Siling San.

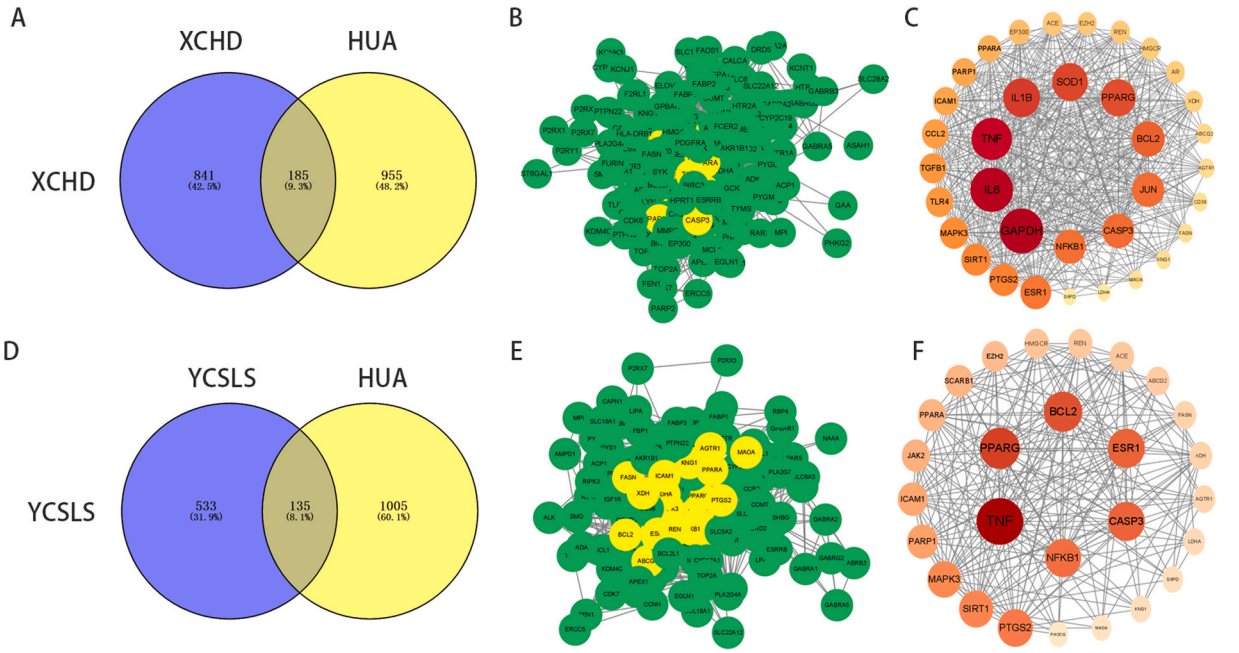

Fig. 4. identify the common targets of XCHD/YCSLS and HUA. (A, D) The intersection of the targets of XCHD/YCSLS and HUA is shown by a Venn diagram. (B, E) XCHD/YCSLS and HUA targets were collated and core targets were identified through the STRING database (The yellow part is the core target site). (C, F) The core targets of XCHD/YCSLS and HUA constructing PPI network, identify the core targets of XCHD/YCSLS and HUA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

NF-κB, implicated in myriad biological processes like immune response, inflammatory reaction, cell apoptosis, and tumorigenesis [30], has been seldom explored in the context of XCHD/YCSLS-mediated HUA inhibition; hence, NF-κB signaling pathway was selected for further analysis.

2.6. Effects of XCHD/YCSLS on TLR4/MyD88/NF-κB signaling pathway in HUA rats

Subsequent to the activation of Toll-like Receptors (TLRs), the downstream factor NF-κB is stimulated, leading to its involvement in the activation and orchestration of the MYD88-dependent pathway, thus consummating the signal transduction process of the inflammatory response [31]. Amalgamating the findings from both GO and KEGG analyses with our initial empirical data, we conjectured that XCHD/YCSLS modulates HUA in rats, thereby reducing inflammatory and oxidative stress through the TLR4/MyD88/NF-κB signaling pathway.

