Chinese Herbal Medicine for Diabetic Kidney Disease: Historical Perspective, Clinical Evidence and New Therapeutic Development

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

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Abbreviations

AA	aristolochic acid
ACCORD trial	Action to Control Cardiovascular Risk in Type 2 Diabetes trial
ACEi	angiotensin converting enzyme inhibitors
ACR	albumin–creatinine ratio
ADME	absorption, distribution, metabolism and excretion
AE	adverse event
ALTITUDE trial	Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints trial
AMED	Allied and Complementary Medicine Database
AMI	acute myocardial infarction
ANZCTR	Australian New Zealand Clinical Trials Registry
AP	activator protein
ARB	angiotensin receptor blockers
BC	betweenness centrality
BIND	Biomolecular Interaction Network Database
BioGRID	Biological General Repository for Interaction Datasets
BP	blood pressure
CAM	complementary and alternative medicine
CAS	Chemical Abstracts Service
CBM	China BioMedical Literature
CC	closeness centrality
CENTRAL	Cochrane Central Register of Controlled Trials
CHF	chronic heart failure
ChiCTR	Chinese Clinical Trial Registry
CHM	Chinese herbal medicine
CI	confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CKD	chronic kidney disease
CM	Chinese medicine
CNKI	China National Knowledge Infrastructure
CQVIP	Chonqing VIP
CVD	cardiovascular disease
DC	degree centrality
DDP- 4	dipeptidyl peptidase-4
DIP	Database of Interacting Proteins
DKD	diabetic kidney disease
DL	drug-likeness
EBM	evidence-based medicine
EC	eigenvector centrality

eGFR	estimated glomerular filtration rate
EMT	epithelial–mesenchymal transition
ESKD	end stage kidney disease
ET1AR	endothelin-1A receptor
EU-CTR	European Union Clinical Trials Register
FBG	fasting blood glucose
FDA	Food and Drug Administration
GAD	Genetic Association Databases
GBM	glomerular basement membrane
GFR	glomerular filtration rate
GGQL	*Ge gen qin lian* formula
GLP-1	glucagon-like peptide-1
GO	gene ontology
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HbA1c	hemoglobin A1c
HDLC	high-density lipoprotein cholesterol
HPRD	Human Protein Reference Database
ICAM-1	intercellular adhesion molecular-1
IDNT	Irbesartan Diabetic Nephropathy Trial
IFTA	interstitial fibrosis and tubular atrophy
ILK	integrin linked kinase
InChIKeys	International Chemical Identifier
IND	investigated new drug
KDOQI	Kidney Disease Outcomes Quality Initiative
LDLC	low-density lipoprotein cholesterol
MD	mean difference
MINT	Molecular INTeraction Database
MRA	mineralocorticoid receptor antagonist
NDRD	non-diabetic renal disease
NF- κ B	nuclear factor kappa light chain enhancer of activated B cells
NIH	US National Institutes of Health
OB	oral bioavailability
OMIN	Online Mendelian Inheritance in Man
PCR	protein-to-creatinine ratio
PKC	protein kinase C
PPI	protein–protein interaction PPI
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QoL	quality of life
RAS	renin–angiotensin system
RCT	randomised controlled trial
RDA	recommended daily allowance

RENAAL trial	Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan trial
RevMan	Review Manager Software
ROB	risk of bias
ROS	reactive oxygen species
RR	risk ratio
Scr	serum creatinine
SGLT2	sodium glucose co-transporter 2
SMD	standardised mean difference
SMILES	simplified molecular input line entry specification
SPSS	Statistical Package for the Social Sciences
TCM Database@Taiwan	Traditional Chinese Medicine Database @Taiwan
TCMSP	Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform
TGF-β	transforming growth factor beta
TRAIL	tumour necrosis factor–related apoptosis-inducing ligand
TTD	Therapeutic Target Database
UAER	urinary albuminuria excretion rate
UKSPD	UK Prospective Diabetes Study
UniProt	Universal Protein resource knowledge base
UP	urine protein excretion
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
WHO	World Health Organization
WMD	weight mean difference
ZHYD	*Zhong Hua Yi Dian* (*Encyclopaedia of Traditional Chinese Medicine*)

Summary

Diabetic kidney disease (DKD) is the foremost microvascular complication of diabetes mellitus, which is characterised as persistent albuminuria and progressive loss of kidney function induced by diabetes. The health burden of DKD is substantial and continues to grow in parallel with the escalating prevalence of diabetes. Despite current pharmacotherapies including hypoglycaemic agents, hypotensive drugs and renin-angiotensin system (RAS) inhibitors, substantial residual risk of DKD initiation and progression remains. Considering the increasing prevalence of DKD, novel renal protective therapeutics are in great need. Chinese herbal medicine (CHM) has been used since antiquity in some countries and regions, and is still being used to treat kidney diseases in combination with contemporary medicine. Guided by traditional knowledge and contemporary practice of herbal application, existing and potentially novel therapeutics for DKD may be evaluated and further developed from CHM. To-date, the development of therapeutics from CHM has been impeded by general lack of clinical evidence, complex chemical profiles and unclear mechanisms of action. Moreover, the conventional drug application of the "one target, one drug" approach has been a limitation when it comes to complex and multi-factorial clinical presentations such as DKD. CHM is a complex intervention that commonly involves a number of herbal ingredients clinically for treating individual patients with DKD.

Objectives

Guided by a "whole evidence" framework, the aims of this research are to:

- Evaluate the classical literature evidence of CHM as a treatment for DKD
- Evaluate the clinical trial evidence of CHM as adjunctive therapy for DKD
- Explore and propose the bioactive compounds and pharmacological mechanisms of promising CHM for DKD

Review of classical literature

A search of the classical Chinese medicine literature was conducted in the *Zhong Hua Yi Dian* (ZHYD, 5th Edition, 2014). A total of 278 DKD-relevant classical citations with treatment information were identified and analysed. These citations were derived

from 68 classical Chinese medicine books spanning from AD 583 to AD 1895. Based on the rating results, there were 23 citations that were most likely DKD. *Ba wei wan*, *Liu wei di huang wan* and *Hui xiang san* were the most frequently cited formulae for DKD. The herbs frequently used were *huang qi* (*Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao), *ren shen* (*Panax ginseng* C. A. Mey.), *wu wei zi* (*Schisandra chinensis* (Turcz.) Baill.), *tian hua fen* (*Trichosanthes kirilowii* Maxim.) and *huang lian* (*Coptis chinensis* Franch.). It was found that citations with positive turbid urine symptoms used *huang qi* more often than other high-frequency herbs.

Systematic reviews of randomised controlled trials

The Cochrane handbook of systematic reviews of interventions (version 5.1.0) guided the methods of the systematic reviews. The first systematic review included 20 randomised controlled trials (RCT) with 2719 DKD patients comparing CHM with placebo. Meta-analysis suggested that CHM reduced greater albuminuria than placebo, regardless of whether RAS inhibitors were concurrently administered. When CHM was used as an adjunct to RAS inhibitors, estimated glomerular filtration rate (eGFR) was improved in the CHM group compared with the placebo group. The adverse events (AE) rates were low and similar between CHM and placebo groups. *Huang qi* was used most frequently among included RCTs.

Accroding to the results of the first systematic review and classical literature review, the herb *huang qi* was selected as a subject for further study. The second systematic review included 66 RCTs employing sole *huang qi* preparations with 4785 DKD participants. Overall, the included studies have substantial risk of bias due to methodological shortfalls. The meta-analysis showed that additional use of *huang qi* injection reduced albuminuria, proteinuria and serum creatinine concentration compared to conventional therapy alone. An anti-albuminuria effect was also reported in the oral *huang qi* preparation group. The safety of *huang qi* prepareations was uncertain because AEs were only reported in one third of included studies. More detailed safety evaluation particularly for *huang qi* injections are needed due to severe allergic reactions after injections have been observed.

Network pharmacology study

Network pharmacology is a novel drug discovery approach that uses data from high-throughput experiments, omics studies and other biological research and integrates and analyses them as a whole. It was applied to visualise and predict the complex relationships underlying the numerous DKD targets and multiple herbal compounds.

The herb *huang qi* was selected for the network pharmacology study based on the results reported above. Searching retrieved 103 distinct human targets related to DKD. Thirty-eight (38) bioactive compounds from *huang* qi were identified, with a corresponding 327 targets. The *huang qi*–DKD PPI network contained 2269 shared targets, and 127 of these were considered to play central communication roles. These key targets were enriched in 174 biological pathways and the most significant pathways were integrin-linked kinase (ILK) signalling, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) signalling, transforming growth factor beta (TGF-β)/Smad2/3 signalling, vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) signalling network and glypican/glypican-1 pathway. Further analysis of the herbal compounds-key targets-pathways network revealed that quercetin, calycosin, formononetin, kaempferol, isorhamnetin, betulinic acid, gamma-sitosterol, (24S)-24-Propylcholesta-5-ene-3beta-ol and bifendate were directly associated with 21 key targets enriched in the top 10 pathways.

Conclusion

By employing a whole evidence strategy, this research systematically evaluated the current best available evidence about CHM as adjunctive therapy for DKD, from both historical and contemporary perspectives. Classical literature evidence indicated that *huang qi* was commonly used in DKD-like disorders, particularly for those presenting with turbid urine (cloudy or foamy urine). With moderate to low quality evidence from RCTs, CHM may have beneficial effects on renal function and albuminuria beyond those reported by conventional treatment alone in adults with DKD. Moreover, adjunctive use of sole *huang qi* preparations with RAS inhibitors appeared to lowering albuminuria/proteinuria, as well as reducing serum creatinine concentration in the short term. The pharmacological actions of *huang qi* could be mediated by ILK signalling,

TGF-β/Smad signalling, NF-κB pathway and glypican/glypican-1 pathway. Eight compounds with direct potential to regulate key targets are provided as new therapeutic development candidates for DKD. Hence, further research is warranted to determine their clinical benefit.

1 Introduction

1.1 Background

Diabetic kidney disease (DKD), previously known as diabetic nephropathy, is the foremost microvascular complication of diabetes mellitus [1, 2]. DKD is a sub-category of chronic kidney disease (CKD) attributed to diabetes. Both primary and secondary diabetes can develop DKD. It often develops in those with a long history of diabetes and poor glycaemic control, which results in kidney complications.

DKD is a global health burden and is increasing due to the large number of people with diabetes. Diabetes patients who develop DKD are at greater risk of progression to end stage kidney disease (ESKD) and cardiovascular disease (CVD) compared to the general population [3, 4]. Due to its inconspicuous onset and progressive nature, DKD creates a large socioeconomic burden leading to significant direct and indirect costs on the health system and individuals. Current treatments are limited to glycaemia management, blood pressure control and anti-albuminuria agents of renin–angiotensin system (RAS) inhibitors [1, 2, 5]. An unmet need exists for novel renoprotective therapeutics in DKD patients who are unresponsive to current pharmacotherapies or those have deteriorating renal function without albuminuria.

In East Asia, Chinese herbal medicine (CHM) is widely used in parallel with conventional therapies to treat several kinds of nephropathies [6]. Traditional Chinese medicine theory uses a holistic approach, and understands and treats disease with a different paradigm to conventional medicine. Due to the multi-component nature of medical plants, the herbal therapies of Chinese medicine (CM) are multi-target and multi-action, and do not specifically focus on one disease pathway or cellular process but affect the person as a whole [7, 8]. Under the pressure of the growing burden of DKD, CHM may be useful in the discovery and development of novel interventions to prevent, slow or even halt the development of DKD.

The predominant “one target, one drug” paradigm of new drug discovery and development is challenged by the complex pathogenic nature of chronic, multi-factorial

diseases like DKD. Potent agents in preclinical studies often fail to show clinical efficacy or safety in human subjects, leading to wasted time and resources [9]. The conventional single target–based approach is inefficient in the field of CHM development as well. The chemicals and molecular mechanism of action corresponding to the effects of CHM are difficult to distinguish due to its multi-component naure. Moreover, the synergric effect of CHM is hard to elucilate at a system level in the conventional approach. The unclear biological mechanisms under CHM raise concerns regarding its effect and safety, especially in patients with kidney impairment [2, 5].

With the advances of systems biology, chemistry and bioinformatics, a novel paradigm for new drug discovery and development named “network pharmacology” was proposed to solve the problem of the low productivity of traditional drug discovery [10]. This new pattern is capable of integrating the high-throughput data generated from “omics” studies, chemical experiments and other pharmacological research. Consequently, the complex interaction relationships among each component within the biological system can be clearly presented and analysed in a network manner. The network pharmacology approach may be helpful in CHM therapeutic development, as the molecular basis of herbal products can be studied in a systematic way [11].

1.2 Aims and research questions

The aim of this research is to identify and assess historical and modern literature evidence regarding the use, efficacy and safety of CHM for DKD. In addition, the common CHM are explored via a network pharmacology method to elucidate the potential targets and action mechanisms for treating DKD. The research questions of this project are:

- Which herbal formulae and individual herbs were used in historical Chinese medicine literature to treat DKD?
- Is CHM effective and safe for DKD, in terms of mortality, disease progression, albuminuria/proteinuria excretion and kidney function?
- What are the proposed pharmacological mechanisms of CHM for DKD?

1.3 Significance of the research

Currently, conventional treatments for DKD focus on risk factor control, while renal protective therapies are limited. The prognosis for DKD patients is poor and they are at high risk of CVD and mortality, especially those who progress to ESKD. Treatments that prevent DKD progression will help to reduce healthcare expenditure and improve patients' quality of life (QoL). With network pharmacology technology, the CHM used in historical Chinese medical literature and in contemporary clinical trials may provide leads for new drug development and therapeutic targets for future DKD research.

1.4 Organisation of the thesis

Chapters One to Three introduce the thesis.

In **Chapter One**, the background, objectives, research questions and significance of this research project as well as the thesis structrure are introduced.

Chapter Two provides an overview of the therapeutic development of CHM. The value of CHM and challenges in the development process are introduced. The conventional approach for new drug discovery and development is reviewed, with discussion of its limitations for complex diseases. A "whole evidence" strategy that combines evidence from historical literature, modern clinical trials and network pharmacology findings is proposed for therapeutic development of CHM.

Chapter Three includes a review of background knowledge of DKD from both conventional medicine and CM perspectives. The epidemiology, burden, risk factors, pathogenesis, pathology, diagnosis and clinical management of DKD in conventional medicine are introduced. The origin, aetiology, pathogenesis and herbal treatments of DKD from the viewpoint of CM are also summarised. Limitations of current pharmacotherapies and the pressing need for more renal protective therapies are highlighted.

Guided by the "whole evidence" strategy, Chapter Four and Five include a systematic evaluation of historical and modern literature on DKD.

Chapter Four presents the results of a text-mining method applied to systematically search and evaluate the use of CHM for DKD in classical CM literature. Three ancient terms of DKD were used and classical citations with varing degrees of relevance to DKD were identified from a database of 1156 classic medical books. Descriptive analysis was conducted to determine the dynasty distribution of citations and search terms. Frequency analysis was performed to determine the most commonly reported formulae and herbs for typical symptoms of DKD.

Chapter Five includes an analysis of the efficacy and safety of CHM by means of systematic review and meta-analysis of randomised, placebo-controlled trials. Findings reported in this chapter provide clinical evidence of CHM for DKD. *This systematic review has been published in a peer-reviewed international medical journal and the chapter reflects the manuscript.*

Evidence from Chapters Four and Five indicates that the herb *huang qi* (Scientific name: *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao) is the most promising treatment for DKD. Therefore, *huang qi* was selected as the research subject for Chapter Six and Seven, a systematic evaluation of evidence of clinical trials and the network pharmacology analysis.

Chapter Six includes a systematic review and meta-analysis of *huang qi* as adjunctive therapy for DKD. Findings reported in this chapter provide clinical evidence about *huang qi* preparations for DKD. *This systematic review has been published in a peer-reviewed international medical journal and the chapter reflects the manuscript.*

Chapter Seven presents the results of the network pharmacology study of *huang qi*. The potential bioactive components and action mechanisms hypothesis of *huang qi* in the treatment of DKD are proposed. Further verified experiments and new drug candidates can be designed based on the findings of this chapter.

Chapter Eight includes the overall findings of the research, as well as limitations and future applications.

2 Therapeutic Development of Chinese Herbal Medicine

2.1 Introduction

In this chapter, the value of Chinese herbal medicine (CHM) and challenges of drug development from CHM are introduced. The conventional approach for new drug discovery, development and limitations is also included. To address current issues, a "whole evidence" strategy that combines evidence of classical literature and modern clinical trials with network pharmacology findings is proposed for therapeutic development of CHM.

2.2 Value of Chinese herbal medicine development

Chinese Medicine (CM) is a traditional medical system rooted in the practices of Chinese culture since antiquity. In the view of CM, disease is caused by the imbalance of two contrary yet complementary forces named *yin* and *yang* in the human body [8]. To restore the harmony of *yin* and *yang*, herbal remedies sourced from plants are one of the dominant interventions, which is known as Chinese herbal medicine (CHM). Dating back to the Han dynasty (AD 220), the earliest *Materia Medica* entitled *Shennong's Classic of Materia Medica* 神农本草经 recorded the medical uses of 252 herbs based on the experience of eminent CM practitioners at that time [12]. In the modern *Chinese Pharmacopoeia*, over 600 herbal medicinals are indexed and under the monitoring and regulation of the Chinese Food and Drug Administration, and are approved to be used in clinical settings in China [13].

Focusing on the whole body rather than the disease itself and using herbs make CHM attractive to consumers. In China, CM is recognised as primary healthcare for advice and treatment, and is used alongside conventional (Western) medicine. It is estimated that almost 20% of health care services are delivered by CM hospitals, and CM therapies can be accessed in half of rural clinics [14, 15]. A survey from the World Health Organization (WHO) showed that the number of member states with herbal medicine regulation rose from 65 to 119 from 1999 to 2012 [16]. The export value of CM products in European markets increased by 19% annually, of which 80% were

herbal products [17]. In the Middle East, a survey conducted in Saudi Arabia showed that approximately 65% of patients held favourable attitudes towards herbal medicines [18]. In North America, up to 35% of adults received complementary therapies at least once a year [19]. As an important component of the Complementary and Alternative Medicine (CAM) system, CM therapies are popular and increasingly used and recognised worldwide.

The extensive use of CHM in China and increasing global acceptance have raised research interest from academia and industry. The multi-component nature of CHM makes it a rich source of natural products with therapeutic potential. A survey conducted over 30-years showed that 34% of newly approved small-molecule drugs were sourced from natural products [7]. The most well-known medicine discovered and developed from CHM is the antimalarial drug artemisinin, which is the bioactive component extracted and isolated from the plant *Artemisia annua* L. (Chinese herb name *qing hao* 青蒿) [20]. The discovery of the antimalarial effect of artemisinin and its derivates successfully solved the drug-resistance crisis in the 1970s and has saved thousands of lives worldwide. Since 2005, artemisinin-based combination therapies have been recommended by WHO as the most effective antimalarial therapy available so far [21].

CHM is not only a chemical pool of therapeutic compounds, but also a rich source of therapeutic leads for single- and multiple-component drug discovery. In the prototype CHM library of the US National Cancer Institute, a total of 3,709 fractions were collected from 80 herbal species, with an average of 45 fractions per herbal sample [22]. Subsequent studies identified a chemical entity with anti-angiogenesis activity from these herbal fractions, which could be the chemical basis for a novel cancer therapy. According to analysis of new chemical entities approved by the US Food and Drug Administration (FDA) between 1981 and 2010, drugs originating from natural products were more diverse in chemical structures and physiochemical features than synthetic drugs [7]. Moreover, natural compounds occupied a larger chemical space than synthetic compounds and covered a different space area, representing diversity in CHM compounds [23, 24]. Since there are fundamental differences between natural and synthetic compounds, using compounds from CHM is a good strategy to enhance the

diversity and integrity of the chemical library for new drug screening and optimisation.

2.3 Conventional approach for new drug discovery and development

2.3.1 Conventional processes

Therapeutic agents, commonly called drugs, are chemical substances used to treat diseases, to relieve symptoms or to aid diagnosis for health-maintenance purposes. Historically, drugs were discovered unintentionally and obtained from medical plants without prior knowledge of action mechanisms. With the advent of chemistry, biology and pharmacology, therapeutic molecules can be synthesised, driven by target-based hypothesis [25]. New drug discovery and development refer to the whole process from therapeutic target identification to new drug marketing. It is a time-consuming, costly and risky process which can be broken down to several phases (**Figure 2-1**).

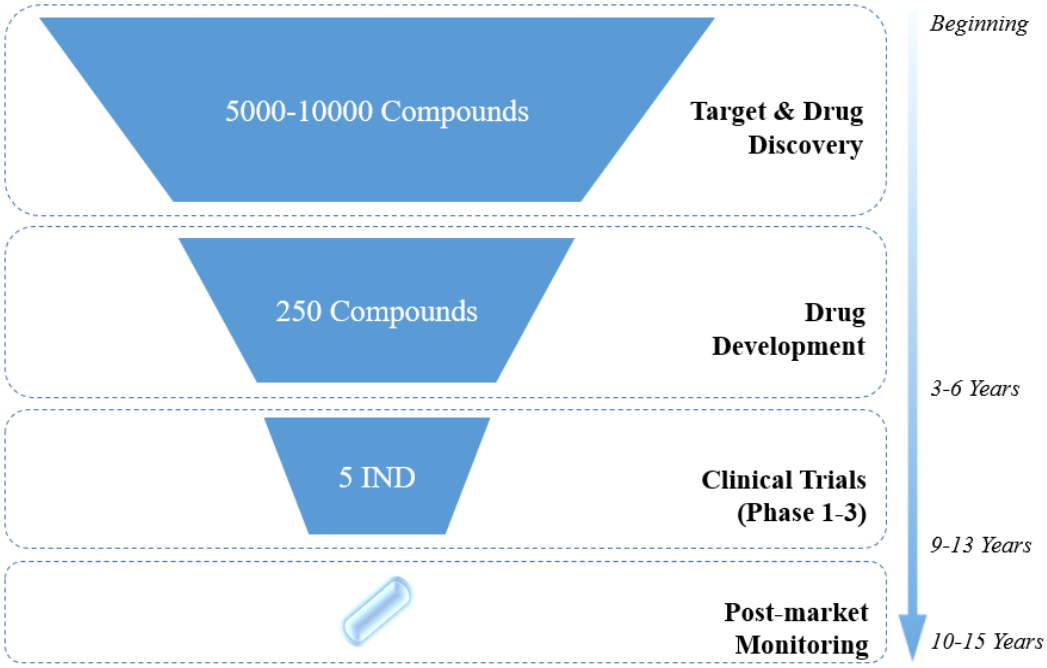

Figure 2-1 Conventional new drug discovery and development process

Abbreviation: IND: investigated new drug. Adapted from Pharmaceutical Research and Manufacturers of America 2007 [26].

The first phase of conventional new drug discovery and development is to identify and validate the biological target implicated in the disease, such as a protein or nucleic acid. It is important to ensure that the candidate target can be modulated by drugs, with sufficient evidence advocating the links between the target and therapeutic hypothesis. The second phase is to screen and optimise chemical leads based on the knowledge of disease targets. In this stage, a group of bioactive compounds responding to the proposed targets (the "hits") are filtered from a chemical library, then narrowed down by *in vivo* activity studies. The chemical structures of promising compounds are then optimised and designed to promote their "druggable" properties. Thirdly, pharmacology experiments are conducted for the candidate compounds to further examine their action mechanisms, efficacy, toxicity and pharmacokinetic features. The candidate drugs that pass through the above stages enter a sequence of clinical trials to test their efficacy and safety in human subjects as the fourth stage. Only after a drug passes through the clinical trials and shows salutary results, will it be reviewed by the FDA as a new therapeutic agent for marketing approval [25].

2.3.2 Limitations of conventional approach

It has been well recognised that the process of new drug discovery and development requires huge investment and close cooperation between the pharmaceutical industry, governments and academia. The overall cost of post-clinical research in the private-sector was estimated at about $1.7-$2.2 billion in the 2000s, and the cost is higher in studies focusing on more recent periods [27]. In a survey including 10 out of the top 50 multinational pharmaceutical firms, the total capitalised cost from clinical trials to approval was up to $2558 million per drug in the early 2010s. Compared to the cost in the 1990s, the cost of later new drug development stages increased with an 8.5% annual growth rate [27]. By publication analysis, a study focused on the public sector revealed that all 210 FDA newly approved drugs during 2010–2016 were directly or indirectly associated with projects funded by the US National Institutes of Health (NIH). These NIH-funded projects consumed $115.3 billion in total, accounting for nearly 20% of the whole NIH budget in the same time interval [28]. Additionally, over 90% of these NIH-funded projects contributed to basic experiments on drug targets rather than chemical entities, which are early stages of the new drug discovery process. The

resources needed for new drug discovery and development are so huge that investment from the private and public sectors is essential.

Despite the high expenditure and resource input from industry and academia, the outputs of novel therapeutics are still unsatisfactory. Even in the top 50 pharmaceutical firms, the success rate of new drug approvals was as low as 7.9%–12.8% [29]. The average annual number of new FDA-approved drugs was 30 in the early half of the 2000s, then reduced to 20.2 in the latter half [30, 31]. Although the mean number of new FDA-approved drugs has slightly rebounded to 44 since 2012, the sale value per drug has been slipping from $1.4 billion in 2014 to $0.9 billion in 2017 [32]. Decreasing projected sale values in recent years have partially resulted from the less common approval of first-in-class drugs, which take 20–25 years from bench to bedside [31, 33]. Collectively, the low productivity and enormous investments are pressing challenges for new drug discovery and development.

To address the problems of low productivity and low success rates, effort has been made to investigate the reasons for the failures in new drug discovery and development. From 2013 to 2015, there were 218 candidate drugs terminated in phase II/III clinical trials. The vast majority, 174 out of the 218, were terminated due to insufficient efficacy and safety, accounting for 52% and 24% of total failures, respectively [9]. A study based on data from the largest four pharmaceutical firms reported consistent findings. Of 812 investigated compounds during 2000–2010, clinical safety was the primary cause of failure for phase I trials and unsatisfactory efficacy for phase II trials [34]. Clinical trials are only conducted after the candidate drugs have passed through the preclinical research phases, and failure at the clinical trial phase significantly impinges on the industry, considering the time and resources that have been invested. The discrepancy between laboratory potency and clinical efficacy/safety of candidate drugs, especially for multifactorial diseases, raises doubts about the conventional “one target, one drug” discovery pattern.

2.4 Challenges of Chinese herbal medicine therapeutic development

CHM has a long history of use and is increasingly used worldwide, but there are

challenges and barriers that impede the development and application of CHM.

Lack of high-quality clinical evidence regarding the efficacy and safety of CHM is one of the most defects. There are a large number of historical books that document the usage, formulation, indications and contraindications of CHM. However, rigorous evaluation of the benefits and harms of CHM by unbiased methods is necessary but currently unavailable. By 2008, 42 systematic reviews with a focus on CHM were recorded in the Cochrane Database of Systematic Reviews. Of these CHM reviews, 55% reported that there were no or insufficient good-quality trials to support the use of CHM. Among the other 19 reviews that suggested possible benefits of CHM, 26% made conclusions based on individual trials instead of meta-analysis. The poor methodological quality and high heterogeneity in the majority of included trials made the conclusions in favour of CHM less convincing [35]. The numbers of published systematic reviews and registered clinical trials of CHM have grown substantially since 2008, but pitfalls in trial design, protocol transparency, search integrity and report quality have reduced the trustworthiness and translation of these CHM evidence into clinical practice [36, 37].

Unclear chemical compositions and action mechanisms are another recurring criticism received during development and application of CHM [38]. As mentioned earlier, the compounds that constitute medical plants including CHM are complex. In addition, chemical compositions of raw herbs may vary due to different origins, harvest seasons and manufacturing procedures [39-41], not to mention that CHM is mostly prescribed in the form of multi-ingredient formulae, whereby several herbs are cooked and taken together to achieve synergistic effects. The typical “one disease, one target, one drug” paradigm is difficult to align with the theory of CM in order to identify the herbal compounds corresponding to specific therapeutic effects and to fully elucidate the pharmacological mechanism of CHM. Novel and advanced approaches addressing the features of CHM are needed to close the knowledge gap between traditional practice and modern pharmacology.

2.5 Whole-evidence strategy for Chinese herbal medicine development

CHM has been practised for thousands of years, with large amounts of classical literature that records the clinical observation of eminent doctors in ancient times. However, rigorous evaluation of efficacy and safety is required for all types of clinical interventions, including CHM. Unclear bioactive components and action mechanisms are the foremost obstacles for CHM assessment and development. Therefore, a "whole evidence" strategy that combines evidence from historical literature and contemporary clinical trials with network pharmacology findings is proposed for CHM development [42].

2.5.1 Evidence from historical literature

CHM and other medical plants are now largely treated as sources of chemical compounds. However, the value of traditional use of CHM to guide new drug discovery and development has been neglected. Traditional use of CHM is a type of empirical knowledge formed and repeatedly tested over generations. Nowadays, traditional knowledge is still used to guide CM clinical practice. The selection of herbal candidates for drug discovery can be informed by the traditional use of herbs, saving time and resources by narrowing scope [43].

Traditional use of herbs for specific diseases can be identified by systematic searching of the classical CM literature. Topics covered in historical literature include medical theory, *Materia Medica*, prescriptions (formulae) and case records. During the development of artemisinin, the breakthrough low-temperature extraction procedure was inspired by an ancient text description on how to deal with the raw herb [20]. The nephrotoxicity of CHM containing aristolochic acids could have been realised earlier if related historical literature regarding traditional use of herbs had been reviewed [44]. Thus, traditional knowledge of CHM, as an alternative evidence source, should be encouraged in new drug discovery and development, particularly evidence suggesting that the same herbs were used in different regions or are still in use in contemporary clinical practice [45].

2.5.2 Evidence from clinical trials

From the perspective of evidence-based medicine (EBM), medical interventions should be carefully examined, regardless of whether they are conventional medicine or CAM. Evidence generated from clinical trials is ranked at a higher level than that from experimental studies due to the substantial interspecies differences [46]. Among different types of clinical trials, randomised controlled trials (RCTs) have been labelled the "gold standard" for evaluating the benefits and harms of tested therapeutics [47]. The prospective randomised allocation and comparative group design of RCT minimise the risk of bias of measurements, which makes it the most reliable approach to demonstrate the causation relation between interventions and treatment outcomes.

As the concept of EBM was introduced to the field of CM, the number of clinical trials investigating CM therapies has increased in the last few decades. Data from the largest clinical trials registry, ClinicalTrial.gov, indicates that the number of registered CM trials was 95 in 2000–2005 and jumped to 779 in 2010–2015. Of the 1270 registered CM clinical trials, 86.5% had randomised designs and 80.2% had comparator settings [37]. It should be noted that the molecular bases and action mechanisms of these CM therapies are largely unclear.

As CHM is already widely used, it is suggested that clinical trials can be the starting point in the new drug discovery pipeline of CHM (**Figure 2-2**). In this efficacy-based approach, *in vivo* and *in vitro* studies are conducted after the efficacy and safety of CHM are confirmed in RCT with the purpose of monitoring chronic toxicity, identifying active components, clarifying action mechanisms and improving preparations [48]. It appears that the results of good-quality RCTs were still recognised as solid evidence even though their molecular and pharmacological bases were not fully understood. The efficacy-based approach can identify ineffective and/or toxic herbal therapies much earlier, saving time and resources spent on herbal remedies doomed to fail.

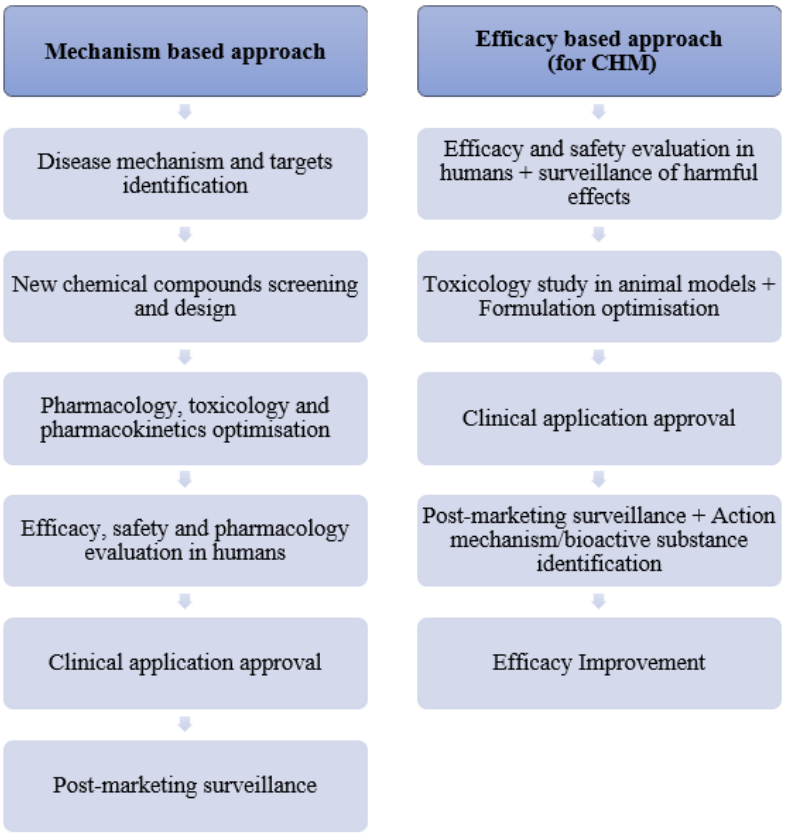

Figure 2-2 Comparison of the conventional mechanism-based approach and CHM specific efficacy-based approach

Adapted from Tang JL 2006 [48].

2.5.3 Exploring action mechanisms using network pharmacology

2.5.3.1 Network features of biology systems

Biology systems refer to a group of interrelated biological objects that function together to maintain homeostasis. Biological systems can be viewed at several scales, spanning the micro scale of cells to the macro scale of organism populations. Within a biological system, no matter whether a yeast or a human body, the biological components are numerous and interdependent. Recent advances in systems biology reveal an abundance of biological components and the complexity of their interactions, which are represented in a network manner [49]. In a biological network, the "nodes" are used to represent molecules, cells, diseases or individuals and the "edges" connecting nodes

represent an interaction relationship [50]. According to the composition and function, there are several types of networks in biological systems, including protein–protein interaction (PPI) networks, gene regulatory networks, signalling networks and metabolic networks [49, 51]. All these networks are interrelated and work together to maintain biological function.

The network feature lead to discovery of the "scale-free" and robust properties of the biological system, which were not revealed in previous studies focusing on individual genes or molecules [50, 52]. In a "scale-free" network, a small proportion of nodes have more connections and higher centrality than most of the nodes. These functionally important but small number of nodes are defined as the "hubs" (**Figure2-3**). Due to the scale-free network structure, biological systems can remain intact even when some nodes (but not the hubs) are randomly removed. These properties were confirmed by the Genome 5000 projects, which found that only 10% of knockout druggable genes resulted in therapeutic relevant phenotype changes [53]. In other words, the biology networks are robust as long as the hubs remain unchanged.

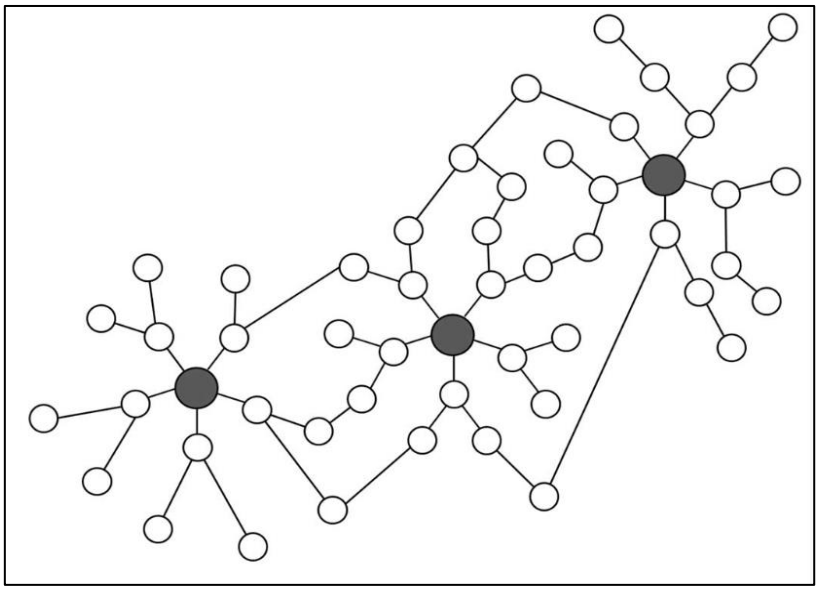

Figure 2-3 Sample of scale-free network

Note: The dark nodes represent the hubs and the white nodes represent common components. Adapted from Seo H et al. 2013 [54].

2.5.3.2 Network Pharmacology as an alternative therapeutic development approach

The findings of the scale-free nature and robustness of biological networks provide fresh perspectives regarding diseases and new drug discovery, potentially eliminating the failure of clinical efficacy and unexpected safety issues with traditional drug discovery. Conventionally, new drug discovery is based on the hypothesis of the single most important target mechanism; therefore, the drug is a highly selective ligand that fits the "disease-causing" target. In contrast, from the view of network biology, diseases are perturbed state of normal biological networks involving changes in a set of key molecules. Absence, overexpression or dysfunction of single key molecules or groups of key molecules can cause diseases by damaging network integrity. Disease can also result from the misregulation, misdirection or inappropriate strength of interactions between the hub components in biological networks [50, 51]. Considering the robustness of biological networks, desired efficacy can only be achieved when a candidate drug modulates the therapeutic hub target(s) and restores the normal network. Moreover, the candidate drug should have little or no effects on other key targets within the same network in order to avoid undesired effects. Guided by the above knowledge, a new paradigm for drug discovery entitled "network pharmacology" was proposed by Andrew Hopkins in 2008 [10].

The network pharmacology approach has been widely used to accelerate and to optimise the preclinical stages of new drug discovery and development processes. Firstly, biology networks enable the integrated representation of genomics, proteomics, metabolomics and interactome information, so the functions of individual molecules can be assessed in a system-level context. The network analysis approach is capable of identifying the functionally important targets from the large number of biological components, reducing the risk of picking irrelevant targets for further study [51]. One example is the ErbB3 as a cancer therapeutic target which was discovered by computational analysis of the ErbB receptor signal network, leading to development of a novel anticancer human monoclonal antibody, MM-121 [55]. In the chemical leads screening and optimisation stage, the pharmacokinetic properties, toxicity and action targets of new chemical entities can be predicted by network pharmacology approaches,

therefore expanding the chemical libraries and accelerating the drug discovery process [51].

2.5.3.3 Chinese medicine network pharmacology

The foundational hypothesis of network pharmacology, "multi-targets, multi-component therapeutics", is highly consistent with the holistic approach of CM. To decode the complex action mechanisms of multi-component interventions like CHM, network pharmacology may be helpful. Multilevel data about components and interactions underlying biological processes can be incorporated and analysed by network pharmacology approaches. In fact, before the concept of network pharmacology was more widely accepted, a network-based CM research framework was already proposed by Li et al., subsequently developed as a new research area of Traditional Chinese Medicine (TCM) network pharmacology [11, 56].

With the framework of TCM network pharmacology, four general steps are involved to reveal the molecular basis and potential action mechanisms of herbs (**Figure 2-4**) [11]. The first step is to identify gene and genes products contributing to specific diseases, and to construct a disease-specific molecular network based on the biomolecular interaction relationships. Meanwhile, the chemical compounds contained in each herb are collected and the target network of these compounds is built based on experimental data and/or *in silico* prediction. Lastly, the herbal target network is mapped to the disease-specific network, followed by qualitative and/or quantitative analysis of molecular interactions to identify the significant bioactive compounds and the hub targets. Potential action mechanisms can then be proposed according to the functional analyses results of the hub targets and further verified by experiments.

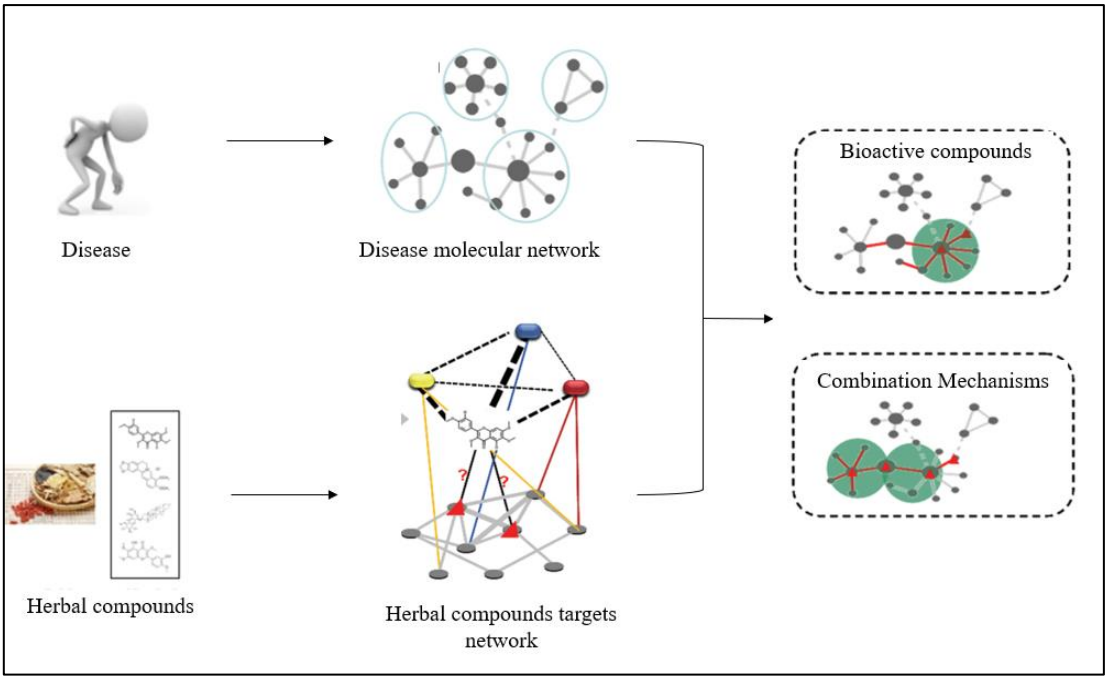

Figure 2-4 Research framework of CM network pharmacology for herbal therapy
Adapted from Li et al. 2012 [11].

Since network pharmacology has been employed in the field of CM, the bioactive components and action mechanisms of several commonly used herbal therapies have been elucidated. For instance, 19 compounds contained in *Ge gen qin lian* (GGQL), a CHM formula, were identified as the bioactive components contributing to its anti-diabetic properties. According to the network analysis, these GGQL bioactive compounds were likely involved in regulating multiple type-2 diabetes biological processes, including glucose homeostasis, glucose transport and glucose metabolism. Subsequently, an *in vitro* study verifed that one of these GGQL bioactive compounds, 4-hydroxymephenytoin, can stimulate insulin secretion, improve insulin resistance and increase glucose consumption [57]. In the same research framework, the bioactive components and action targets of the *Ma zi ren wan* formula for functional constipation, *Ma xing shi gan* decoction for asthma, *Angelica sinensis* for acute myocardial infarction (AMI) and others have been successfully deciphered [58-60].

2.6 Summary

CHM has a long history of use in China and is increasingly used worldwide. Due to its multi-component nature, CHM is a significant source for novel chemical compounds with therapeutic potential. However, the development and application of CHM are impeded due to a lack of clinical evidence, complex chemical profiles and unclear action mechanisms. These issues cannot be addressed by the conventional new drug development paradigm of “one disease, one target, one drug”. Therefore, a “whole evidence” strategy that integrates evidence from traditional use documented in historical CM literature, contemporary clinical trials and network pharmacology findings is proposed for CHM development.