Further exploration was carried out to check the expression of TLR4, MYD88, and p–NF–κB in kidneys of HUA rats models. When contrasted with kidney tissues from rats treated with PBS, the expression of TLR4, MYD88, and p–NF–κB were found to be notably attenuated in those treated with XCHD and YCSLS (Fig. 6 A). Moreover, the expression of p–NF–κB in rats treated with XCHD/YCSLS were appreciably lower than those in PBS-treated counterparts (Fig. 6B). These observations lend support to the hypothesis that XCHD/YCSLS may serve to impede HUA progression via the intricate mechanisms of the TLR4/MyD88/NF-κB signaling pathway.

3. Discussion

Concurrent with the enhancement of human living standards and alterations in dietary structure, the prevalence of HUA in China continues to escalate, progressively emerging as another substantial factor imperiling human health. HUA is predisposed to renal complications and has been empirically validated as an independent risk determinant for the progression of renal diseases [32]. Though pharmacological interventions remain the principal therapeutic approach to HUA, their serious side effects and the necessity for chronic medication necessitate the identification of novel targets and strategies to mitigate these adverse consequences. XCHD exhibits properties including antiviral effects, immunomodulatory enhancement, anti-inflammatory actions, hepatic protection, and inhibition of platelet aggregation; YCSLS possesses anti-oxidative, immunoregulatory, and hepatoprotective functions. Contemporary investigations demonstrate that XCHD and YCSLS mitigate uric acid concentrations in HUA patients and alleviate renal damage consequent to HUA, but the underlying molecular targets and mechanisms remain inadequately elucidated [33,34].

Within the ambit of our research, we initially ascertained the inhibitory influence of XCHD and YCSLS in HUA rats. Through a rigorous examination of their roles in the HUA rat model, our data elucidated that XCHD and YCSLS markedly ameliorated weight loss in HUA Rats, arrested the increase of SUA and BUN, and attenuated associated renal damage. And the data from ELISA、IHC and qRT-PCR proved that XCHD and YCSLS can dramatically suppress inflammatory and oxidative stress in HUA rats via reducing the

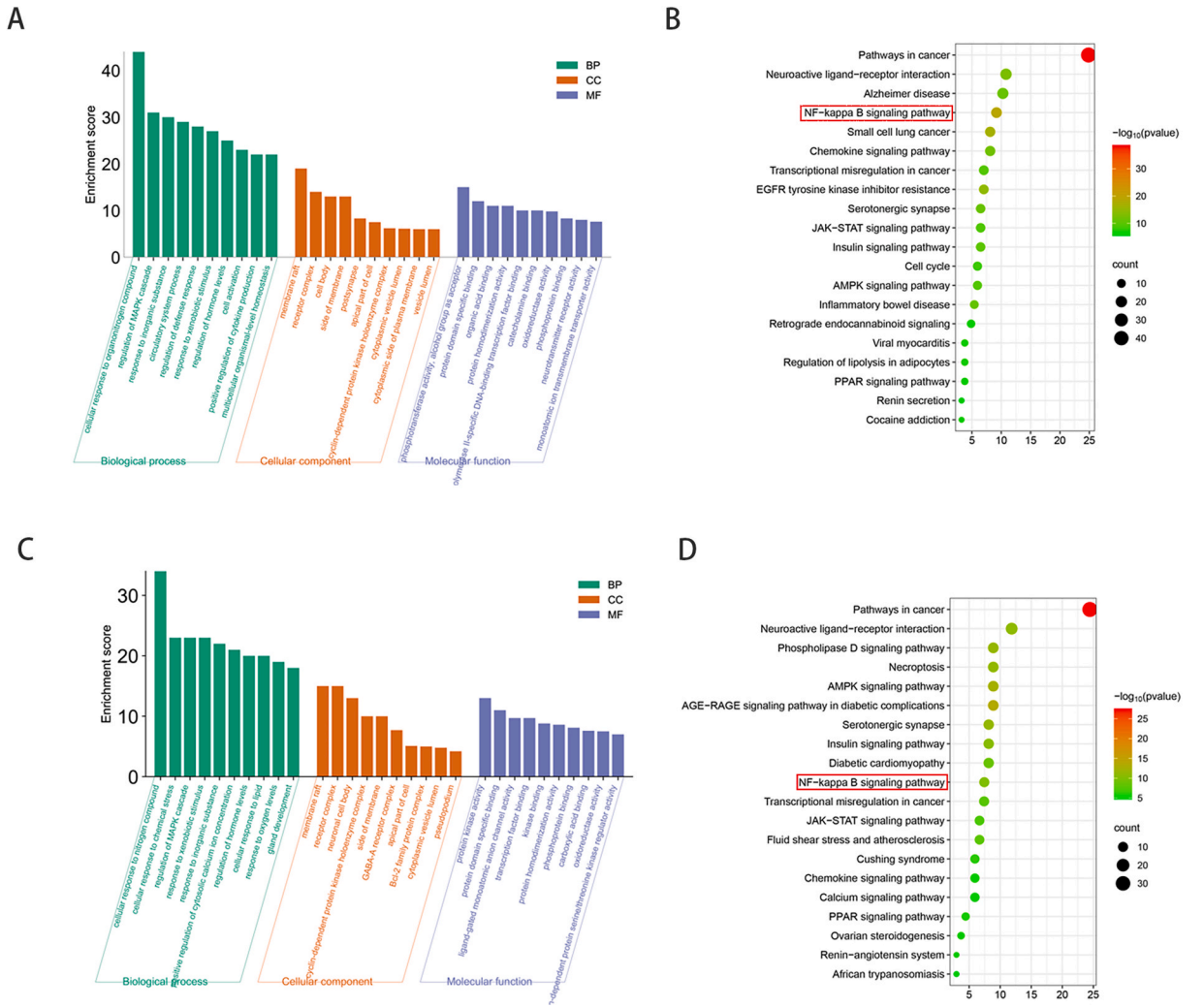

Fig. 5. KEGG and GO analysis. (A, C) GO analysis of the common target genes. (B, D) Enrich top 20 KEGG pathways.

expression level of Molecules associated with the inflammation (IL-1β、TNF-α、ICAM-1、IL-6) and oxidative stress (MDA、SOD、GSH-PX).

Although extant literature has posited that XCHD and YCSLS may modulate myriad targets and signaling pathways in HUA, a comprehensive perspective on their mechanisms of action continues to be nebulous [35,36]. In scenarios of this nature, network pharmacology emerges as a particularly efficacious methodology, exploring the multifaceted interactions of proteins and networks within a disease background. In our research, we amalgamated data from public databases to prognosticate interactions between XCHD/YCSLS and their prospective targets in HUA, in conjunction with the implicated signaling pathways. Through this integrative approach, common targets of XCHD/YCSLS and HUA were discerned and subjected to subsequent analysis, revealing a connection throughout these targets.These targets were primarily concentrated in the inflammatory and oxidative stress signaling pathways, as confirmed by both KEGG and GO analyses.