3 Overview of Diabetic Kidney Disease

3.1 Introduction

This chapter presents an overall background to diabetic kidney disease (DKD), including up-to-date knowledge of epidemiology, risk factors, pathology, pathogenesis and clinical management.

DKD is the foremost microvascular complication of diabetes mellitus [1, 2]. It is a sub-category of chronic kidney disease (CKD) caused by diabetes, with unique features compared with other CKD aetiologies. Both insulin-dependent (type 1) and non-insulin-dependent diabetes (type 2) can develop DKD. It often develops in those with long-term diabetic history and poor glycaemic control, which results in kidney complications. The primary clinical feature of DKD is persistent albuminuria/proteinuria and progressive loss of kidney function. The typical pathological changes include mesangial expansion, basement membrane thickening and glomerular sclerosis.

3.2 Prevalence and burden

The prevalence of renal damage (defined as albuminuria or declined glomerular filtration rate) in diabetic populations is reported to be between 12.3% and 70% [61, 62]. The variable DKD prevalence could be due to differences in study locations, study subjects, research dates etc. A global cross-sectional study including 24,151 type 2 diabetic patients from 33 countries indicated that 49% were comorbid with albuminuria [63]. In a prospective study in the UK, the prevalence of albuminuria among the 5000 participants was 30.2% ten years after of diagnosis of type 2 diabetes [64]. Similar stable prevalence was observed in the USA (27%) and Norway (31%) despite more frequent use of glycaemic-control medications and renin–angiotensin system (RAS) inhibitors during the past two decades [65-67].

Although the prevalence of DKD has been stable in some regions in recent years, the epidemic magnitude of diabetes still poses a global healthcare challenge. By 2045, it is

estimated the prevalence of diabetes will be 9.9%, equalling about 628 million people worldwide [68]. Moreover, the majority of newly diagnosed cases are from developing countries and areas with large populations but limited healthcare resources, such as China, India Africa and Latin America [69]. Currently, nearly 50% of type 2 diabetic patients in India are diagnosed with CKD and 20% of Chinese diabetic patients already suffer from significant CKD [70, 71]. Even more concerning, the number of patients with DKD is rising in both hospital and general populations in China, which makes DKD the leading cause of kidney disease [72]. The increasing prevalence of DKD is also reported in North America among people with glomerular diseases [73]. If the trend continues, the prevalence of DKD is very likely to grow, leading to greater demands on healthcare resources [74].

The health burden of DKD is heavy, largely due to the close relationship between DKD, CVD and the development of ESKD. Diabetes has been the leading cause of ESKD in developed countries for decades [74, 75]. It accounts for approximately 45% of new dialysis patients in the USA, 37% in Australia and 43% in Japan [75-77]. Both patients with DKD and those with ESKD are at higher risk of mortality than the general population and CVD is the primary cause of death [78-80]. For DKD patients secondary to type 2 diabetes, fatal events are more likely to happen even before the progression to ESKD [81].

In North America, medical costs for patients with CKD and diabetes rose 70.2% between 2008 and 2012, nearly $25,000 per patient-year, while similar costs for patients without CKD, diabetes or chronic heart failure (CHF) increased by only 4.1% [4]. In Australia, DKD accounts for a substantial economic burden. The annual cost for renal replacement therapy was almost three times the cost for acute CVD [82]. The total direct health care costs reach almost $1 billion per annum for early stage DKD and $300 million for ESRD patients.

3.3 Risk factors

Predisposing genetic features, gender, ageing, ethnicity and a family history of diabetes are irreversible risk factors for DKD. Several meta-analyses indicate that susceptibility

to DKD is related to polymorphism in the angiotensin-converting enzyme (ACE) gene, methylenetetrahydrofolate reductase gene and endothelial nitric oxide synthase gene [83-85]. But the identified genetic variants can not fully explain the family cluster phenomenon of DKD. Male diabetes patients seem to be more susceptible to DKD, but the sexual difference could be the result of inconsistent definitions of DKD. In the UK Prospective Diabetes Study (UKSPD), male sex was the independent risk factor for albuminuria while females were at greater risk of a low glomerular filtration rate (GFR) [86]. African-Americans are more likely to develop DKD and the rate of renal function decline is accelerated in Indo-Asians more than other ethnic groups [87, 88].

Hyperglycaemia is a widely recognised risk factor for DKD which plays a major role in both disease initiation and progression. Long duration of diabetes, poor control of glycaemia, insulin resistance and advanced glycation end products are all closely associated with DKD [89, 90]. Thus, blood glucose management is crucial for diabetes patients to prevent and defer DKD progression. Hypertension and dyslipidemia are also putative risk factors for DKD, irrespective of type 1 or type 2 diabetic status [86, 91]. These three risk factors constitute the cornerstone of DKD management strategy.

Modifiable risk factors of DKD in terms of lifestyle include smoking, low physical activity and high salt intake [89, 90, 92]. In addition, anaemia, a procoagulant state, obesity, an inflammation state, endotoxins, vitamin D deficiency and hypomagnesaemia may also be risk factors for DKD [89, 90, 93, 94].

3.4 Pathogenesis

The pathogenesis of DKD is complex and not completely understood. Hyperglycaemia is confirmed as a prerequisite for the development of DKD which is compounded by subsequent haemodynamic alterations [95, 96]. The cascades induced by metabolic and haemodynamic pathways, including oxidative stress and inflammation, overlap, interact and feed back to the two main pathways, leading to the development of DKD.

High blood glucose triggers the initiation of DKD via several pathways: non-enzymatic glycosylation, which generated advanced glycation end products (AGEs); activation of

the protein kinase C (PKC) pathway; acceleration of the aldose reductase (polyol) pathway; and stimulation of the hexosamine pathway [97]. As a result, pathological products accumulated from the above processes induce oxidative stress and the release of pro-inflammatory and pro-fibrotic mediators, accelerating the development of DKD [98, 99].

From the haemodynamic aspect, the alternation in the glomerular haemodynamic is the result of RAS activation, which is mainly triggered by increased angiotensin II and upregulated expression of endothelial-1 [96]. Prostanoids, nitric oxide and TGF-β 1 induced by hyperglycaemia contribute to the change in arteriole resistance in glomeruli [95]. As the glomerular afferent arterioles dilate, the efferent arterioles constrict and the inter-glomerular pressure increases, resulting in hyperfusion and hyperfiltration. The enforced intra-glomerular mechanical strain stimulates the release of cytokines and growth factors, overlapping with metabolic and other pathways again [95, 100].

Abnormal metabolic, haemodynamic and subsequent oxidative stress and inflammatory processes eventually lead to functional and structural changes in the kidneys. Both proliferation and hypertrophy are observed in mesangial cells, together with overproduction of extracellular matrix expansion in the mesangial area. In addition, mesangial cells take part in maintaining the normal structures of glomerulus vasculature. The abnormality of mesangial cells and vascular endothelial damage in the glomerulus are associated with nodule lesion formation. In addition to the damage in the glomerulus, the fibrotic cytokines, growth factors and inflammatory mediators also cause fibrotic lesions in the tubule-interstitial. Podocyte loss and impaired integrity are also observed [89]. All these pathogenic processes and structural changes occur before the clinical manifestations of excess albuminuria and declining GFR.

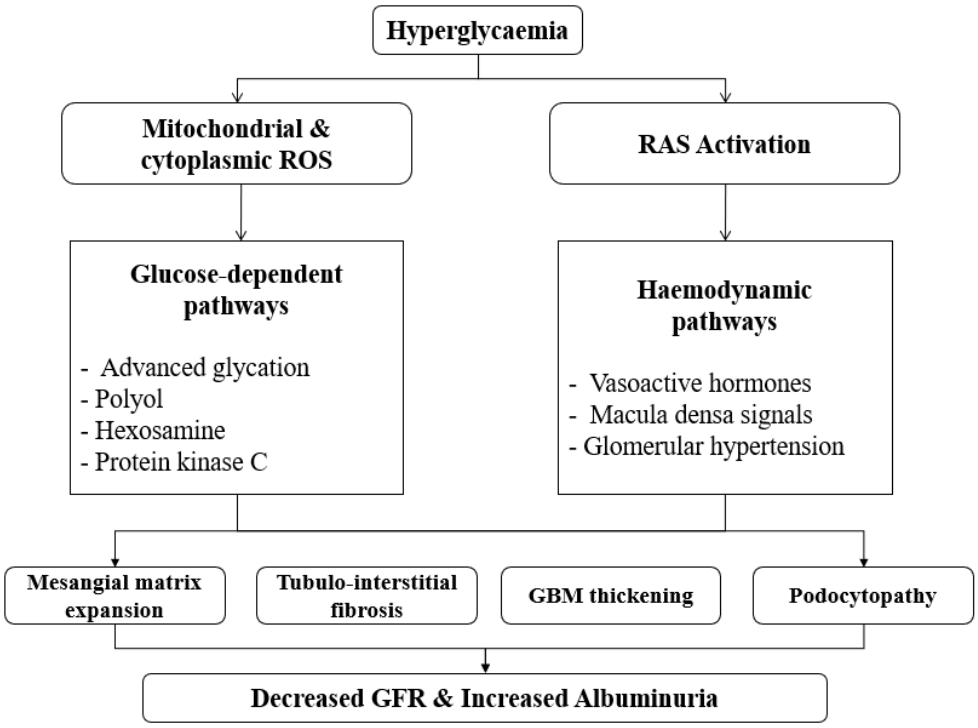

Figure 3-1 Pathogenesis of DKD

Abbreviations: GBM: glomerular basement membrane; RAS: renin-angiotensin system; ROS: reactive oxygen species.

3.5 Pathology

Renal biopsy often shows structural changes in the kidneys of people with DKD before clinical manifestations such as increased albuminuria (formerly termed microalbuminuria). The predominant histologic changes in the glomeruli in DKD are mesangial expansion, glomerular basement membrane thickening and glomerular sclerosis [101, 102]. In some cases, the glomerular sclerosis may manifest the characteristic nodular appearance named "the Kimmelstiel-Wilson lesion (K-W nodules)" [103]. The US Renal Pathology Society developed a classification for DKD in 2010, in which glomerular lesions were defined and the severities of interstitial and vascular lesions were scored (**Table 3-1**) [104].

Although this pathological classification of DKD was reported to have good reproducibility, its clinical utility has not been fully clarified. Associations between

pathological classifications and the renal endpoint events, such as initiation of renal replacement therapy, progression to ESKD or doubling of serum creatinine, were investigated in two Chinese studies with similar designs. Both studies indicated that the severity of glomerular lesions was closely related to the renal outcomes, while the interstitial inflammation was not an independent predictor. Conflicting results were found regarding the levels of interstitial fibrosis and tubular atrophy [105, 106]. In order to improve the prediction of renal survival based on the pathological findings, an overall pathological risk score (D-score) was developed, but its validity still needs to be examined in diverse populations [107].

Table 3-1 Renal Pathology Society classification of DKD

Glomerular lesions	
Class I	Isolated glomerular basement membrane thickening. Basement membranes >430 nm in males (>age 9) and 395 nm in females. No evidence of mesangial expansion, increased mesangial matrix or global glomerulosclerosis involving >50% of glomeruli.
Class II	Mild (class IIa) or severe (class IIb) mesangial expansion. A lesion is considered severe if areas of expansion > the mean area of a capillary lumen are present in >25% of the total mesangium.
Class III	At least one Kimmelstiel–Wilson lesion (nodular intercapillary glomerulosclerosis) is observed and <50% global glomerulosclerosis.
Class IV	Advanced diabetic sclerosis. There is >50% global glomerulosclerosis.
Interstitial and vascular lesions	
Interstitium	0: no areas of IFTA; 1: areas of IFTA <25%; 2: areas of IFTA 25–50%; 3: areas of IFTA >50%.
Immunocyte infiltrate	0: no T lymphocytes or macrophage infiltrate present. Scores of 1 or 2 are assigned if infiltrate is limited to the area surrounding atrophic tubules or if infiltrate is not limited, respectively.
Vascular	Scores of 0, 1 or 2 are assigned if no arteriolar hyalinosis, one arteriole or more than one arteriole with hyalinosis is present. The most severely affected arteriole is assigned a score of 0, 1 or 2 if there is no intimal thickening, intimal thickening < thickness of the media or intimal thickening > thickness of the media.

Abbreviations: IFTA: interstitial fibrosis and tubular atrophy. Adapted from Tevaert et al. 2010 [104].

3.6 Diagnosis

DKD is asymptomatic in the early stages and usually identified when patients are assessed for diabetes. In advanced DKD, physical examination may reveal hypertension,

oedema or symptoms of other diabetic complications such as blurry vision and numbness in the extremities. In patients who develop advanced DKD, ESKD symptoms of uremia may be the chief complaint [108].

In cases with an unclear diagnosis or when other causes of CKD are suspected, renal biopsy can be used to definitively diagnose diabetic glomerulopathy. However, in patients with type 2 diabetes and microalbuminuria, 30% of them may have normal or near-normal biopsy results [2]. In order to distinguish kidney diseases from other underlying causes in the diabetic population, the term DKD, instead of diabetic nephropathy, is applied as a presumptive diagnosis of kidney disease caused by diabetes. Meanwhile, the term 'diabetic glomerulopathy' is reserved for biopsy-proven kidney disease caused by diabetes [2].

The clinical diagnosis of DKD is based on abnormal urinary albumin excretion and diabetic history [2]. Albuminuria is the early indicator of DKD, and is categorised as microalbuminuria and macroalbuminuria. In individuals with diabetes and showing macroalbuminuria, with or without microalbuminuria, plus diabetic retinopathy, this indicates a probable diagnosis of DKD. In addition, individuals with type 1 diabetes for at least 10 years who present with microalbuminuria were considered to have DKD [2]. In the recent Chinese guideline, the diagnostic criteria was modified so that diabetes patients with diabetic retinopathy and any stage of CKD will be considered to have DKD [109]. This modification was made to avoid misdiagnosis of those with declined eGFR (estimated GFR) but without albuminuria.

Excretion of albuminuria can be measured differently including a spot test of the urinary albumin-creatinine ratio (ACR) or a timed test of the urinary albuminuria excretion rate (UAER). Infections, fever, recent vigorous exercise and other factors could falsely elevate albumin excretion; therefore, they need to be differentiated. Abnormal increase of urinary albumin excretion is defined as over 30 mg/g creatinine measured by ACR or over 20 μg/min by UAER (**Table 3-2**). Although the terms 'microalbuminuria' and 'macroalbuminuria' have been widely used to indicate disease severity, it should be noted that these cut-off points are arbitrary. Changes of albuminuria level within either

normal or abnormal ranges are associated with risk of cardiovascular events and renal function loss, due to its continous nature [1].

Table 3-2 Abnormality of albumin excretion

Category	Spot collection (mg/g creatinine)	24-hour collection (mg/24h)	Timed collection (µg/min)
Normal	<30	<30	<20
Microalbuminuria	30-300	30-300	20-200
Macroalbuminuria	>300	>300	>200

References: [2, 5]

Along with quantitative urinary albumin assessment, serum creatinine should be measured to estimate the GFR. Although albuminuria is an early indicator of DKD, studies have found a substantial percentage of type 2 diabetes patients with decreased eGFR without increased urinary albumin excretion. Therefore diabetes patients with declining GFR, especially those accompanied with diabetes retinopathy, after excluding other underlying causes of kidney disease, would be considered to have impaired kidney function due to DKD [109].

Since the number of people with diabetes is large and albuminuria can be seen in several conditions, other underlying causes of kidney diseases in diabetic patients should be taken into consideration when making a diagnosis, especially in the following circumstances:

- Absence of diabetic retinopathy
- Low or rapidly decreasing GFR
- Rapidly increasing proteinuria or presence of nephrotic syndrome
- Refractory hypertension
- Presence of active urinary sediment
- Signs or symptoms of other systemic disease; or
- >30% reduction in GFR within 2–3 months after initiation of angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) medications.

Evidence from a systematic review of 48 studies suggested that non-diabetic renal disease (NDRD) and mixed forms of DKD and NDRD are commonly seen in diabetic patients [110]. It should be noted that judgements based on history and clinical parameters alone may lead to misdiagnoses. To preclude other potential diseases, renal biopsy should be considered.

3.7 Treatment and management

3.7.1 Non-pharmacotherapies

Lifestyle modification is one of the cornerstone interventions in the management of both diabetes and DKD. Smoking cessation is recommended to prevent DKD progression [2, 109]. At least 150 minutes of moderate-intensity physical activity per week benefits the non-dialysis DKD population, but adverse events induced by inappropriate sports should be avoided by making an individualised sports plan [109]. Obesity is a potential promotor for DKD deterioration, thus DKD patients are recommended to maintain their body mass index (BMI) in the normal range [2].

Diet modification includes reducing salt intake to reduce hypotension [111]. As for daily protein intake, the recommended daily allowance (RDA) is 0.8 g/kg body weight for diabetic patients with CKD stage 1–4 and higher levels for those on dialysis [1]. There were reports of kidney function improvement with an extremely low protein diet (0.6 g/kg bodyweight) in the DKD population, but the benefits for renal outcomes and nutrition status need more evidence [112, 113].

3.7.2 Pharmacotherapies

Current treatment strategy for DKD is mainly focused on the primary disease (diabetes) and risk factor management. In addition, the discovery of renal-protective effects of RAS inhibitors and their wide application have significantly reduced the incidence and progression of DKD among diabetic populations. Yet effective therapies to prevent or stop DKD deterioration are still limited, especially medications restoring kidney injury. Novel agents targeting specific pathogenesis pathways of DKD have been developed, some of which have failed and others require further testing.

3.7.2.1 Glycaemia control

As the primary initiator and promotor, hyperglycaemia is the putative treatment target of DKD. It has been confirmed by several large-scale clinical trials with five to ten years of follow-up that diabetes patients with lower glycaemic levels are less likely to develop albuminuria and ESKD [114-116]. The benefits of tight glycaemic control persisted after the termination of intervention, known as metabolic memory [117].

However, higher death rates were observed in the more intensive glycaemic control group (targeting haemoglobin A1c [HbA1c] at 6%) in the Action to Control Cardiovascular Risk in Type 2 Diabetes (ACCORD) trial [118]. Compared to less intensive glycaemic control, pooled analysis showed stringent glycaemic control had no benefits in decreasing risk of important clinical outcomes such as ESKD, death and major CVD [5]. As a result, a target HbA1c of around 7% was suggested for diabetes patients [5]. Meanwhile, individualised glycaemic targets are recommended to optimise blood glucose management, particularly in the elderly, patients with limited life expectancy and those at risk of hypoglycaemia [1, 5].

Since some classes of hypoglycaemia drugs are metabolised or directly excreted by the kidneys, hypoglycaemia agents for patients with kidney impairment should be cautiously selected [119, 120]. For example, the first-line hypoglycaemia medicine metformin should be used under close monitoring in patients with eGFR less than 45 mL/min and is contraindicated once eGFR drops below 30 mL/min due to the risk of lactic acidosis resulting from impaired renal function [121]. The second generation sulfonylureas, which are the alternative first-line medication for type 2 diabetes, should also be avoided in people with eGFR <45 mL/min. Only pioglitazone, rosiglitazone and insulin require no dose adjustment in DKD patients [119, 120].

There are some new glucose-lowering agents that appear to be renal protective independent of hypoglycaemia pathways, including dipeptidyl peptidase-4 (DDP-4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists and sodium glucose co-transporter 2 (SGLT2) inhibitors. Both DDP-4 inhibitors and GLP-1 receptor agonists are incretin-based therapies and show anti-albuminuria properties [122-124]. But the

evidence of incretin-based therapies in terms of renal clinical outcomes is contradictory. Meta-analysis of RCTs with treatment duration longer than 12 weeks favoured GLP-1 receptor agonists compared to placebo to prevent incident nephropathy in type 2 diabetes patients, while pooled results of RCTs with 200 or more participants suggested no difference regarding DKD onset [125, 126]. For DKD patients with substantial renal injury, it is unclear whether incretin-based therapies affect the progression to ESKD [127].

As for SGLT2 inhibitors, both canagliflozin and empagliflozin decreased by about 40% the risk of renal endpoints, such as initiation of renal replacement therapy, doubled levels of serum creatinine and renal deaths in individual large-scale placebo-controlled trials [128, 129]. But it should be noted that all these trials only enrolled type 2 diabetic patients with substantial risk of CVD and the renal effect was analysed as a secondary outcome. Additionally, a higher risk of amputation with canagliflozin raised safety concerns. Whether SGLT2 inhibitors can prevent or retard the progression of DKD and their safety profile still needs further study with kidney-related primary outcomes.

3.7.2.2 Blood pressure control

Hypertension is commonly seen in diabetes patients and plays an important role in the development and deterioration of DKD. In type 2 diabetes patients who failed to maintain blood pressure (BP) less than 140/85 mmHg, the risk of incidence of DKD was 38% higher than for those who achieved target BP [130]. In diabetes patients with established DKD, the risk of ESKD and the rate of eGFR decline progressively increased as mean systolic BP rose from 120 to 150 mmHg and dialytic BP rose from 80 to over 90 mmHg [131]. Meta-analysis showed an association of every 10 mmHg decrease in systolic BP with a 13% and 17% risk reduction in death and albuminuria in type 2 diabetes patients with baseline BP over 140 mmHg [132]. But the benefits of aggressive BP lowering strategies, especially in normotensive diabetes patients, remain controversial [133]. Thus, a relatively conservative treatment BP target of less than 130/80 mmHg and tailored according to individuals' CVD and renal risks is recommended in current guidelines [1, 2].

As for hypotensive agents for the DKD population, the RAS blockages of either ACEi or ARB are the putative drug of choice [1, 5]. Systematic reviews suggested a 20% risk reduction of ESKD in DKD patients treated with ARB and 20 – 30% lower risk of doubling serum creatinine in those received either ACEi or ARB therapy [134, 135]. In diabetes patients without renal injury, there was evidence that ACEi was superior to placebo in terms of preventing the onset of albuminuria and reducing the risk of death, irrespective of hypertension [136]. In addition, the renoprotection of ACEi and ARB was independent of its hypotensive properties, and may be mediated by reducing urinary albumin leakage, thus preventing the subsequent cascade reaction. A network meta-analysis comparing the efficacy and safety of different classes of hypotensive agents as treatment for DKD, and the ARB monotherapy or combination used of ACEi and ARB were showed to have the greatest benefits regarding the outcome of progression to ESKD [134].

Although the discovery of extra renoprotective properties of ACEi and ARB was a major step forward in the treatment of DKD, limitations exist in the use of RAS inhibitors. Serval RCTs were conducted to evaluate the efficacy and safety of dual blockage in RAS in combination with use of ACEi and ARB. Unfortunately, the combination therapy not only increased the risks of death, dialysis and doubling of serum creatinine, but also had more adverse events, including hypotension, hyperkalemia and acute kidney injury [137, 138]. As a result, this combination strategy is not recommended clinically [139, 140].

After the failure of the combination of ACEi and ARB, the add-on effect of a more complete RAS inhibitor, the direct renin inhibitor (aliskiren), together with ACEi or ARB was examined. Aliskiren combined with losartan decreased albuminuria more than losartan alone, and was independent of blood presure lowering effect [141]. However, the later Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints (ALTITUDE) trial including 8,000 DKD participants was prematurely terminated due to more adverse events and higher incidences of stroke in the aliskiren plus ACEi or ARB arm [142] and no additional renal benefit effect was observed in the combination therapy group. A systematic review suggested the risk of

hyperkalaemia was 58%–67% higher when adding aliskiren to ACEi or ARB than monotherapy, regardless of whether hypertensive patients had diabetes [143].

Except for the direct renin inhibitors ACEi and ARB, the mineralocorticoid receptor antagonists (MRA) are the remaining agents with RAS manipulation effect (**Figure 3-2**). For those already on ACEi or ARB, the 'aldosterone escape' was observed and it was considered to be responsible for the suboptimal therapeutic effect [144]. Therefore, both steroidal (spironolactone and eplerenone) and non-steroidal (finerenone) MRA have been tested to assess their effect and safety in the DKD population based on other RAS inhibitors. A systematic review including 15 RCTs of spironolactone, two RCTs of eplerenone and one RCT of finerenone showed lower albuminuria levels in the adjunctive MRA group, but no differences in eGFR compared to ACEi or ARB alone [145]. Moreover, the risk of hyperkalaemia was significantly increased in the MRA arm. In a pilot RCT of the non-steroidal MRA finerenone, it appeared to be tolerated in DKD patients with moderately impaired renal function (40% of participants with baseline GFR <60mL/min) and it reduced albuminuria in a dose-dependent manner [146]. Further studies to validate these findings are ongoing.

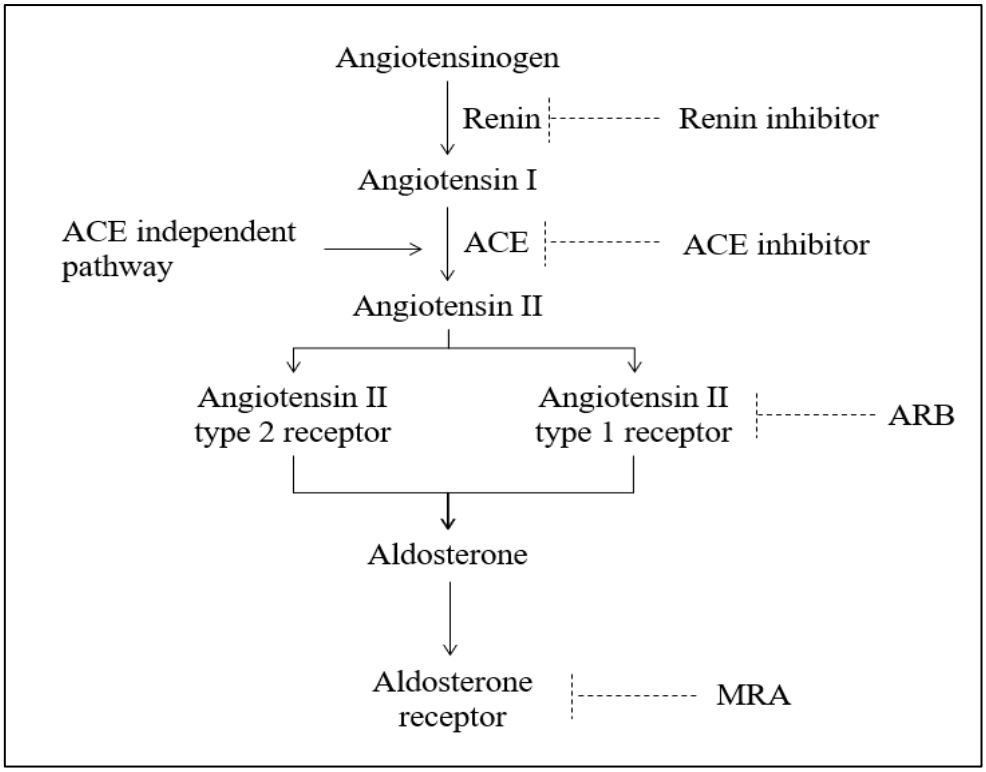

Figure 3-2 The renin-angiotensin system (RAS) and related inhibitors

Abbreviations: ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blockers; MRA: mineralocorticoid receptor antagonists. Note: The solid lines represent activated effect and the dotted lines represent inhibited effects.

3.7.2.3 Dyslipidaemia management

Albuminuria, declined eGFR, diabetes and dyslipidaemia are all strong, independent risk factors for CVD [147, 148]. In cases of DKD, risk of cardiovascular events is much higher than for general population. A substantial number of DKD patients prematurely die even before progressing to ESKD. In current guidelines, the initial goal of lipid-lowering therapy is to reduce the risk of cardiovascular events in this high-risk subgroup [5, 149].

Association between dyslipidaemia and incident albuminuria or declined renal function in either type 1 or type 2 diabetes patients is supported by recent research [150, 151]. However, it is still unclear if lipid-lowering therapy is valuable for this group of patients. Systematic reviews show that statin therapy has positive effects on reducing

albuminuria excretion, but no difference on eGFR or serum creatinine versus placebo and limited evidence regarding renal clinical outcomes [152, 153]. In addition to systematic alternations of lipid profiles, there is accumulating evidence that abnormal lipid metabolism also contributes to DKD [154]. The role lipids play in the development and progression of DKD still needs further investigation.

3.7.3 Novel therapies

Novel therapeutic strategies which aim to address the underlying pathogenesis and potential risk factors of DKD are being developed. As stated above, inflammation and oxidative stress induced by hyperglycaemia are critical in the pathogenesis of DKD. Theoretically, antioxidant or anti-inflammatory agents targeting the kidney could be beneficial to prevent or delay progression of DKD. However, the clinical evidence is less promising: bardoxolone methyl failed due to increased incidence of CVD, worse BP and albuminuria excretion [155]; vitamin B derivatives showed no effect on creatinine clearance, GFR or BP [156]; and vitamin D receptor activators' effect on albuminuria was uncertain and they appeared to be less tolerated [157]. Pentoxifylline used to be applied for claudication but was then tested as a potential treatment for DKD because of its anti-inflammatory properties. Integrated data from eight RCTs indicated anti-albuminuria effect of pentoxifylline when used with ACEi or ARB in the DKD population, but there is a lack of evidence for long-term renal outcomes [158].

In addition to the inflammation and oxidative stress pathways, there are new therapies targeting haemodynamic mechanisms. Sulodexide is an anti-thrombotic agent composite of heparin and dermatan sulfate which has albuminuria- and proteinuria-reducing properties [159]. But its efficacy in DKD has been controversial since a phrase III clinical trial with 1248 participants observed no extra benefits in ESKD and doubling of serum creatinine endpoints [160].

Another new agent targeting haemodynamic pathways reported inconclusive results with the endothelin-1A receptor (ET1AR) blockade. It down regulated the activity of endothelin-1, leading to reduction of system and glomerular hypertension. An early RCT of avosentan was terminated after four months' follow-up due to more

cardiovascular events and deaths in the avosentan arm [161]. To avoid cardiovascular adverse events, the new ET1AR blockade atrasentan was applied instead. A 12-week RCT showed 35%–38% lower levels of ACR in the atrasentan group, while more participants withdrew due to adverse events in the high-dose atrasentan group [162]. A phrase III trial of atrasentan with the primary outcomes of renal endpoints (doubling of serum creatinine, ESKD and renal death) was terminated early due to low endpoint event rates, leaving inconclusive the efficacy and safety of atrasentan in the DKD population.

3.7.4 Limitations of current management

Although the risk factor control strategy and the application of ACEi and ARB have significantly reduced the risks of mortality and ESKD over the past decades, an unmet need exists for new therapies for the DKD population. For example, the choice of glycaemia-lowering drugs is limited for those in advanced stages, who are the same subgroup with high risks of rapid disease progression and hypoglycaemia events. For BP control, it should be noted that BP targets are hard to achieve and may require combinations of different classes of anti-hypertensive drugs. Moreover, the milestone therapy of RAS inhibitors may fail in some diabetes and DKD patients. A risk prediction model based on data from the Irbesartan Diabetic Nephropathy Trial (IDNT) and the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial indicated that the absolute risk reduction of ARB was 3.4%, leading to a residual 4.7% risk of ESKD and 25.8% risk of CVD and death [163]. The varing treatment effects resulted in a substantial proportion of DKD patients remaining at high risk for ESKD, CVD and mortality despite RAS inhibitor therapy. Novel therapies for DKD targeting different pathogenic pathways are being developed. Some promising treatments failed to show efficacy and safety in clinical trials, and some remain underexamined.

3.8 Diabetic kidney disease in Chinese Medicine

3.8.1 Origin and development

DKD is a modern disease definition based on laboratory tests and renal pathological

examination. Therefore, the exact term DKD cannot be found in the historical Chinese medicine (CM) literature. Yet a large number of texts describing symptoms and signs highly consistent with DKD can be found in the classical literature. As early as the Han dynasty, there were classical texts describing polyuria in patients with diabetic symptoms in the CM medical book named *Synopsis of Prescriptions of the Golden Chamber* 金匮要略. During the Shui and Tang dynasties, physicians at that time already observed that ulcer and oedema could develop in patients suffering from long-term diabetic symptoms (from the books: *Treatise on the Pathogenesis and Manifestations of All Diseases* 诸病源候论 and *Medical Secrets of an Official* 外台秘要). In a book published in the Ming dynasty (*Tips of Syndrome Diffitiation and Treatments* 证治要诀), detailed records of urinary changes (sweet, turbid, cloudy and oil-like) in patients with a long diabetic-like history can be seen.

The treatment of DKD symptoms can be traced back to ancient CM disease name(s) such as *Xiao shen* 消肾, *Shen xiao* 肾消 and *Xia xiao* 下消. Although these three terms were used interchangeably in classical literature, the concept of *Xia xiao* was considered more general than the other two terms. According to the classical literature, typical symptoms covered by these three terms included muscle dystrophy in the lower half of the body, bone and joint pain, being thirsty with or without drinking a lot, frequent urination and disturbed and sweet urine [164, 165].

3.8.2 Aetiology and pathogenesis

Based on the theory of Cm, both internal causes and external factors contribute to the development of DKD. It should be noted that the concept of Kidney in CM is not aligned with the physical kidney known in conventional medicine. Constitutional insufficiency and deficiency in the five *zang* organs (especially Kidney deficiency) serve as the internal causes of DKD as *Miraculous Pivot* 灵枢 says “People with five *zang* organ deficiency are vulnerable to the disease *Xiao dan* 消瘅”. In this susceptible population, DKD is very likely to be initiated by dietary irregularities and liquor addiction, as *Peaceful Holy Benevolent Prescriptions* 太平圣惠方 accounts: “*San xiao* 三消 is rooted from constitutional kidney deficiency and triggered by daily unhealthy

greasy food intake". Additionally, DKD can result from emotional disorders, internal damage due to overexertion and fatigue, untimely or erroneous treatment, or overconsumption of medicinals with warm and dry flavours.

The pathogenesis of DKD from a CM perspective is streamlined as the following dynamics. In healthy status, the physiological function of the body (summarised as *yang*) is maintained by the support of biological components (summarised as *yin*). In the initial stage, Kidney deficiency leads to an internal dryness-heat status and fluid-humour depletion, undermining the *yin* components in the human body. Thus, patients may experience thirst even with excess fluid intake, fatigue and lack of strength, dry eyes with blurred vision or numbness of the limbs under this *qi* and *yin* depleting stage, all of which could be explained by the failure to nourish the *zang* organs and meridians. As the disease continues to deteriorate, the long-term detriment to the *yin* component begins to affect the *yang* function.

In the Kidney, the diminished storage and astringency function results in essence draining, which symptomatically produces turbid and oily urine. Moreover, impaired Kidney *qi* transformation function causes retention of waste fluid, so patients manifest with lower-limb oedema. The spleen and Liver may also be dysfunctional due to their close relation with the Kidney. In the advanced stage, this further develops to dual damage of the *yin* and *yang* and congestion of dampness turbidity and static blood. Additionally, symptoms and signs indicating pathological changes in blood stasis and dampness turbidity are also frequently observed in DKD patients. Symptoms and signs suggesting severe disease conditions, including oliguria, pitting oedema, nausea and vomiting can be observed.

3.8.3 Chinese herbal medicine

Due to the different stages of DKD in CM theory, treatments should be tailored to individuals based on their clinical manifestations and underlying pathological changes, which is known as the principle of "syndrome differentiation and treatment" in CM. As the traditional knowledge of DKD has been passed down and evolved, the CM syndromes most frequently seen in DKD patients are summarised in the experts'

consensus-based guidelines and textbooks, accompanied by corresponding herbal treatments (**Table 3-3**).

In clinical practice, physicians usually modify the recommended formulae to fit the personal needs of each patient; therefore the herbal ingredients can be different in prescriptions for DKD patients under the same syndrome.

Although there are published systematic reviews of specific formulae and CHM preparations, the poor methodological and reporting quality of these reviews makes them unreliable and not informative enough in clinical decision-making [166]. High-quality evidence regarding the overall efficacy and safety of CHM as treatment for DKD is still lacking.

Table 3-3 Chinese medicine syndromes and treatments for DKD

Syndrome Differentiation	CHM formulae	Function of Formulae	Herbal Ingredients
Dual deficiency of *qi* and *yin*	*Shen qi di huang tang* 参芪地黄汤	Tonify *qi* and *yin*	*dang shen* 党参, *huang qi* 黄芪, *fu ling* 茯苓, *shu di huang* 熟地黄, *shan yao* 山药, *shan zhu yu* 山茱萸, *mu dan pi* 牡丹皮, *ze xie* 泽泻
Liver-Kidney *yin* deficiency	*Qi Ju Di huang wan* 杞菊地黄丸	Enrich the Kidney and nourish the Liver	*gou qi zi* 枸杞子, *ju hua* 菊花, *shu di huang* 熟地黄, *shan zhu yu* 山茱萸, *shan yao* 山药, *fu ling* 茯苓, *ze xie* 泽泻, *mu dan pi* 牡丹皮
Spleen-Kidney *yang* deficiency	*Fu zi li zhong wan* 附子理中丸 with *Zhen wu tang* 真武汤	Warm the Kidney and fortify the Spleen	*fu zi* 附子, *sheng jiang* 生姜, *dang shen* 党参, *bai zhu* 白术, *fu ling* 茯苓, *bai shao* 白芍, *gan cao* 甘草
Dual deficiency of *qi* and Blood	*Dang gui bu xue tang* 当归补血汤 with *Ji sheng shen qi wan* 济生肾气丸	Tonify *qi* and Blood	*huang qi* 黄芪, *dang gui* 当归, *fu zi* 附子, *rou gui* 肉桂, *shu di huang* 熟地黄, *shan yao* 山药, *shan zhu yu* 山茱萸, *fu ling* 茯苓, *mu dan pi* 牡丹皮, *ze xie* 泽泻, *che qian zi* 车前子, *niu xi* 牛膝
Dual deficiency of *yin* and *yang*	*Jin gui shen qi wan* 金匮肾气丸	Tonify both *yin* and *yang*	*gui zhi* 桂枝, *fu zi* 附子, *shan yao* 山药, *shan zhu yu* 山茱萸, *shu di huang* 熟地黄, *fu ling* 茯苓, *ze xie* 泽泻, *mu dan pi* 牡丹皮

References: [167-169].

4 Text-mining the Chinese Classical Literature for Diabetic Kidney Disease

4.1 Introduction

The long history of use and development of CM has produced and accumulated large amounts of medical literature. The historical medical books provide traditional knowledge of the aetiology, diagnosis, treatment, and prognosis of many disorders. Potentially effective therapies for specific diseases may be found or inspired by reviewing the historical literature. One of the most famous cases of new drug development through CM historical literature was the discovery of the anti-malaria drug artemisinin. The herb- processing approach that unlocked the true potential of artemisinin and propelled the drug discovery was recorded in a book written in the Han Dynasty (AD 317–420). The book noted the extraction procedure of the herb so the bioactive compounds can be used [20].

Although diabetes and its complications are contemporary diseases, there are many descriptions highly consistent with the symptoms and signs of diabetes in the classical CM literature [170]. For example, the clinical syndrome of diabetes characterised by thirsting, excessive hunger, weight loss, and sweet and frequent urination was identified in classical literature, naming as *Xiao Ke* disease (In Chinese: 消渴) [171]. The earliest descriptions of *Xiao Ke* disease written in the book *The Yellow Emperors' Classic of Internal Medicine* stated that, *Xiao Ke* disease was seen in rich and obese people, and was induced by overconsumption of sweet and fatty foods. According to the pediatric monograph (*Compendium of Pediatrics*) published in the Qing dynasty, *Xiao Ke* disease can also occur in children and adolescents. The Long-term complications of *Xiao Ke* disease included gangrenous necrosis of the foot, blindness, edema etc. were also recorded in medical books from different dynasties [172]. In the digital collections of historical CM literature (*Zhong Hua Yi Dian*), citations related to *Xiao Ke* disease were identified in 477 out of 1156 books[173].

The inconsistency bewteen the plentiful CM literature of *Xiao Ke* disease and the low

prevalence of diabetes and its complications in ancient China may be caused by the "sampling bias". Famous CM physicians in ancient China were more likely to serve royal families and upper-class patients rather than the general population. These patients usually had much higher food intake, better living conditions and longer life span that allow diabetes and its complications develop. Thus, the ancient experience and knowledge about diabetes were recorded and preserved by the famous doctors' monographs and then passing down for generations.

As an important source of evidence, traditional knowledge about the CM treatments for diabetes and its renal complication has been passed down via the historical literature and still influences contemporary CM practice. Therefore, a systematic and comprehensive review of the classical literature to identify potential herbal therapies for DKD is necessary. Further studies can be planned based on the findings and may lead to novel therapeutics.

In this chapter, a text-mining method for systematic evaluation of classical literature is reported; it determined which formulae and herbs had been frequently used to treat DKD in CM.

4.2 Methods

The methods used in this section follow the principles and standard operating procedures developed by the China-Australia International Research Centre for Chinese Medicine (a collaborative partnership between RMIT University and the Guangdong Provincial Hospital of Chinese Medicine) and the work of Dr Brian May [174, 175].

4.2.1 Search strategies

4.2.1.1 Database

Search of the CM historical literature was conducted in the 5th Edition of the *Zhong Hua Yi Dian* 中华医典 (ZHYD, *Encyclopedia of Traditional Chinese Medicine*). The ZHYD is the largest collection of CM historical literature with a searchable feature

[174]. It is in CD-ROM format and contains the contents of 1156 books in the field of CM [176]. All books in the ZHYD are categorised according to their bibliographical properties and grouped as Theory Classics, Diagnostics, Materia Medica, Formularies (Prescriptions), Acupuncture and Moxibustion etc., and a search scope can be specified in one or more of the above groups. Searching can be performed in three ways: a search of headings, a search of book names, and a search of body texts (**Figure 4-1**).

Figure 4-1 Search interface of *Zhong Hua Yi Dian*

4.2.1.2 Search terms

The definition and diagnosis of DKD are based on modern laboratory tests and the term 'DKD' is not available in the historical literature. Zhang et al. developed a set of classical terms which are representative of DKD [170]. Briefly, the method that Zhang et al. used to develop the set of terms included collecting information from medical dictionaries, CM textbooks, DKD monographs and publications about classical terms of DKD. The frequency of 31 classical terms in different sources was calculated and summarised. Then, a questionnaire survey was conducted among 35 nephrologists from tertiary CM hospitals across China, to obtain their opinions on the consistency between each candidate term and DKD. Finally, eight out of the 31 terms recommended by over

50% of nephrologists were cross checked for their corresponding modern diseases in modern CM textbooks, monographs and dictionaries.

Among the eight expert-recommended terms, ancient expressions about the symptoms of oedema (*Shui zhong* 水肿), turbid urine (*Niao zhuo* 尿浊), fatigue (*Xu lao* 虚劳) and anuria with vomiting (*Guan ge* 关格) were not representative enough for DKD as they also refer to other kidney or internal diseases in the modern literature. Another expert-recommended term, the *Xiao Ke* (消渴), referrs to the whole process of diabetes therefore it is too broad for the concept of DKD. The classical terms that best represent DKD, as it is known today, is *Shen Xiao* 肾消*, Xia Xiao* 下消*, and Xiao Shen* 消肾. There was consensus by the nephrologists that these three terms were suitable, and they only referred to DKD in the modern textbooks and monographs. Considering their sensitivity and specificity, the terms *Shen Xiao, Xia Xiao* and *Xiao Shen* were adopted as search terms in this research.

4.2.2 Data collection and screening

4.2.2.1 Data collection

Searching was conducted by looking up the three search terms in body texts. The hit number for each search term was recorded. The passages of text containing the search terms were copied into a Microsoft Excel spreadsheet with information about identified term, location in ZHYD, book name and whether it provided treatment data. After removing duplicates, the preliminary dataset of DKD relevant historical literature was obtained.

The second step was searching for literature with herbal treatment information from the preliminary dataset. Historical literature with treatment data but in the form of case reports were excluded due to their different nature. A citation referred to one formula with its treated symptoms/signs. If a passage of text mentioned more than one formula, two or more citations were generated. All citations were retrieved and documented in a new spreadsheet with information about the identified term, book name, original texts, symptoms/signs description, formula name and herbal ingredients. For the purpose of characteristics analysis, information including author, publication dynasty and

publication year was added for each citation. The reference sources and procedures were adapted from the methods of May et al. [177].

For those citations that only mentioned the formulae name without ingredients, additional sources were consulted in order to find the missing ingredients. Firstly, the formula name was used as a search term to identify its ingredient descriptions within the same book. If text of ingredient descriptions was not found in the same book, then other books written by the same author were searched. If ingredients were still not found, then the corresponding formulae were excluded from the frequency analysis of ingredients. As a result, DKD treatment data from historical literature was developed in the form of a citations pool.

4.2.2.2 Data screening

Citations that met one of the following criteria were excluded:

- Originating from books published after the modernisation of CM, defined as after 1949 [178]
- Originating from the *Prescriptions* book (方剂书), which was written to assist memory of formulae ingredients (e.g. *Prescriptions in Rhymes* 汤头歌诀);
- Describing exclusive aetiologies or symptoms/signs of DKD such as priapism (original text: 强中) and haematuria (original text: 小便赤似血色).

The screening was first finished by one researcher and then was checked by another. Each excluded citation was marked in a separate column in the spreadsheet with a justification. Any disagreement was resolved by consulting a CM nephrologist who was experienced in historical literature.

4.2.3 Data rating

Since searches in ZHYD may find many references, a rating system was developed to categorise citations with varing degrees of relevance to modern DKD.

4.2.3.1 Rating system development

Firstly, current diagnosis criteria of DKD in the guidelines and textbooks of contemporary medicine were reviewed [2, 5, 108]. Long-term diabetic history, typical symptoms/signs of diabetes and manifestations of kidney injury were found to be the key aspects in modern clinical diagnosis of DKD. Thus, the rating categories include presentation of typical symptoms/signs, duration of condition, symptoms/signs of retinopathy and exclusive symptoms for differential diagnosis. The details of the rating items are shown in **Table 4-1**.

Numerical codes were used in each rating item according to:

- Code 0: the citation did not mention the relevant information
- Code 1: the citation included relevant information but was not explicit
- Code 2: the citation included the target expression.

Table 4-1 Rating categories, items and possible expression in CM literature

Category and items	Possible expression (Pin yin & Chinese)
History of diabetes	
Terms relating to diabetes	*Xiao Ke* 消渴, *Nei Xiao* 内消, *San Xiao* 三消, *Shang Xiao* 上消, *Xiao Zhong* 消中, *Zhong Xiao* 中消
Profuse drinking and profuse urine	*Yin yi sou yi* 饮一溲一, *Yin yi sou er* 饮一溲二
Sweet urine	*Tian* 甜, *Gan* 甘, *Tang* 糖, *Mi* 蜜
Symptoms of diabetes	
Frequent urination	*Shuo* 数, *Duo* 多, *Da li* 大利, *Wu du* 无度, *Bu jin* 不禁
Increased thirst	*Ke* 渴, *Duo yin* 多饮, *Yin shui* 饮水, *Shan yin* 善饮
Increased appetite	*Xiao Shi* 消食, *Ji* 饥, *Yu shi* 欲食, *Shan shi* 善食
Weight loss	*Shou* 瘦, *Wei* 痿, *Xi* 细
Symptoms of kidney injury	
Turbid urine (protein urine)	*Bai zhuo* 白浊, *Zhi ye* 脂液, *Gao you* 膏油, *Gao* 膏
Oedema of lower limbs	*Shui zhong* 水肿, *Tui zhong* 腿肿
Disease duration	
Chronic or long-term	*Jiu* 久, *Bu cuo* 不瘥, *Bu yu* 不愈
Symptoms/signs of retinopathy	
Blurred vision	*Mu hun* 目昏, *Shi zhan hun miao* 视瞻昏渺, *Shi wu mo hu* 视物模糊
Exclusive symptoms	
Haematuria	*Niao xie* 尿血, *Xiao bian chi* 小便赤

Secondly, a hierarchy of relevance to DKD in the rating system was constructed based on the following criteria (**Table 4-2**): (1) history of diabetes; (2) symptoms/signs of diabetes; and (3) symptoms/signs of kidney injury. Citations fulfilling all three criteria were most likely to refer to DKD as it is known today. Citations that satisfied the third criterion plus the first or second criteria were possibly DKD. Citations only meeting the third criterion were conditional DKD. Other citations were unclear as to their relevance to DKD. The overall score ranged from 0 to 3 representing a low to high degree of similarity of the historical citations to modern DKD.

Table 4-2 Hierarchy of relevance to DKD

Relevance to DKD	Score	Score criterion
Unclear	0	Does not include any symptoms of kidney injury or not enough information to judge
Conditional DKD	1	Only includes symptoms of kidney injury
Possible DKD	2	Includes diabetes history and symptoms of kidney injury, or symptoms of diabetes and symptoms of kidney injury
Most likely DKD	3	Includes diabetes history and symptoms of diabetes and symptoms of kidney injury

4.2.3.2 Data rating procedure

Each rating category and its items were listed in separate columns in the spreadsheet for each citation. Based on the original texts for each citation, codes were allocated to each rating item under different categories. Judgements of relevance scoring were made based on the coding results of all rating categories. Four citations pools with different relevance to DKD were obtained.

The rating procedure was independently conducted by two reviewers. Before all citations were rated, a pilot trial was done to test the consistency of coding results between the two reviewers and to test the validity of the rating system. Any disagreement on rating and relevance scoring was resolved by discussion with a third CM nephrologist or by consulting CM dictionaries.

4.2.4 Data analysis

Data analysis included two parts. The first part was a descriptive analysis of the citation characteristics to present an overview of DKD records in historical literature. The second part was a frequency analysis of the formulae and herbal ingredients, leading to discovery of the most frequently used formulae and herbs for DKD in antiquity. The analysis was performed in the Statistical Package for the Social Sciences (SPSS) software (version 21, IBM).

4.2.4.1 Descriptive analysis of citation characteristics

The dynasty distributions of citations and search terms were analysed for all treatment

citations. The dynasty of each citation and term was determined based on the written or publication years of the cited CM books. In addition, the hit frequency of each search term in ZHYD and different citation pools was calculated.

4.2.4.2 Frequency analysis of formulae and herbs

The herb names were standardised before analysis, to ensure the same herb with multiple names was calculated altogether (**Appendix 1** includes a list of herbs and their corresponding Pin yin and scientific names that are referenced in this thesis). For example, *shan yao* 山药 and *huai shan* 淮山 were both considered to be the same herb; thus it was standardised to be the same name. Formulae were evaluated and standardised based on formulae names and composite ingredients. If two formulae had the same names but different ingredients, a number was added after the formulae names, for instance *Ba wei wan 1* 八味丸 1 and *Ba wei wan 2* 八味丸 2. If two formulae shared identical ingredients but with different names, the name that appeared earlier was kept. The changes were separately documented in the spreadsheet.

After herbs and formulae standardisation was completed, frequency analysis was conducted for the "treatment citation", "conditional DKD citation", "possible DKD citation" and "most likely DKD citation" pools separately. Frequency analysis of herbs for turbid urine and oedema was also conducted based on different citation pools.

4.3 Results

4.3.1 Search, screening and rating results

In total, the three search terms *Shen Xiao, Xia Xiao,* and *Xiao Shen* were mentioned in 1214 citations in classical CM literature. After duplicate, non-treatment paragraph and case report removal, the number of citations which contained treatment information was 331.

Among the 331 treatment citations, 53 citations were excluded for satisfying at least one exclusion criteria. One citation retrieved from *Brief Summary of Materia Medica Prescriptions* 本草简要方 was excluded since the book was published in 1965. Another citation from *Prescriptions in Rhymes* 汤头歌诀 was also excluded. Another 51

citations were excluded because of symptoms/signs descriptions indicating non-DKD aetiologies. For instance, original texts indicating haematuria (赤似血色, 小便数或赤, 小便赤黄, 赤白浊, 如血色), medication misuse (饵金石, 服金石药) and priapism (不交精出, 茎长而坚, 阴茎强) were found in these excluded citations. After screening, 278 treatment citations remained for rating of relevance to DKD.

Among the 278 treatment citations, the number of citations scored with 0, 1, 2 and 3 points were 194, 24, 37 and 23, respectively. As a result, the most likely DKD citations pool included 23 citations with a rating score of 3. In the same manner, the possible DKD citations pool included 37 citations and the conditional DKD citations pool included 24 citations (see **Figure 4-2**).

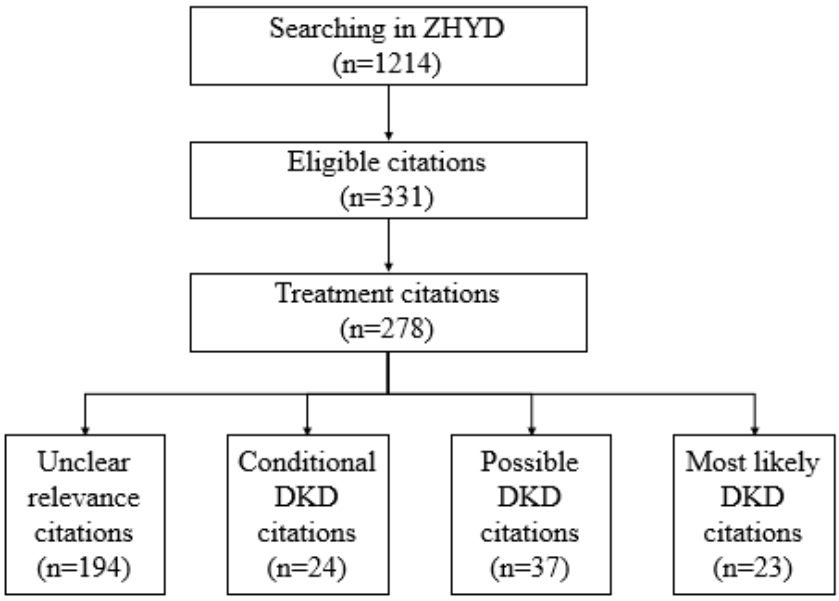

Figure 4-2 Searching, screening and rating results of historical literature

4.3.2 Citation characteristics

4.3.2.1 Publication dynasty

The citations were retrieved from 68 distinct historical CM books which were written from AD 583 to 1895. The earliest document was found in the *Variorum of Experiential Prescriptions* 集验方 published in the Han Dynasty and the most recent record was from the *Minor formulae of Difficult and Emergency Issues* 疑难急症简方 published

in the Qing Dynasty.

Table 4-3 shows the dynastic distribution of treatment citations. The number of treatment citations motably increased after the Tang Dynasty. The official prescription monograph published in the Song Dynasty, *Peaceful Holy Benevolent Prescriptions* 太平圣惠方 together with another prescriptions monograph (*Ji Feng Prescriptions for Universal Relief* 鸡峰普济方) in the same period contributed 13.7% (38 citations) of the total treatment citations. In the Yuan Dynasty, there was a significant drop in the number of citations. Then the number of citations increased in the Ming and Qing dynasties, accounting for over 70% of total citations. The book that contributed the most citations (40 citations) with a proportion of 14.4%, was the *Prescriptions for Universal Relief* 普济方, which was the most comprehensive pharmacopoeia in CM history and written in the Ming Dynasty.

Table 4-3 Dynastic distribution of treatment citations

Dynasty	No. of treatment citations (%)
Before Tang Dynasty (before 618)	1 (0.4%)
Tang and 5 Dynasties (618–960)	8 (2.9%)
Song and Jin Dynasties (960-1279)	66 (23.7%)
Yuan Dynasty (1271–1368)	4 (1.4%)
Ming Dynasty (1368–1644)	128 (46.0%)
Qing Dynasty (1644–1911)	71 (25.5%)
Ming Guo/Republic of China (1912–1949)	0 (0%)
Total	278 (100%)

4.3.2.2 Dynasty of search terms

The dynastic distribution pattern of each search term was different (**Figure 4-4**). The term *Shen Xiao* appeared in CM books across different dynasties. The citations before the Tang and in the Tang and Yuan dynasties were mainly located by this term. As for the term *Xiao Shen*, it was mentioned frequently since the Song and Jin dynasties. Lastly, the term *Xia Xiao* was not used in the classical texts with treatment information until the Ming Dynasty.

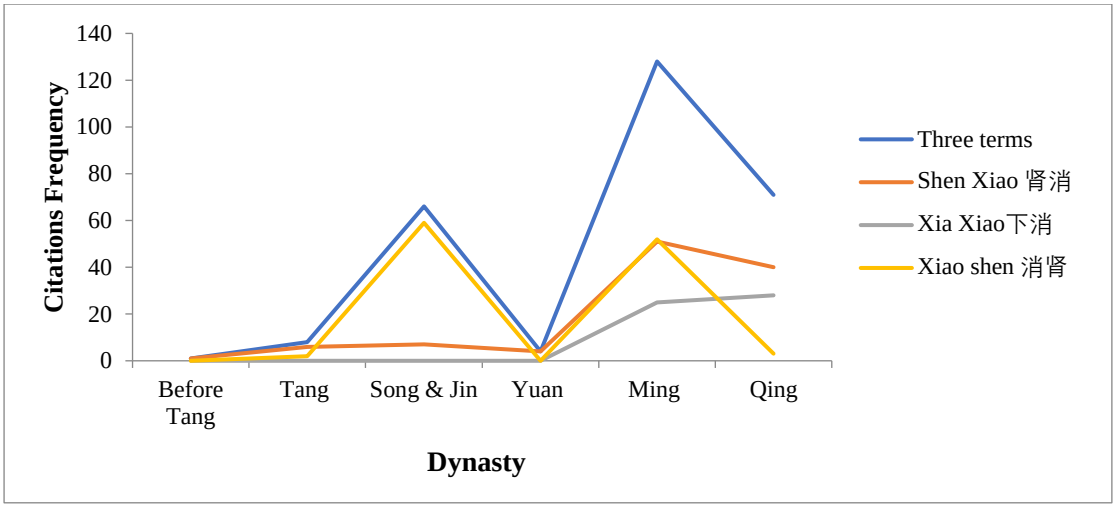

Figure 4-3 Dynastic distribution of search terms

Citation frequency and proportion of each term are presented based on different citation pools (**Table 4-4**). In the ZHYD, the term *Xiao Xia* located nearly twice the number of citations as the other two terms. In the treatment citations pool, the number of citations identified by *Xiao Xia* was half of those identified by *Shen Xiao* or *Xiao Shen*. In the three DKD relevant citation pools, the terms retrieving the largest number of citations were different. *Shen Xiao* was mentioned in 58.3% of citations in the conditional pool, while 67.6% of citations in the possible pool recorded *Xiao Shen*. In the most likely citations pool, the three search terms found almost the same number of citations.

Table 4-4 Citation frequency of each search term in different pools

Search term	Citation frequency (n, %)				
	ZHYD (n=1,214)	Treatment citations pool (n=278)	Most likely citations pool (n=23)	Possible citations pool (n=37)	Conditional citations pool (n=24)
Shen Xiao	324 (26.7)	109 (39.2)	8 (34.8)	9 (24.3)	14 (58.3)
Xia Xiao	556 (45.8)	53 (19.1)	8 (34.8)	3 (8.2)	6 (25)
Xiao Shen	334 (27.5)	116 (41.7)	7 (30.4)	25 (67.6)	4 (16.7)

4.3.3 Most common formulae

4.3.3.1 Formulae in treatment citations pool

A total of 194 citations without enough information for judgements of relevance to DKD were included in the "treatment citations" pool. In this citation pool, 128 distinct formulae consisting of 143 different herbs were reported in the 278 citations. The formulae were diverse, as more than half were cited once and the remaining 30% were mentioned less than three times. The top ten most commonly cited formulae and their ingredients are listed in **Table 4-5**.

Table 4-5 Common formulae in treatment citations pool

Formulae name	Herb ingredients	No. of citations
Ba wei wan 八味丸	*fu zi* 附子, *rou gui* 肉桂, *shu di huang* 熟地黄, *shan zhu yu* 山茱萸, *ze xie* 泽泻, *mu dan pi* 牡丹皮, *shan yao* 山药, *fu ling* 茯苓	16
Liu wei di huang wan 六味地黄丸	*shu di huang* 熟地黄, *shan zhu yu* 山茱萸, *ze xie* 泽泻, *mu dan pi* 牡丹皮, *shan yao* 山药, *fu ling* 茯苓	15
Hui xiang san 茴香散	*xiao hui xiang* 小茴香, *chuan lian zi* 川楝子	12
Bai fu ling wan 白茯苓丸	*fu ling* 茯苓, *fu pen zi* 覆盆子, *huang lian* 黄连, *ren shen* 人参, *tian hua fen* 天花粉, *shu di huang* 熟地黄, *bi xie* 萆薢, *xuan shen* 玄参, *ji nei jin* 鸡内金, *shi hu* 石斛, *she chuang zi* 蛇床子	9
Lu jiao (single herb) 鹿角	*lu jiao* 鹿角	7
Modified Ba wei wan 八味丸加减	*wu wei zi* 五味子, *shu di huang* 熟地黄, *shan zhu yu* 山茱萸, *ze xie* 泽泻, *rou gui* 肉桂, *mu dan pi* 牡丹皮, *shan yao* 山药, *fu ling* 茯苓	6
Ge gen wan 葛根丸	*ge gen* 葛根, *gua lou* 瓜蒌, *qian dan* 铅丹, *fu zi* 附子	6
Huang qi wan 黄芪丸	*huang qi* 黄芪, *shan zhu yu* 山茱萸, *ren shen* 人参, *wu wei zi* 五味子, *shu di huang* 熟地黄, *ji nei jin* 鸡内金, *rou cong rong* 肉苁蓉, *niu xi* 牛膝, *bu gu zhi* 补骨脂, *lu rong* 鹿茸, *mai dong* 麦冬, *di gu pi* 地骨皮, *fu ling* 茯苓, *xuan shen* 玄参	5
Ping bu wan 平补丸	*tu si zi* 菟丝子, *shan zhu yu* 山茱萸, *dang gui* 当归, *yi zhi ren* 益智仁, *chuan lian zi* 川楝子, *niu xi* 牛膝, *hu lu ba* 葫芦巴, *du zhong* 杜仲, *ba ji tian* 巴戟天, *rou cong rong* 肉苁蓉, *ru xiang* 乳香	5
Shen li wan 肾沥丸	*ji nei jin* 鸡内金, *yuan zhi* 远志, *ren shen* 人参, *huang qi* 黄芪, *sang piao xiao* 桑螵蛸, *ze xie* 泽泻, *shu di huang* 熟地黄, *rou gui* 肉桂, *dang gui* 当归, *long gu* 龙骨, *gan cao* 甘草, *mai dong* 麦冬, *wu wei zi* 五味子, *ci shi* 磁石, *fu ling* 茯苓, *chuan xiong* 川芎, *xuan shen* 玄参	5

Note: The use of some herbs may be restricted in some countries; readers are advised to comply with relevant regulations.

Ba wei wan 八味丸 was the most frequently cited formula in the treatment citations pool, followed by *Liu wei di huang wan* 六味地黄丸. The herb ingredients of these two formulae were highly homologous. The earliest record of the formula *Ba wei wan* was found in the book *Synopsis of Prescriptions of the Golden Chamber* 金匮要略 written in the Han Dynasty. It was named *Shen qi wan* 肾气丸 and was indicated for diabetic patients who were comorbid with obvious thirst and profuse urine (original text: 男子

消渴, 小便反多, 以饮一斗, 小便一斗, 肾气丸主之). The *fu zi* 附子 and *rou gui* 肉桂 in *Ba wei wan* were used to generate *yang qi* in the Kidney in order to decrease the excessive urine. *Liu wei di huang wan* derived from *Ba wei wan* was created for children in the Song Dynasty. Dr *Zhu Danxi* 朱丹溪 from the Yuan Dynasty made use of its nourishing *yin* feature to treat turbid urine caused by pathogenic fire in Kidney (original text: 火盛于下，为肾消。病则烦躁，小便浊，淋如膏油之状。论云：焦烦水易亏者是也。六味地黄丸主之).

Besides *Liu wei di huang wan*, there was another formula modified based on *Ba wei wan* and frequently cited in this pool. The *fu zi* in the original *Ba wei wan* was replaced by *wu wei zi*. This formula was named *Modified ba wei wan*. This small modification was suggested by Dr *Zhang Zihe* 张子和 to avoid the excess warm-dryness induced by taking *fu zi* in summer. The sour and sweet flavours of *wu wei zi* are good for *yin* formation, in addition to its securing essence and reducing urination.

The formula *Hui xiang san* 茴香散 was one of the three formulae with a citation frequency greater than ten in this pool. It was first mentioned as a treatment for *Shen Xiao* 肾消 with initial symptoms of turbid urine by Dr *Liu Wansu* 刘完素 in the Jin Dynasty. This citation was repeatedly cited by pharmacopeia in the Ming and Qing dynasties, such as the *Compendium of Materia Medica* 本草纲目 and *Prescriptions for Universal Relief* 普济方. The composition of *Hui xiang san* is simple and only contained two herbs: *xiao hui xiang* 小茴香 and *chuan lian zi* 川楝子. The x*iao hui xiang* can warm the Kidney and Bladder, while *chuan lian zi* reinforces the action by regulating *qi*.

Another formula that appeared nine times was *Bai fu lin wan* 白茯苓丸. It has been cited in several pharmacopeia since the Song Dynasty. This formula was designed for patients with a long-term diabetic history and therefore at high risk of kidney injury. The prominent symptoms were muscle atrophy and weakness in the lower limbs (original text: 治消肾, 因消中之后, 胃热入肾, 消烁肾脂, 令肾枯燥, 遂致此疾, 即两腿渐细, 腰脚无力, 白茯苓丸方).

The formula *Ge gen wan* 葛根丸 was first described by Dr *Zhang Zihe* 张子和 for patients with *Xiao Shen* 消肾 who suffered from thirst and were drinking large amounts of water daily. The herb *gua lou* 瓜蒌 was one of the ingredients in the records from the Song and Jin dynasties. But in the citations in the Ming Dynasty, *gua lou* was removed and replaced with *tian hua fen* 天花粉. These two herbs are from the same plant but different parts. *Tian hua fen* is good for nourishing *yin* and thus may exert better effect in relieving thirst.

The formulae mentioned in five citations each were *Huang qi wan* 黄芪丸, *Ping bu wan* 平补丸, and *Shen li wan* 肾沥丸. The indications of these three formulae were similar, for diabetic patients with polyuria, muscle wasting and weakness in the lower extremities. The majority of ingredients contained in these formulae replenish Kidney *yang* function.

4.3.3.2 Formulae in most likely DKD citations pool

The citations that metioned diabetic history, diabetic symptoms and kidney injury symptoms were considered most likely to be DKD. Although the number of citations included in the most likely DKD pool was only 23, there were 14 different formulae recorded. The highest citation frequency was three and 50% of formulae only appeared once. The formulae mentioned more than once in this most likely pool are listed in **Table 4-6.**

Table 4-6 Common formulae in most likely DKD citations pool

Formula name	Herb ingredients	No. of citations
Liu wei di huang wan 六味地黄丸	*shu di huang* 熟地黄, *shan zhu yu* 山茱萸, *ze xie* 泽泻, *mu dan pi* 牡丹皮, *shan yao* 山药, *fu ling* 茯苓	3
Sao si tang 缲丝汤	*jian si* 茧丝	3
Gu ben wan 固本丸	*sheng di huang* 生地黄, *shu di huang* 熟地黄, *tian dong* 天冬, *mai dong* 麦冬	2
Hua cong rong wan 花苁蓉丸	*hua cong rong* 花苁蓉, *ze xie* 泽泻, *wu wei zi* 五味子, *ba ji tian* 巴戟天, *di gu pi* 地骨皮, *gua lou* 瓜蒌, *ci shi* 磁石, *ren shen* 人参, *chi shi zhi* 赤石脂, *gan jiang* 干姜, *yu yu liang* 禹余粮, *sang piao xiao* 桑螵蛸, *mang xiao* 芒硝	2
Ren shen fu ling san 3 人参茯苓散 3	*ren shen* 人参, *bai zhu* 白术, *dang gui* 当归, *chi shao* 赤芍, *da huang* 大黄, *zhi zi* 栀子, *ze xie* 泽泻, *lian qiao* 连翘, *tian hua fen* 天花粉, *ge gen* 葛根, *fu ling* 茯苓, *rou gui* 肉桂, *mu xiang* 木香, *huo xiang* 藿香, *han shui shi* 寒水石, *gan cao* 甘草, *shi gao* 石膏, *hua shi* 滑石, *mang xiao* 芒硝	2
Tian hua wan 天花丸	*huang lian* 黄连, *bai bian dou* 白扁豆, *zhu sha* 朱砂, *fu ling* 茯苓, *mu li* 牡蛎, *zhi mu* 知母, *ku shen* 苦参, *tian hua fen* 天花粉, *tie fen* 铁粉, *lu hui* 芦荟, *jin bo* 金箔, *yin bo* 银箔	2
Zhi bo ba wei wan 知柏八味丸	*zhi mu* 知母, *huang bo* 黄柏, *shu di huang* 熟地黄, *shan zhu yu* 山茱萸, *ze xie* 泽泻, *mu dan pi* 牡丹皮, *shan yao* 山药, *fu ling* 茯苓	2

Note: The use of some herbs may be restricted in some countries; readers are advised to comply with relevant regulations.

The most common formulae were *Liu wei di huang wan* 六味地黄丸 and *Sao si tang* 缲丝汤. Citations in this pool clearly described that *Liu wei di huang wan* was used for turbid urine resulting from diabetes progression (original text: 肾消者，饮一溲二。其溲如膏油，即膈消、消中之传变). The *Sao si tang* that originated from books in the Ming Dynasty, also known as Reeling silk decoction, shared the same citation frequency as *Liu wei di huang wan* in this pool. It contained only one ingredient, that is, *jian si* 茧丝 (silk cocoon or silk floss). The citation stated that *Sao si tang* was extremely effective as a treatment for turbid urine, thirst and weight loss caused by diabetes. Diet restriction by avoiding salty foods with *Sao si tang* was described in the citations as well (original text: 缲丝汤治肾消，白浊，及上中二消、饥渴不生肌肉，其效如神...忌食盐物).

Gu ben wan 固本丸, *Hua cong rong wan* 花苁蓉丸, *Tian hua wan* 天花丸, *Ren shen fu lin san* 人参茯苓散 and *Zhi bo ba wei wan* 知柏八味丸 were mentioned in multiple citations in this pool. These formulae were all created for long-term diabetes with severe thirst and frequent and turbid urine, except for *Hua cong rong wan* which was prescribed for diabetes with lower extremity oedema.

4.3.3.3 Formulae in possible DKD citations pool

The possible DKD citations pool consist of 37 citations that mentioned a history or symptons of diabetes, in addition to the symptoms of kidney diseases. The possible DKD citations pool included 22 different formulae. The most common formulae were cited four times, while more than half of the formulae were used once. Formulae in this pool mentioned more than once are shown in **Table 4-7**.

Table 4-7 Common formulae in possible DKD citations pool

Formula name	Herb ingredients	No. of citations
Hui xiang san 茴香散	*xiao hui xiang* 小茴香, *chuan lian zi* 川楝子	4
Ci shi tang 磁石汤	*ci shi* 磁石, *huang qi* 黄芪, *du zhong* 杜仲, *ren shen* 人参, *wu wei zi* 五味子, *shu di huang* 熟地黄	3
Tu si zi san 菟丝子散	*tu si zi* 菟丝子, *pu huang* 蒲黄, *ci shi* 磁石, *huang lian* 黄连, *rou cong rong* 肉苁蓉, *wu wei zi* 五味子, *ji nei jin* 鸡内金	3
Shen fu tang 参附汤	*ren shen* 人参, *fu zi* 附子, *qin dai* 青黛	2
Huang lian wan 黄连丸	*huang lian* 黄连, *tian hua fen* 天花粉, *shen di huang* 生地黄	2
Huang qi san 2 黄芪散 2	*huang qi* 黄芪, *ji nei jin* 鸡内金, *wu wei zi* 五味子	2
Lu rong wan 鹿茸丸	*lu rong* 鹿茸, *rou cong rong* 肉苁蓉, *huang qin* 黄芩, *ren shen* 人参, *tu gua gen* 土瓜根, *ji nei jin* 鸡内金, *tu si zi* 菟丝子	2
Ren shen fu ling 2 人参茯苓散 2	*ren shen* 人参, *bai zhu* 白术, *ze xie* 泽泻, *tian hua fen* 天花粉, *jie geng* 桔梗, *zhi zi* 栀子, *lian qiao* 连翘, *ge gen* 葛根, *huang qin* 黄芩, *da huang* 大黄, *bo he* 薄荷, *fu ling* 茯苓, *gan cao* 甘草, *shi gao* 石膏, *hua shi* 滑石, *han shui shi* 寒水石, *sha ren* 砂仁	2
Tie fen wan 铁粉丸	*tie fen* 铁粉, *shen di huang* 生地黄, *ji nei jin* 鸡内金, *mu li* 牡蛎, *huang lian* 黄连	2
Yuan tu dan 元菟丹	*fu ling* 茯苓, *tu si zi* 菟丝子, *lian zi* 莲子, *wu wei zi* 五味子, shan yao 山药	2

Note: The use of some herbs may be restricted in some countries; readers are advised to comply with relevant regulations.

Hui xiang san 茴香散 was the most frequently cited formula in this pool. The citations that mentioned *Hui xiang san* in this pool were all retrieved from pharmacopeia published in the Ming and Qing dynasties.

Ci shi tang 磁石汤 and *Tu si zi san* 菟丝子散 were both cited in three different citations in prescription monographs from the Song and Ming dynasties. The formula *Ci shi tang* was prescribed to diabetic patients with severe turbid urine and weight loss or weakness. The appearance of urine was described as greasy like fat (original text: 治消肾, 小便白浊如凝脂, 形体羸瘦, 磁石汤方). Lamb kidney soup should be added to boil with the other ingredients in *Ci shi tang*. Therefore, *Ci shi tang* was also called *Shen li tang* 肾沥汤 which highlighted the combination of herbs and lamb kidney. Likewise, *Tu si*

zi san was indicated for diabetic patients with turbid urine as well as frequent urine (original text: 治消肾，小便多，白浊，或不禁，菟丝子散方).

The other formulae mentioned twice in this pool were *Shen fu tang* 参附汤, *Huang lian wan* 黄连丸, *Huang qi san 2* 黄芪散 2, *Lu rong wan* 鹿茸丸, *Ren shen fu lin san 2* 人参茯苓散 2, *Tie fen wan* 铁粉丸 and *Yuan tu dan* 元菟丹. These formulae were all targeted for turbid urine with or without thirst, polyuria, muscle wasting and weakness following by diabetes.

4.3.3.4 Formulae in conditional DKD citations pool

For citations that only contained search terms and descriptions of kidney injury symptoms, they were included in the conditional DKD citations pool. In the conditional DKD citations pool, 14 distinct formulae were identified from 24 citations, only three of which were cited more than once (**Table 4-8**).

Table 4-8 Common formulae in conditional DKD citations pool

Formula name	Herb ingredients	No. of citations
Hui xiang san 茴香散	*xiao hui xiang* 小茴香, *chuan lian zi* 川楝子	7
Huang qi san 1 黄芪散 1	*huang qi* 黄芪, *mai dong* 麦冬, *fu shen* 茯神, *long gu* 龙骨, *tian hua fen* 天花粉, *shu di huang* 熟地黄, *ze xie* 泽泻, *bai shi zhi* 白石脂, *sang piao xiao* 桑螵蛸, *gan cao* 甘草	2
Sang piao xiao wan 桑螵蛸丸	*sang piao xiao* 桑螵蛸, *tu si zi* 菟丝子, *shu di huang* 熟地黄, *shan zhu yu* 山茱萸, *huang lian* 黄连	2

Note: The use of some herbs may be restricted in some countries; readers are advised to comply with relevant regulations.

The formula *Hui xiang san* 茴香散 again, was cited the highest number of times. *Hui xiang san* in this pool was cited seven times, much more than the other formulae. A formula named *Modified hui xiang san* 加减茴香散 which was similar but had slightly different herbal ingredient was also found in this pool. The composition of *Modified Hui xiang san* was *xiao hui xiang* 小茴香, *chuan lian zi* 川楝子 plus *wu wei zi* 五味子.

It was first cited in a book written by Dr *Zhu Danxi* 朱丹溪 and had the same indications as *Hui xiang san*, which was to treat turbid urine in *Shen Xiao* 肾消 disease.

In this pool, *Huang qi san 1* 黄芪散 1 and *Sang piao xiao wan* 桑螵蛸丸 were both cited twice. Both formulae were given to those with turbid urine. But *Huang qi san 1* contained ingredients with cool properties and nourishing *yin* action, therefore was more suitable for those with comorbidity and agitation. *Sang piao xiao wan* included *sang piao xiao* 桑螵蛸 as the main medicinal ingredient for its strong astringent effect to reduce longstanding urinary essence (protein) excretion (original text: 治消肾，小便白浊，久不瘥，桑螵蛸丸方).

4.3.4 Most common herbs

The common herbs from each citation pool are summarised as follows (**Table 4-9)**.

Table 4-9 Common herbs in different citation pools

Citation pool	Herb name (pin yin)	English name	No. of citations
Treatment citations	*fu ling* 茯苓	Poria	129
	shu di huang 熟地黄	Rehmanniae Radix Praeparata	121
	ze xie 泽泻	Alismatis Rhizoma	105
	ren shen 人参	Ginseng Radix Et Rhizoma	94
	shan zhu yu 山茱萸	Corni Fructus	93
	shan yao 山药	Dioscorea Rhizoma	73
	mu dan pi 牡丹皮	Moutan Cortex	71
	huang qi 黄芪	Astragali Radix	67
	tian hua fen 天花粉	Trichosanthis Radix	62
	wu wei zi 五味子	Schisandrae Chinensis Fructus	61
Most likely DKD citations	*fu ling* 茯苓	Poria	12
	ze xie 泽泻	Alismatis Rhizoma	12
	shu di huang 熟地黄	Rehmanniae Radix Praeparata	11
	shan zhu yu 山茱萸	Corni Fructus	9
	mu dan pi 牡丹皮	Moutan Cortex	8
	shan yao 山药	Dioscorea Rhizoma	8
	ren shen 人参	Ginseng Radix Et Rhizoma	6
	tian hua fen 天花粉	Trichosanthis Radix	5
	zhi mu 知母	Anemarrhenae Rhizoma	5
	huang lian 黄连	Coptidis Rhizoma	4
	jian si 茧丝	Silk cocoon, Silk floss	4
	wu wei zi 五味子	Schisandrae Chinensis Fructus	4
Possible DKD citations	*ren shen* 人参	Ginseng Radix Et Rhizoma	14
	wu wei zi 五味子	Schisandrae Chinensis Fructus	14
	fu ling 茯苓	Poria	12
	huang lian 黄连	Coptidis Rhizoma	10
	ji nei jin 鸡内金	Galli Gigerii Endothelium Corneum	9
	shu di huang 熟地黄	Rehmanniae Radix Praeparata	9
	huang qi 黄芪	Astragali Radix	8
	mu li 牡蛎	Ostreae Concha	7
	tu si zi 菟丝子	Cuscutae Semen	7
	ci shi 磁石	Magnetitum	6
	shan yao 山药	Dioscorea Rhizoma	6
	ze xie 泽泻	Alismatis Rhizoma	6
Conditional DKD citations	*shu di huang* 熟地黄	Rehmanniae Radix Praeparata	11
	chuan lian zi 川楝子	Toosendan Fructus	8
	shan zhu yu 山茱萸	Corni Fructus	8
	xiao hui xiang 小茴香	Foeniculi Fructus	8
	gan cao 甘草	Glycyrrhizae Radix Et Rhizoma	6
	shan yao 山药	Dioscorea Rhizoma	6
	ze xie 泽泻	Alismatis Rhizoma	6
	dang gui 当归	Angelicae Sinensis Radix	5
	fu ling 茯苓	Poria	5

Citation pool	Herb name (pin yin)	English name	No. of citations
	huang bo 黄柏	Phellodendri Chinensis Cortex	5
	huang qi 黄芪	Astragali Radix	5

Note: The use of some herbs may be restricted in some countries; readers are advised to comply with relevant regulations.

4.3.4.1 Herbs in treatment citations pools

In the treatment citations pool, 143 different herbs were identified from almost 300 formulae. The frequency of herbs varied from one to 129 and the median frequency was 6. The summarised frequency of the top 10 herbs occupies 39.5% of the cumulative percentage. Thus, the top 10 herbs were regarded as the common herbs of the treatment citations pool.

The most commonly used herbs of the treatment citations pool were *fu ling* 茯苓, followed by *shu di huang* 熟地黄 and *ze xie* 泽泻. Each of these top three herbs was used in over 100 of the formulae. The formulae ingredients of *Liu wei di huang wan* 六味地黄丸 were all included in the top ten list, suggesting that *Liu wei di huang wan* may be the core treatment formula. Other high-frequency herbs tonified the Kidney *qi* (*ren shen* 人参 and *huang qi* 黄芪), replenished *yin* (*tian hua fen* 天花粉) and secured the Kidney essence (*wu wei zi* 五味子). These commonly cited herbs correspond with the CM theory that the basic mechanism of DKD is Kidney deficiency and essence leakage.

4.3.4.2 Herbs in most likely DKD citations pool

In the most likely DKD citations pool, there were 61 unique herbs used in 14 formulae. The highest frequency of herbs was 12 citations while the lowest was one and the median frequency was 2. The top 7 herbs were considered the common herbs in this pool because these herbs contributed a cumulative percentage of 48.9%.

Fu ling, *shu di huang* and *ze xie* were the most common herbs. The herbs constituting *Liu wei di huang wan* occupied the top 6 positions in this pool. The herbs *ren shen*, *wu wei zi* and *tian hua fen* were also found in the list. Additionally, the ingredient of the

single-herb formula *jian zi* 茧丝 was also in the common herbs list.

The herbs *zhi mu* 知母 and *huang lian* 黄连 targeted the Kidney meridians and cleared heat, purged fire and nourished the *yin* function. Its frequent application in the most likely pool indicated that heat or fire pathogen in the Kidney may play an important role in the disease process.

4.3.4.3 Herbs in possible DKD citations pool

Among the possible DKD citations, 65 different herbs constituted the 22 formulae in this pool. The herb frequency ranged from 14 to one, with a median of 2. There were 12 unique herbs in the high-frequency herbs list, which represented 46.2% of herb frequency.

The top 3 most commonly used herbs were *ren shen*, *wu wei zi* and *fu ling*. In addition to the majority ingredients of *Liu wei di huang wan*, herbs with the action of securing essence and reducing urination (*ji nei jin* 鸡内金 and *tu si zi* 菟丝子) were frequently cited. Herbs used to subdue Kidney *yang* were commonly reported in this pool as well, including *ci shi* 磁石 and *mu li* 牡蛎. This implied that reinforcing *yang* in the Kidney was important in treatment for DKD.

4.3.4.4 Herbs in conditional DKD citations pool

In the conditional DKD citations pool, the total number of unique herbs identified was 46. About half of the herbs were cited once or twice and the highest frequency was 11. The summarised frequency of the top four herbs occupied 52.9% of the cumulative percentage of frequency, thus these herbs were regarded as the common herbs.
Although the most commonly cited formula in this pool was the *Hui xiang san* 茴香散, the top herb with a frequency of 11 times was *shu di huang*. Then the ingredients of *Hui xiang san* (*xiao hui xiang* 小茴香 and *chuan lian zi* 川楝子) together with *shan zhu yu* 山茱萸 shared second place. In this common herb list, herb with blood-activating action (*dang gui* 当归) was found, which correspond to the blood stasis syndromes in some DKD patients.

4.3.5 Common herbs for DKD symptoms/signs

In total, 73 citations reporting the presentation of turbid urine were included in analysis. The number of citations in each pool and the most cited herbs are shown in **Table 4-10**. The most cited herbs for turbid urine were highly consistent with the common herbs lists for DKD (seen in **Table 4-9**) except for *huang qi* 黄芪, which commonly appeared in citations mentioning turbid urine but was not listed in the common herbs of the conditional DKD pool, which indicated that *huang qi* may be specific for symptom of turbid urine.

Oedema was reported in three citations using the formulae *Huang cong rong wan* 花苁蓉丸 and *modified ba wei wan* 加减八味丸. Frequency analysis of these herbs was not performed due to the limited number of citations.

Table 4-10 Common herbs for turbid urine

Citation pool (citations included)	Herb name (pin yin)	English name	No. of citations
Most likely DKD citations (n=20)	*fu ling* 茯苓	Poria	12
	shu di huang 熟地黄	Rehmanniae Radix Praeparata	11
	ze xie 泽泻	Alismatis Rhizoma	10
	shan zhu yu 山茱萸	Corni Fructus	9
	mu dan pi 牡丹皮	Moutan Cortex	8
	shan yao 山药	Dioscorea Rhizoma	8
	tian hua fen 天花粉	Trichosanthis Radix	5
	zhi mu 知母	Anemarrhenae Rhizoma	5
	huang lian 黄连	Coptidis Rhizoma	4
	jian si 茧丝	Silk cocoon, Silk floss	4
Possible DKD citations (n=35)	*ren shen* 人参	Ginseng Radix Et Rhizoma	14
	wu wei zi 五味子	Schisandrae Chinensis Fructus	14
	fu ling 茯苓	Poria	12
	huang lian 黄连	Coptidis Rhizoma	10
	ji nei jin 鸡内金	Galli Gigerii Endothelium Corneum	9
	shu di huang 熟地黄	Rehmanniae Radix Praeparata	9
	huang qi 黄芪	Astragali Radix	8
	mu li 牡蛎	Ostreae Concha	7
	tu si zi 菟丝子	Cuscutae Semen	7
	ci shi 磁石	Magnetitum	6
	shan yao 山药	Dioscorea Rhizoma	6
	ze xie 泽泻	Alismatis Rhizoma	6
Conditional DKD citations (n=18)	*shu di huang* 熟地黄	Rehmanniae Radix Praeparata	11
	shan zhu yu 山茱萸	Corni Fructus	8
	gan cao 甘草	Glycyrrhizae Radix Et Rhizoma	6
	shan yao 山药	Dioscorea Rhizoma	6
	ze xie 泽泻	Alismatis Rhizoma	6
	chuan lian zi 川楝子	Toosendan Fructus	5
	dang gui 当归	Angelicae Sinensis Radix	5
	fu ling 茯苓	Poria	5
	huang qi 黄芪	Astragali Radix	5

Abbreviation: n: number of citations. Note: The use of some herbs may be restricted in some countries; readers are advised to comply with relevant regulations.

4.4 Discussion

4.4.1 Summary of findings

In the field of CM, historical medical literature spans more than 2500 years and is still of great value in guiding modern clinical practice. In the case of DKD, although there was no ancient disease name directly corresponding to the modern definition of DKD, descriptions consistent with clinical manifestations of DKD can be located by specific terms. These terms were basically typical symptoms and signs of diabetes and its complications.