NF-κB, a protein initially identified by David Baltimore, binds to the κ light chain enhancer of B cells, orchestrating the expression of genes. Emerging evidence reveals that NF-κB ubiquitously permeates various cellular contexts [37,38]. Functioning as a central mediator, NF-κB actively engages in immune and inflammatory response mechanisms. Upon activation, NF- κB p65 enters the nucleus and promotes the synthesis of inflammatory factors, thus assuming a cardinal role in the regulation of immune regulation and inflammatory response [39]. Comprised of a dimer originating from p65 and p50, NF-κB unites with the inhibitory protein IkB to configure a trimer in a latent state. The phosphorylation and subsequent dissociation of IkB from the trimer facilitate the elevation of cellular NF-κB concentrations. Concurrently, the nuclear localization sequence (NLS) of the NF-κB dimer becomes accessible, enabling NF-κB's nuclear transference, DNA binding, transcriptional promotion of pertinent genes, and modulation of diverse cellular physiological functions [40,41]. TLR, an instrumental pattern recognition receptor within the innate immune framework, activates NF-κB via TLR4. NF-κB, in turn, partakes in the MYD88-dependent pathway's activation and transposition, culminating in the inflammatory

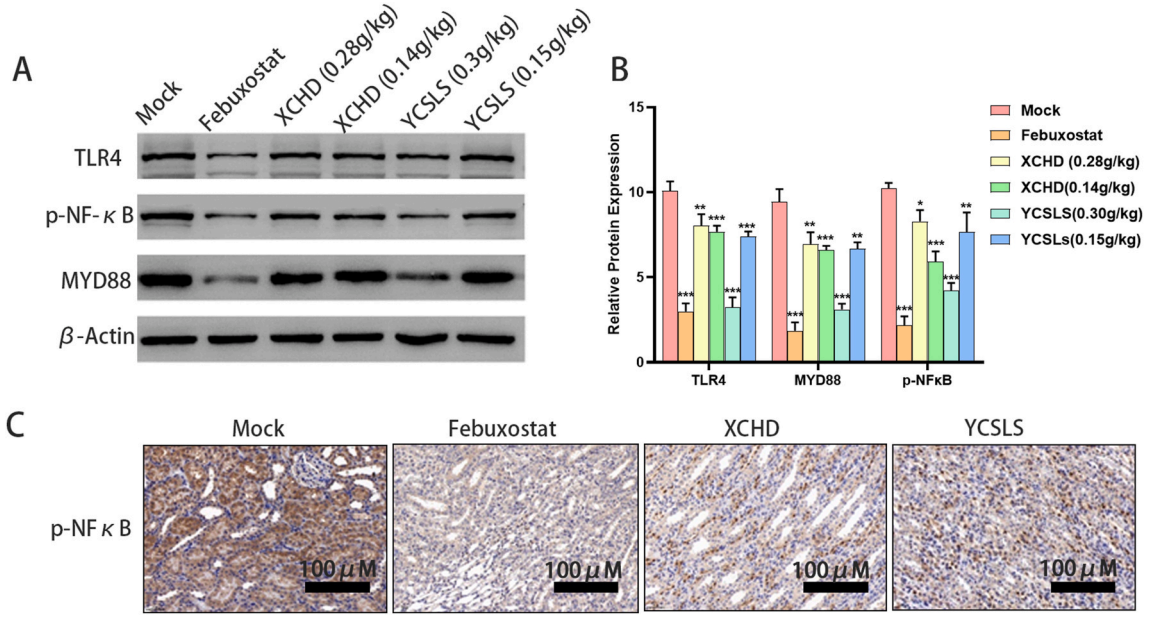

Fig. 6. XCHD and YCSLS inhibits HUA development by regulation of TLR4/MYD88/NF-κB pathway. (A) The expression of TLR4, MYD88 and p–NF–κB in the serum of HUA rats were determined by Western Bolt, β-Actin was used as loading control. (B) Grayscale value quantification of TLR4, MYD88 and p–NF–κB protein level from five rats. Data represent the mean ± SD of three separate studies. **p < 0.01, ***p < 0.001, compare to Mock group. (C) Expression of p–NF–κB in kidney was determined by immunochemistry. Scale bar: 100 μm. Decreased expression of p–NF–κB was detected in rat treated with XCHD and YCSLS. Data represent the mean ± SD (n = 5), **P < 0.01 vs. Mock group. Mock: PBS, XCHD: Xiao Chai Hu Decoction, YCSLS: Yinchen Siling San.