The three terms employed as search terms in this study were *Shen Xiao*, *Xia Xiao* and X*iao Shen*. Nearly 300 citations with treatment information were identified by these search terms. Comparing the dynasty distribution and hit frequency in different citation pools of the three terms, it was observed that none of the terms was superior to the others in terms of identifying historical literature about DKD. Therefore, citations retrieved by any of the three terms were equally important as information sources.

Citations spanned from the Tang to the Qing dynasties in Chinese history. However, most citations were found in books written or published in the Ming Dynasty (1369–1644) and Qing Dynasty (1645–1911), most of which were official prescription monographs and medical encyclopaedias.

All included citations provided treatment information related to internal administration of herbal medicine. A variety of formulae with different indications were identified in the citations. Comparing the common formulae identified in different citations pools, *Liu wei di huang wan* and *Hui xiang san* were the most frequently cited formulae. The former and its modified formulae, such as *Shen qi di huang tang* 参芪地黄汤, *Qi ju di huang wan* 杞菊地黄丸 and *Ji shen shen qi wan* 济生肾气丸, have been widely used and are recommended in CM guidelines today [167-169]. This confirmed that information from CM historical literature can inform contemporary clinical practice.

But another commonly cited formula, *Hui xiang san*, was seldom applied or mentioned as treatment for DKD today. The reason could be the potential toxicity of *chuan lian zi*

川楝子 raising concerns in modern practice. There was a report of toxic hepatitis after taking the medicinal *ku lian* 苦楝, which has the same genus as *chuan lian zi* [179, 180]. Thus, the use of *Hui xiang san* is limited today.

There were approximately 150 unique herbs found in the treatment citations. The herbs commonly reported in different citation pools were mainly tonics and astringents with meridian entry into the Kidney. Several heat-clearing herbs and blood-activating herbs were also commonly mentioned. This indicated that elimination of pathogens and reinforcement of body essence were both important and should be considered together in the treatment of DKD. By comparing the common herbs list of the diseases with the lists of symptoms, *huang qi* 黄芪 was identified as specific for turbid urine.

Although the majority of common herbs in the historical literature have been used up until today, some herbs should be used with caution, especially in those with kidney impairment. For example, the ingredient *ci shi* 磁石 requires special processing from the raw material and it cannot be boiled in iron cookware.

4.4.2 Limitations

There were several limitations which may impact on the results. First of all, the search scope was limited by the search terms. Despite the selected terms being relatively specific to find the citations consistent with DKD more than other ancient terms, they were not broad enough to retrieve all citations potentially related to DKD. The number of included citations was relatively small. Future development of Boolean search functions in the ZHYD may enlarge the treatment citations pool and provide a more comprehensive evaluation.

Secondly, the lack of direct evidence to confirm the consistency between ancient terms and modern DKD is an unavoidable limitation in classical CM literature study. Although citations describing exclusive aetiologies or symptoms/signs of DKD were excluded and a rating system of relevance to DKD was developed, it cannot be assured that disorders described in the classical citations were equal to modern DKD.

Lastly, frequency analysis of formulae and herbs was conducted to find the most common treatment methods. But it should be noted that the most commonly cited formulae or herbs may not be the most effective interventions. It should also be noted that formulae and herbs identified from this study may not be specific for DKD. Some of them can be prescribed as treatments for other diseases. In the CM theory, some diseases with different causes may share the same pathogenesis, therefore they can be treated by identical formulae. For example, the formula *Liu Wei Di Huang Wan* is constructed with the function of replenishing *yin*, especially targeting on the Kidneys. Thus, *Liu Wei Di Huang Wan* is a treatment option for various diseases involved with Kidney *yin* deficiency.

4.5 Summary

The ancient disease terms *Shen Xiao, Xia Xiao* and *Xiao Shen* were useful in locating historical literature possibly related to DKD. The most frequently cited formulae in the classical literature were *Ba wei wan*, *Liu wei di huang wan* and *Hui xiang san*. Furthermore, the commonest herbs in classical literature for DKD treatment were those with tonifying Kidney, securing essence, clearing heat and activating blood action. The herb *huang qi* was frequently appeared in citations relating to turbid urine, indicating its potency in targeting relief of proteinuria.

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5 Chinese Herbal Medicine for Diabetic Kidney Disease: A Systematic Review and Meta-analysis of Randomised Placebo-controlled Trials

5.1 Introduction

Diabetic kidney disease (DKD) is one of the most common complications of diabetes. As the prevalence of diabetes continues to grow globally, it is estimated that the number of DKD patients will double by 2025 [181]. Since patients with DKD are at markedly higher risks of progression to end stage kidney disease (ESKD) and cardiovascular disease (CVD), the socioeconomic and public health burden of DKD is significant [3, 4]. Effective therapies in preventing and treating DKD are therefore of critical importance.

Glycaemic management, blood pressure (BP) control and renin–angiotensin system (RAS) inhibitors are the mainstay of treatment for DKD and has been successful in reducing risk of disease onset or progression [2, 5]. However, unmet needs exist in diabetes patients intolerant of or unresponsive to current pharmacotherapies, and those DKD patients with deteriorating renal function yet normo-albuminuria [66, 182, 183]. Some promising therapies addressing novel targets, such as sulodexide and bardoxolone methyl, have been found to be ineffective and/or harmful, while several others, including sodium-glucose cotransporter 2 (SGLT-2) inhibitors and mineralocorticoid receptor antagonist and phosphodiesterase inhibitors, are still under evaluation [155, 160, 184].

To facilitate the discovery of new therapeutic agents for patients with diabetes and impaired renal function, screening candidates from natural products including Chinese herbal medicine (CHM) which have been traditionally used for symptoms associated with this indication may offer insights into a more targeted approach for therapeutic development. With respect to CHM, relevant records of treatment of DKD symptoms in Chinese classical literature date back to the Han Dynasty (AD 202–220) and it has evolved to contemporary literature including randomised controlled trials (RCTs)

concerning the use of CHM for diabetes and its complications [170]. Multi-ingredient herbal decoctions, and manufactured products of *Abelmoschi Corolla* and *Cordyceps* have been recommended for patients with DKD in the clinical practice guidelines of Chinese Medicine (CM) [168, 185]. However, these guidelines were based on experts' consensus rather than outcomes of systematically evaluated, best available clinical evidence. Moreover, safety concerns exist due to the potential for aristolochic-acid nephrotoxicity with some herbal products [5, 186]. Even though legislation and quality control have been reinforced in recent years, the general lack of information regarding the safety profiles of some herbal formulae due to their multi-compound nature has limited their application [5, 6].

In recent years, there have been a growing number of clinical trials and systematic reviews of CHM for DKD but not of placebo-controlled trials. We therefore undertook a systematic review and meta-analysis of randomised, placebo-controlled trials to evaluate the efficacy and safety of oral CHM as adjunctive treatment for DKD.

5.2 Methods

This systematic review was conducted following the *Cochrane Handbook of Systematic Reviews of Interventions* and reported in accordance with the PRISMA guidelines [187, 188]. The protocol was registered in the PROSPERO database and can be accessed online (Registry number: CRD42015029293).

5.2.1 Search strategy

A comprehensive search was conducted in the following databases irrespective of publication status or language: MEDLINE, EMBASE, Cumulative Index of Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials (CENTRAL), Allied and Complementary Medicine Database (AMED), China BioMedical Literature (CBM), China National Knowledge Infrastructure (CNKI), Chonqing VIP (CQVIP) and Wanfang. The former five databases were in English while the latter four were in Chinese. Databases were searched from inception to May 2018. The US National Institutes of Health register (ClinicalTrials.gov), the Australian New Zealand Clinical Trial Registry (ANZCTR), the Chinese Clinical Trial Registry

(ChiCTR) and the European Union Clinical Trials Register (EU-CTR) were searched for completed but unpublished trials. Further, reference lists of related systematic reviews were reviewed for additional publications.

Search terms included “diabetic nephropathy”, “diabetic kidney disease”, “albuminuria”, “Traditional Chinese Medicine”, “randomised controlled trial” and their synonyms. All terms were mapped to controlled vocabulary (where applicable) in addition to being searched as keywords. The MEDLINE search strategy is provided in **Appendix 2**.

5.2.2 Eligibility criteria

Eligible studies had to fulfill the following criteria: (1) randomised controlled trial design; (2) included primary diabetes adults with persistent albuminuria/proteinuria, which was defined as an albumin excretion rate (AER) more than 20 μg/min, an albumin-to-creatinine ratio (ACR) larger than 30 mg/g [2, 5] or 24-hour proteinuria over 0.5 g/d (the overt DKD stage defined by Mogensen and used as in DKD diagnostic criteria in China) [189, 190]; (3) oral CHM as intervention which could have been either single or multiple ingredients in any form (decoction, granules, capsules etc.); (4) CHM-matched placebo was applied in the control group; (5) both intervention and control groups received the same conventional treatments of DKD, including comprehensive management of glycaemia, BP, serum lipid level, life-style and nutrition in accordance with Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines’ recommendation [2, 5]; and (6) the study reported at least one of the primary outcomes. Studies including patients with albuminuria that was not caused by diabetes, patients who already had ESKD or those receiving renal replacement therapy were excluded.

5.2.3 Outcomes of interest

Primary outcomes of interest included albuminuria/proteinuria, kidney function, number of participants progressing to ESKD, all-cause mortality and adverse events, at the end of treatment or follow-up. Progression to ESKD was defined as initiation of renal replacement therapy or estimated GFR (eGFR) lower than 15 mL/min/1.73m^2.

Kidney function was reflected by the measurement of serum creatinine (Scr) concentration and glomerulus filtration rate (GFR). Likewise, quantitative measurement of albuminuria and proteinuria included urinary albumin excretion rate (AER), albumin-to-creatinine ratio (ACR), 24-hour urine protein excretion (UP) and protein-to-creatinine ratio (PCR).

Secondary outcomes included cardiovascular mortality, all-cause hospitalisation, quality of life (QoL) measured by validated scales, indicators of risk factor control (such as fasting blood glucose, glycated haemoglobin [HbA1c], BP, total cholesterol, triglycerides, low-density lipoprotein cholesterol [LDLC], and high-density lipoprotein cholesterol [HDLC]). All outcomes were reported with specified units at the end of treatment or at the end of follow-up.

Safety outcomes included numbers and type of adverse events and serious adverse events during the study period.

5.2.4 Study selection and data extraction

Titles and abstracts identified in searching were screened by one reviewer and then checked by another (LZ and XQ) against the predefined criteria. After title and abstract screening, possibly relevant studies underwent full-text review by LZ and were cross-checked by LY to confirm their eligibility. Any disagreement was resolved by consensus and discussion with a third reviewer (JS or ALZ).

Two reviewers (LZ and LY) independently extracted data from eligible studies into a pre-designed spreadsheet. A third reviewer (JS) cross-checked the data. Study design characteristics, trial locations, demographic features (age, types of diabetes, baseline albuminuria, kidney function, etc.), intervention and control protocol (herbal ingredients, dosage, frequency, treatment duration, follow-up period, etc.) and outcome measures were recorded. Authors of studies with missing data were contacted by email or telephone to obtain additional data.

5.2.5 Data synthesis and analysis

All studies satisfying the eligibility criteria were included for qualitative synthesis. For continuous variables, mean and standard deviation of each study were obtained and pooled as mean difference (MD) or standardised mean differences (SMD) with a 95% confidence interval (CI). SMD was used in the meta-analysis of albuminuria and proteinuria outcomes due to the different scales used in the included studies such as microgram per minute (μg/min), milligram to gram (mg/g) and milligram per day (mg/24 hours). For dichotomous data, risk ratios (RR) were calculated with a 95% CI. Considering the diversity of interventions and potential heterogeneity among included studies, a random-effect model was applied in all meta-analyses. Review Manager Software (RevMan, version 5.3) was used to perform the statistical analysis [191].

Pre-defined subgroup analysis included baseline DKD severity and CHM formulae. Heterogeneity between studies was detected by using the Cochrane Q statistic and I^2 test. For outcomes with substantial heterogeneity (I^2 levels >50%), subgroup analyses were performed to explore potential sources, whereby results were stratified by factors such as different measured approaches for the same outcome. Sensitivity analysis was performed by excluding studies with high/unclear risk of bias in the domain of random sequence generation. Publication bias was explored when 10 or more studies were included in one meta-analysis by visual inspection of funnel plots for asymmetry.

5.2.6 Quality assessment

The methodological quality of each individual study was assessed by two reviewers (LZ and LY) in parallel according to the Cochrane Risk of Bias (ROB) Tool [192]. For the domain of other sources of bias, baseline imbalance and conflicts of interest were evaluated. Each domain was judged as high, low or unclear risk of bias with justifications. The consistency was checked by a third reviewer (LZ) and disagreements were resolved by discussion with methodologists (ALZ and XG).

To evaluate the overall quality of evidence for primary outcomes, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was applied [193]. A panel group was formed to make the GRADE evaluation, which

included methodologists, CM practitioners and conventional medicine physicians. The assessments of evidence started at “high quality” and were downgraded when significant risk of bias, indirectness, inconsistency, imprecision of estimated effect or publication bias were detected.

5.2.7 Patient and public involvement

Patients and the public were not directly involved in this systematic review.

5.3 Results

5.3.1 Description of studies

The comprehensive search retrieved over 50,000 citations and 7255 of them were examined in full-text (**Figure 5-1**). Eighty-five percent of the studies were excluded due to lack of a placebo control. As a result, 20 eligible studies with 23 publications involving 2719 DKD participants were included [194-216]. For studies with multiple reports, the most recent publication or the one with primary outcomes was used, and complementary outcomes data from other reports was extracted and merged.

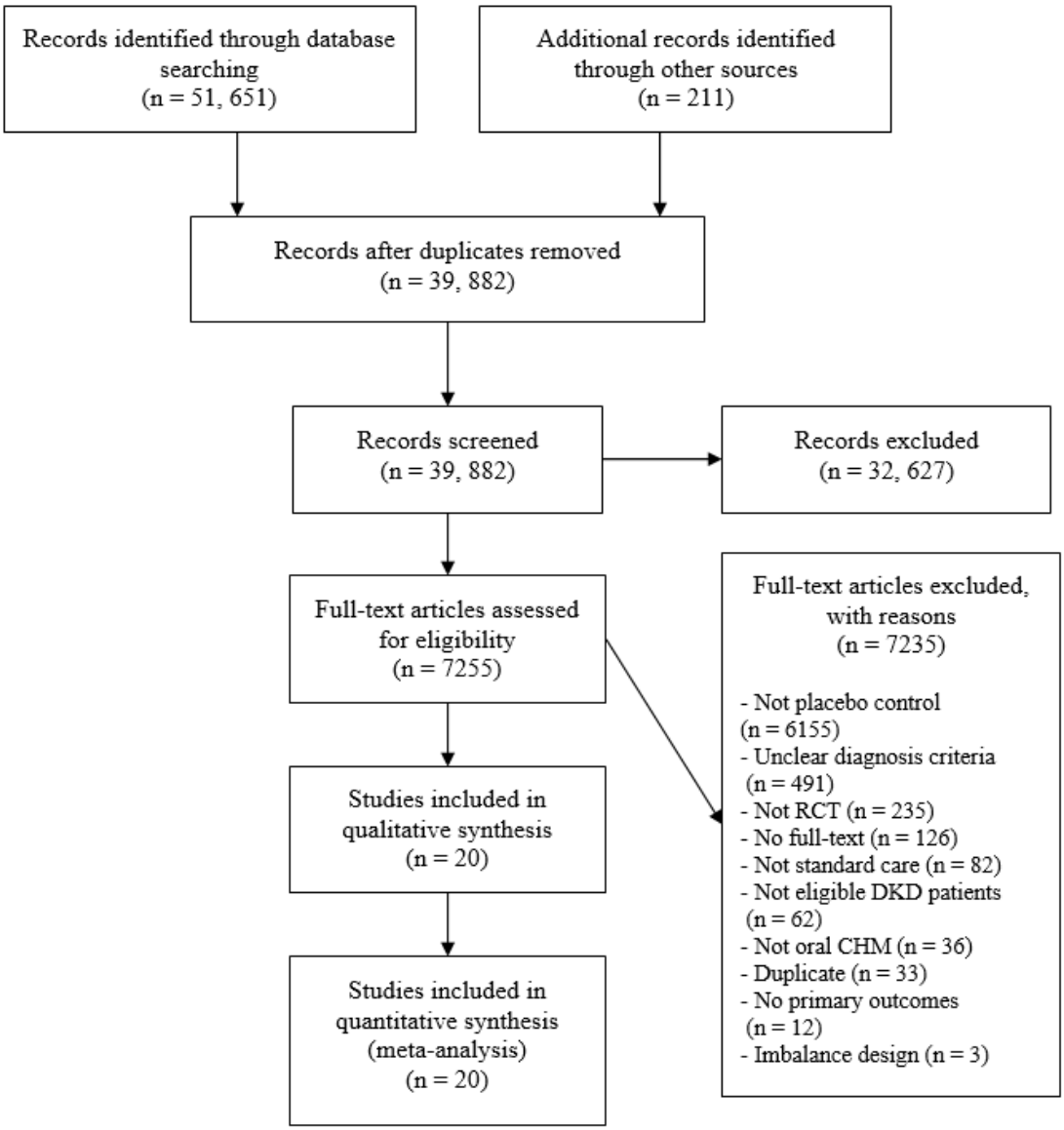

Figure 5-1 PRISMA flowchart

Abbreviation: CHM: Chinese herbal medicine; DKD: diabetic kidney disease; RCT: randomised controlled trial.

Characteristics of the included studies are summarised in **Table 5-1**. All 20 studies were conducted in China. Except for one study [194] written in English, all others were published in Chinese language between 2000 and 2017. Enrolled participants were all diabetic patients with persistent albuminuria or proteinuria but varied in terms of baseline kidney function. The mean age was 55.1 years old (range 20 to 79 years). Three studies [204, 205, 207] used herbal compounds or a single herb as intervention while

the remaining 17 studies used CHM formulae with multi-ingredients. The ingredients of CHM used in each study are provided in **Table 5-2**. The most common herbal ingredients used by ten or more studies was *Astragali radix* (15 trials), *Rehmanniae radix* (11 trials) and *Rhei radix et rhizoma* (10 trials). All studies applied CHM-matched placebo, except for one [202] which made Captopril (comparator) identical in appearance to CHM (intervention). Treatment duration ranged from 4 weeks to 2 years (median 3 months). There was no outcome data with respect to cardiovascular mortality and all-cause hospitalisation among the included studies.

Table 5-1 Characteristics of included studies

Study	Sample size (M/F)	Age	Diabetes type	Inclusion criteria of kidney function	Intervention and control protocol	Duration	Reported outcomes
Fan, 2010 [195]	61 (28/33)	59.6	2	Albuminuria 30–300 mg/g or 30–300 mg/24h	T: Qi Kui granule 1 bag bid C: placebo	12m	UAE; FBG
Jia, 2012 [200]	60 (29/31)	58.3	2	Proteinuria <3.5 g/24h; Normal Scr level	T: Qi Wei granule 4.5g tid C: placebo	3m	UAE; 24hUP
Ma, 2011a [204]	414 (186/ 228)	56.6	NS	Proteinuria ≤4.5 g/24h; Scr ≤190 μmol/L	T: Arctiin granule 1 bag tid C: placebo	2m	UAE; 24hUP
Ma, 2011b [205]	186 (78/108)	55.3	NS	Proteinuria ≤3.5 g/24h; Scr <176 μmol/L	T1: Arctiin granule 2 bag bid + placebo 2 bag qd T2: Arctiin granule 1 bag tid + placebo 1 bag tid C: placebo 2 bag tid	2m	UAE; 24hUP
Wei, 2012 [207]	56 (24/32)	50.6	NS	Albuminuria 30-300 mg/24h; Scr ≤105 μmol/L	T: Xue Zhi Kang capsule 0.6g tid C: placebo	3m	UAE; TC; TG; LDLC; HDLC
Wei, 2016 [208]	41 (32/9)	61.8	2	Albuminuria >30 mg/g and Proteinuria ≤3.5 g/24h GFR ≥30 mL/min	T: Gan Di capsue 3 pieces tid C: placebo	6m	Scr; FBG; A1C; TC; TG; LDLC; HDLC
Xie, 2011 [209]	67 (30/37)	62.3	2	Albuminuria 30–299 μg/mg	T: Liu Wei Di Huang pill 3g tid + Ginkgo biloba tablet 19.2mg tid C: LWDHW placebo + GBT placebo	24m	UAE; FBG; A1C; TC; TG; LDLC; HDLC; SBP; DBP
Yang, 2014 [210]	142 (80/62)	48.5	NS	Albuminuria 30–300 mg/24h; Normal Scr level	T: Qi Ming granule 4.5g tid C: placebo	3m	UAE; FBG; TC; TG; LDLC; HDLC
Zhou, 2014 [215]	48 (27/21)	58.5	2	Proteinuria ≤3.5 g/24h; Normal Scr level	T: Qi Wei granule 6g tid C: placebo	3m	UAE; 24hUP; Scr; GFR; FBG; A1C; SBP; DBP
Li, 2015	180	59.0	2	Albuminuria >20 μg/min or	T: Tang Shen granule 8g bid +	6m	UAE; 24hUP; Scr;

Study	Sample size (M/F)	Age	Diabetes type	Inclusion criteria of kidney function	Intervention and control protocol	Duration	Reported outcomes
[194]	(100/80)			Proteinuria 0.5–2 g/24h GFR 60–130 mL/min	ACEi/ARB C: placebo + ACEi/ARB		GFR; A1C; TC; TG; LDLC; HDLC; SBP; DBP; QoL
Liu, 2015 [203]	60 (NS)	20–70	2	Albuminuria 20–200 μg/min or Proteinuria ≤3.5 g/24h GFR >60 mL/min	T: Qi Huang capsule 1.9g tid + losartan C: placebo + losartan	6m	24hUP; Scr
Ni, 2013 [206]	224 (112/112)	54.7	NS	Albuminuria 20–200 μg/min or Proteinuria ≤3.5 g/24h GFR 60–130 mL/min	T: Qi Yao Xiao Ke capsule 2.4g tid + benazepril C: placebo + benazepril	3m	UAE; 24hUP; Scr; GFR; FBG; A1C; TC; TG; LDLC; HDLC
Yang, 2017 [211]	25 (23/2)	59.3	2	Albuminuria 20–200 μg/min or 30–300 mg/24h	T: Qi Zhu granule 1 bag bid + irbesartan C: placebo + irbesartan	6m	UAE; Scr; GFR FBG; A1C; TC; TG; LDLC; HDLC
Zhang, 2006 [212-214]	221 (119/102)	61.9	NS	Proteinuria <10 g/24h; Scr 133-354 μmol/L or Ccr 30–70 mL/min	T 1: Modified Qi Wei granule 1 bag bid + losartan T 2: Modified Qi Wei granule 1 bag bid + losartan simulant C: placebo + losartan	3m	24hUP; Scr; GFR; QoL
Gao, 2006 [196]	90 (NS)	35-70	2	Albuminuria 20–200 μg/min or 30–300 mg/24h	T: Tang Shen Ning granule 5g tid + benazepril simulant C: placebo + benazepril	2m	UAE; Scr;
Gao, 2017 [197]	250 (116/134)	52.3	2	Albuminuria 30–300 mg/24h	T: Tang Shen Ning granule 8g tid + losartan simulant C: placebo + losartan	3m	UAE; Scr; FBG; A1C
Han, 2014 [198]	104 (NS)	30-78	2	Proteinuria ≥0.5 g/24h Scr <265 μmol/L	T1: Bao Shen pill 1 bag bid + Tripterygium glycosides 20mg tid T2: Bao Shen pill 1 bag bid C: BS placebo + valsartan	1m	24hUP; Scr

Study	Sample size (M/F)	Age	Diabetes type	Inclusion criteria of kidney function	Intervention and control protocol	Duration	Reported outcomes
Jia, 2015 [199]	56 (31/25)	59.6	NS	Proteinuria <10 g/24h; Scr <265 μmol/L	T: San Huang Yi Shen granule 1 bag bid + irbesartan simulant C: placebo + irbesartan	3m	GFR
Li, 2012 [201, 216]	315 (194/121)	58.1	NS	Proteinuria <10 g/24h; Scr <265 μmol/L or GFR >40 mL/min;	T: Modified Qi Wei granule 4.5g bid C: placebo + irbesartan	24m	Mortality; Composite endpoints; QoL
Lin, 2000 [202]	119 (46/73)	55.3	NS	Proteinuria <0.5 g/24h; Normal Scr level	T: Tang Wei Kang capsule 2g tid C: Captopril (same appearance as herbal capsule)	3m	UAE; FBG; A1C; TC; TG; HDLC

Abbreviation: 24hUP: 24-hour proteinuria; A1C: glycated haemoglobin; bid: twice daily; C: control group; Ccr: creatinine clearance rate; DBP: diastolic blood pressure; FBG: fasting blood glucose; GFR: glomerular filtration rate; HDLC: high-density lipoprotein cholesterol; LDLC: low-density lipoprotein cholesterol; m: months; M/F: male versus female; NS: not specified in the original reports; qd: once daily; QoL: quality of life; SBP: systolic blood pressure; Scr: serum creatinine concentration; T: tested group; TC: total cholesterol; TG: triglycerides; tid: thrice daily; UAE: urinary albuminuria excretion.

Table 5-2 Herbal ingredients used in included studies

Study	Formulae name	Ingredients
Fan, 2010 [195]	Qi Kui granule	Astragali radix; polygoni multiflori radix; abelmoschi corolla
Jia, 2012 [200]	Qi Wei granule	Astragali radix; rehmanniae radix; rhei radix et rhizoma; prunellae spica; curcumae rhizoma; euonymus alatus; notoginseng radix et rhizoma
Ma, 2011a [204]	Arctiin granule	Arctii fructus
Ma, 2011b [205]	Arctiin granule	Arctii fructus
Wei, 2012 [207]	Xue Zhi Kang capsule	Fermentum rubrum*
Wei, 2016 [208]	Gan Di capsue	Scutellariae radix; astragali radix; corni fructus; rehmanniae radix phylianthi fructus; leonuri herba leonuri herba; bombyx batryticatus; sophorae flos (stir fry processed)
Xie, 2011 [209]	Liu Wei Di Huang pill Ginkgo biloba tablet	Rehmanniae radix; corni fructus; dioscoreae rhizoma; alismatis rhizoma; moutan cortex; poria; ginkgo folium
Yang, 2014 [210]	Qi Ming granule	Astragali radix; puerariae lobatae radix; rehmanniae radix; lycii fructus; cassiae semen; leonuri fructus; typhae pollen; hirudo
Zhou, 2014 [215]	Qi Wei granule	Astragali radix; rehmanniae radix; rhei radix et rhizoma; prunellae spica; curcumae rhizoma; euonymus alatus; notoginseng radix et rhizoma
Li, 2015 [194]	Tang Shen granule	Astragali radix; rehmanniae radix; rhei radix et rhizoma; notoginseng radix et rhizoma; euonymus alatus; corni fructus; aurantii fructus
Liu, 2015 [203]	Qi Huang capsule	Astragali radix; rehmanniae radix; ligustri lucidi fructus; hirudo; bombyx batryticatus; eupolyphaga steleophaga; rhei radix et rhizoma; *gymnema sylvestre**; sinomenii caulis; plantaginis semen
Ni, 2013 [206]	Qi Yao Xiao Ke capsule	Panacis quinquefolii radix; astragali radix; rehmanniae radix; dioscoreae rhizoma; corni fructus; lycii fructus; ophiopogonis radix; anemarrhenae rhizoma; trichosanthis radix; puerariae lobatae radix; schisandrae chinensis fructus schisandrae chinensis fructus; galla chinensis
Yang, 2017 [211]	Qi Zhu granule	Astragali radix; ligustri lucidi fructus; atractylodis macrocephalae rhizoma; abelmoschi corolla; rosae laevigatae fructus dioscoreae spongiosae rhizoma; paeoniae radix rubra; coptidis rhizoma
Zhang, 2006 [212-214]	Modified Qi Wei granule	Astragali radix; rehmanniae radix; prunellae spica; rhei radix et rhizoma; euonymus alatus; epimedii folium; corni fructus; curcumae longae rhizoma

Study	Formulae name	Ingredients
Gao, 2006 [196]	Tang Shen Ning granule	Astragali radix; rehmanniae radix; euryales semen; corni fructus; rhei radix et rhizoma; chuanxiong rhizoma
Gao, 2017 [197]	Tang Shen Ning granule	Astragali radix; euryales semen; rosae laevigatae fructus; rhei radix et rhizoma; chuanxiong rhizoma
Han, 2014 [198]	Bao Shen pill; Tripterygium glycosides	Not given.
Jia, 2015 [199]	San Huang Yi Shen granule	Astragali radix; curcumae longae rhizoma; rhei radix et rhizoma; chuanxiong rhizoma; angelicae sinensis radix; salviae miltiorrhizae radix et rhizoma; cervi cornu; anemarrhenae rhizoma; arctii fructus
Li, 2012 [201, 216]	Modified Qi Wei granule	Astragali radix; rehmanniae radix; prunellae spica; rhei radix et rhizoma; euonymus alatus; epimedii folium; corni fructus; curcumae longae rhizoma
Lin, 2000 [202]	Tang Wei Kang capsule	Astragali radix; ligustri lucidi fructus; rhei radix et rhizoma

Note: All ingredients are the standardised pharmaceutical name from the Chinese Pharmacopoeia 2015. * *Monascus purpureus* Went. (Red Rice Yeast); pharmaceutical name not included in Chinese Pharmacopoeia 2015.

5.3.2 Quality of studies

Generally, the quality of included studies was fair with low or unclear risk of bias, especially regarding blinding and outcome data completeness (**Figure 5-2**). Two studies were judged at high risk of bias with respect to blinding of patients and personnel because blinding may have been compromised by prescription of unequal numbers/amounts of medication between groups [198, 201]. Twelve studies reported correct procedures for random sequence generation [194, 195, 198-200, 204-206, 208, 211, 214], whereas eight studies did not provide adequate details. For the domain of allocation concealment, one study did not conceal the allocation to researchers, thus was judged at high risk of bias [211]. Seven studies were considered to have high risk of selection reporting bias (mainly incomplete reporting in secondary outcomes) [195, 196, 199, 201, 203, 208, 214], with13 studies were at unclear risk because protocols were not found. Other bias asseassment included baseline balance and conflict of interest. Two studies included pharmaceutical industry employees as co-authors without statements regarding their roles in the study, therefore these two trials were judged as high risk for potential conflicts of interest [204, 205]. Seven studies without baseline statistical test results or without information regarding sources of funding were judged to be at unclear risk [196, 198, 201, 206, 207, 209, 210].

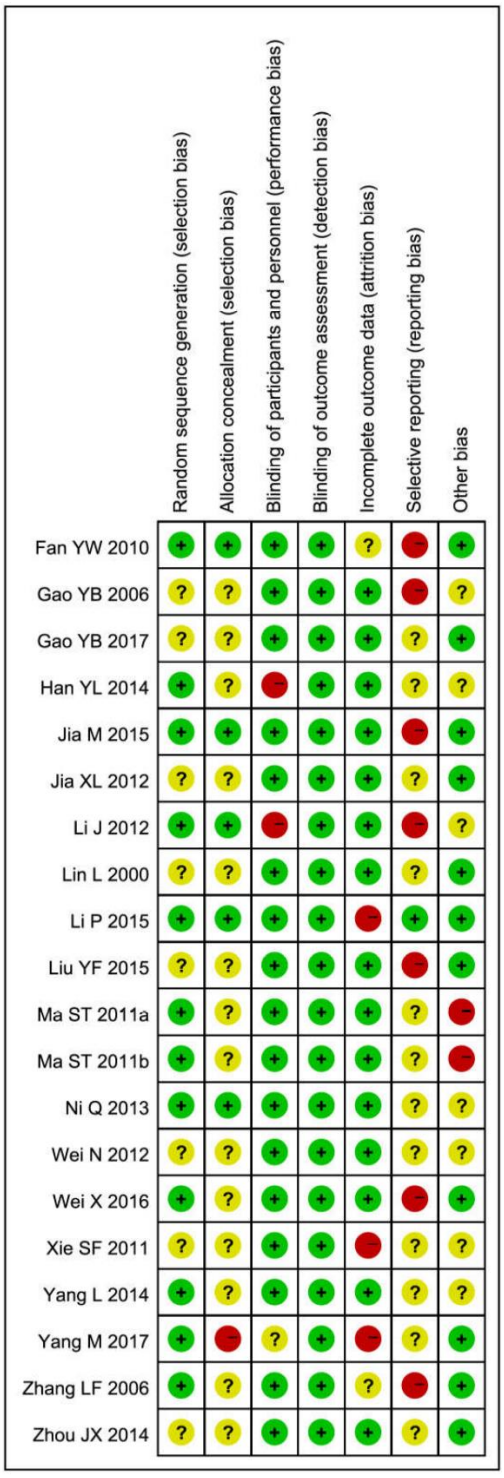

Figure 5-2 Risk of bias of included studies

Note: The red, yellow and green dots represent high, unclear and low risk of bias of the included study in each domain, respectively.

5.3.3 Efficacy of Chinese herbal medicine

Considering the use of RAS blockage may affect the primary outcomes, studies were categorised and separated into three groups according to trial application of RAS blockade (angiotensin-converting enzyme inhibitors [ACEi] and/or angiotensin receptor blockers [ARB]) in each arm prior to meta-analysis. It should be noted that conventional concurrent treatments of DKD recommended by guidelines were applied equally in both groups in all included studies, such that these conventional treatments are not separately mentioned henceforth. The three groups were:

- CHM versus placebo [195, 200, 204, 205, 207-210, 215];
- CHM plus ACEi/ARB versus placebo plus ACEi/ARB [194, 203, 206, 211, 214]; and
- CHM versus placebo plus ACEi/ARB [196-199, 201, 202, 214, 216].

5.3.3.1 Mortality and progression to ESKD

Although all-cause mortality was measured in a study [201] comparing CHM with matched placebo plus Irbesartan, no deaths were observed among the 315 participants during the two-year follow-up (**Table 5-3**). Within the same trial, the number of patients that progressed to ESKD was reported as part of a composite outcome, measured the number of patients with microalbuminuria progressing to macroalbuminuria, doubling of serum creatinine from baseline or initiating of dialysis. Compared with placebo plus Irbesartan, the risk of experiencing this composite outcome may be 66% lower in the CHM group over the two-year period (RR: 0.34, 95% CI [0.15, 0.77], p =0.01; low-quality evidence).

Table 5-3 Summary of findings table

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)
	Risk with placebo	Risk with CHM			
Comparison 1: CHM versus Placebo					
Albuminuria follow-up: range 2 to 12 months	–	SMD 0.92 lower (1.35 lower to 0.51 lower)	–	1021 (8 RCTs)	⊕⊕⊕◯ MODERATE [a, b]
24-hour proteinuria follow-up: range 2 to 3 months	–	SMD 1.34 lower (2.18 lower to 0.51 lower)	–	699 (4 RCTs)	⊕⊕◯◯ LOW [a, b, c]
Serum creatinine (Scr) follow-up: range 3 to 6 months	The mean Scr was 77.41 μmol/L	The mean Scr in the intervention group was 5.75 μmol/L higher (2.06 lower to 13.57 higher)	–	85 (2 RCTs)	⊕⊕⊕◯ MODERATE [a, d]
Estimated glomerular filtration rate (eGFR) follow-up: mean 3 months	The mean eGFR was 96.24 mL/min	The mean eGFR in the intervention group was 10.71 mL/min lower (23.93 lower to 2.51 higher)	–	44 (1 RCT)	⊕⊕◯◯ LOW [a, d]
Comparison 2: Placebo + ACEi/ ARB versus CHM +ACEi/ARB					
Albuminuria follow-up: range 3 to 6 months	–	SMD 0.56 lower (1.04 lower to 0.08 lower)	–	330 (3 RCTs)	⊕⊕⊕◯ MODERATE [d, e]
24h-proteinuria follow-up: range 3 to 6 months	–	SMD 0.15 lower (0.52 lower to 0.23 higher)	–	489 (4 RCTs)	⊕⊕◯◯ LOW [b, d, e]
Serum creatinine (Scr) follow-up: range 3 to 6 months	The mean Scr was 88.13 μmol/L	The mean Scr in the intervention group was 4.02 μmol/L lower (7.81 lower to 0.23 lower)	–	595 (5 RCTs)	⊕⊕⊕◯ MODERATE [a, c]
Estimated glomerular filtration rate (eGFR) follow-up: range 3 to 6 months	The mean eGFR was 79.27 mL/min	The mean eGFR in the intervention group was 6.28 mL/min higher (2.42 higher to 10.14 higher)	–	535 (4 RCTs)	⊕⊕⊕◯ MODERATE [c, e]
Comparison 3: CHM versus Placebo + ACEi/ ARB					
All-cause mortality follow-up: mean 24 months	0 per 1000	0 per 1000 (0 to 0)	not estimable	315 (1 RCT)	⊕⊕⊕◯ MODERATE [f]
Composite end-points events follow-up: mean	133 per 1000	45 per 1000 (20 to 102)	RR 0.34 (0.15 to 0.77)	315 (1 RCT)	⊕⊕◯◯ LOW [d, g]

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of particip ants (studies)	Quality of the evidence (GRADE)
	Risk with placebo	Risk with CHM			
24 months					
Albuminuria follow-up: mean 3 months	–	SMD 6.38 lower (9.01 lower to 3.75 lower)	–	499 (3 RCTs)	⊕◯◯◯ VERY LOW [a, b, d]
24h-proteinuria follow-up: range 1 to 3 months	–	SMD 0.00 lower (0.32 lower to 0.32 higher)	–	260 (2 RCTs)	⊕⊕⊕◯ LOW [d, h]
Serum creatinine (Scr) follow-up: range 1 to 3 months	The mean Scr was 105.52 μmol/L	The mean Scr in the intervention group was 4.05 μmol/L lower (6.09 lower to 2.01 lower)	–	590 (4 RCTs)	⊕⊕⊕◯ MODERATE [a, c]
Estimated glomerular filtration rate (eGFR) follow-up: range 1 to 3 months	The mean eGFR was 97.24 mL/min	The mean eGFR in the intervention group was 0.57 mL/min lower (11.01 lower to 9.88 higher)	–	542 (4 RCTs)	⊕⊕◯◯ LOW [a, b, c]

Abbreviations: CI: confidence interval; MD: mean difference; RR: risk ratio; SMD: standardised mean difference.

Note: *The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). GRADE justification: a. Unclear risk of bias of randomisation and allocation concealment; b. Significant heterogeneity; c. Wide confidence interval; d. Small sample size and wide confidence interval; e. High or unclear risk of attrition bias; f. Low events rate leading to imprecise estimation and small simple size; g. Number of patients progressing to ESRD were included in composite outcomes, not solely reported; h. Unclear risk of attrition bias and potential selecting report bias.

5.3.3.2 Albuminuria

Fourteen studies reported albuminuria at the end of treatment (**Figure 5-3)**. Based on meta-analysis of eight studies [195, 200, 204, 205, 207, 209, 210, 215] involving 1021 participants, use of CHM probably lowered end-of-study albuminuria compared to

placebo over 2 to 12 months' intervention (SMD -0.92, 95% CI [-1.35, -0.51], I^2=87%, p <0.0001; moderate-quality evidence). Subgroup analysis suggested different CHM formulae could be the sources of heterogeneity (**Appendix 3**). The estimate of effect with the least heterogeneity was observed in the Qi Wei granule CHM subgroup [200, 215] in which albuminuria was 70.06 mg/24h lower compared to placebo after 3 months (95% CI [-88.84, -51.28], I^2=0%, p <0.0001). Likewise, the Arctiin granule [204, 205] reduced albuminuria more than placebo after 2 months' intervention (SMD -0.38, 95% CI [-0.56, -0.20], I^2=0%, p <0.0001).

When used in combination with ACEi/ARB, a slightly lower end-of-treatment albuminuria level was still observed in the CHM relative to the placebo group over a 3 to 6 months interval (SMD -0.56, 95% CI [-1.04, -0.08], I^2=64%, p =0.002; moderate-quality evidence) [194, 206, 211]. However, although lower albuminuria excretion was reported in the CHM group [196, 197, 202], the effect of CHM in decreasing albuminuria compared to ACEi/ARB was uncertain because of the very low quality of evidence (**Table 5-3**).

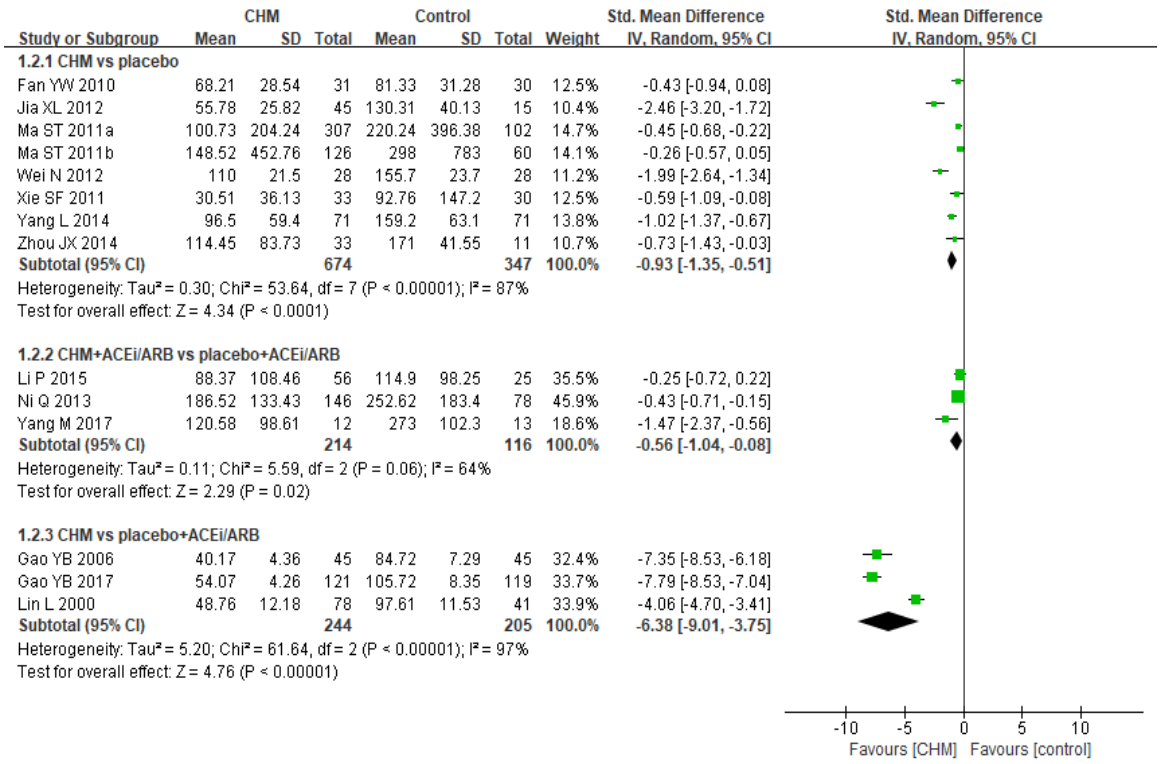

Figure 5-3 Forest plot of albuminuria outcomes

Abbreviations: ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; CHM: Chinese herbal medicine.

5.3.3.3 Proteinuria

Nine studies measured end-of-treatment 24-hour proteinuria (**Figure 5-4**). The pooled estimated effect showed CHM may reduce proteinuria compared to placebo after 2 to 3 months' intevention, although heterogeneity was high (SMD -1.34, 95% CI [-2.18, -0.51], I^2=94%, p =0.002; low-quality evidence) [200, 204, 205, 215]. Subgroup analysis revealed that different formulae and proteinuria scales may have been the source of heterogeneity (**Appendix 3**). Pooled estimates of effects of Qi Wei granule [200, 215] and Arctiin granule [204, 205] both showed that CHM may lead to greater reductions in proteinuria than placebo. Subgroup of measurement unit of milligram per 24-hour showed proteinuria was 324.42 mg/24h lower (95% CI, [-485.15, -163.69]; I^2=30%; p <0.0001) in the CHM group than the placebo group [204, 205, 215].

When used in combination with ACEi/ARB, meta-analysis of four studies with 489 participants [194, 206, 214] showed that CHM may make little or no difference to proteinuria compared to placebo after 3 to 6 months' intervetions (SMD -0.15, 95% CI [-0.52,0.23], I^2=72%, p =0.44; low-quality evidence). Sources of heterogeneity were not identified (**Appendix 3**). Likewise, low-quality evidence suggested that CHM may make no differences to end-of-treatment proteinuria compared to placebo plus ACEi/ARB after 1 to 3 months' intervention (**Table 5-3**) [198, 214].

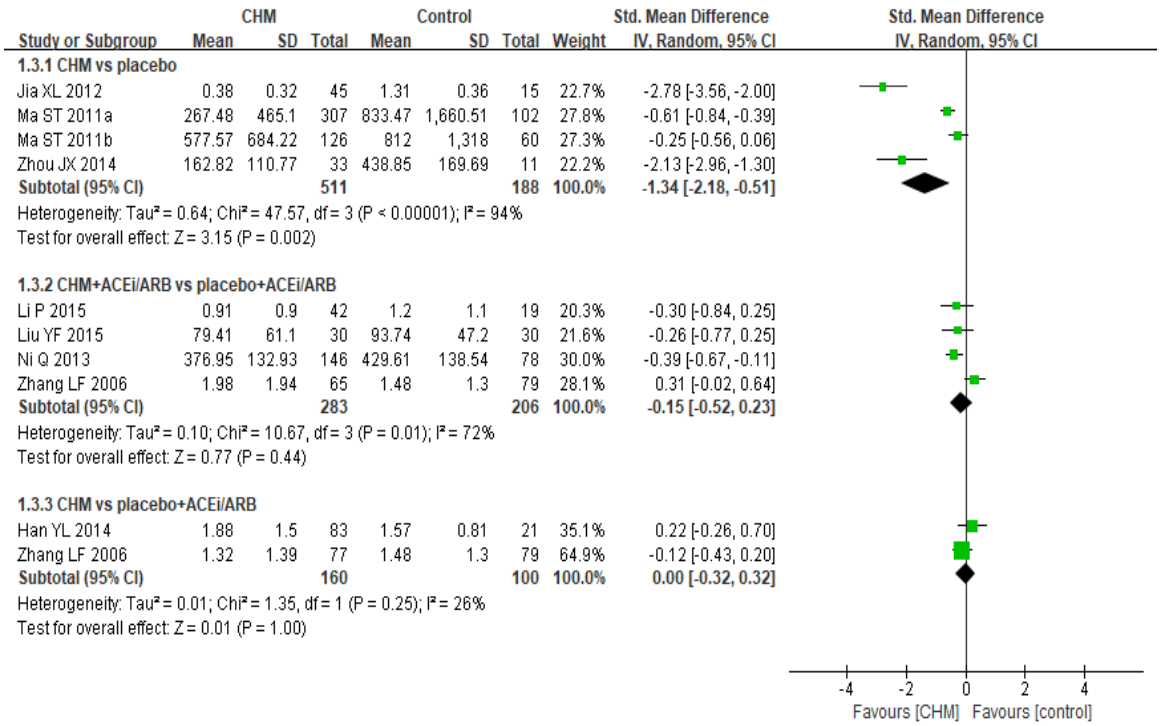

Figure 5-4 Forest plot of proteinuria outcomes

Abbreviations: ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; CHM: Chinese herbal medicine.

5.3.3.4 Serum creatinine level

Ten studies provided end-of-treatment data of serum creatinine (Scr) level (**Figure 5-5**). Pooled estimation of two small studies [208, 215] showed that the additional CHM

intervention probably made little difference to Scr level compared with placebo after 3 to 6 months (MD 5.75 μmol/L, 95% CI [-2.06, 13.57], I^2=0%, p =0.15; moderate-quality evidence). When used in combination with ACEi/ARB, end-of-treatment Scr level was slightly lower in the CHM than the placebo group over 3 to 6 months, but was not clinically significant (MD -4.02 μmol/L, 95% CI [-7.81, -0.23], I^2=0%, p =0.15; moderate-quality evidence) [194, 203, 206, 211, 214]. Subgroup analysis found that the lowering Scr effect of CHM was evident in patients with abnormal baseline Scr after 3 months' intervention (MD -9.99 μmol/L, 95% CI [-17.71, -2.26], I^2=0%, p =0.01) [206, 214].

Slightly lower Scr levels were observed in the CHM group compared to placebo plus ACEi/ARB group after 1 to 3 months' intervention, but the difference was not clinically significant [196-198, 214]. A similar effect was found in the subgroup analysis of Tang Shen Ning formula compared to placebo plus ARB in 2 to 3 months' treatment (MD -3.96 μmol/L, 95% CI [-6.13, -1.78], I^2=6%, p =0.0004) [196, 197].

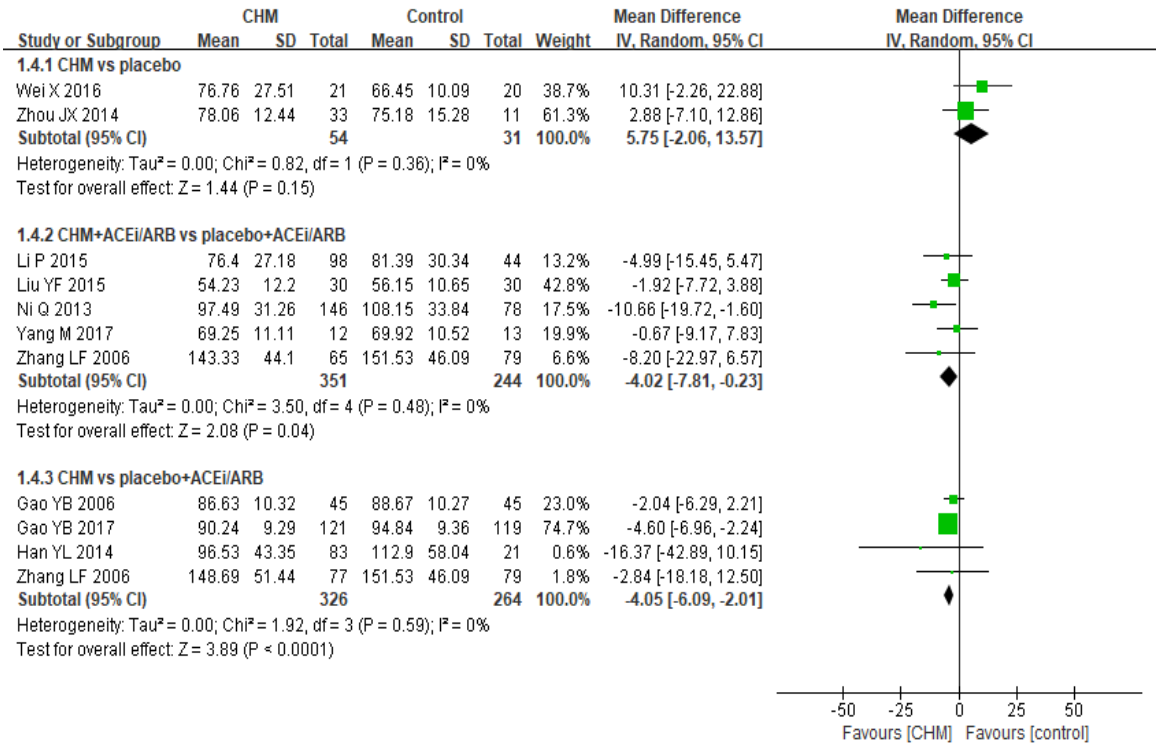

Figure 5-5 Forest plot of serum creatinine outcomes

Abbreviations: ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; CHM: Chinese herbal medicine.

5.3.3.5 Estimated glomerular filtration rate

Of the eight studies, the GFR was estimated by either the Cockcroft-Gault equation or other serum creatinine-based equations (**Figure 5-6**). When CHM was used in combination with ACEi/ARB, the end-of-treatment eGFR was slightly higher in the CHM group compared to the placebo group after 3 to 6 months' intervention (MD 6.28 mL/min, 95% CI [2.42, 10.14], I^2=0%, p =0.001; moderate-quality evidence) [194, 206, 211, 214]. Subgroup analysis of specific formulae showed that the end-of-treatment eGFR was 5.22 mL/min higher (95% CI [0.69, 9.74], I^2=0%, p =0.02) in the Tang Shen Fang formula plus ACEi/ARB group than the placebo plus ACEi/ARB group [194, 214]. It should be noted that Cockcroft-Gault equation may overestimate eGFR, leading to 10-20% higher value in pooled estimation of eGFR than the actual eGFR and these

positive results should be interpreted cautionsly.

One small study (44 participants) provided low-quality evidence that CHM made no difference to placebo in terms of eGFR after 3 months' intervention (**Table 5-3**) [215]. When compared CHM to placebo plus ACEi/ARB, meta-analysis results indicated that no significant difference in eGFR over 1 to 3 months' treatment (low-quality evidence; **Table 5-3**) [196, 197, 199, 214].

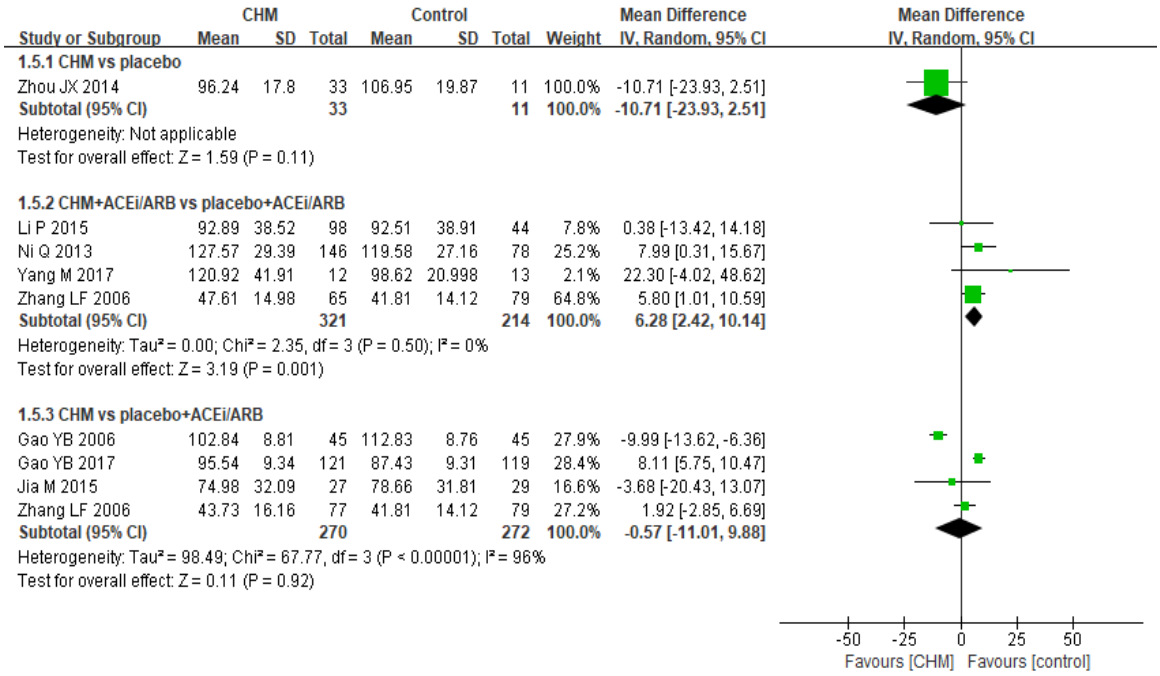

Figure 5-6 Forest plot of estimated glomerular filtration rate outcome

Abbreviations: ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; CHM: Chinese herbal medicine.

5.3.3.6 Secondary outcomes

Meta-analysis results of secondary outcomes are summarised in **Table 5-4**. When compared to placebo, the pooled estimated effects for both fasting blood glucose (FBG) [195, 197, 202, 206, 208-211] and HbA1c [194, 197, 202, 206, 208, 209, 211, 215] did

not show additional benefit of CHM in lowering blood glucose. Likewise, summarised effects from three studies showed no statistical differences between the CHM and placebo groups for systolic and diastolic BP [194, 209, 215]. CHM resulted in lower levels of total cholesterol [194, 202, 206-211], triglycerides [194, 202, 206-211] and LDLC [194, 206-211], although HDLC levels [194, 202, 206-211] were not statistically significant compared to placebo. However, the results were limited by substantial heterogeneity and the reason was not found. Three studies [194, 212, 216] measured patients' QoL by questionnaire at the end of treatment but only two studies used the Diabetes QoL tool and provided usable data. The pooled estimation suggested no statistically significant differences between the CHM and the placebo groups regarding QoL [194, 216].

Table 5-4 Meta-analysis results of secondary outcomes

Outcome	Studies	Participants	Effect estimate (95% CI)	I^2	*p* value
Fasting blood sugar	9	962	-0.45 [-1.15, 0.25]	93%	*p* =0.21
Haemoglobin A1c	8	901	0.04 [-0.17, 0.24]	59%	*p* =0.73
Total cholesterol	8	815	-0.96 [-1.70, -0.21]	95%	*p* =0.01
Triglyceride	8	815	-0.60 [-1.01, -0.19]	90%	*p* =0.004
LDLC	7	696	-0.51 [-0.93, -0.09]	92%	*p* =0.02
HDLC	8	815	0.14 [-0.04, 0.33]	93%	*p* =0.12
Systolic BP	3	252	0.64 [-0.90, 2.17]	0%	*p* =0.43
Diastolic BP	3	252	0.14 [-2.02, 2.29]	52%	*p* =0.90
Diabetes QoL score	2	461	0.07 [-3.87, 4.00]	54%	*p* =0.97

Abbreviation: BP: blood pressure; CI: confidence interval; HDLC: high-density lipoprotein cholesterol; LDLC: low-density lipoprotein cholesterol; QoL: quality of life.
Note: All outcomes analysed with mean difference.

5.3.4 Safety evaluation of Chinese herbal medicine therapy

Data on adverse events was provided in 14 studies. Of these, 7 studies stated no adverse events (AEs) were observed during the study period [197, 199, 200, 202, 203, 208, 215].

In total, 53 cases of AEs were reported in 7 studies with 1445 participants. Except for Li's study [201], details of AEs in each group were reported. The most common AE of CHM was digestive system disorders (18 cases), including abdominal pain and diarrhea or sloppy stools [204, 205, 211]. Both the CHM and control groups reported a modest number of cases of elevated liver enzyme levels (11 cases), infection (2 cases) or anaemia (3 cases) [194, 198, 214]. In a three-arm study [214], one case of hypertension in the CHM group, one case of hypotension in the losartan group and one case of hyperkalaemia in the CHM plus losartan group were reported. All participants who experienced the above AEs recovered after discontinuation of the tested interventions. Three cases of serious AEs, including two cases of death and a case of acute myocardial infarction (AMI), were reported in Li's trial [194]. One participant in the CHM group died due to subarachnoid haemorrhage while another participant died after AMI. The researchers reported that these serious AEs were not related to the study agent.

5.3.5 Sensitivity and subgroup analysis

Sensitivity analysis by excluded studies with substantial risk of bias regarding randomisation showed consistent results with the primary analysis, except for the comparison of CHM versus placebo plus ACEi/ARB in terms of Scr level (**Appendix 4**). Subgroup analysis indicated that baseline kidney function, different CHM formulae and outcome measurment scale could partially explain the variant treatment effect of primary outcomes (**Appendix 3**). Publication bias was not evaluated due to the limited number of studies included in each outcome.

5.4 Discussion

This review included 20 RCTs involving 2719 participants and evaluated the effects and safety of CHM in addition to conventional therapies for DKD. As an adjunctive therapy, CHM may decrease proteinuria (either measured as urinary albumin or protein

excretion) in DKD patients compared with placebo, regardless of concomitant use of ACEi/ARB. When CHM and ACEi/ARB were used simultaneously, eGFR improved compared to ACEi/ARB alone but studies had measurement shortfalls that may have overestimated the effect. CHM appeared to be well tolarated in DKD patients and no significant adverse events causal to CHM interventions were reported. These results suggest potential short-term renal benefit by adding CHM to conventional pharmacotherapies in DKD populations. However, due to the short follow-up periods and small numbers of clinical events in terms of mortality and progression to ESKD the long-term benefit of CHM is yet to be determined.

This study demonstrated that CHM may be applied as an add-on treatment for DKD to achieve better renal outcomes. From the clinical perspective, the short-term albuminuria/proteinuria reduction effect of CHM identified in this review is moderate when compared to placebo. In patients with chronic kidney disease (CKD), the early reduction in albuminuria is associated with lower risk of incident ESKD or doubling of Scr level, particulally in those patients with baseline albuminuria greater than 30mg/g [217]. Therefore, for the subgroup of DKD patients who are contraindicated for ACEi/ARB, CHM may offer some benefit. When used in combination with ACEi/ARB, the lowering albuminuria/proteinuria effect of CHM is mild to moderate from a clinical perspective. Considering the failure of dual RAS inhibitor therapy, CHM could be a potential option for those DKD patients who are on ACEi/ARB to achieve greater albuminuria/proteinuria reduction in the short term. The combination of CHM and ACEi/ARB may also be beneficial in improving eGFR, especially for patients who experienced acute drop of eGFR after early RAS inhibitor initiation.

Findings from this review are in line with those of previous reviews focusing on single herbs or particular formulae. Li et al. reviewed the clinical effect of preparations of *Astragali radix* in DKD patients, finding that *Astragali* injection lowered Scr, increased

eGFR and reduced proteinuria based on data from 21 RCTs and 4 non-randomised controlled trials [218]. In published reviews of *Ginkgo folium* extract and *Xue Zhi Kang* capsules, lower FBG and HbA1c levels in the CHM group were reported [219, 220]. The inconsistency in terms of the glycaemic outcomes may have been due to differences in ingredients among the included studies. In our review, only two trials applied either *Ginkgo folium* extract or *Xue Zhi Kang* capsules as interventions. The glycaemic control effect may have been diluted by other trials using various herbal ingredients which targeted the kidney rather than glycaemic control. It should also be noted that the studies included in the previous reviews of *Ginkgo folium* extract and *Xue Zhi Kang* capsules resulted in significant risk of bias (including publication bias). Thus, rigorous and large-scale clinical trials are needed to confirm the glycaemic control effects of CHM in DKD patients.

The renal protective effect of CHM may be related to particular bioactive compounds contained in the herbal ingredients included in these RCTs. The most frequently used herb was *Astragali radix*. Both *in vitro* and *in vivo* studies have indicated that chemical components of *Astragali radix,* such as Astragaloside IV and Astragalus saponin I, exert anti-oxidant and anti-inflammatory properties in diabetic models [221, 222]. These chemicals can prevent and restore kidney tissue injury related to oxidative stress. Additionally, Astragaloside IV can reduce endoplasmic reticulum stress and increase podocyte integrity, which is the therapeutic target for decreasing albuminuria [223, 224]. The second most frequently used herb, *Rehmanniae Radix*, also upregulates anti-inflammatory and antioxidant effects in diabetic rats [225]. Furthermore, anti-diabetic properties were observed in its constituent compound (catalpol) and ethanolic extract [226]. Although the glucose-lowering effect of *Rehmanniae Radix* was not superior to metformin, its use was associated with higher anti-inflammatory activity, lower oxidative stress levels and restoration of diabetes-induced kidney lesions. The third most frequent herb was *Rhei Radix et Rhizoma*. Active compounds of *Rhei Radix et*

Rhizoma, including anthraquinones (rhein and emodin) and phenolic acids (gallic acid and ferulic acid), have been shown to protect the kidneys by reducing oxidative stress, inflammation, fibronectin and extracellular matrix accumulation [227-229]. Furthermore, *in vitro* experiments have demonstrated that extracts of *Rhei Radix et Rhizoma* can inhibit lipid peroxidation and lower serum lipid levels, which are risk factors for diabetes and DKD progression [230, 231].

Renal toxicity induced by aristolochic acid (AA) has been a concern since a series of renal failure cases caused by AA-contaminated products were reported [232, 233]. In our review, the CHM used in included studies appeared to be well-tolerated and safety signals were not identified. This could be related to the fact that all herbal ingredients investigated were free from AA and some of the studies mentioned strict quality control processes regarding the CHM raw materials and manufacturing procedures [194, 201, 214]. Mortality risk reduction effect of non-AA prescribed CHM was indicated in a CKD population study, but for DKD patients, the long-term safety of CHM requires further studies to confirm [234].

Although this review was conducted in a systematic and comprehensive manner, there are limitations that should be taken into account when interpreting the findings. Firstly, the number of included studies was relatively small and few studies measured and reported the same outcomes consistently. This caused difficulty in meta-analysis and introduced heterogeneity across studies and led to downgrading the quality of evidence. Even meta-analyses with low heterogeneity may not be reliable because there were only a very small number of included studies in the subgroup analyses (less than or equal to three studies in each subgroup). In addition, the positive effect of CHM in eGFR outcomes is dominated by a study using the Cockcroft–Gault equation (64.8% weight), leading to possible overestimation of eGFR value, as it is less precise than the guideline recommended equations [235]. Core outcome sets with standardised measurements are

needed in future studies to rigorously assess the effect of CHM. Secondly, most of the studies had short follow-up periods (1–3 months) and small sample sizes, leading to imprecision of the estimated effect and low centainty with regards to long-term benefit and effect on renal function and clinical outcomes. Thirdly, more than half of the included studies did not provide information on randomisation and allocation procedures, such that the impact of potential selection bias was unclear. Although the CHM formulae were processed as granules or capsules in order to achieve blinding, quality assurance information for each CHM preparation was not provided in most of the studies. Further studies are strongly encouraged to report following the CONSORT reporting guidelines with sufficient details regarding the manufacture and quality control of investigated CHM [236-238]. Finally, although we did not limit the CHM interventions in terms of herbal composition, five included studies shared highly homologous CHM ingredients synthesis [194, 200, 201, 214, 215], thereby limiting the diversity of CHM treatments evaluated.

Since the participants in most included trials were older adults with a GFR greater than 60 mL/min, the renal protective effect of CHM in younger individuals and in advanced kidney disease is uncertain. Moreover, all included studies were conducted in China, such that the effect of CHM reported in this review may not be generalisable to other population groups. It should further be noted that, in most of the included studies, the forms of CHM used were multi-ingredient herbal formulae which were constructed based on traditional CM theory and experts' clinical experience. While indicative of pharmacological studies, the most frequently used ingredients discussed above may not necessarily be relevant to the observed effects reported in this study.

5.5 Conclusion

In conclusion, combination of CHM with conventional RAS inhibitors showed promise as an adjunctive treatment for improving renal function and decreasing urinary albumin

and protein excretion in patients with DKD. The rate of occurrence of AE was low and the tested CHM appeared to be well-tolerated. This systematic review also provided potential candidate formulae and frequently used herbs for further investigation. Well-designed RCTs following reporting guidelines with adequate sample sizes and longer follow-up periods are warranted to confirm the long-term efficacy and safety of CHM, especially with respect to patient-oriented outcomes such as mortality, disease progression and QoL.

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6 *Astragalus Membranaceus* (*Huang Qi*) as an Adjunctive Therapy for Diabetic Kidney Disease: An Updated Systematic Review and Meta-analysis

6.1 Introduction

Diabetic kidney disease (DKD) develops in approximately one-third of diabetic patient and is asymptomatic in the early stage but rapidly deteriorates [1]. It has been the leading cause of chronic renal failure in developed countries for decades and is increasingly prevalent in developing regions [72, 79, 239]. DKD causes a large socioeconomic burden, as diabetes and impaired renal function relate to higher risks of mortality and cardiovascular events [3, 78, 80]. The angiotensin-converting enzyme inhibitor (ACEi) and angiotensin II receptor blockers (ARB) are cornerstone therapies for DKD, with recent advances of renoprotection in certain sodium glucose co-transporter 2 (SGLT2) inhibitors [1, 5, 240]. However, current pharmacotherapies are compromised by the potential harms of acute kidney injury, hyperkalaemia, hypoglycemia, diabetic ketoacidosis and lower limb amputation [241-243]. As diabetes increases around the world, novel therapies to limit DKD progression are needed.

Chinese herbal medicine (CHM) has been extensively used in east Asia for centuries. In existing historical literature, descriptions highly consistent with symptoms of DKD as it is understood in contemporary clinical practice were commonly seen, and some medical terms specifically referred to DKD-like manifestations [170]. Text-mining of ancient texts found that *Astragalus membranaceus* (Fisch.) BGE. var. *mongholicus* (BGE.) Hsiao (family name: Fabaceae; common name: astragalus root or milk-vetch root; Chinese name: *Huang qi*) was frequently used for DKD-like discomfort [244]. According to Chinese medicine (CM) theory, *A. membranaceus* is capable of raising

yang qi and tonifying the Spleen and Lung *qi*, therefore facilitating urination and reducing oedema [245]. The chemical constitutions of *A. membranaceus* are diverse with over 100 compounds isolated from the crude herb, including flavonoids, saponins, polysaccharides, amino acids and some trace elements [245, 246]. A systematic review of 11 animal studies provided preliminary evidence that *A. membranaceus* preparations (single-herb decoction or extract) were promising in lowering glycaemia and albuminuria excretion, relieving glomerular hyperfiltration and reducing glomerular membrane thickness for early stages of DKD [247]. Clinically, *A. membranaceus* is still widely prescribed for DKD today and is recommended in several experts' consensus-based practice guidelines in China [167, 185]

Despite *A. membranaceus* being used for a long time for DKD in Chinese medicine, it is still unclear as to the true extent of its efficacy and safety. Generally, herbal supplements are not recommended for patients with chronic kidney disease (CKD) in conventional medicine guidelines due to the lack of high-quality evidence, nephrotoxicity of some herbal compounds and unclear herb–drug interaction [2, 248]. Although benefits of *A. membranaceus* in improving kidney function and reducing albuminuria were reported in a systematic review of 25 controlled trials, the certainty of evidence was compromised by the low quality of included studies, high heterogeneity and imprecise meta-analysis results. Additionally, the previous review failed to assess different types of *A. membranaceus* preparations, to clarify the concurrent uses of ACEi or ARB and to sufficiently report adverse events (AEs) [218]. Considering the large consumption of *A. membranaceus* preparations in China, it is imperative to rigorously evaluate the efficacy and safety of *A. membranaceus* as adjunctive therapy to conventional treatments for DKD.

6.2 Methods

This systematic review followed the methods of the *Cochrane Handbook of Systematic*

Reviews and complied with the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) reporting guideline [187, 188]. The review protocol was registered with PROSPERO before commencement (CRD: 42017081403).

6.2.1 Information sources and searches

Five English and four Chinese databases were searched from inception to October 2017. Databases included MEDLINE, Embase, Cumulative Index of Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials (CENTRAL), Allied and Complementary Medicine Database (AMED), China Biomedical Literature (CBM), China National Knowledge Infrastructure (CNKI), Chonqing VIP (CQVIP) and Wanfang. There was no limitation on language and publication status. References of published systematic reviews with similar topics were examined for relevant studies. Clinical trial registries of the WHO, USA, China, European Union, Australia and New Zealand were searched for unpublished or ongoing trials. Terms and synonyms representing the concepts of DKD and *A. membranaceus* were used in the database searches (search strategy is shown in **Appendix 5**).

6.2.2 Study selection and eligibility criteria

Two reviewers independently performed the title/abstract and full-text screening process, and a third reviewer checked for consistency. Disagreements of screening results were resolved by discussion with methodologists. Randomised controlled trials (RCTs) that enrolled adults (aged 18 years and over) diagnosed with DKD secondary to primary diabetes and used *A. membranaceus* preparations in addition to conventional therapies as intervention were eligible. All forms of *A. membranaceus* preparations including boiled decoction, extracts, granules and injections were eligible. Studies that assessed *A. membranaceus* combined with other herbal ingredients or complementary therapies were not included. All participants received conventional therapies according

to clinical practice guidelines, including ACEi or ARB.

The primary outcomes were all-cause mortality, number of patients progressing to end stage kidney disease (ESKD), change in albuminuria and proteinuria excretion, change in kidney function and AEs. Secondary outcomes included quality of life (QoL), glycaemia level, blood pressure (BP) and blood lipids profile. Studies that failed to report at least one of the above primary outcomes were excluded. In addition, studies including participants with non-diabetic albuminuria, patients already in ESKD or those undertaking renal replacement therapy were excluded.

6.2.3 Data collection and quality assessment

Two reviewers independently extracted data from eligible studies, using a pre-designed Microsoft Excel spreadsheet. Information regarding study characteristics (participants' age, gender, diabetes types, baseline kidney function, DKD severity, sample size, follow-up period etc.), intervention protocol of *A. membranaceus* (administration route, dosage, frequency, and duration), concurrent conventional therapies (hypoglycaemia drugs, use of renin–angiotensin system [RAS] inhibitors etc.) and outcome data was collected. Consistency of extracted data was examined by a third reviewer. When necessary, authors of the included studies were contacted by email or telephone to clarify details or to acquire additional information about their trials.

Two reviewers evaluated the methodological quality of included studies in parallel based on the Cochrane Risk of Bias Tool [192]. Methodologists were consulted when consensus was not reached. For individual studies, each domain of the risk of bias tool was graded as high, unclear or low risk of bias with justifications.

6.2.4 Data synthesis and analysis

Studies fulfilling the eligibility criteria with valid outcome data were included in the analysis. Dichotomous data is presented as risk ratio (RR) with 95% confidence intervals (CI) and continuous data is presented as mean difference (MD) or standardised mean difference (SMD) with 95% CI. A random effects model was used in meta-analysis. Statistical analysis was performed in RevMan 5.3 and Stata software 11.0 (Stata Corp. College Station, Texas, USA) [191]. A p value less than 0.05 was considered statistically significant.

Treatment effects were analysed separately based on different administration routes. Heterogeneity across studies was detected by using the Cochrane Q statistic and I^2 test. An I^2 greater than 50% was considered to represent substantial heterogeneity, and subgroup analysis and random effects univariate and multivariate meta-regression was conducted to explore potential sources of variants. The pre-specified subgroups were baseline kidney function, treatment duration and outcome measured methods, and the post-hoc covariate was dosage of *A. membranaceus* preparation. Sensitivity analysis including studies with low risk of bias in randomisation was conducted to assess robustness of the results. Publication bias was also examined by visual inspection of funnel plots for asymmetry and Egger's linear regression test for outcomes with ten or more included studies.

6.3 Results

6.3.1 Study selection

There were 7872 citations retrieved from databases and an extra 143 citations collected from the reference lists of published systematic reviews (**Figure 6-1**). Among the 436 full-text articles screened, a total of 66 eligible studies were identified [249-314]. The most common reasons for exclusion were non-randomized controlled study design

(35.5%), neither ACEi nor ARB used (23.8%) and combined use of other investigated agents (11.7%). All included studies were performed in China and published in Chinese language medical journals from 2000 to 2017.

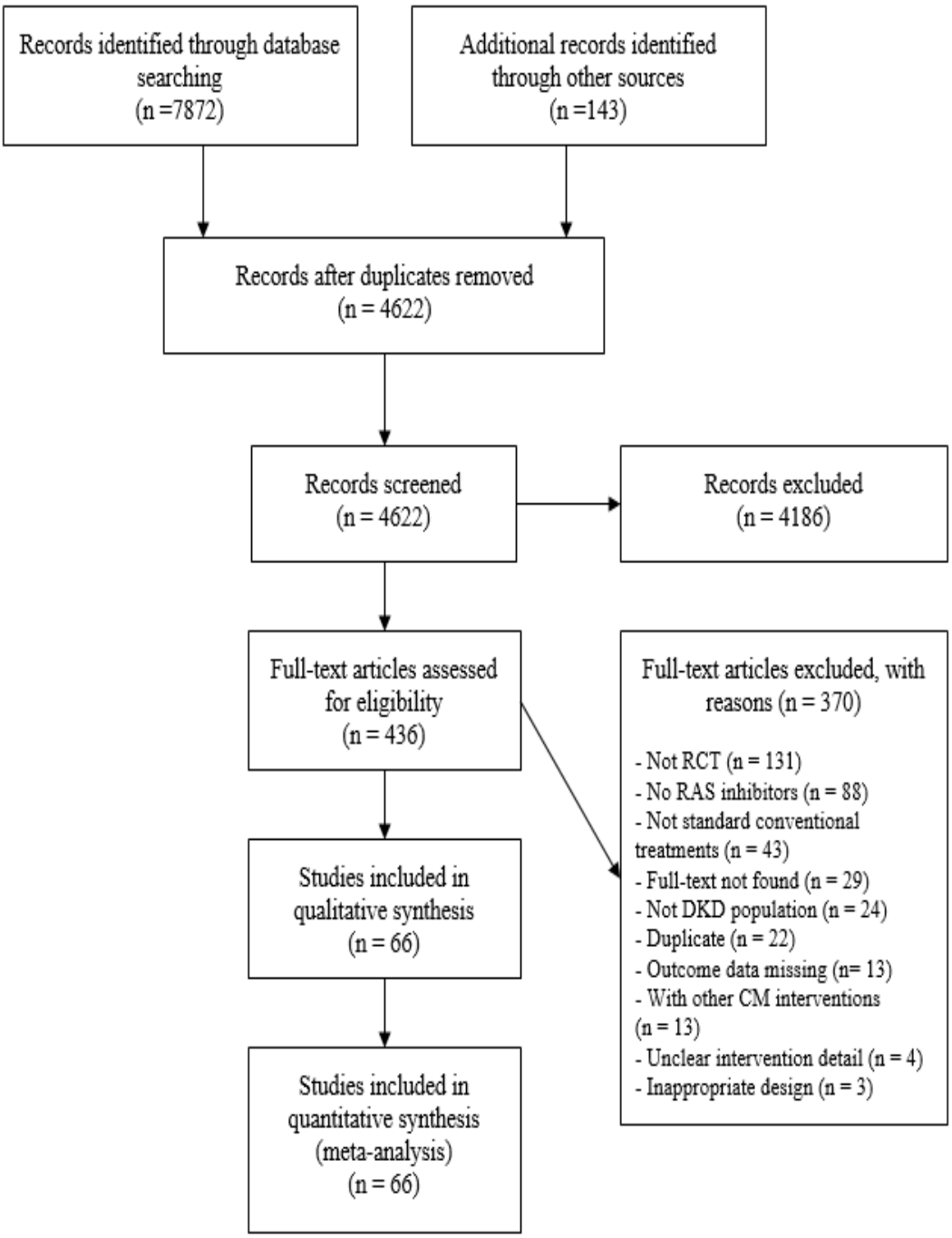

Figure 6-1 PRISMA flowchart

6.3.2 Study characteristics

Details of study characteristics are presented in **Table 6-1**. A total of 4785 DKD participants were included in the 66 eligible studies, with a mean age of 53.5 years (range from 18 to 84). The numbers of male and female participants were similar (2,725 versus 2072, respectively). Twenty-seven studies only enrolled participants with type 2 diabetes [249, 253, 256-259, 263, 264, 269, 270, 272, 278, 280, 282, 283, 285, 290, 295, 297, 298, 301, 303, 307, 311], two studies enrolled both type 1 and type 2 diabetic patients [296, 312] and other studies did not specify type but only that participants had a diagnosis of diabetes [250-252, 254, 255, 260, 262, 265-268, 271, 273, 274, 276, 277, 279, 281, 284-289, 291, 293, 294, 299, 300, 302, 304-306, 308-310, 313, 314]. Thirty-two studies recruited DKD patients in the early stage (micro-albuminuria) [249-252, 256-261, 264, 267-271, 275, 276, 282, 285, 286, 291, 295, 297, 298, 301, 303, 304, 306, 309, 311, 313], while six studies also included those with overt nephropathy (macro-albuminuria) [253, 289, 290, 295, 312]. Five studies included DKD patients presented that with mild to moderate declined kidney function [255, 272, 278, 283, 299].

In most of the studies (61 RCTs), *A. membranaceus* preparation was administered in the form of intravenous drip injection, which is a sterilised water extract of *A. membranaceus* [249-257, 259-288, 290-294, 296-300, 302-309, 311-314]. The dose of *A. membranaceus* injection varied from 20 to 60 mL per day and 40 mL was the most common dose. Oral *A. membranaceus* preparations were applied in five studies, including the forms of tablet [258], granule [295], decoction [289, 310] and extract liquid [301]. Treatment duration was on average four weeks, ranging from one to 12 weeks. Both participants of intervention and controlled groups received matched conventional therapies of DKD, including glycaemic and BP control, dyslipidaemia regulation, and either ACEi or ARB. Most of the studies reported at least one laboratory test outcome of albuminuria, proteinuria or kidney function, but none of them measured the clinical outcomes of mortality or number of patients progressing to ESKD.