signal transduction process, thereby stimulating the synthesis of cytokines [42]. Concurrently, NF-κB augments ICAM-1 expression, magnifying the adhesion and recruitment of inflammatory leukocytes, extending their infiltration duration, and inducing oxygen free radical production. These radicals, capable of activating NF-κB, induce pro-inflammatory cytokines like ICAM-1 and TNF-α, thus forging an autocrine amplification circuit, and engendering a pernicious cycle of oxidative stress and inflammation [43].

Few studies have explored the role of the TLR4-MYD88-NF-κB signaling pathway in XCHD/YCSLS-induced HUA depression, exploring the connection between TLR4-MYD88-NF-κB signaling and HUA have significantly value. Our in vivo evidence delineates that XCHD and YCSLS considerably abated the expression of TLR4, MYD88, and p–NF–κB proteins in HUA rats model, underscoring the salience of the TLR4/MyD88/NF-κB signaling pathway in HUA rats. Pertaining to XCHD, notwithstanding its efficacious mitigation of HUA progression, it manifested superior efficacy at lower concentrations. This suggests that certain factors attenuate the anti-HUA effect of XCHD at elevated concentrations, more research is needed to understand how XCHD and YCSLC modulate TLR4/MYD88/NF-κB activation and how this pathway inhibits HUA progression. Our study preliminarily shows that XCHD and YCSLS can inhibit the progression of HUA through NF-κB, but the specific mechanism of action is still unknown, and subsequent experiments can further verify the target nature of NF-κB by knockdown or overexpression of NF-κB.

4. Conclusion

The present study integrates Network Pharmacology and in vitro experimental models to investigate the mechanisms of XCHD/YCSLS in preventing HUA progression. Our data indicate that TLR4/MYD88/NF-κB signaling is one of critical pathways involved in XCHD/YCSLS-induced HUA inhibition which deserves further investigation.

5. Materials and methods

5.1. Reagents and antibodies

Xiao Chai Hu Decoction and Yinchen Siling San was purchased from TianJiang Pharmaceutical (China National Pharmaceutical Group Corporation, China). Febuxostat was buyed from Wanbang Biopharmaceuticals (Jiangsu, China). Hypoxanthine was purchased from Shanghai Yuanye Biotechnology Corporation (Shanghai, China). Potassium oxonate was purchased from Shanghai Yuanye Biotechnology Corporation (Shanghai, China). Primary antibodies included anti-β-Actin (Affinity Biosciences, USA), anti-GAPDH (Affinity Biosciences, USA), anti-TLR4 (Beijing bioss Biotechnology Corporation, China), anti-MYD88 (ImmunoWay Biotechnology Company, USA), anti-p–NF–κB (Affinity Biosciences, USA) . The secondary antibodies included goat anti-mouse IgG and goat anti-rabbit IgG (MultiSciences Biotechnology Corporation, China).

5.2. Animal experiments

The Institutional Animal Ethics Committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine approved all animal studies of this study (approval number: 2021DL-009). One week after the end of adaptive feeding, 35 SD (200g ± 20g) male rats were randomly divided into 7 groups(n = 5). They were given hypoxanthine 500 mg/kg by gavage and potassium oxyzincate 200 mg/kg subcutaneously to create a model of hyperuricemia. The rats were then given various doses of XCHD (0.28 g/kg, 0.14 g/kg)/YCSLS (0.3 g/kg, 0.15 g/kg). After 9 days of medication therapy, the animals were sacrificed and their blood and kidneys were collected.

5.3. Quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, USA), the cDNA was obtained by reverse transcribing the RNA from SuperScript III (Invitrogen, Carlsbad, USA). Then we used the qReal-time PCR experiments to detect mRNA levels of the target genes, GAPDH was used as internal reference, Primer used in PCR were listed in Table 1.