Table 6-1 Study characteristics

Study	Diabetes type	Baseline albuminuria and renal function	Participants (male/female)	Age mean (range)	*A. membranaceus* preparations, dose & frequency	RAS inhibitors, dose & frequency	Treatment duration
Chen, 2010 [251]	NS	microalbuminuria	112(58/54)	46.73 (NS)	injection, 20 mL, qd	Irbesartan 150 mg, qd	28 days
Chen, 2015 [252]	NS	NS	100(59/41)	63.19 (38-78)	injection, 30 mL, qd	Telmisartan (dose not given)	2 weeks
Chen, 2008 [253]	T2DM	macroalbuminuria	60(34/26)	56.5 (51-78)	injection, 20 mL, qd	Benazepril 10 mg, qd	4 weeks
Chen, 2012 [254]	NS	microalbuminuria	78(35/43)	47.71 (NS)	injection, 40 mL, qd	Benazepril 10 mg, qd	6 weeks
Cong, 2013 [255]	NS	mild-moderate renal function impaired	98(60/38)	43.3 (18-65)	injection, 40 mL, qd	Enalapril 10 mg, qd or Valsartan 80 mg, bid	4 weeks
Cui, 2011 [257]	T2DM	microalbuminuria; Normal renal function	60(34/26)	50.6 (24-68)	injection, 30 mL, qd	Benazepril 10 mg, qd or bid	12 weeks
Cui, 2009 [256]	T2DM	microalbuminuria	72(49/23)	51.2 (35-70)	injection, 50 mL, qd	Captopril 25 mg, tid	4 weeks
Dou, 2014 [258]	T2DM	microalbuminuria; Normal renal function	48(25/23)	52.5 (NS)	tablet, 4 pieces, bid	Valsartan 80 mg, qd	12 weeks
Geng, 2010 [259]	T2DM	microalbuminuria	60(26/34)	58 (39-73)	injection, 60 mL, qd	Benazepril 10 mg, qd	4 weeks
Hu, 2010 [260]	NS	microalbuminuria; Normal renal function	40(27/13)	NS (51-72)	injection, 40 mL, qd	Valsartan 80 mg, qd	4 weeks
Huan, 2010 [261]	T2DM	microalbuminuria	40(21/19)	68.95 (54-82)	injection, 30 mL, qd	Losartan 100 mg, qd	4 weeks
Huang, 2013 [262]	NS	NS	100(NS)	67.05 (43-82)	injection, 20 mL, qd	Losartan 50 mg, qd	4 weeks
Huang, 2004 [263]	T2DM	NS	58(34/24)	57.71 (42-74)	injection, 40 mL, qd	Benazepril 10-30 mg, qd	4 weeks
Jiang, 2005 [265]	NS	Normal renal function	62(34/28)	57.11 (43-74)	injection, 30 mL, qd	Benazepril 10 mg, qd	1 month
Jiang, 2010 [264]	T2DM	microalbuminuria	98(47/51)	62.98 (NS)	injection, 30 mL, qd	Telmisartan 80 mg, qd	3 months
Kong, 2015 [266]	NS	NS	78(37/41)	53 (NS)	injection, 20 mL, qd	Benazepril 10 mg, qd	3 weeks
Li, 2008 [268]	NS	microalbuminuria	78(42/36)	60.6 (38-76)	injection, 30 mL, qd	Captopril 6.25-50 mg, qd	1 month

Study	Diabetes type	Baseline albuminuria and renal function	Participants (male/female)	Age mean (range)	*A. membranaceus* preparations, dose & frequency	RAS inhibitors, dose & frequency	Treatment duration
Li, 2017 [269]	T2DM	microalbuminuria	110(65/45)	NS (48-73)	injection, 50 mL, qd	Irbesartan 150 mg, qd	1 month
Li, 2007 [267]	NS	microalbuminuria; Normal renal function	76(49/27)	NS (48-71)	injection, 40 mL, qd	Captopril 25 mg, tid	2 weeks
Li, 2011 [270]	T2DM	microalbuminuria; Normal renal function	60(48/42)	60 (40-83)	injection, 60 mL, qd	Irbesartan 150-300 mg, qd	6 weeks
Li, 2010 [271]	NS	microalbuminuria	70(44/26)	57.52 (31-72)	injection, 50 mL, qd	Telmisartan 40 mg, qd	4 weeks
Liang, 2002 [272]	T2DM	mild-moderate renal function impaired	59(42/17)	53.82 (50-70)	injection, 30 mL, qd	Benazepril 5-20 mg, qd	4 weeks
Liu, 2004 [273]	NS	NS	36(21/15)	NS (23-70)	injection, 20 mL, qd	Captopril 12.5 mg, tid	4 weeks
Liu, 2013 [274]	NS	NS	56(32/24)	59.6 (47-65)	injection, 40 mL, qd	Valsartan 80 mg, qd	30 days
S. Liu, 2015 [275]	T2DM	microalbuminuria; Serum creatinine level < 133 μmol/L	56(29/27)	67.25 (NS)	injection, 40 mL, qd	Irbesartan 150 mg, qd	4 weeks
Liu, 2010 [277]	NS	Normal renal function	43(25/18)	62.5 (NS)	injection, 40 mL, qd	Irbesartan 150 mg, qd	15 days
X. Liu, 2015 [276]	NS	microalbuminuria	72(41/31)	53.9 (NS)	injection, 50 mL, qd	Benazepril 10 mg, qd	30 days
Liu and Yu, 2004 [278]	T2DM	Serum creatinine level < 265 μmol/L	56(32/24)	72 (56-84)	injection, 40 mL, qd	Valsartan 80 mg, qd	4 weeks
Ma, 2006 [279]	NS	NS	168(98/70)	53.2 (40-76)	injection, 40 mL, qd	Enalapril 10 mg, qd	4 weeks
Ma, 2016 [280]	T2DM	NS	86(49/37)	43.65 (35-76)	injection, 40 mL, qd	Valsartan 80 mg, qd	1 week
Qi, 2017 [281]	NS	NS	138(68/70)	39.49 (30-60)	injection, 30 mL, qd	Captopril 12.5 mg, bid	4 weeks
Qiu, 2011 [282]	T2DM	microalbuminuria	120(70/50)	47.98 (32-65)	injection, 50 mL, qd	Valsartan 80 mg, bid	4 weeks
Shen, 2006 [283]	T2DM	mild-moderate renal function impaired	56(34/22)	56.5 (40-73)	injection, 40 mL, qd	Benazepril 10-30 mg, qd	4 weeks

Study	Diabetes type	Baseline albuminuria and renal function	Participants (male/female)	Age mean (range)	*A. membranaceus* preparations, dose & frequency	RAS inhibitors, dose & frequency	Treatment duration
Shi, 2015 [284]	NS	NS	84(48/36)	42.98 (34-78)	injection, 40 mL, qd	Valsartan 80 mg, qd	1 week
Sun, 2014 [285]	T2DM	microalbuminuria	76(40/36)	52.24 (39-75)	injection, 20 mL, qd	Telmisartan 40 mg, qd	2 weeks
Tang, 2016 [286]	NS	microalbuminuria	144(87/57)	52.31 (41-68)	injection, 20 mL, qd	Valsartan 80 mg, qd	4 weeks
Tian, 2009 [287]	NS	NS	80(39/41)	49.75 (NS)	injection, 50 mL, qd	Enalapril 10 mg, qd	2 months
Tuo, 2011 [288]	NS	NS	51(47/51)	54.6 (32-76)	injection, 40 mL, qd	Quinapril 10 mg, qd	4 weeks
Wang, 2012 [289]	NS	micro-macroalbuminuria	26(14/12)	NS (42-70)	decoction, 30 grams, bid	Enalapril 5 mg, bid	30 days
Wang, 2003 [290]	T2DM	micro-macroalbuminuria	62(28/34)	NS (49-78)	injection, 30 mL, qd	Losartan 50 mg, qd	12 weeks
Wang, 2008 [291]	NS	microalbuminuria; Normal renal function	69(39/30)	NS (39-71)	injection, 40 mL, qd	Irbesartan 150 mg, qd	1 month
Wang, 2010 [292]	T2DM	NS	48(26/22)	42.6 (25-68)	injection, 20 mL, qd	ACEi or ARB	2 weeks
Wen, 2004 [293]	NS	NS	57(40/17)	54.96 (38-72)	injection, 30 mL, qd	Captopril 12.5 mg, bid or tid	4 weeks
Wu, 2005 [294]	NS	NS	53(58/34)	53 (40-68)	injection, 20 mL, qd	Captopril 25 mg, tid	3 weeks
Xiang, 2016 [295]	T2DM	microalbuminuria	67(31/36)	57.96 (NS)	granules, 4 grams, bid	Valsartan 80 mg, qd	3 months
G. Xu, 2008 [296]	T1 & T2DM	macroalbuminuria	52(28/24)	53.16 (26-72)	injection, 20 mL, qd	Telmisartan 80 mg, qd	6 weeks
X. Xu, 2008 [297]	T2DM	microalbuminuria	80(47/33)	49.22 (39-70)	injection, 60 mL, qd	Losartan 50 mg, qd	4 weeks
Xu, 2009 [298]	T2DM	microalbuminuria	56(27/25)	49.2 (34-68)	injection, 40 mL, qd	Valsartan 80 mg, qd	4 weeks
R. Yang, 2006 [299]	NS	micro-macroalbuminuria; 34 cases renal insufficiency	92(56/36)	54.04 (35-68)	injection, 20 mL, qd	Captopril 12.5 mg, tid	6 weeks
Y. Yang, 2006 [300]	NS	NS	62(43/19)	NS (56-82)	injection, 40-50 mL, qd	Losartan 50-100 mg, qd	4 weeks
Yao, 2015 [301]	T2DM	microalbuminuria	60(40/20)	51.2 (35-70)	extract liquid, 20 mL, qd	Captopril 25 mg, tid	8 weeks
Zeng, 2010 [249]	T2DM	microalbuminuria	52(31/21)	50.55 (35-74)	injection, 40 mL, qd	Enalapril 10 mg, qd	4 weeks

Study	Diabetes type	Baseline albuminuria and renal function	Participants (male/female)	Age mean (range)	*A. membranaceus* preparations, dose & frequency	RAS inhibitors, dose & frequency	Treatment duration
Zeng, 2007 [250]	NS	microalbuminuria; Normal renal function	80(46/34)	56.23 (47-71)	injection, 20 mL, qd	Benazepril 10 mg, qd	45 days
D. Zhang, 2014 [302]	NS	NS	60(33/27)	57 (40-73)	injection, 40 mL, qd	Benazepril 10 mg, qd	3 weeks
Zhang, 2007 [303]	T2DM	microalbuminuria	50(28/22)	58.2 (NS)	injection, 30 mL, qd	Candesartan 4 mg, qd	30 days
Q.Zhang, 2014 [304]	NS	microalbuminuria	56(33/23)	51.33 (41-70)	injection, 60 mL, qd	Losartan 50 mg, qd	4 weeks
Zhang, 2009 [306]	NS	microalbuminuria; Normal renal function	68(39/29)	NS (51-72)	injection, 30 mL, qd	Valsartan 80 mg, qd	4 weeks
Zhang, 2000 [305]	NS	NS	35(16/19)	NS (21-63)	injection, 20 mL, qd	Captopril 12.5 mg, tid	4 weeks
Zhang, 2001 [307]	T2DM	NS	60(41/19)	56 (42-76)	injection, 40 mL, qd	Benazepril 5 mg, qd	4 weeks
Zhang, 2017 [308]	NS	NS	178(123/55)	54.95 (32-80)	injection, 20 mL, qd	Valsartan 80 mg, qd	3 weeks
Zhang, 2012 [309]	NS	microalbuminuria	60(29/31)	51.65 (NS)	injection, 20 mL, qd	Valsartan 80 mg, qd	3 weeks
Zhao, 2011 [310]	NS	NS	61(35/26)	53.02 (38-63)	decoction, 60 grams, qd	Benazepril 10 mg, qd	NS
Zhao, 2013 [311]	T2DM	microalbuminuria	112(65/47)	45.6 (31-59)	injection, 40 mL, qd	Irbesartan 150 mg, qd	1 month
Zhao, 2003 [312]	T1 & T2DM	macroalbuminuria	48(29/19)	53.6 (26-72)	injection, 20 mL, qd	Enalapril 10 mg, qd	45 days
Zheng, 2013 [313]	NS	microalbuminuria	51(28/23)	44.86 (25-72)	injection, 60 mL, qd	Losartan 50 mg, qd	4 weeks
Zhong, 2002 [314]	NS	NS	91(42/49)	NS (24-72)	injection, 30 mL, qd	Benazepril 5 mg, qd	1 month

Abbreviations: bid: twice a day; mg: milligram; mL: millilitre; NS: not specified; qd: once a day; T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus; tid: thrice a day.

6.3.3 Quality assessment

The overall methodology quality of included studies was low, because of unmasked participants and personnel, unclear randomised and allocated procedures, and potential risk of selective reporting (**Table 6-2**). None of the included studies used blinding methods or placebo control to reduce performance bias. Details of approaches to generate a random sequence were only provided in nine studies [252, 256, 259, 276, 284, 286, 299, 304, 313] and 65 out of 66 eligible studies did not report how they concealed allocation sequence. All studies were judged at low risk of bias in terms of blinding of assessment because of the nature of laboratory test outcomes. Over 70% of studies were judged at low risk of attrition bias because of no withdrawals or losses to follow-up during the study period.

Table 6-2 Summary of risk-of-bias assessment of included studies

Risk of Bias Domain	Low risk	Justification	Unclear risk	Justification	High risk	Justification
Random sequence generation	13.6% (9)	Random number chart or coin tossing	86.4% (57)	Details not given	0	–
Allocation concealment	0	–	98.5% (65)	Not mentioned	1.5% (1)	Coin toss
Blinding of participants and personnel	0	–	0	–	100% (66)	Blinding not used
Binding of outcome assessment	100% (66)	Laboratory outcomes unlikely influenced by not blinding	0	–	0	–
Incomplete outcome data	72.7% (48)	No withdrawals or losses to follow-up	27.3% (18)	Attrition number not mentioned	0	–
Selective reporting	0	–	97.0% (64)	Protocol not found	3.0% (2)	Missing outcome data

6.3.4 Effects of *A. membranaceus* on albuminuria and proteinuria

Change of albuminuria was measured in 32 studies using *A. membranaceus* injection [249, 250, 254, 256, 257, 259, 263-265, 267, 270, 271, 275, 276, 279, 280, 282-285, 287, 288, 291, 292, 297, 298, 303, 304, 307, 309, 312, 313] and 4 studies using oral *A. membranaceus* preparation [258, 295, 301, 310]. The pooled estimation indicated that additional use of either intravenous (SMD: -2.05 [-2.49, -1.61], I^2=94%) or oral *A. membranaceus* preparations (SMD: -1.27 [-1.82, -0.73], I^2=73%) reduced albuminuria more than conventional therapies alone (**Figure 6-2**).

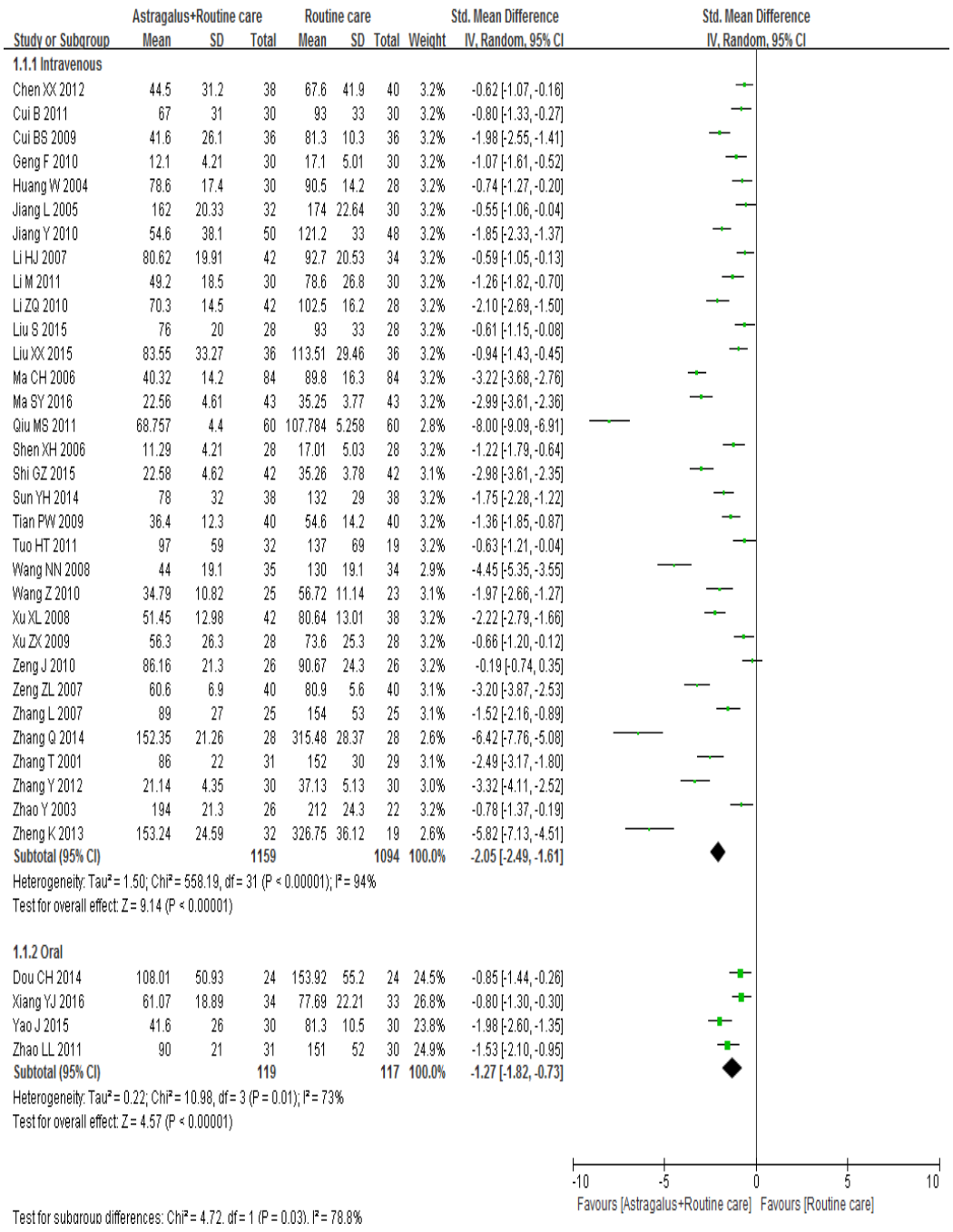

Figure 6-2 Forest plot of albuminuria outcome

As for proteinuria, the meta-analyses result of 26 studies [251, 253, 254, 259, 262, 268, 269, 272-274, 277, 278, 283, 286, 288, 290, 292, 294, 296, 299, 302, 305, 309, 311, 312, 314] also suggested lower proteinuria in the *A. membranaceus* injection group (SMD: -1.85 [-2.34, -1.37], I^2=95%), albeit with substantial heterogeneity (**Figure 6-3**).

Sensitivity analysis of low risk-of-bias studies found consistent results of both albuminuria (SMD: -2.87 [-4.25, -1.50], I^2=96%) [256, 259, 276, 284, 304, 313] and proteinuria (SMD: -2.79 [-4.31, -1.27], I^2=95%) outcomes [259, 286, 299]. But the sources of heterogeneity were not identified by subgroup analysis of baseline kidney function, treatment duration, outcome measurement methods or different doses of *A. membranaceus* injection (**Appendix 6).** Univariate meta-regression analysis showed that baseline albuminuria excretion partially contributed to the heterogeneity of albuminuria outcome (Adjusted R^2 = 18.37%, p = 0.009). Likewise, baseline proteinuria excretion was part of the source of heterogeneity for proteinuria outcome (adjusted R^2= 22.34%, p= 0.013). Other sources of heterogeneity were not identified.

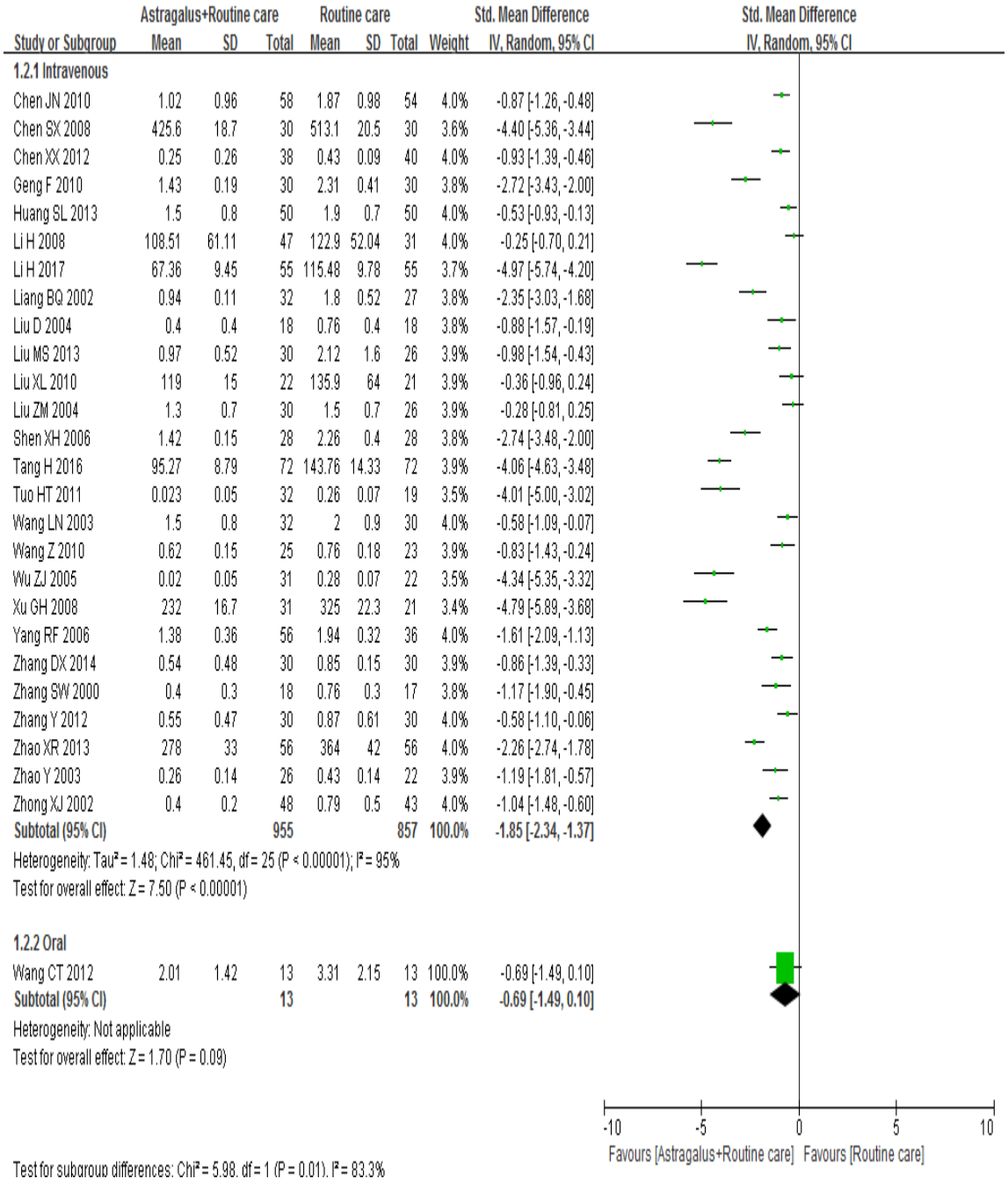

Figure 6-3 Forest plot of proteinuria outcome

6.3.5 Effects of *A. membranaceus* on kidney function

Kidney function was reflected by the measurement of serum creatinine concentration (Scr) and glomerular filtration rate (GFR) in included studies. Of 36 studies employing *A. membranaceus* injection [249, 251-253, 255-257, 261, 264-272, 274-276, 278, 279, 282, 284-286, 290, 292, 297, 298, 302, 304, 307, 308, 311, 313], the end-of-study estimated Scr was −14.78 μmol/L (95% CI [-19.22, -10.33]) lower in the *A. membranaceus* group than the conventional therapies group, although heterogeneity was significant (I^2=97%, **Figure 6-4**). In terms of GFR, three studies measured it using creatinine clearance [277, 287, 312], while one study estimated it based on Scr [296]. Although the GFR of the *A. membranaceus* group tended to be higher, the effect of add-on *A. membranaceus* injection on GFR was uncertain (223 participants; MD: 6.64 mL/min [-2.11, 15.39], I^2=74%).

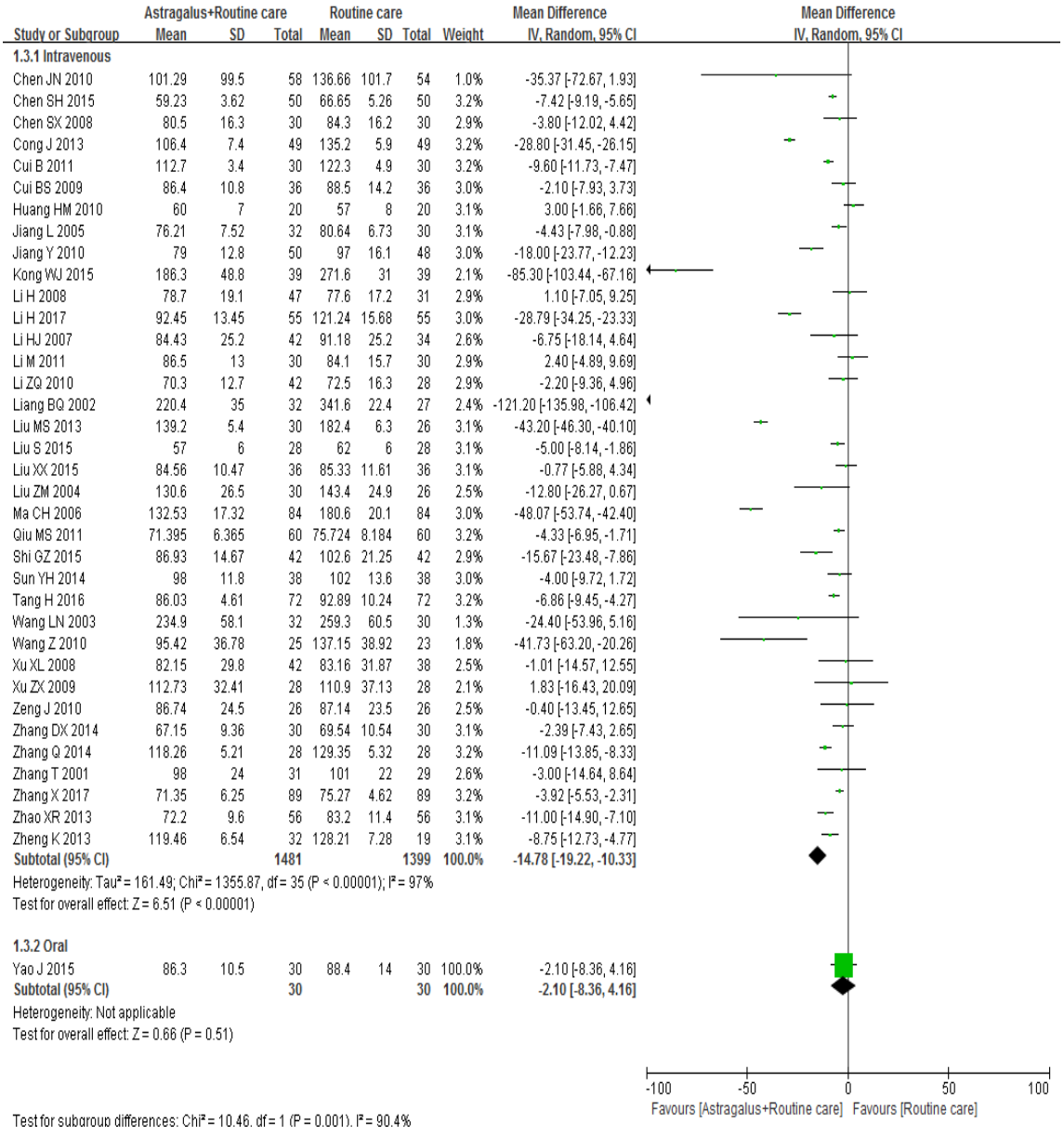

Figure 6-4 Forest plot of serum creatinine concentration outcome

The decreasing Scr effect of *A. membranaceus* injection was consistent in sensitivity analysis (567 participants; MD: -8.31 μmol/L [-10.38, -6.24], I^2=69%) %) [252, 256, 276, 284, 286, 304, 313]. The substantial heterogeneity was not explained by pre-defined subgroup analysis (**Appendix 6**). Meta-regression of Scr suggested that baseline Scr variants could be the main source of heterogeneity (adjusted R^2 =94.08%, p <0.0001), in which greater Scr reduction was associated with higher baseline Scr

values (**Figure 6-5**).

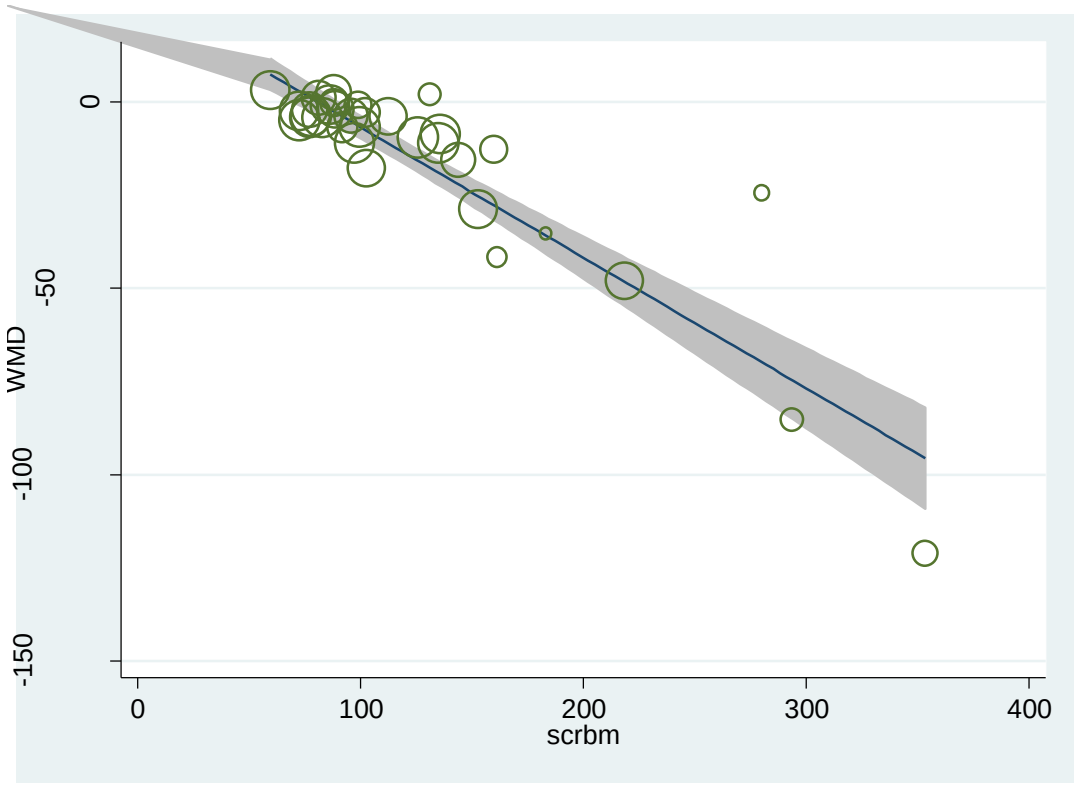

Figure 6-5 Meta-regression of serum creatinine concentration outcome

Abbreviations: scrbm: baseline serum creatinine concentration; WMD: weight mean differences. Note: the meta-regression outcome of serum creatinine concetration was based on the baseline serum creatinine level in each study.

6.3.6 Effects of *A. membranaceus* on DKD risk factors

Meta-analysis showed that FBG and HbA1c levels of the additional *A. membranaceus* injection group was lower than the conventional therapies alone group, but no statistical differences regarding postprandial blood glucose were observed (**Table 6-3**). Patients receiving additional *A. membranaceus* injection had lower blood lipid concentration, except for non-significant changes in HDLC (**Table 6-3**). In terms of BP, there were no evidence of effect of adjunctive use of *A. membranaceus* compared to use ACEi or ARB alone (**Table 6-3**).

Table 6-3 Meta-analysis results of secondary outcomes

Outcome (Unit)	Administration route	Study	Participants	Statistical method	Effect estimate [95% CI]	I^2	*p* value
Fasting Blood Glucose (mmol/L)	Intravenous drip	23	1497	Mean Difference	-0.54 [-0.83, -0.24]	94%	p =0.0004
	Oral	3	176	Mean Difference	-0.06 [-0.31, 0.19]	0%	p =0.65
Postprandial blood glucose	Intravenous drip	4	318	Mean Difference	0.03 [-0.40, 0.46]	0%	p =0.89
Glycated haemoglobin (HbA1c, %)	Intravenous drip	9	522	Mean Difference	-0.20 [-0.39, -0.02]	31%	p =0.03
	Oral	2	115	Mean Difference	-0.05 [-0.37, 0.27]	0%	p =0.74
Total cholesterol (mmol/L)	Intravenous drip	15	1008	Mean Difference	-1.35 [-1.86, -0.84]	96%	p <0.0001
	Oral	1	61	Mean Difference	-0.26 [-0.71, 0.19]	NA	p =0.26
Triglycerides (mmol/L)	Intravenous drip	15	1056	Mean Difference	-0.82 [-1.04, -0.60]	91%	p <0.0001
	Oral	1	61	Mean Difference	0.05 [-0.35, 0.45]	NA	p =0.80
LDLC (mmol/L)	Intravenous drip	1	84	Mean Difference	-2.65 [-3.23, -2.07]	NA	p <0.0001
HDLC (mmol/L)	Intravenous drip	3	246	Mean Difference	0.55 [-0.07, 1.16]	97%	p =0.08
Systolic BP	Intravenous drip	18	1273	Std. Mean Difference	-0.07 [-0.21, 0.06]	28%	p =0.27
	Oral	1	60	Std. Mean Difference	0.00 [-0.51, 0.51]	NA	p =1.00
Diastolic BP	Intravenous drip	18	1273	Std. Mean Difference	-0.11 [-0.25, 0.03]	33%	p =0.11
	Oral	1	60	Std. Mean Difference	-0.09 [-0.59, 0.42]	NA	p =0.74

Abbreviations: BP: blood pressure; CI: confidence interval; HDLC: high-density lipoprotein cholesterol; LDLC: low-density lipoprotein cholesterol; mmol/L: millimoles per litre; NA: not applicable; Std: standard.

6.3.7 Safety outcomes

Safety outcomes were reported in 20 out of 66 included studies. Fifteen studies stated no AEs during the treatment period [256, 257, 261, 262, 265, 270, 276, 278, 287, 288, 294, 295, 297, 301, 309]. The most common AEs reported in five studies were dry cough (18 cases), Scr elevated more than 30% from baseline (15 cases) and dizziness (13 cases) [250, 263, 282, 283, 290]. Three cases of angioedema and a case of hyperkalaemia were observed in two studies [282, 290]. All AEs were relieved spontaneously and there was no difference of incidence across the groups.

6.3.8 Publication Bias

Publication bias was suspected in relation to outcomes of albuminuria (p <0.001) and proteinuria (p <0.001), but not detected in Scr (p =0.287). Funnel plots of both albuminuria and proteinuria outcomes were asymmetric, suggesting a lack of studies with negative results **(Figure 6-6**).

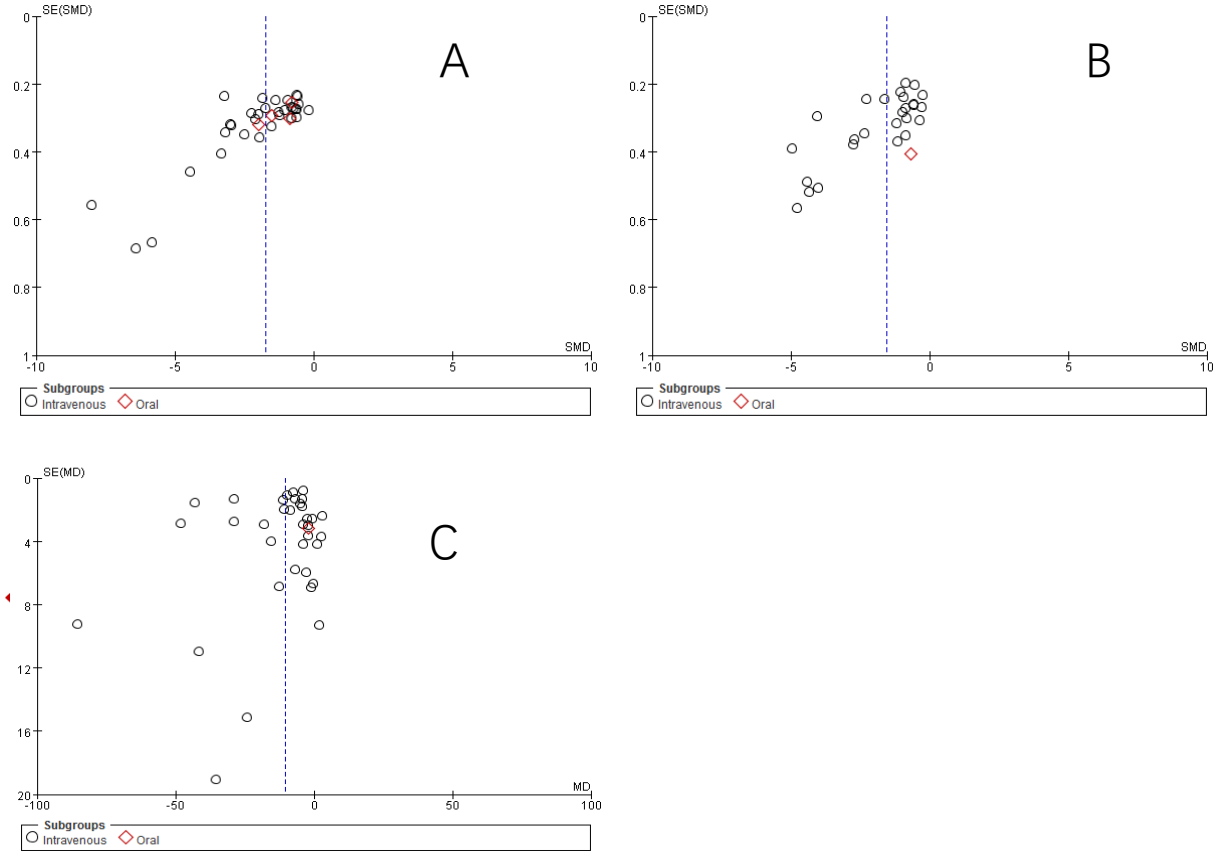

Figure 6-6 Funnel plot of albuminuria, proteinuria and serum creatinine outcomes

Note: Panel A. albuminuria outcome; Panel B. proteinuria outcome; Panel C. serum creatinine outcome

6.4 Discussion

6.4.1 Summary of findings

This systematic review and meta-analysis indicated that adjunctive use of *A. membranaceus* preparations with conventional therapies was probably beneficial in the short-term reduction of urinary albumin/protein excretion and lowering Scr level for adult DKD patients. Additionally, the combination of *A. membranaceus* preparations and current pharmacotherapies appeared to be tolerated. However, considering the overall low quality of clinical trials, large variant of effect size and potential publication bias, application of *A. membranaceus* preparations should be with caution.

From clinical perspective, the magnitude of urinary albumin/protein reduced by *A. membranaceus* injection was moderate. Based on the results of different measurement subgroups, additional uses of *A. membranaceus* injection resulted in -50.21 mg/day (−30.86 ug/min) reduction of albuminuria and −59.64 mg/day (−0.43 g/day) decrease of proteinuria. Compared to a previous systematic review, the estimated effect size of proteinuria was similar (SMD: -1.85 versus -1.78, respectively). The albuminuria outcome could not be compared due to missing measurement units [218]. The moderate anti-albuminuria effect was also observed in studies using oral *A. membranaceus* preparations, but the effect on proteinuria was uncertain due to a limited number of studies. As albuminuria/proteinuria are established indicators of DKD progression, cardiovascular events and deaths, the anti-albuminuria/proteinuria effect of *A. membranaceus* preparations might be helpful in improving these patient-important outcomes [315, 316].

The Scr-lowering effect of *A. membranaceus* injection was also considerable from the clinical point of view, but the effect of oral *A. membranaceus* preparations was unclear since only one small study was included. The Scr-lowering effect of *A. membranaceus* injection was more apparent in the study subgroup of DKD participants with mild to moderate declined kidney function. This finding was confirmed by the meta-regression analysis that the higher the baseline Scr level, the greater reduction of end-of-trial Scr. Since sudden elevated Scr is a common side effect leading to discontinuation of ACEi/ARB, *A. membranaceus* injection could be a complementary therapy particularly

for advanced DKD patients intolerant to RAS inhibitors in initial stages.

The add-on effect of *A. membranaceus* preparations on GFR was uncertain based on current meta-analysis results. In a previous review, GFR measured as creatinine clearance was 14.02 mL/min higher in the *A. membranaceus* group than the conventional therapies alone group [218]. The inconsistency of GFR outcomes could be partly explained by different baseline kidney function. The pooled estimation in the previous review was dominated (weight 96%) by a trial of DKD participants with moderately impaired renal function, in contrast to the baseline GFR of included studies in our review which were all over 60 mL/min. Moreover, the use of RAS inhibitors in each arm was not clarified in the previous review, therefore the increased GFR could have resulted from the acute effect of ACEi/ARB rather than the therapeutic effect of *A. membranaceus* injections.

Although all included studies used single-herb preparations of *A. membranaceus* as intervention, the bioactive constituents and action mechanisms corresponding to the therapeutic effect were not fully clarified. The preparations are extracts of *A. membranaceus* raw herb rather than isolated chemical entities, and contain multi-components and have synergistic functions. Some action mechanisms have been proposed by studies focusing on specific *A. membranaceus* constituents. For example, attenuated podocyte apoptosis by administration of astragaloside IV was observed in two different diabetic mouse models [317, 318]. An *in vitro* study showed that astragaloside IV also reversed the high glucose-induced adhesion dysfunction in mouse podocyte [319]. As podocyte injuries play a major role in urinary protein leakage, the anti-proteinuria properties of *A. membranaceus* may be partially mediated by the podocyte protective effect of astragafloside IV. In addition, *in vitro* experiments suggested that *Astragalus* saponin I suppressed the oxidative and inflammatory activity in high-glucose mesangial cells, thus reducing the accumulation of extracellular matrix [320]. Such chemicals contained in *A. membranaceus* preparations attenuated renal injuries secondary to diabetes via multiple targets and multiple pathways, supplementary to the RAS inhibitory pathway of ACEi/ARB.

6.4.2 Limitations

This review was intended to be comprehensive and unbiased by performing an exhaustive search and pre-defined protocol focusing on the effect of *A. membranaceus* preparations on top of conventional therapies. But limitations existed when interpreting and generalising the findings. Firstly, the confidence in the beneficial effect of *A. membranaceus* preparations was compromised due to the unblinded study designs, unexplained heterogeneity and potential publication bias. Additionally, the effect of *A. membranaceus* preparations could be exaggerated as larger effects were observed in clinical trials published in Chinese compared to those in English [321]. Secondly, there was not enough data to completely confirm the safety of *A. membranaceus* preparations. More detailed evaluation and reporting of chemical profiles and AE is required since severe allergic reactions have been observed after high-dose oral preparations or injections [245]. Thirdly, the long-term effect of *A. membranaceus* preparations (over 3 months), particularly for patient-important outcomes, was inconclusive due to the lack of evidence.

Clinically, oral *A. membranaceus* preparations may be preferred over injection for its convenience and non-invasive nature. Unfortunately, the effect and safety of oral *A. membranaceus* preparations were less certain due to the limited number of studies and various forms of products. For the same reasons, we failed to compare the injected *A. membranaceus* preparations with oral ones to determine which forms achieved better therapeutic outcomes. In addition, all included studies were conducted in China with adult Chinese participants. Treatment effect may vary when applied to children or other ethnic groups.

6.4.3 Implications for future research

Further studies are needed to better understand the therapeutic effect and safety profile of *A. membranaceus* preparations. Application of placebo is highly recommended to reduce the risk of performance bias. RCT with adequate sample sizes and long follow-up periods in addition to measuring clinically relevant outcomes are warranted. The hard outcomes, such as mortality, disease progression and QoL, are more relevant to

patients and more informative in clinical decision-making than the surrogate laboratory outcomes. Furthermore, well-designed clinical trials of oral *A. membranaceus* preparations are preferred.

In terms of study reporting, it is encouraged to provide information about the manufacturing and quality control processes of *A. membranaceus* preparations. Considering the fact that chemical constitutents are affected by the raw material source, cultivation methods and manufacturing approach, consistent high-quality *A. membranaceus* preparations are necessary. Lastly, clinical trials with "negative outcomes" should also be reported [237, 238, 322]. The included studies evaluating *A. membranaceus* for DKD are overall very positive, but it is likely that negative result studies have been undertaken but not reported.

6.5 Conclusion

This updated systematic review and meta-analysis suggests that adjunctive use of *A. membranaceus* preparations probably reduced albuminuria, proteinuria and serum creatinine levels compared to conventional therapies alone in people with DKD. Adverse herb–drug interactions were not found. The overall quality of evidence was low and the findings should be interpreted with caution. Well-designed RCTs are needed to confirm the long-term efficacy and safety of *A. membranaceus* preparations, particularly with respect to outcomes of mortality, disease progression and QoL.

7 A Network Pharmacology Approach to Explore the Active Compounds and Possible Mechanisms of *Huang qi* in Diabetic Kidney Disease

7.1 Introduction

Over the past 20 years, traditional drug development has failed to yield a sufficient number of new drugs despite increasing investment [323, 324]. This brings into question the ongoing validity of conventional drug discovery processes and the pharmaceutical industry. The overriding reason for research and design (R&D) failure is lack of efficacy and safety revealed in the clinical trial stages [325, 326]. In the field of diabetic kidney disease (DKD), two widely known cases of drug failure during development are documented, namely, sulodexide and bardoxolone methyl. Sulodexide showed a lack of efficacy when compared to placebo during clinical testing, while bardoxolone methyl was associated with higher rates of cardiovascular events [155, 160]. It led to early termination of their Phase III (in human efficacy assessment) trials. Considering the huge effort invested before entering the clinical trial phase, this kind of failure is a major setback to both industry and the academic field.

Reducing the discrepancy between promising pre-clinical trends and subsequent disappointing clinical results is pivotal for new drug development. Conventional drug discovery involves compounds highly selective to disease-specific targets chosen as potential candidates for drug development research. However, recent advances in systems biology reveal that biological systems are complex and robust. The network relationships of genes, gene products and other biological components are important organism features and a single compound–target drug can be counteracted by after-effects of other pathways in the same network. The complex nature of biological systems and multi-factorial characteristics of chronic diseases like DKD require novel methods for new drug discovery and development [327, 328].

In the face of these challenges, a novel drug discovery paradigm named “network pharmacology” was advocated in 2008 by Hopkins [10]. This novel drug design and discovery approach was based on the development of chemistry, systems biology and

network analysis techniques. Different from the traditional “one target, one drug” mode, network pharmacology was developed to address the limitations in the traditional drug discovery pipeline that focus on selective ligands to act on individual drug targets. In contrast to the conventional approach, potential therapy in network pharmacology was assumed to modify the hub target in the disease network or regulate multiple targets simultaneously, leading to restoration of the biology network back to normal status.

One application of the network pharmacology approach is in the assessment of mechanisms of herbal medicine. Due to the complex chemical compositions of medical plants, multiple targets and pathways may be affected by a single herb. The bioactive compounds and action mechanisms corresponding to the therapeutic effects of Chinese herbal medicine (CHM) are difficult to fully elucidate using traditional research techniques because they have multiple components and multiple targets. The advent of network pharmacology approach may help to shed light on CHM and assist with the therapeutic development, as the molecular basis of herbal products can be studied in a systematic way [11]. Due to its unique advantages, the network pharmacology approach has been increasing applied in the context of Chinese medicine (CM) and specific tools tailored for CM have been developed [329].

This chapter reports on a network pharmacology approach that was used to decipher the action mechanisms of the selected herb, *huang qi* 黄芪 (*Astragalus Membranaceus*), in the treatment of DKD. This herb was selected because it is the most commonly studied herb in clinical trials of DKD and has been consistently used in historical CM literature, indicating it is the most promising herb for the treatment of DKD. The findings of this network pharmacology study provide insight into bioactive compounds and a corresponding action mechanism hypothesis related to DKD for future experimental confirmation.

7.2 Methods

The methods used in this study are adapted from the research framework of CM network pharmacology proposed by Li et al. [11]. Briefly, herbal chemicals are collected and then predicted or searched for corresponding herbal targets. The herbal

DKD targets are assembled and constructed into separate protein–protein interaction (PPI) networks. The herbal and DKD networks are combined and intersections shown in a network configuration. The network undergoes analysis, and active chemicals and action mechanisms are revealed. The main steps are shown in **Figure 7-1**.

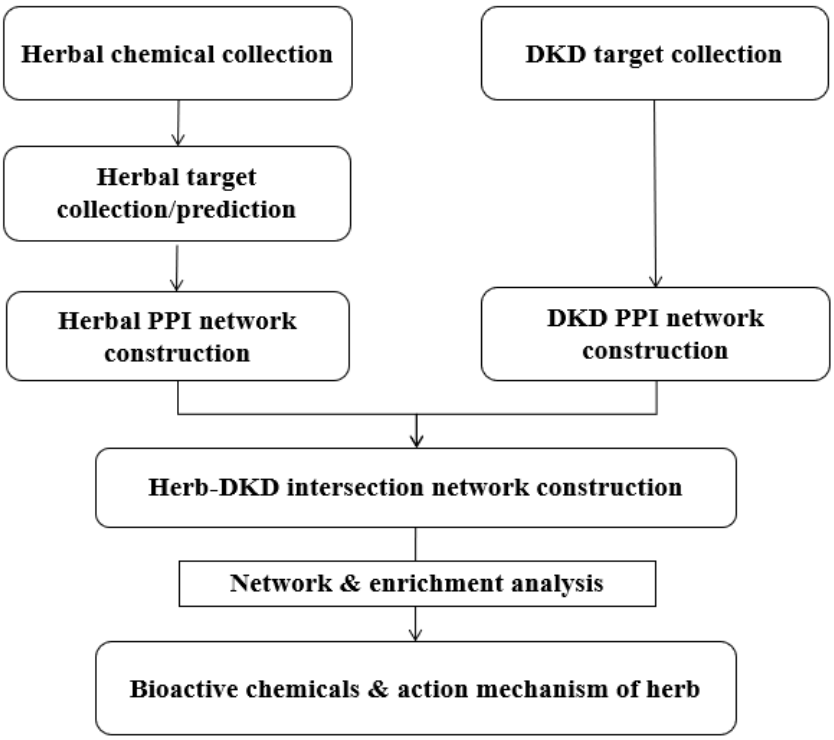

Figure 7-1 Flowchart of network pharmacology study process

Abbreviations: DKD: diabetic kidney disease; PPI: protein-protein interaction.

7.2.1 DKD target collection

DKD-related targets were collected from four databases: Online Mendelian Inheritance in Man (OMIN), Genetic Association Databases (GAD), Therapeutic Target Database (TTD) and DrugBank (**Table 7-1**). These four databases are recognised as the most important when collecting disease targets. Together, they provide enough known target information related to specific diseases to use in network analysis [330, 331]. The OMIN and GAD databases are repositories of data associated with known genes–diseases, while the TTD and DrugBank databases provide "druggable" targets for specific diseases. In all databases, "diabetic nephropathy" and "diabetic kidney disease"

were used as search terms. The retrieved sources, target names and gene symbols of each DKD target were collected in a Microsoft Excel spreadsheet. The DKD targets that were duplicates and identified in more than one database were removed. The remaining targets were examined to exclude non-homogeneous species targets.

Table 7-1 Databases for DKD target collection

Database (Website)	Introduction	Update Frequency
Online Mendelian Inheritance in Man, OMIN [332] www.omim.org	Primary information repository of over 15,500 genes and 7,800 genetic phenotypes, with a focus on the gene-phenotype relationship	Initiated in the early 1960s and updated daily
Genetic Association Databases, GAD [331] geneticassociationdb.nih.gov	Provides genetic association information of complex diseases	Last updated in September 2014
Therapeutic Target Database, TTD [333] db.idrblab.org/ttd/	Provides information about known and explored therapeutic targets, with correlated disorders and drugs data	Updated annually
DrugBank [334] www.drugbank.ca	Provides chemical data on 11,680 drugs and 5,129 protein sequences corresponding to these drugs	The latest version 5.0 was released in 2018

7.2.2 Herbal chemical collection

7.2.2.1 Database

The chemical composition of *huang qi* was collected from two databases: the *Traditional Chinese Medicine Database @Taiwan* (TCM Database @Taiwan) and *the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform* (TCMSP) [335, 336]. Since the same chemical compounds may have various synonyms in different data sources, the herbal compounds collected from TCM Database@Taiwan and TCMSP were standardised with unique labels. The PubChem and ChEMBL (version 24) public repositories were used as references [337, 338].

The TCM Database @Taiwan includes not only herbs but also medical minerals and animal products recorded in classical literature and dictionaries, claiming to be the largest and most comprehensive non-commercial database in the field of CM. A total

of 32,364 compounds originating from 453 different Chinese medicinals are indexed. All compounds were gathered manually from published literature, with molecule structures illustrated for docking purposes [336], therefore ensuring herbal compounds and chemical structures can be obtained from the TCM Database @Taiwan.

As for the TCMSP, it provides three types of information: (1) herbal compounds with absorption, distribution, metabolism and excretion (ADME) related properties; (2) integrated compounds, targets and disease information sourced from PubChem, DrugBank and other databases; (3) computed compounds–target relationships and target–disease relationships. In total, the TCMSP includes 499 herbs indexed in the *Chinese Pharmacopoeia* (2010 version), 29,834 compounds contained in these herbs, 3311 targets and 837 associated diseases. One of the advantages of the TCMSP is that ADME information is provided, so herbal chemicals with greater druggable possibility can be filtered out for further study [335].

PubChem is the largest open-access archive of chemical information, established by the US National Institutes of Health in 2004. It consists of three linked databases (Substance, Compound and BioAssay databases). In the PubChem Compound database, an aggregated view of a particular compound is provided after standardisation of substance descriptions from different data sources [337]. As of August 2018, there were over 96 million records of compounds with unique chemical structures which were mainly identified by high-throughput screening experiments [337]. On the Compound webpage, information about specific compounds includes, but is not limited to, names and identifiers, chemical and physical properties, chemical structure, bioactivities and related literature (**Figure 7-2**).

Figure 7-2 Snapshot of PubChem Compound summary page for berberine

The other compound source is ChEMBL. This is a freely accessible large-scale bioactivity database maintained by the European Bioinformatics Institute. It includes extracted and curated bioactivity data from published literature at regular time intervals, with a manual annotation process to ensure data quality [338]. In the latest version of ChEMBL (version 24), it covers over 1.8 million distinct compounds, 15 million bioactivity records and 12,091 targets extracted from nearly 70 thousand publications and corresponding databases. Although there are more compounds recorded in

PubChem, ChEMBL provides a larger proportion of active compounds with dose–response properties validated in published literature [339]. Likewise, there are overlaps and differences regarding the target datasets of PubChem and ChEBML. Thus, the two open repositories are complementary to each other and used together in this research.

7.2.2.2 Chemicals collection procedure

The Chinese names of *huang qi* were used as search terms. In the TCM Database @Taiwan, since the compound records were manually extracted from chemistry experiment results, all retrieved herbal compounds were considered reliable, therefore could be directly included in the *huang qi* chemical profile. In contrast, herbal compounds identified in the TCMSP went through an additional ADME screening step to search for chemicals with potential bioactivity. Herbal compounds with oral bioavailability (OB) higher than 30% and drug-likeness (DL) larger than 0.18 were eligible for selection [340, 341].

Herbal compounds gathered from the TCM Database @Taiwan and eligible compounds from the TCMSP were recorded in a Microsoft Excel spreadsheet as the huang qi chemical profile. Molecular names of herbal compounds were standardised in accordance with PubChem or ChEMBL databases. Additional compound identifiers and chemical information such as Chemical Abstracts Service (CAS) registry number, International Chemical Identifier (InChIKeys), molecular formulae and simplified molecular input line entry specification (SMILES) notation were collected and recorded in the spreadsheet.

7.2.3 Herbal target collection and prediction

Targets corresponding to the herbal chemicals were generated by two approaches: bioactivity database searching and *in silico* prediction. Bioactive database searching was efficient for finding experimental verified targets, while the *in silico* prediction was useful for exploring potentially related targets. Therefore, both methods were used to collect targets possibly related to the therapeutic effect of herbal compounds.

7.2.3.1 Known herbal target collection

The PubChem BioAssay database and ChEBML contain structural bioactive data from published literature thus they were used as sources of targets validated by laboratory experiments [338, 342]. By inputting the compounds' identifiers, the bioactivity data of interaction targets can be downloaded from the compounds' webpages. Only targets labelled "Active" in PubChem databases and those with bioactivities larger than 3000 in ChEBML were included in the *huang qi* target profile. Non-homogeneous species targets were excluded.

7.2.3.2 Potential herbal target prediction

The *in silico* technique was used to predict potential target interaction with given herbal compounds. This prediction was based on the chemical similarity theory that chemicals with similar molecular structures were likely to act on the same targets [343]. As a result, molecular interaction relationships of uncharacterised compounds can be assumed. The web server of SwissTargetPrediction (www.swisstargetprediction.ch) was used to predict the potential targets of herbal compounds. Over 280 thousand ligands and 2686 target are contained in SwissTargetPrediction for chemical similarity comparison and target prediction [344].

The SMILES notation of each herbal compound was entered into the SwissTargetPrediction web interface, then a 2D structure was automatically synchronised (**Figure 7-3**). After setting target prediction limited to human organisms, the computation was performed. Except for the targets predicted based on homology, all putative targets were added to the *huang qi* target profile.

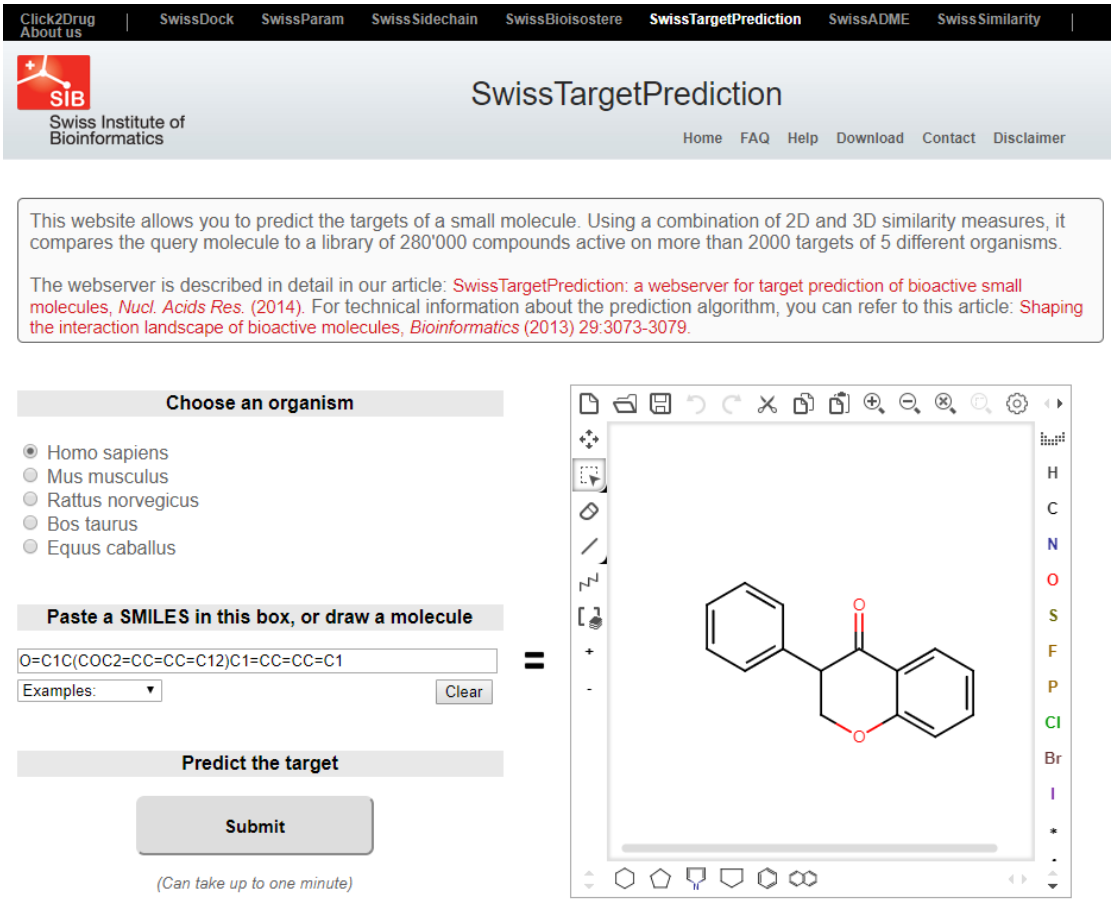

Figure 7-3 Example working interface of SwissTargetPrediction

7.2.3.3 Herbal target profile construction

Both *huang qi* known targets and putative targets were assembled and recorded in a spreadsheet. The targets' name and identifiers were standardised based on the official gene symbols in the Universal Protein Resource (UniProt) knowledge base (www.uniprot.org), which covers over 60 million protein sequences and associated annotation [345]. Duplicate targets were removed.

7.2.4 Construction of Herb–DKD protein–protein interaction network

In a living organism, intentional physical contact between proteins are essential in both biological processes and disease status, and is defined as protein–protein interaction (PPI) [346]. These PPI relationships can be detected by experiment and the proven PPIs have been manually curated in primary databases by different research groups. In this

study, five primary databases were searched to explore human PPIs of collective targets, including the Biological General Repository for Interaction Datasets (BioGRID), the Biomolecular Interaction Network Database (BIND), the Molecular INTeraction Database (MINT), the Human Protein Reference Database (HPRD) and the Database of Interacting Proteins (DIP) [347-351]. The plugin app of Cytoscape software named BisoGenet was used to retrieve data from these databases simultaneously [352].

The complex PPI relationships of huang qi and DKD targets were visualised as a network by Cytoscape software (version 3.6.1) [353]. The PPI networks of *huang qi* targets and DKD were built separately. In a PPI network, a node represents a target and the edge represents an interaction link between nodes. To explore the action mechanism of *huang qi* in terms of DKD pathogenesis, the herbal PPI network and DKD PPI network were merged to find overlapping targets. As a result, a herb–DKD network of shared targets was constructed.

7.2.5 Network and enrichment analysis

7.2.5.1 Network analysis of key targets

Based on graph theory, the importance of each node in a network can be evaluated by centrality indices from a topological point of view. Four basic centrality measures were calculated to identify key targets in the herb–DKD PPI network, including degree centrality (DC), betweenness centrality (BC), closeness centrality (CC) and eigenvector centrality (EC). The topological meanings of these centrality indices are listed in **Table 7-2** with interpretations in a biological context. The CytoNCA app in the Cytoscape software was used for central indices calculation [354]. Targets with DC, BC, CC and EC quantitative values ranked in the top 10 per cent among the whole herb–DKD targets list were defined as key targets and selected for further analysis.

Table 7-2 Definition of central indices

Index	Definition	Biological interpretation
Degree centrality	The number of nodes directly connected to a given node, corresponding to the number of adjacent links	Target with higher degree centrality links to more targets, suggesting a central regulatory role
Betweenness centrality	The number of times a given node bridges the shortest path between two other nodes	Target with higher betweenness centrality communicates more distant targets, indicating an organising regulatory role
Closeness centrality	The average length of the shortest path between the node and all other nodes in the network	Target with higher closeness centrality is closer to all other targets, suggesting a central regulatory role
Eigenvector centrality	The number of high degree centrality nodes connected to a given node	Target with higher eigenvector centrality interacts with more central targets

References:[355, 356].

7.2.5.2 Enrichment analysis of key targets

To understand the biological role of the key targets, gene ontology and pathway functional enrichment analysis were performed. Gene ontology (GO) is a dynamic, structured, well-defined, controlled vocabulary produced by biologists to describe knowledge of gene and gene products in organisms [357]. In addition to descriptions of gene functions, the relations between gene functions are also provided. However, due to the complexity of biological systems, one gene or gene product may be involved in different biological processes, and therefore labelled with many inter-linked GO terms. For a given gene set, enrichment analysis can identify the GO terms where these genes are markedly present. Similarly, the over-represented pathways of specific gene sets can be revealed by pathway enrichment analysis.

The GO and pathway enrichment analysis were conducted in the FunRich Functional Enrichment Analysis Tool (version 3.1.3) [358, 359]. The gene symbols of the key targets of the herb–DKD PPI network were entered in the software, with default analysis gene background. Default parameters were used for enrichment analysis, with an adjusted Bonferroni *p*-value <0.01 considered the significance cut-off. The relationships and cross-talk among the top 10 enriched pathways were further analysed based on the sub-pathway searching results in the PathwayCommons database

(www.pathwaycommons.org/pc/home.do) [360].

7.3 Results

7.3.1 Characteristics of DKD targets

Searching in OMIN, GAD, TTD and *DrugBank* databases retrieved a total of 118 human targets related to DKD. After duplicate removal, 103 DKD-related targets remained (**Table 7-3**). Among these DKD-related targets, angiotensin-converting enzyme (ACE) appeared in all four databases. Mitochondrial superoxide dismutase (SOD2), advanced glycosylation end product-specific receptor (AGER), C-C chemokine receptor 2 (CCR2), C-C chemokine receptor 5 (CCR5), transforming growth factor beta receptor 1 (TGFBR1), Type-1 angiotensin II receptor (AGTR1), Vascular endothelial growth factor A (VEGFA) and methylenetetrahydrofolate reductase (MTHFR) were identified in two different databases.

Table 7-3 Known DKD targets

Uniprot entry	Gene symbol	DKD rarget name
P08183	ABCB1	Multidrug resistance protein 1
Q92887	ABCC2	Canalicular multispecific organic anion transporter 1
Q9UNQ0	ABCG2	ATP-binding cassette sub-family G member 2
P12821	ACE	Angiotensin-converting enzyme
Q15848	ADIPOQ	Adiponectin
Q9H2P0	ADNP	Activity-dependent neuroprotector homeobox protein
P18825	ADRA2C	Alpha-2C adrenergic receptor
P08588	ADRB1	Beta-1 adrenergic receptor
P07550	ADRB2	Beta-2 adrenergic receptor
Q15109	AGER	Advanced glycosylation end product-specific receptor
P01019	AGT	Angiotensinogen
P30556	AGTR1	Type-1 angiotensin II receptor
P02768	ALB	Serum albumin
Q6Q788	APOA5	Apolipoprotein A-V
P02656	APOC3	Apolipoprotein C-III
P02649	APOE	Apolipoprotein E
P56817	BACE1	Beta-secretase 1
P06276	BCHE	Cholinesterase
P46663	BDKRB1	B1 bradykinin receptor
P04040	CAT	Catalase
P41597	CCR2	C-C chemokine receptor type 2
P51681	CCR5	C-C chemokine receptor type 5
P23141	CES1	Liver carboxylesterase 1

Uniprot entry	Gene symbol	DKD rarget name
P29279	CTGF	Connective tissue growth factor
P05177	CYP1A2	Cytochrome P450 1A2
P10632	CYP2C8	Cytochrome P450 2C8
P11712	CYP2C9	Cytochrome P450 2C9
P33261	CYP2C19	Cytochrome P450 2C19
P10635	CYP2D6	Cytochrome P450 2D6
P08684	CYP3A4	Cytochrome P450 3A4
P20815	CYP3A5	Cytochrome P450 3A5
O15528	CYP27B1	25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial
P43146	DCC	Netrin receptor DCC
P05305	EDN1	Endothelin-1
P25101	EDNRA	Endothelin-1 receptor
P01588	EPO	Erythropoietin
P09488	GSTM1	Glutathione S-transferase Mu 1
Q30201	HFE	Hereditary hemochromatosis protein
P20823	HNF1A	Hepatocyte nuclear factor 1-alpha
P00738	HP	Haptoglobin
P05362	ICAM1	Intercellular adhesion molecule 1
P08833	IGFBP1	Insulin-like growth factor-binding protein 1
P01584	IL1B	Interleukin-1 beta
P18510	IL1RN	Interleukin-1 receptor antagonist protein
P08887	IL6R	Interleukin-6 receptor subunit alpha
P05106	ITGB3	Integrin beta-3
P23458	JAK1	Tyrosine-protein kinase JAK1
O60674	JAK2	Tyrosine-protein kinase JAK2
P05412	JUN	Transcription factor AP-1
P11150	LIPC	Hepatic triacylglycerol lipase
P01374	LTA	Lymphotoxin-alpha
P09960	LTA4H	Leukotriene A-4 hydrolase
Q99683	MAP3K5	Mitogen-activated protein kinase kinase kinase 5
P53778	MAPK12	Mitogen-activated protein kinase 12
Q9UBB5	MBD2	Methyl-CpG-binding domain protein 2
P02686	MBP	Myelin basic protein
P08473	MME	Neprilysin
P03956	MMP1	Interstitial collagenase
P08253	MMP2	72 kDa type IV collagenase
P14780	MMP9	Matrix metalloproteinase-9
P42898	MTHFR	Methylenetetrahydrofolate reductase
Q96PU5	NEDD4L	E3 ubiquitin-protein ligase NEDD4-like
P35228	NOS2	Nitric oxide synthase, inducible
P29474	NOS3	Nitric oxide synthase, endothelial
P08235	NR3C2	Mineralocorticoid receptor
P16860	NPPB	Natriuretic peptides B
O95427	PIGN	GPI ethanolamine phosphate transferase 1
P27169	PON1	Serum paraoxonase/arylesterase 1
P37231	PPARG	Peroxisome proliferator-activated receptor gamma
P23219	PTGS1	Prostaglandin G/H synthase 1

Uniprot entry	Gene symbol	DKD rarget name
Q13464	ROCK1	Rho-associated protein kinase 1
P34741	SDC2	Syndecan-2
P05121	SERPINE1	Plasminogen activator inhibitor 1
P05120	SERPINB2	Plasminogen activator inhibitor 2
Q9UIV8	SERPINB13	Serpin B13
Q6FHJ7	SFRP4	Secreted frizzled-related protein 4
Q9NRM0	SLC2A9	Solute carrier family 2, facilitated glucose transporter member 9
P23975	SLC6A2	Sodium-dependent noradrenaline transporter
P46059	SLC15A1	Solute carrier family 15 member 1
Q16348	SLC15A2	Solute carrier family 15 member 2
Q4U2R8	SLC22A6	Solute carrier family 22 member 6
Q9Y694	SLC22A7	Solute carrier family 22 member 7
Q8TCC7	SLC22A8	Solute carrier family 22 member 8
Q96S37	SLC22A12	Solute carrier family 22 member 12
P46721	SLCO1A2	Solute carrier organic anion transporter family member 1A2
Q9Y6L6	SLCO1B1	Solute carrier organic anion transporter family member 1B1
Q9NPD5	SLCO1B3	Solute carrier organic anion transporter family member 1B3
Q6EEV6	SUMO4	Small ubiquitin-related modifier 4
P22309	UGT1A1	UDP-glucuronosyltransferase 1-1
P35503	UGT1A3	UDP-glucuronosyltransferase 1-3
Q9HAW8	UGT1A10	UDP-glucuronosyltransferase 1-10
P16662	UGT2B7	UDP-glucuronosyltransferase 2B7
O75795	UGT2B17	UDP-glucuronosyltransferase 2B17
Q9UKP6	UTS2R	Urotensin-2 receptor
P15692	VEGFA	Vascular endothelial growth factor A
P01137	TGFB1	Transforming growth factor beta-1 proprotein
P36897	TGFBR1	TGF-beta receptor type-1
P37173	TGFBR2	TGF-beta receptor type-2
P01375	TNF	Tumor necrosis factor
Q86Y38	XYLT1	Xylosyltransferase 1
Q9H1B5	XYLT2	Xylosyltransferase 2
Q8N4Q0	ZADH2	Prostaglandin reductase 3

7.3.2 Chemical and target profile of *huang qi*

There were 22 compounds from *huang qi* identified in the TCM Database @Taiwan and 87 compounds in the TCMSP. After ADME property screening and cross-checking against PubChem/ChEMBL databases, 38 compounds remained as potential bioactive chemicals of *huang qi*.

The chemical information of the 38 compounds is summarised in Table 7-4. The potential bioactive compounds include flavonoids, terpenes, saponins and alkaloids. The molecular weights of compounds range from 117 to 947 g/mol and the molecular

masses of 26 compounds were no greater than 500 g/mol. The oral bioavailability (OB) and drug-likeness (DL) data are available for 29 compounds. Among the bioactive compounds identified from TCM Database @Taiwan, four compounds had an OB less than 30% and the DL of five compounds was smaller than 0.18.

Table 7-4 Chemical information of bioactive compounds of *huang qi*

PubChem ID	Compoundname	Chemical classification	Molecular formula	Molecular weight (g/mol)	Oral bioavailability (%)	Drug-likeness	No. of targets
5280343	Quercetin	Flavonoids	$C_{15}H_{10}O_7$	302.238	46.43	0.28	194
5280863	Kaempferol	Flavonoids	$C_{15}H_{10}O_6$	286.239	41.88	0.24	89
5280378	Formononetin	Flavonoids	$C_{16}H_{12}O_4$	268.268	69.67	0.21	40
64971	Betulinic Acid	Triterpenoids	$C_{30}H_{48}O_3$	456.711	55.38	0.78	38
6037	Folic Acid	Vitamin B family	$C_{19}H_{19}N_7O_6$	441.404	68.96	0.71	35
5280448	Calycosin	Flavonoids	$C_{16}H_{12}O_5$	284.267	47.75	0.24	22
5281654	Isorhamnetin	Flavonoids	$C_{16}H_{12}O_7$	316.265	49.6	0.31	20
2837663	8-(2,3-Dihydroxy-3-methylbutoxy)-4-methoxy-1-methylquinolin-2-one	Alkaloids	$C_{16}H_{21}NO_5$	307.346	NA	NA	14
247	Betaine	Alkaloids	$C_5H_{11}NO_2$	117.148	24.8	0.55	13
143	Calcium Folinate	Vitamin B family	$C_{20}H_{23}N_7O_7$	473.446	23.6	0.74	13
21160900	Chrysanthemaxanthin	Terpenoids	$C_{40}H_{56}O_3$	584.885	38.72	0.58	13
23135	Candicine	Alkaloids	$C_{11}H_{18}NO^+$	180.271	38.7	0.04	13
5316874	Pinosylvin Dimethyl Ether	Stilbenoids	$C_{16}H_{16}O_2$	240.302	NA	NA	12
457801	gamma-Sitosterol	Triterpenoids	$C_{29}H_{50}O$	414.718	NA	NA	11
15976101	(24S)-24-Propylcholesta-5-ene-3beta-ol	Triterpenoids	$C_{30}H_{52}O$	428.745	36.23	0.78	10
5316760	3,9-Dimethoxy-6H-[1] benzofuro [3,2-c]chromene-1,7-diol	Pterocarpene	$C_{17}H_{14}O_6$	314.293	39.05	0.48	10
5317378	Kumujancine	Alkaloids	$C_{13}H_{10}N_2O_2$	226.235	NA	NA	10
71306915	Astragaloside-II	Saponins	$C_{41}H_{66}O_{15}$	798.964	46.06	0.13	8
21680050	2-Hydroxy-3-methoxystrychnine	Alkaloids	$C_{22}H_{24}N_2O_4$	380.444	NA	NA	8
73299	Hederagenin	Triterpenoids	$C_{30}H_{48}O_4$	472.71	36.91	0.75	8
160767	Isoflavanone	Flavonoids	$C_{15}H_{12}O_2$	224.259	109.99	0.3	8
14077830	Astrapterocarpan	Flavonoids	$C_{17}H_{16}O_5$	300.31	64.26	0.42	7
108213	Bifendate	Heterocyclic compounds	$C_{20}H_{18}O_{10}$	418.354	31.1	0.67	7

PubChem ID	Compoundname	Chemical classification	Molecular formula	Molecular weight (g/mol)	Oral bioavailability (%)	Drug-likeness	No. of targets
5318035	DSTCZBGJCUOFLM-COECVNONSA-N	Terpenoid	$C_{36}H_{58}O_6$	586.854	NA	NA	7
15689653	Isomucronulatol 7,2'-di-O-glucoside	Flavonoid glucosides	$C_{29}H_{38}O_{15}$	626.608	49.28	0.62	7
5318869	Jaranol	Flavonoids	$C_{17}H_{14}O_6$	314.293	50.83	0.29	7
13943297	n-Benzyloxycarbonyl-1-aminocyclopropanecarboxylic acid	Saponins	$C_{41}H_{68}O_{14}$	784.981	17.74	0.15	7
10380176	NQRBAPDEZYMKFL-NSHDSACASA-N	Flavonoids	$C_{17}H_{18}O_5$	302.326	67.67	0.26	7
9986231	Suffruticoside A	Paeonol glycosides	$C_{27}H_{32}O_{16}$	612.537	13.9	0.2	7
13996685	Astragaloside I	Saponins	$C_{45}H_{72}O_{16}$	869.055	46.79	0.11	6
441905	Astragaloside III	Saponins	$C_{41}H_{68}O_{14}$	784.981	31.83	0.1	6
71448939	Astragaloside V	Saponins	$C_{47}H_{78}O_{19}$	947.122	NA	NA	6
71448940	Astragaloside VI	Saponins	$C_{47}H_{78}O_{19}$	947.122	NA	NA	6
14241100	Astragaloside VII	Saponins	$C_{47}H_{78}O_{19}$	947.122	NA	NA	6
15689655	3,9-Di-O-methylnissolin	Flavonoids	$C_{18}H_{18}O_5$	314.337	53.74	0.48	5
15689652	7-O-Methylisomucronulatol	Flavonoids	$C_{18}H_{20}O_5$	316.353	74.69	0.3	4
101679160	Astrapterocarpan Glucoside	Flavonoid glucosides	$C_{23}H_{26}O_{10}$	462.451	36.74	0.92	4
15689654	SRVGYVIWVOOXQO-BKCJKFSYSA-N	Flavonoids	$C_{29}H_{38}O_{16}$	642.607	41.72	0.69	4

Abbreviation: NA: not available (data not found).

Of the 38 potential bioactive compounds, 16 had experiments that verified targets according to the PubChem BioAssay and ChEBML databases. After removal of duplicates and non-human targets, 303 targets corresponding to the *huang qi* bioactive compounds were identified from the two databases. In addition, 138 potential targets were obtained by the *in silico* prediction. The Venn diagram (**Figure 7-4**) shows that 71.6% of the herbal targets can be found in only one of the sources, indicating the supplementary relationship of the target collection methods. In total, the *huang qi* target profile contains 327 unique targets.

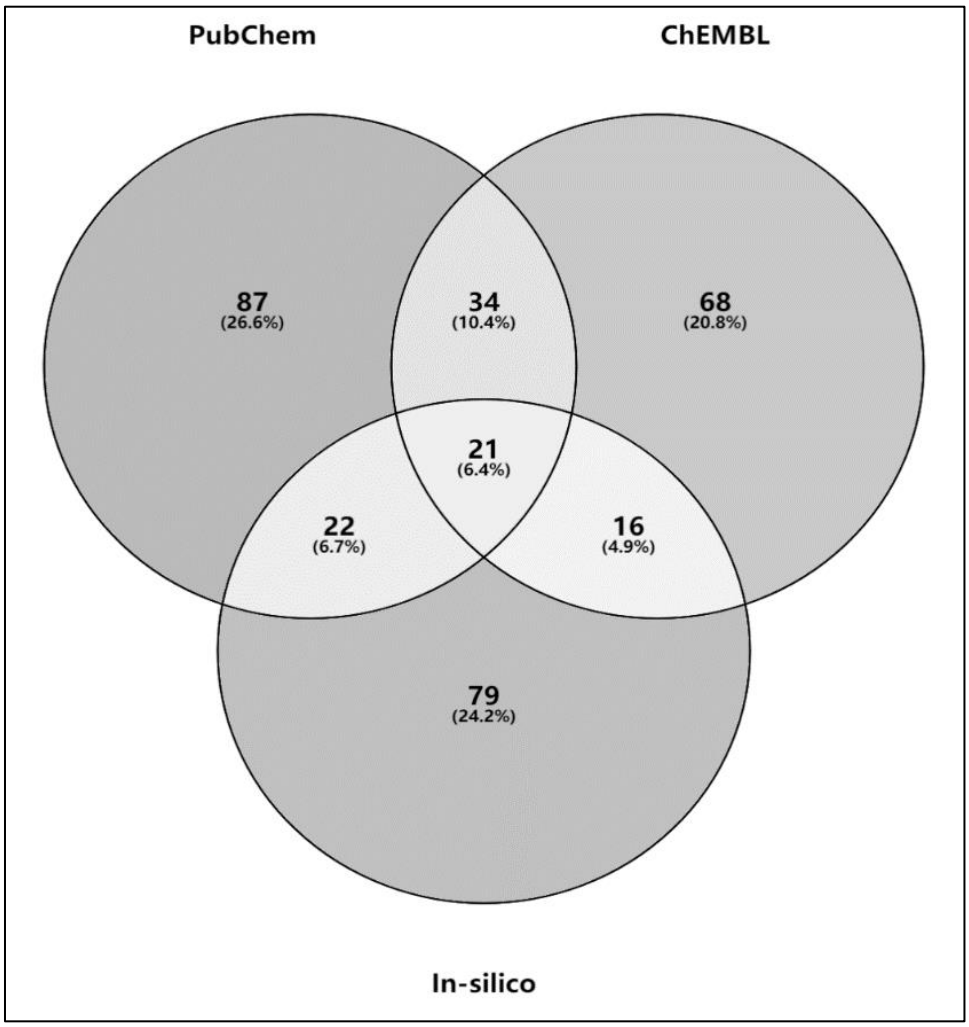

Figure 7-4 Venn diagram of *huang qi* targets from different sources

The number of targets gained from each of the 38 bioactive compounds varied from 4 to 194 (**Table 7-4**). Nearly 90% of compounds (32 out of 36) were thought to interact with less than 20 targets. Quercetin and kaempferol contributed many more targets than other compounds, with 194 and 89 related targets, respectively. The complex relationships between the 38 bioactive compounds and their interactive targets were then represented in a network manner (**Figure 7-5**). Notably, there were shared targets across different compounds and unique targets corresponding to specific compounds.

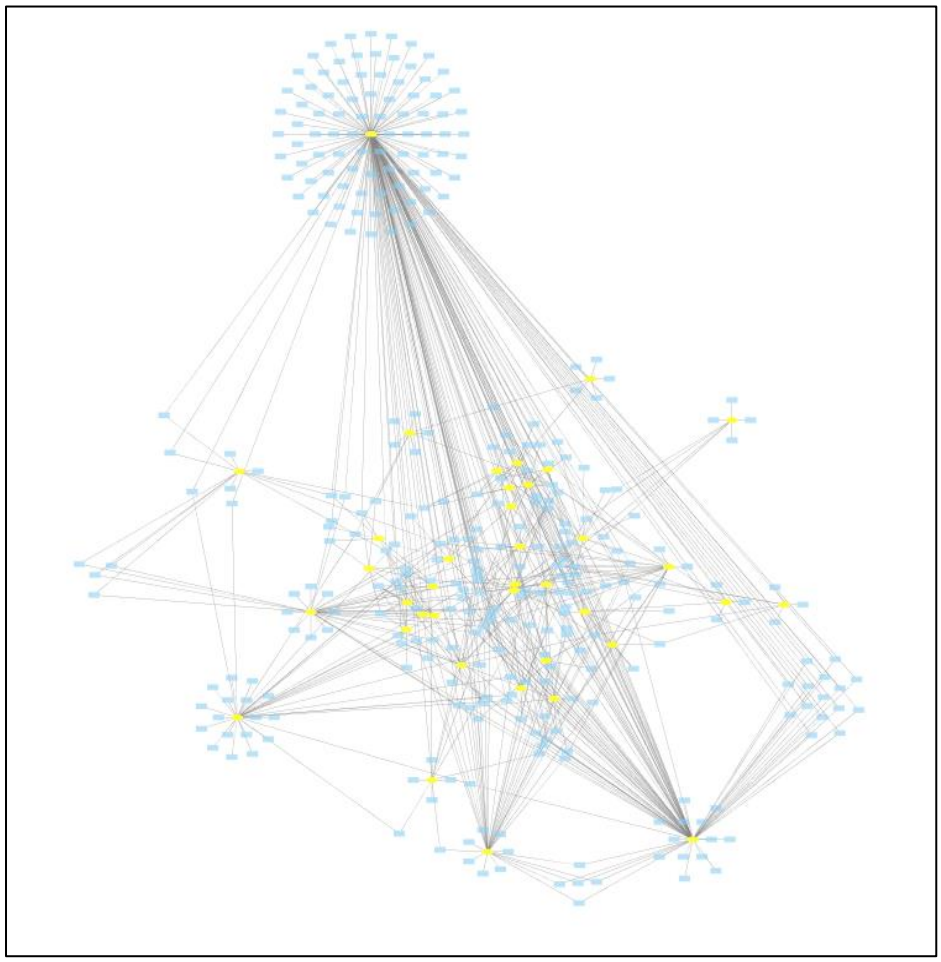

Figure 7-5 *Huang qi* compounds–targets network

Note: Yellow nodes represent bioactive compounds, blue nodes represent related targets, edges represent association relationships.

7.3.3 Network analysis of *huang qi*–DKD PPI network

7.3.3.1 Characteristics of huang qi–DKD PPI network

The PPI network for DKD was constructed based on 103 disease-related targets in the Cytoscape software, resulting in a DKD-specific PPI network with 2738 nodes and 53,925 edges. The PPI network for *huang qi* was generated by using the 327 putative targets by the same method, and a herbal PPI network with 8817 nodes and 195,294 edges was obtained. Then, a *huang qi*–DKD shared targets network was built, which consisted of 2269 nodes and 50,304 edges. Over 83% of targets in the disease PPI network were covered by the herbal PPI network.

7.3.3.2 Key targets of huang qi–DKD PPI network

The network analysis of the *huang qi*–DKD shared PPI network revealed that the top 10% cut-off values of centrality indices were 107 for DC, 0.00139859 for BC, 0.4606947 for CC and 0.03383857 for EC. Consequently, 118 key targets from the *huang qi*–DKD PPI network with all centrality indices higher than the cut-off values were identified (**Table 7-5**). The four centrality indices of each key target are provided in **Appendix 7**.

Table 7-5 Key targets of *huang qi*–DKD PPI network

Uniprot entry	Gene symbol	*Huang qi*–DKD key target name
P00519	ABL1	Tyrosine-protein kinase ABL1
P60709	ACTB	Actin, cytoplasmic 1
P07550	ADRB2	Beta-2 adrenergic receptor
P31749	AKT1	RAC-alpha serine/threonine-protein kinase
P05067	APP	Amyloid-beta A4 protein
P10275	AR	Androgen receptor
P49407	ARRB1	Beta-arrestin-1
P32121	ARRB2	Beta-arrestin-2
O14965	AURKA	Aurora kinase A
P38398	BRCA1	Breast cancer type 1 susceptibility protein
Q9Y297	BTRC	F-box/WD repeat-containing protein 1A
P0DP23	CALM1	Calmodulin-1
P0DP24	CALM2	Calmodulin-2
P0DP25	CALM3	Calmodulin-3
Q86VP6	CAND1	Cullin-associated NEDD8-dissociated protein 1

Uniprot entry	Gene symbol	*Huang qi*–DKD key target name
P22681	CBL	E3 ubiquitin-protein ligase CBL
Q9H0W5	CCDC8	Coiled-coil domain-containing protein 8
Q16543	CDC37	Hsp90 co-chaperone Cdc37
P06493	CDK1	Cyclin-dependent kinase 1
P38936	CDKN1A	Cyclin-dependent kinase inhibitor 1
Q00610	CLTC	Clathrin heavy chain 1
Q92905	COPS5	COP9 signalosome complex subunit 5
Q92793	CREBBP	CREB-binding protein
P68400	CSNK2A1	Casein kinase II subunit alpha
Q8NEV1	CSNK2A3	Casein kinase II subunit alpha 3
P35222	CTNNB1	Catenin beta-1
Q13617	CUL2	Cullin-2
Q93034	CUL5	Cullin-5
Q14999	CUL7	Cullin-7
O75530	EED	Polycomb protein EED
P68104	EEF1A1	Elongation factor 1-alpha 1
P00533	EGFR	Epidermal growth factor receptor
Q09472	EP300	Histone acetyltransferase p300
P03372	ESR1	Estrogen receptor
Q01844	EWSR1	RNA-binding protein EWS
Q9NRD1	FBXO6	F-box only protein 6
P21333	FLNA	Filamin-A
P02751	FN1	Fibronectin
P35637	FUS	RNA-binding protein FUS
P04406	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
P62993	GRB2	Growth factor receptor-bound protein 2
P16104	H2AFX	Histone H2AX
Q13547	HDAC1	Histone deacetylase 1
Q92769	HDAC2	Histone deacetylase 2
Q9UQL6	HDAC5	Histone deacetylase 5
Q9UBN7	HDAC6	Histone deacetylase 6
P68431	HIST1H3A; HIST1H3B; HIST1H3C; HIST1H3D; HIST1H3E; HIST1H3F; HIST1H3G; HIST1H3H; HIST1H3I; HIST1H3J	Histone H3.1
P09651	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1

Uniprot entry	Gene symbol	*Huang qi*–DKD key target name
P61978	HNRNPK	Heterogeneous nuclear ribonucleoprotein K
Q00839	HNRNPU	Heterogeneous nuclear ribonucleoprotein U
P07900	HSP90AA1	Heat shock protein HSP 90-alpha
P08238	HSP90AB1	Heat shock protein HSP 90-beta
P34932	HSPA4	Heat shock 70 kDa protein 4
P11021	HSPA5	Endoplasmic reticulum chaperone BiP
P11142	HSPA8	Heat shock cognate 71 kDa protein
P38646	HSPA9	Stress-70 protein, mitochondrial
P04792	HSPB1	Heat shock protein beta-1
P10809	HSPD1	60 kDa heat shock protein, mitochondrial
Q7Z6Z7	HUWE1	E3 ubiquitin-protein ligase HUWE1
Q14164	IKBKE	Inhibitor of nuclear factor kappa-B kinase subunit epsilon
Q9Y6K9	IKBKG	NF-kappa-B essential modulator
Q12906	ILF3	Interleukin enhancer-binding factor 3
P46940	IQGAP1	Ras GTPase-activating-like protein IQGAP1
P05412	JUN	Transcription factor AP-1
Q92993	KAT5	Histone acetyltransferase KAT5
Q13233	MAP3K1	Mitogen-activated protein kinase kinase kinase 1
P28482	MAPK1	Mitogen-activated protein kinase 1
P49736	MCM2	DNA replication licensing factor MCM2
Q00987	MDM2	E3 ubiquitin-protein ligase Mdm2
P01106	MYC	Myc proto-oncogene protein
P35579	MYH9	Myosin-9
P19338	NCL	Nucleolin
P19838	NFKB1	Nuclear factor NF-kappa-B p105 subunit
P06748	NPM1	Nucleophosmin
P04150	NR3C1	Glucocorticoid receptor
P04629	NTRK1	High affinity nerve growth factor receptor
O75147	OBSL1	Obscurin-like protein 1
Q504Q3	PAN2	PAN2-PAN3 deadenylation complex catalytic subunit PAN2
P09874	PARP1	Poly [ADP-ribose] polymerase 1
P12004	PCNA	Proliferating cell nuclear antigen
P30153	PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
P78527	PRKDC	DNA-dependent protein kinase catalytic subunit
O60260	PRKN	E3 ubiquitin-protein ligase parkin
P25788	PSMA3	Proteasome subunit alpha type-3
P63244	RACK1	Receptor of activated protein C kinase 1
Q04206	RELA	Transcription factor p65
Q99496	RNF2	E3 ubiquitin-protein ligase RING2

Uniprot entry	Gene symbol	*Huang qi*–DKD key target name
P62979	RPS27A	Ubiquitin-40S ribosomal protein S27a
Q9UBS0	RPS6KB2	Ribosomal protein S6 kinase beta-2
P29353	SHC1	SHC-transforming protein 1
Q96EB6	SIRT1	NAD-dependent protein deacetylase sirtuin-1
Q9NRC8	SIRT7	NAD-dependent protein deacetylase sirtuin-7
Q15796	SMAD2	Mothers against decapentaplegic homolog 2
P84022	SMAD3	Mothers against decapentaplegic homolog 3
P51532	SMARCA4	Transcription activator BRG1
Q9HCE7	SMURF1	E3 ubiquitin-protein ligase SMURF1
P08047	SP1	Transcription factor Sp1
Q13501	SQSTM1	Sequestosome-1
P12931	SRC	Proto-oncogene tyrosine-protein kinase Src
Q9UNE7	STUB1	E3 ubiquitin-protein ligase CHIP
Q13148	TARDBP	TAR DNA-binding protein 43
P04637	TP53	Cellular tumor antigen p53
Q9Y4K3	TRAF6	TNF receptor-associated factor 6
Q71U36	TUBA1A	Tubulin alpha-1A chain
P07437	TUBB	Tubulin beta chain
P0CG48	UBC	Polyubiquitin-C [Cleaved into: Ubiquitin]
P63279	UBE2I	SUMO-conjugating enzyme UBC9
P11441	UBL4A	Ubiquitin-like protein 4A
P55072	VCP	Transitional endoplasmic reticulum ATPase
P40337	VHL	von Hippel-Lindau disease tumor suppressor
P08670	VIM	Vimentin
O14980	XPO1	Exportin-1
P12956	XRCC6	X-ray repair cross-complementing protein 6
P31946	YWHAB	14-3-3 protein beta/alpha
P62258	YWHAE	14-3-3 protein epsilon
P61981	YWHAG	14-3-3 protein gamma
P27348	YWHAQ	14-3-3 protein theta
P63104	YWHAZ	14-3-3 protein zeta/delta

7.3.4 Enrichment analysis of key targets

All 127 key targets obtained from network analysis of the *huang qi*–DKD PPI network were included for GO and pathway enrichment analysis in the FunRich tool. But one target (Receptor of activated protein C kinase 1, RACK1) was not indexed in the FunRich default databases, therefore it was excluded from enrichment analysis.