5.4. Western blot assay

Proteins were separated by eletrophresis through 8–10 % SDS-polyacrylamide gel, electrotransfer was performed and the proteins were transferred to the PVDF membrane (Millipore, USA). At room temperature (RT), Membranes were blocked by incubation in 5 % (w/v) skim milk for 1 h, then incubated overnight at 4 °C in primary antibody (1:1000), incubated in HRP-conjugated secondary antibodies for 1 h at RT. Using ECL chemiluminescence substrate (Biosharp, GuangZhou Sopo Biological Technology Corporation, China).

5.5. ELISA detection

The ELISA kits for the Rat ICAM-1 and TNF-α, IL-1β test were purchased from Wuhan Elabscience Biotechnology Corporation (Wuhan, China.). and the experiments were carried out as directed by the kit instructions.

5.6. Immunohistochemistry

After paraffin-embedded tissue sections (3–5 μm) were dried overnight at 37 °C, deparaffinized and rehydrated were performed, they were processed for antigen repair by boiling for 30 min in antigen retrieval buffer and placed until the temperature reached RT. 5 % BSA was closed for 2 h at RT, overnight incubation with primary antibody at 4 °C, the sections were incubated with HRP conjugated secondary antibody for 1 h at RT, after development with DAB kit (ZSGB-BIO, Beijing, China), collect images by Eclipse TE2000 Inverted Microscope (Nikon, Tokyo, Japan).

5.7. Pathological examination

The collected tissues were fixed in 4 % paraformaldehyde, It was subsequently dehydrated, waxed and embedded, then get sectioned (3-5μm), processed for tissue pathology analysis by hematoxylin and eosin (H&E) staining.

5.8. Search potential targets for YCSLS and XCHD in HUA via network pharmacology

First of all, the GeneCards Database was used to find the targets of HUA, the candidate targets of XCHD and YCSLS were analyzed through the PharmMapper Database and the Swiss target prediction Database. To identify the key cross-point targets between the HUA and XCHD/YCSLS target networks, use Venny 2.1.0. The cross-target genes were constructed into a PPI network through the STRING database. Finally, targets with node centrality and high number of adjacent nodes ($P < 0.01$) were evaluated as core genes in the PPI network by Cytoscape 3.8.2. The GO and KEGG identified possible targets of XCHD/YCSLS in HUA.

5.9. Statistical analysis

Data are expressed as mean ± SD (standard deviation). The student-t test was used to compare the difference between two groups, and the one-way ANOVA was used to compare the differences between more than 2 groups. Data were analyzed with the Prism 8 (GraphPad Software, San Diego, USA). At $p < 0.05$, differences between groups were deemed statistically significant.

Data availability statement

The authors affirm that the data supporting the study's conclusions are available in the article.

Table 1
Primer sequences.

Gene	Sequence
MDA	F: 5′-TTCACGGAGCAACTCCTAAGA-3′ R: 5′-GCACTGCCCAAAACAAGAAGA-3′
SOD	F: 5′-AATCAGCGTTCTCACTGACAG-3′ R: 5′-TGCGTAAATCCCAAGCAAAGT-3′
GSH-PX	F: 5′-CAGCCTTATCTTCCGTAACGC-3′ R: 5′-GCCCATACATGCCATTCTGAT-3′
GAPDH	F: 5′-ACAACTTTGGCATTGTGGAA-3′ R: 5′-GATGCAGGGATGATGTTCTG-3′

Funding

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Ethics declarations

This study was reviewed and granted permission by the institutional Animal Ethics Committee at the Affiliated Hospital of Chengdu University of TCM (2021DL-009).

CRediT authorship contribution statement

Yining Luo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **Ping Huang:** Resources, Methodology, Investigation, Data curation. **Jiaxue Chen:** Resources, Methodology, Investigation, Data curation. **Ping Ma:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33416.

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