7.3.4.1 GO enrichment analysis

The GO enrichment analysis showed that the 126 key targets of the *huang qi*–DKD PPI network were enriched in three biological processes and four molecular functions (**Table 7-6**). All enriched biological processes and the molecular functions of ubiquitin-specific protease activity are related to metabolic processes. The other three enriched molecular functions are associated with DNA and protein binding.

Table 7-6 Enriched biological process and molecular function of key targets

	GO Term	No. of genes input/ in background	Percentages of genes	Fold enrichment	*p*-value (Bonferroni method)
Biological process	Protein metabolism	30/1323	24.2	3.315129	6.51E-07
	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	38/2828	30.6	1.96433	0.00332
	Regulation of gene expression, epigenetic	5/66	4.0	11.0924	0.015752
Molecular function	Receptor signalling complex scaffold activity	10/322	8.1	4.543455	0.016648
	Ubiquitin-specific protease activity	17/377	13.7	6.594365	1.82E-07
	DNA binding	17/654	13.7	3.801382	0.000513
	Chaperone activity	8/126	6.5	9.290713	0.000548

7.3.4.2 Pathway enrichment analysis

As for the biological pathway analysis, the 126 key targets of the *huang qi*–DKD PPI network were found to be enriched in 174 pathways. The top 10 most significantly enriched biological pathways are shown in **Figure 7-7**, ranked by ascending logarithmic *p*-values.

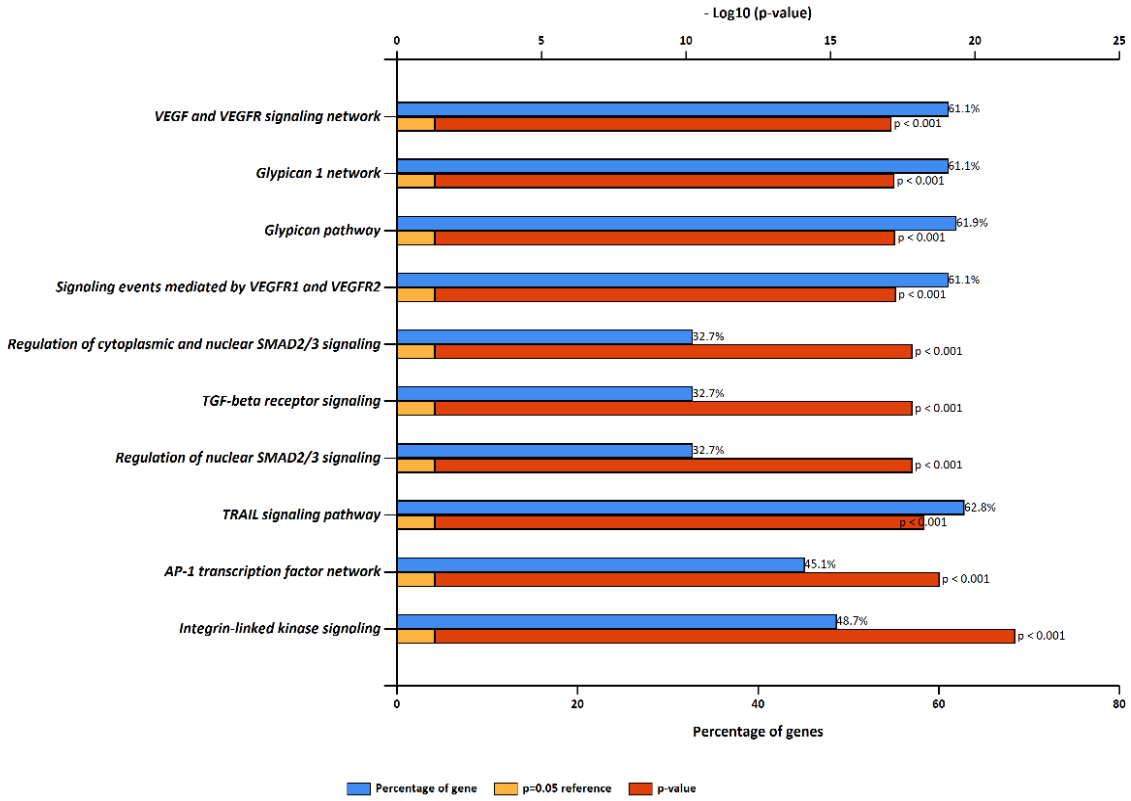

Figure 7-6 Top 10 enriched pathway of key targets

Abbreviations: AP: activator protein; TGF- β : transforming growth factor beta; TRAIL: tumour necrosis factor-related apoptosis inducing ligand; VEGF(R): vascular endothelial growth factor (receptor).

Among the pathways highlighted, cross-talk and internal linkage exist (**Figure 7-8**). Based on the relations, two groups can be distinguished among the top 10 enriched pathways. The first group includes the up-stream pathways of the integrin-linked kinase (ILK) signalling pathway, glypican pathway and activator protein (AP) 1 transcription factor network which impact on the downstream transforming growth factor beta (TGF- β) receptor signalling pathway and subsequent Smad 2/3 signal regulation. The second group of enriched upstream pathways include the vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) signalling network and the tumour necrosis factor-related apoptosis inducing ligand (TRAIL) signalling pathway. Both share the downstream pathway of the

nuclear factor kappa light chain enhancer of activated B cells (NF-κB) signalling pathway, which was also enriched but not in the top 10 list.

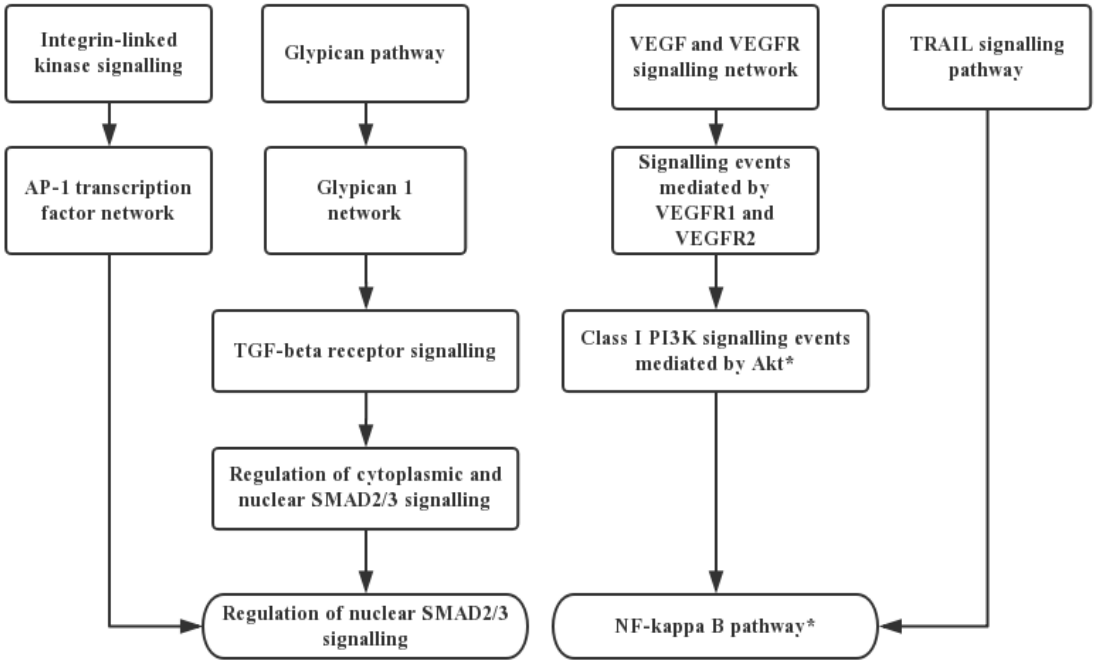

Figure 7-7 Relationship between top 10 enriched pathways

Abbreviations: Akt: protein kinase B; AP: activator protein; NF-κ: nuclear factor kappa light chain enhancer of activated B cells TGF-β: transforming growth factor beta; PI3K: phosphoinositide 3-kinases; TRAIL: tumour necrosis factor-related apoptosis inducing ligand; VEGF(R): vascular endothelial growth factor (receptor). Note: *indicates pathways not included in the top 10 list. Arrows represent sub-pathway relationships. For example, the glypican 1 network is the sub-pathway of the glypican pathway.

7.3.5 Interaction network of *huang qi* key compound–target–pathway

To further elucidate the potential new drug candidates, an interaction network of the *huang qi* key bioactive compounds, related targets and corresponding pathways was constructed (**Figure 7-9**). Only chemicals directly interacting with at least 2 targets contained in the

enriched pathways were shown in the network. Therefore, quercetin, betulinic acid, calycosin, formononetin, kaempferol, isorhamnetin, bifendate, gamma-Sitosterol and (24S)-24-Propylcholesta-5-ene-3beta-ol were included in this compound–target–pathway network. Quercetin connected with the largest number of key targets (18), followed by kaempferol (8).

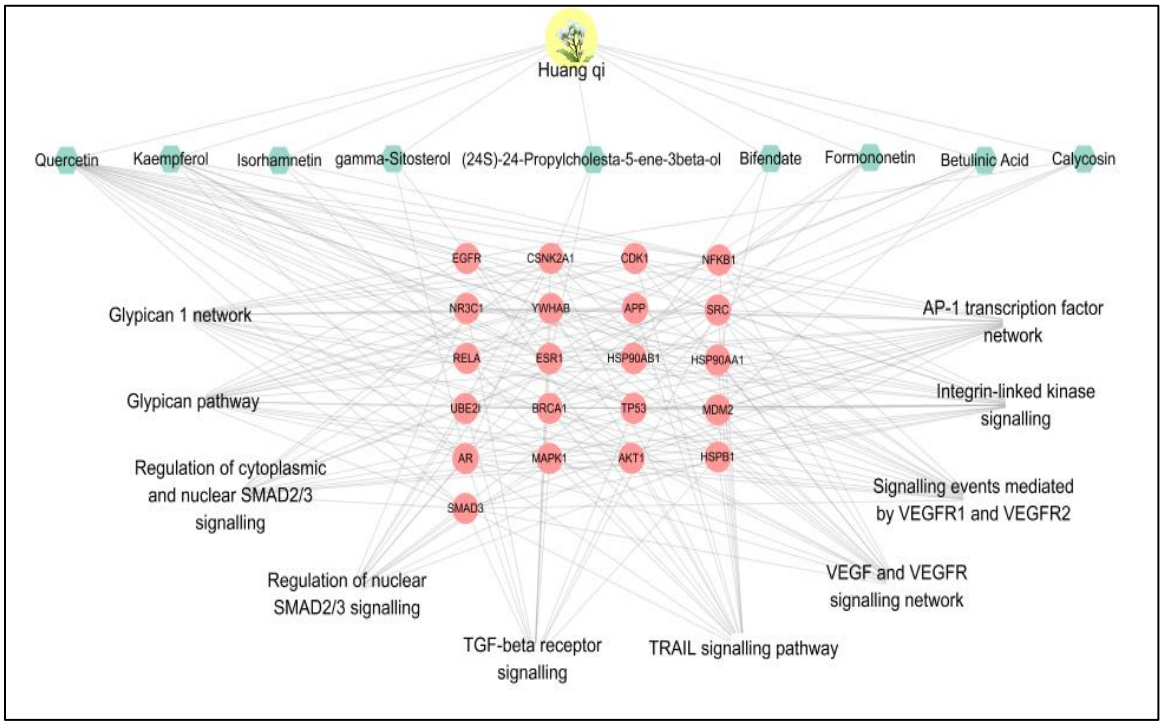

Figure 7-8 Interaction network of *huang qi* key compounds, key targets and top ten pathways

Note: Yellow node represent the herb *huang qi*; green nodes represent the bioactive compounds with multiple direct connections to key targets; pink nodes represent targets shared by *huang qi* compounds and top 10 enriched pathways.

7.4 Discussion

Huang qi is used in clinical practice to treat a range of conditions including DKD. In the field of nephrology, *huang qi* was reported to be effective in reducing proteinuria and improving renal function in patients with membranous nephropathy or chronic kidney disease [361, 362]. By systematically evaluating the evidence of both classical and

contemporary literature, it was revealed that *huang qi* was the most promising herb for the treatment of DKD. However, a lack of information regarding its action mechanism(s) raises safety concerns. especially in the renal-impaired population [2, 5, 248]. It is imperative to elucidate the molecular basis corresponding to the therapeutic effect of *huang qi*. In this network pharmacology study, 38 bioactive compounds of *huang qi* and a group of key targets are proposed. The treatment effect of *huang qi* appeared to be mainly mediated by the anti-oxidation, anti-inflammation and apoptosis related pathways according to the functional analysis results.

7.4.1 Bioactive compounds of *huang qi*

The chemical properties of bioactive compounds of *huang qi* identified in this study are slightly different from previous knowledge about drug chemistry profiles. Of the 38 bioactive compounds, twelve of them (32%) are large molecules with a weight over 500 g/mol, which does not fit Lipinski's rule of five indicating it has drug-likeness. But three of these large molecular compounds still satisfy OB and DL parameters, contributing 4 to 13 targets to the herbal targets network. Likewise, each of the 8 compounds that failed to meet the OB or DL criteria are associated with at least 6 targets. Seven compounds with exceptional molecular weight, OB or DL parameters were found to be directly influential on some of the key targets. It should be noted that the value of conventional chemistry parameters is not compromised by the above exceptions, as the 9 bioactive compounds with direct connection to multiple key targets all fit in the criteria.

7.4.1.1 Flavonoids

Among the 38 herbal compounds, 9 have direct connection with two or more key targets in the top 10 enriched pathways, indicating their important roles in the *huang qi*–DKD interaction network. Of the 9 compounds, quercetin is associated with far more targets than other compounds, in addition to direct interaction with 18 key targets of predominant pathways. Quercetin is a plant-source flavonoid and is ubiquitous in food, with anti-oxidative, anti-inflammatory and anti-proliferation properties [363]. *In vivo* studies reported that quercetin significantly reduced serum creatinine level and urinary protein

excretion in streptozotocin (STZ)-induced diabetic rats/mice [364-366]. But the effect of quercetin on controlling plasma glycaemic level was uncertain [365-367]. Renal pathological changes of glomerular swelling, glomerulosclerosis, tubulointerstitial fibrosis and renal cortex lymphocytes infiltration were attenuated after quercetin treatment [364, 366]. Quercetin alleviated oxidative stress injury in kidney tissues, as the activity of superoxide dismutase (SOD) was increased and reactive oxygen species (ROS) production was reduced in these animal studies. Moreover, it suppressed the neutrophil adhesion and intercellular adhesion molecular-1 (ICAM-1) expression in endothelial cells to exert anti-inflammation properties [364]. The renal fibrosis lesions in diabetic rats were also attenuated by administration of quercetin, mediated by inhibition of fibrogenic cytokine overexpression and epithelial–mesenchymal transition (EMT) in renal tubular proximal epithelial cells [367, 368].

Although the above experimental studies suggested that quercetin possessed a variety of protective activities in different parts of the renal system, the safety data regarding long-term use of quercetin in human subjects is limited. Quercetin has been tested in a small sample of healthy people and patients with obesity, chronic hepatitis C, chronic pelvic pain syndrome or prostatic disease [369]. In previous clinical studies, oral quercetin was used at up to 1000 mg/day with a treatment duration no longer than 12 weeks. In general, the adverse events attributed to administration of quercetin was mild and rare, and no significant change was observed in terms of kidney and liver function. It should be noted that the dose of quercetin used in these studies was low to moderate compared to daily diet intakes (about 25-250 mg/day). A chronic toxicity study reported that chronic nephropathy was developed in male F344/N rats after being fed with mild- and high- dose of quercetin for 2 years and the incidence of renal tubule adenoma or adenocarcinoma was higher in the high-dose male rats group (corresponding to 140 g/day intake of quercetin in a 70 kg individul) [370]. Eventhough the renal tubule tumor development of the chronic toxicity study was considered no relevance for extrapolation to humans, more data is needed to confirm the safety of long-term and high-dose supplement of quercetin, especially in patients with exiting renal lesions [371].

For other flavonoids which directly linked to the key DKD targets, experimental evidence related to anti-DKD bioactivities of kaempferol, isorhamnetin, calycosin and formononetin was also identified. Kaempferol, isorhamnetin and calycosin exhibited anti-inflammatory, anti-oxicant effects in high glucose-induced glomerular mesangial and/or endothelial cells, mediated by the NF-κB pathway [372-374]. In addition, calycosin decreased endothelial cell apoptosis and dysfunction induced by advanced glycation end products (AGE) [375]. Calycosin and formononetin reduced nitric oxide production and promoted endothelial nitric oxide synthase expression, resulting in regulation of endothelium-dependent vasodilation [376]. Kaempferol reduced the expression of profibrotic factors and inflammatory cytokines by inhibiting RhoA/Rho-kinase in both rat and human renal tubular epithelial cells [377]. Anti-hyperglycaemic effects were observed in animal experiments of formononetin [378, 379]. In a rat model of type 2 diabetes, lower serum creatinine level, improved creatinine clearance and decrease glycaemia was observed after 16 weeks of formononetin treatments [380].

However, smiliar to quercetin, toxicity information of flavonoids is insuffient to confirm their clinical safety. In vitro studies suggested that kaempferol may promote mixed lineage leukemia translocations in blood stem cells and increase risk of reproductive tract and estrogen-dependent tumors (such as breast cancer) due to its estrogenic activity [381]. But in animal studies, genotoxic and carcinogenic effects of kaempferol was not observed, and meta-analysis of epidemiologic studies indicated that the risk of breast cancer was about 20% lower in women with high intake of flavonols and flavones compared with women with low intake [382, 383]. More toxicology testing regarding the long-term use and supplementary dose of kaempferol, isorhamnetin, formononetin and calycosin are needed for rigorous safety evaluation.

7.4.1.2 Triterpenoids

Of the 9 candidate compounds, betulinic acid, gamma-sitosterol and (24S)-24-propylcholesta-5-ene-3beta-ol are triterpenoids. Betulinic acid is a natural product isolated from the bark of birch trees and is known for its anti-HIV, antineoplastic and anti-

inflammatory properties [384]. Lowering glycaemia and insulin effect of betulinic acid was reported in a STZ-induced diabetic rat model, along with amelioration of renal histopathological changes [385, 386]. Both studies indicated that betulinc acid exerted its renal protective effect via surpression of NF-κB pathway acticity in kidney tissues, which is consistent with the findings of network pharmacology. Experiments found that betulinic acid appeared to be safe at a dose of 100 mg/kg in rats, and a phase I/II clinical trial indicated that betulinic acid treatments for 20 days was well tolerated in HIV patients with a single oral dose of 250 mg/day [387, 388]. However, clinical data regarding the efficacy and safety of betulinic acid in renal impariment patients is not available and further research is needed.

Gamma-sitosterol also showed anti-diabetic proterties in STZ-induced diabetic rats in addition to hyperlipidemia regulation effect, therefore it was considered to be a protential therapeuitic agent for diabetes [389]. A subsequent toxicity study revealed that gamma-sitosterol induced DNA damage in human peripheral blood mononuclear cells so it is discourage to be consumped as a natural supplement [390].

7.4.1.3 Bifendate

Bifendate was discovered as an intermediate during the synthetic process of Schisandrin C [391]. Because of its alanine aminotransferase (ALT) lowering effect, bifendate has been adjuvantly used for patients with chronic hepatitis or other liver injuries to reduce the serum level of ALT in China [392, 393]. But the anti-hepatitis B virus and anti-hepatic fibrosis effect of bifendate is still controversial, and it is unclear whether lowering ALT by bifendate leads to improvement of clinically relevant outcomes [394, 395]. In the field of nephology, bifendate has been studied in kidney transplant patients for decreasing the plasma concertration of immunosuppressants [396, 397]. Evidence of bifendate as a potential therapy for diabetes and its complications have not been identified.

The evidence from network pharmacology results and existing publications suggests that some of the chemicals contained in *huang qi*, including quercetin, kaempferol,

isorhamnetin, formononetin, calycosin and betulinic acid could be the bioactive components corresponding to its therapeutic effect.

7.4.2 Action mechanisms of *huang qi*

Notably, there were two distinct action patterns of *huang qi*'s bioactive compounds. One is the direct regulation of targets involved in disease processes, as the putative action targets of *huang qi* shared 21 known DKD-related targets. In contrast, the alternative pattern is to modulate the molecular interaction network rather than a single target. By exploring the PPI relationship, a complex DKD network consisting of thousands of targets was identified. This could be the reason why current target-based agents are partially effective. Although only a quarter of DKD targets directly interacted with *huang qi*'s bioactive compounds (the first action pattern), over 80% of targets in the DKD–PPI network were covered by the *huang qi*–PPI network. The wide overlap area of PPI networks implied the network regulation function of *huang qi*. The second action pattern also confirmed the multi-component, multi-target features of herbal products.

Although the target number for *huang qi* is extensive, it seems that the key targets of the *huang qi*–DKD PPI network are markedly enriched in biological pathways related to signal transduction. Induced by the haemodynamic and metabolic changes, abnormal activation of diverse signal transduction pathways in renal cells has been observed and is closely related to the pathogenesis of DKD [398]. The TGF-β receptor signalling pathway is involved in angiogenesis, extracellular matrix production, immunosuppression and apoptosis induction, and it is a recognised pathogenic pathway of kidney diseases [399]. The TGF-β/Smad signalling pathway is highly activated in DKD, contributing to glomerular and tubulointerstitial cell fibrosis mediated mainly by Smad3 [400]. TGF-β 1 and high ambient glucose also stimulate ILK expression in podocytes and the upregulation of ILK signalling promotes podocytes EMT, migration and ultimately protein leakage through podocytes [401]. *In vivo* studies showed that proteinuria can be attenuated by inhibitions of TGF-β 1/ILK signalling pathways [401, 402].

The NF-κB signalling pathway is another well-known element of DKD pathogenesis which can be activated by hyperglycaemia or haemodynamic stretch [403]. Increased activity of the NF-κB pathway is mainly observed in diabetic tubular epithelial cells and podocytes. The activated NF-κB pathway induces production of cytokines, chemokines, adhesion molecules and proliferative proteins, resulting in circulating inflammatory cells infiltrating into the kidneys and initiating intra-renal inflammatory injuries, leading to DKD [403, 404]. The role of these signal transduction pathways in the development of DKD is well established based on existing experimental evidence, and the treatment effect of *huang qi* may be mediated by these pathways synergistically based on the findings of the enrichment analysis.

The roles of the VEGF-VEGFR signalling pathway and TRAIL signalling pathway in the pathogenesis of DKD have not been completely elucidated. The VEGF-VEGFR signalling pathway is known to be related to proliferation, migration and permeability of vascular endothelial cells [405]. It has been reported that VEGF signalling is abnormal, increased in early DKD and decreased in later stages. Elevated VEGF resulted in glomerulus endothelial cell proliferation and reduction led to cell apoptosis and glomerulosclerosis formation [398]. As for the TRAIL pathway, TRAIL was expressed most in tubular epithelial cells in DKD patients' renal samples and the expression level was correlated to the severity of tubulointerstitial pathological changes. However, in a study using TRAIL gene knock-out mice model, deficiency of TRAIL exacerbated inflammation, renal damage and insulin resistance, indicating a protective role of TRAIL [406]. It is uncertain whether the interaction between *huang qi*'s compounds and these two pathways exerts protective or detrimental effects.

7.4.3 Limitations

The potential bioactive compounds and action mechanism of *huang qi* explored by this network pharmacology approach have several limitations. Firstly, some chemical compounds with actual bioactivity may be missing due to the absorption, distribution,

metabolism and excretion (ADME) screening criteria. As techniques develop, new bioactive compounds of *huang qi* may be identified based on other parameters. Secondly, considering the herbal putative targets were collected from experimental evidence and *in silico* prediction, it is possible that a different set of targets will be found by new experiments or other prediction methods [407]. Finally, only PPI was explored in this study, whereas other interactions including generic regulatory networks, RNA networks and metabolic networks were not studied [408]. Collectively, the key bioactive compounds and action mechanisms of *huang qi* have been systematically explored in this study.

7.4.4 Implication for future studies

The multi-compound, multi-target, multi-pathway characteristics of *huang qi* in the treatment of DKD are presented in this study. In addition to the larger target number for *huang qi* than for DKD (327 versus 103, respectively), the size of the herbal PPI network is much bigger than the DKD PPI network. The broad effects and complex action mechanisms of *huang qi* bring concerns about potential toxicity and adverse herb–drug interactions. To reduce the potential risk, herbal therapy should be refined based on network pharmacology findings, called "component-based Chinese medicine" [409]. In this study, 9 herbal compounds of *huang qi* have been identified having direct regulation potential for key DKD targets. Some of these herbal compounds have also shown promise in pre-clinical experiments. However, only one ongoing phase II clinical trial evaluating the effects of quercetin plus Dasatinib in advanced DKD patients has been found [410]. Further research evaluating dosage, effect, safety and possible combinations of the 8 herbal compounds (excluding the gamma-sitosterol) may lead to novel poly-compound drugs for DKD.

The finding about the glypican pathway involved in the action mechanism of *huang qi* provides new insight with regard to the pathogenesis of DKD. Glypicans are a family of heparin sulfate proteoglycans anchored in the outer surface of the cell membrane. In the kidneys, glomerular endothelial cells mainly express glypican-1 while the epithelial cells express the glypican-5. A knockdown model of zebrafish revealed that deficiency in

glypican-1 caused endothelial cell injuries and loss of glypican-5, resulting in focal podocyte effacement, eventually manifested as oedematous phenotypes [411]. In a separate *in vivo* study, increased expression of glypican-5 and enhanced signal activity were observed in diabetic mesangial regions [412]. The role of glypicans and their related pathways in DKD need to be further confirmed by human cohort data.

7.5 Conclusion

The network pharmacology approach identified 38 bioactive compounds of *huang qi*, with 8 compounds promising for new drug development. The bioactive compounds of *huang qi* exert synergistic therapeutic effects by regulating the disturbed DKD molecular network, probably involving the TGF-β/Smad signalling pathway, ILK signalling pathway and NF-κB pathway. Glypican pathways may contribute to the pathogenesis of DKD and are potentially regulated by *huang qi*. Further experiments are required to confirm the action hypothesis generated in this study.

8 General Discussion and Conclusions

8.1 Summary of the research

As one of the microvascular complications of diabetes, DKD is prevalent and particularly in the context of the global diabetes epidemic. DKD has been the leading cause of end stage kidney disease (ESKD) for decades and substantially increases the risk of cardiovascular events and mortality. Current pharmacotherapies for DKD such as glycaemia management, BP control and renin-angiotensin system (RAS) inhibitors are only partially effective. An unmet need exists for novel or improved treatments targeting diabetic kidney injury. Chinese herbal medicine (CHM) has been used for treating symsptoms related to kidney disorders for many centuries. Knowledge from traditional use of CHM for symptoms relevant to those identified in modern diagnosis of DKD may provide new leads and innovative therapies. In this research project, evidence of classical and modern literature with regards to the use of CHM for DKD has been rigorously gathered and evaluated. Promising herbal candidates identified from literature evidence were further studied using a network pharmacology approach to decipher the bioactive compounds and potential action mechanisms related to DKD. With this "whole evidence" framework, herbal compounds with new drug development potential are proposed for future studies.

8.1.1 Classic Chinese medicine literature

The assessment of ancient evidence was performed by text-mining classical CM books. In a collection of 1156 CM books, three ancient disease terms of DKD (*Shen Xiao, Xia Xiao,* and *Xiao Shen*) retrieved 278 classical citations with treatment information. As early as the Han Dynasty, descriptions highly consistent with DKD already existed in the book *Variorum of Experiential Prescriptions* 集验方. Based on the historical text content and the contemporary DKD definition, the classical citations were divided into four groups to classify their similarity to DKD. Frequency analysis revealed that *Ba wei wan*, *Liu wei di huang wan* and *Hui xiang san* were the most commonly mentioned formulae for DKD among citations of different pools. The six herbal ingredients of *Liu wei di huang wan* were

frequently cited in all citation pools. Additionally, *huang qi* (*Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao), *ren shen* (*Panax ginseng* C. A. Mey.), *wu wei zi* (*Schisandra chinensis* (Turcz.) Baill.), *tian hua fen* (*Trichosanthes kirilowii* Maxim.) and *huang lian* (*Coptis chinensis* Franch.) were high-frequency herbs repeatedly appearing in different citation pools. Notably, the herb *huang qi* was commonly used in citations with turbid urine descriptions, indicating a potential role of *huang qi* for proteinuria.

8.1.2 Contemporary Chinese medicine literature

To evaluate the contemporary evidence, a systematic review assessed the overall efficacy and safety of CHM as adjunctive therapy for DKD. Due to the large number of clinical trials in this field, this review only included RCT of oral CHM compared with matched placebo to reduce the potential risk of bias. A comprehensive search in both English and Chinese databases identified 20 eligible RCTs with 2719 adult DKD patients involved. Seventeen studies investigated herbal formulae with multiple ingredients and five showed highly similar compositions. *Huang qi*, *di huang* and *da huang* were the top three most commonly used herbs of included RCTs. Low to moderate quality of evidence suggests that CHM is favourable in decreasing albuminuria/proteinuria, irrespective of concurrent use of ACEi/ARB. When used in combination with ACEi/ARB, CHM preserved eGFR (moderate-quality evidence). All CHM used in included studies were aristolochic acid (AA)-free and safety concerns related to CHM were not identified. The findings of this systematic review provide preliminary modern evidence of renal protective potential of CHM in general.

Comparing the common CHM in ancient literature and those in contemporary clinical trials, preservation of traditional use and innovative development are both observed. *Huang qi* as the herb specific for turbid urine in classical literature was applied in 15 out of 20 included RCTs. The second most common herb in clinical trials was *di huang*, which was the primary component of the classical formula *Liu wei di huang wan*. Although a substantial proportion of herbs used in included RCTs were commonly cited in classical literature, only one randomised, placebo-controlled trial of a traditional formulae was found in the

systematic review. The herbal composition of formulae in other collected RCTs were constructed and modified based on researchers' preferences, rather than direct use of classical formulae. The herbs commonly cited in classical literature are highly consistent with the herbs most frequenly used in contemporaty RCTs. But the formulae identified in classical literature and modern RCTs are different. The consistency of herbs and variations in formulae between classical and modern evidence may imply a process of both inheritance and evolution of CHM knowledge for DKD.

Since evidence of classical and contemporary literature both pointed out the frequent application of *huang qi* in the treatment for DKD and its typical symptoms, a systematic review focusing on *huang qi* preparations was performed to evaluate its efficacy and safety as adjunctive therapy. Consequently, 66 RCTs with 4785 DKD patients employing sole *huang qi* preparations without any concurrent use of herbal products was assessed. Adding *huang qi* preparations to current pharmacotherapies appeared to be beneficial and well-tolerated in short-term reduction of albuminuria/proteinuria and serum creatinine (Scr) concentrations in adult DKD patients. Notably, positive results for *huang qi* were dominated by the 61 RCTs using injected preparations. Although a promising trend for *huang qi* was observed, confidence in this finding was compromised due to the overall low methodological quality of included RCTs, imprecision of estimated effect and potential publication bias.

8.1.3 Network pharmacology

A network pharmacology approach was used in order to better understand the molecular basis corresponding to the therapeutic effect of *huang qi* in the context of DKD. In total, 38 potential bioactive compounds of *huang qi* were identified with 327 putative interactive targets. The network analysis of the *huang qi*–DKD PPI network revealed the 127 key targets involved in the mechanisms of action of *huang qi*, which are markedly enriched in signal transduction pathways including but not limited to TGF-β/Smad, ILK and NF-κB pathways. The complex interactions among the herbal bioactive compounds, multiple key targets and diverse related pathways underline the synergic effect of *huang qi*, clearly

demonstrated in the network approach. Moreover, quercetin, calycosin, formononetin, kaempferol, isorhamnetin and betulinic acid are proposed as chemical candidates for future anti-DKD new drug development.

Based on the case of *huang qi*, the network pharmacology approach showed its unique advantages in studying herbal medicine and complex diseases. First and foremost, the network analysis technique facilitated the rapid identification of the key bioactive compounds from the raw herbs and the corresponding targets, narrowing down the subsequent experimental scope. In addition, the protein-protein interaction networks provided a comprehensive understanding of the spectrum of biological effects exerted by the herbal components. The overall action mechanisms and toxicity profile of specific herbs can therefore be predicted and explored. Potential detrimental herbal compounds can be excluded during the development stage to avoid unexpected adverse effects. [409]. With the advances of network pharmacology, raw herbs with diverse components can be further simplified and developed as a group of bioactive compounds with fixed proportion and clear active mechanisms, leading to better quality control, efficacy and safety of herbal therapy.

8.2 Overall limitations of the research

In this research, a “whole evidence” strategy was employed with the attempt to identify and provide potential CHM as new therapeutic candidates for DKD. Several limitations need to be considered when interpreting the results. Firstly, the subject of the network pharmacology study (*huang qi*) was selected based on the available classical CM and contemporary CM evidence. With regards to the evidence from the classical literature, some DKD-related records are likely to be missing in the current analysis since only ancient disease terms were used to retrieve citations. More citations may be yeiled if ancient terms of the symptoms for diabetes and kidney diseases can be searched in combination. The commonly known formulae and herbs in classical literature may increase if the citation pool is expanded. Additional herbal candidates may be identified from network pharmacology studies of other high-frequency formulae and herbs.

In addition, the quality of current clinical evidence of *huang qi* preparations is low and evidence regarding its long-term benefit and safety, especially on clinically relevant outcomes, is lacking. In toxicity studies based on animal models, acute and sub-chronic toxicity of *huang qi* was not observed [246]. In clinical practice, the most frequently reported adverse effect of *huang qi* is allergic reactions when it used in high doses [245]. The actual effect and safety of *huang qi* preparations for DKD is inconclusive. When it comes to *huang qi* injection, some herbal compounds may be detrimental when intravenously administrated [245, 413].

Moreover, the included clinical trials did not provide information regarding the herbal chemical profile. The chemical constiuents of *huang qi* and the concentration of each compound employed in these clinical trials were unknown and could be different from the bioactive compounds used for network pharmacology study. Collectively, the bioactivity and pharmacological actions of the candidate compounds identified in *huang qi* need to be confirmed by further biological and pharmacological experiments.

8.3 Directions for future research

Currently, the formulae and their herbal ingredients identified in classical and contemporary CM research were only analysed for frequency. Advanced analysis methods such as cluster analysis may reveal the underlying relationships across ingredients (such as synergistic effect) within a formula and the prescription patterns between individual DKD symptoms and specific herbs [414, 415].

In current CM guidelines and textbooks, herbal therapies derived from the classical formulae *Ba wei wan* and *Liu wei di huang wan* have been recommended for DKD without support from clinical evidence [167-169]. As mentioned above, the number of high-quality RCTs evaluating the effect and safety of classical formulae is limited. The knowledge gap between traditional formulae and their actual clinical effects and safety for DKD needs to be established through adequately powered, well-conducted RCTs using validated primary

outcome measures.

Eight chemicals contained in *huang qi* showed potential therapeutic relevance to DKD. Subsequent experiments to test their individual and combined effects in both *in vivo* and *in vitro* DKD models are necessary for novel therapeutic agent development. Experimental testing for safety and toxicity of the herbal compound candidates is also required, particularly examination of potential nephrotoxicity and hepatoxicity.

According to the pathway analysis results, in addition to established pathways related to inflammation, oxidative stress and apoptosis, the renal protective properties of *huang qi* could be mediated via the glypican pathway. Decreased glypican-1 level in skin blood vessels is observed in diabetic patients, and exogenous supplements of glypican-1 improved perfusion and vessel formation in ischaemic diabetic mice [416]. Glypican-4, which is known as an insulin sensitiser, increased in the impaired glucose tolerance stage and then decreased after progressed to established diabetes [417, 418]. It remains unclear whether regulation of the glypican pathway may impact on the onset and progression of DKD, thus further studies are needed.

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Appendices

Appendix 1 Herbs included in the thesis

Pin yin name	Simplified Chinese characters	Scientific name	Pharmaceutical name
Ba ji tian	巴戟天	1. *Morinda officinalis* How	Morindae Officinalis Radix
Bai bian dou	白扁豆	1. *Dolichos lablab* L.	Lablab Semen Album
Bai zhu	白朮	1. *Atractylodes macrocephala* Koidz.	Atractylodis Macrocephalae Rhizoma
Bo he	薄荷	1. *Mentha haplocalyx* Briq.	Menthae Haplocalycis Herba
Bu gu zhi	补骨脂	1. *Psoralea corylifolia* L.	Psoraleae Fructus
Che qian zi	车前子	1. *Plantago asiatica* L. 2. *Plantago depressa* Willd.	Plantaginis Semen
Chi shao	赤芍	1. *Paeonia lactiflora* Pall. 2. *Paeonia veitchii* Lynch	Paeoniae Radix Rubra
Chi shi zhi	赤石脂	1. Hydrated aluminium silicate	Halloysitum Rubrum
Chong wei zi	茺蔚子	1. *Leonurus japonicus* Houtt.	Leonuri Fructus
Chuan lian zi	川楝子	1. *Melia toosendan* Sieb. et Zucc.	Toosendan Fructus
Chuan xiong	川芎	1. *Ligusticum chuanxiong* Hort.	Chuanxiong Rhizoma
Ci shi	磁石	1. Ferroferric oxide	Magnetitum
Da huang	大黄	1. *Rheum palmatum* L. 2. *Rheum tanguticum* Maxim. ex Balf. 3. *Rheum officinale* Baill.	Rhei Radix et Rhizoma
Dan shen	丹参	1. *Salvia miltiorrhiza* Bge.	Salviae Miltiorrhizae Radix et Rhizoma
Dang gui	当归	1. *Angelica sinensis* (Oliv.) Diels	Angelicae Sinensis Radix
Di gu pi	地骨皮	1. *Lycium chinense* Mill. 2. *Lycium barbarum* L.	Lycii Cortex
Di huang	地黄	1. *Rehmannia glutinosa* Libosch.	Rehmanniae Radix
Du zhong	杜仲	1. *Eucommia ulmoides* Oliv.	Eucommiae Cortex

Pin yin name	Simplified Chinese characters	Scientific name	Pharmaceutical name
E zhu	莪术	1. *Curcuma phaeocaulis* Val. 2. *Curcuma kwangsiensis* S. G. Lee et C. F. Liang 3. *Curcuma wenyujin* Y. H. Chen et C. Ling	Curcumae Rhizoma
Fu ling	茯苓	1. *Poria cocos* (Schw.) Wolf	Poria
Fu pen zi	覆盆子	1. *Rubus chingii* Hu	Rubi Fructus
Fu shen	茯神	1. *Poria cocos* (Schw.) Wolf	Poriae Sclerotium Pararadicis
Fu zi	附子	1. *Aconitum carmichaelii* Debx.	Aconiti Radix Lateralis Praeparata
Gan cao	甘草	1. *Glycyrrhiza uralensis* Fisch. 2. *Glycyrrhiza inflata* Bat. 3. *Glycyrrhiza glabra* L.	Glycyrrhizae Radix et Rhizoma
Gan jiang	干姜	1. *Zingiber officinale* Rosc.	Zingiberis Rhizoma
Ge gen	葛根	1. *Pueraria lobata* (Willd.) Ohwi	Puerariae Lobatae Radix
Gou qi zi	枸杞子	1. *Lycium barbarum* L.	Lycii Fructus
Gua lou	瓜蒌	1. *Trichosanthes kirilowii* Maxim. 2. *Trichosanthes rosthronii* Harms	Trichosanthis Fructus
Han shui shi [a]	寒水石	1. Calcite 2. Gypsum rubrum	Glauberitum
He shou wu	何首乌	1. *Polygonum multiflorum* Thunb.	Polygoni Multiflori Radix
Hong qu	红曲	1. *Monascus purpureu*s Went.	Fermentum Rubrum
Hu lu ba	胡芦巴	1. *Trigonella foenum-graecum* L.	Trigonellae Semen
Hua shi	滑石	1. Hydrated magnesium silicate	Talcum
Huai mi tan	槐米炭	1. *Styphnolobium japonicum* (L.) Schott.	Flos Sophorae Immaturus
Huang bo (Huang bai)	黄柏	1. *Phellodendron chinense* Schneid.	Phellodendri Chinensis Cortex
Huang lian	黄连	1. *Coptis chinensis* Franch. 2. *Coptis deltoidea* C. Y. Cheng et Hsiao 3. *Coptis teeta* Wall.	Coptidis Rhizoma
Huang qi	黄芪	1. *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao 2. *Astragalus membranaceus* (Fisch.) Bge.	Astragali Radix

Pin yin name	Simplified Chinese characters	Scientific name	Pharmaceutical name
Huang qin	黄芩	1. *Scutellaria baicalensis* Georgi	Scutellariae Radix
Huang shu kui hua	黄蜀葵花	1. *Abelmoschus manihot* (L.) Medic.	Abelmoschi Corolla
Huo xiang	藿香	1. *Pogostemon cablin* (Blanco) Benth. 2. *Agastache rugosa* (Fisch. & Mey.) O. Ktze.	Pogostemonis/Agastaches Herba
Ji nei jing	鸡内金	1. *Gallus gallus domesticus* Brisson	Galli Gigerii Endothelium Corneum
Jiang can	僵蚕	1. *Bombyx mori* Linnaeus. 2. *Beauveria bassiana* (Bals.) Vuillant	Bombyx Batryticatus
Jiang huang	姜黄	1. *Curcuma longa* L.	Curcumae Longae Rhizoma
Jie geng	桔梗	1. *Platycodon grandiflorum* (Jacq.) A. DC.	Platycodonis Radix
Jin ying zi	金樱子	1. *Rosa laevigata* Michx.	Rosae Laevigatae Fructus
Jue ming zi	决明子	1. *Cassia obtusifolia* L. 2. *Cassia tora* L.	Cassiae Semen
Ku shen	苦参	1. *Sophora flavescens* Ait.	Sophorae Flavescentis Radix
Lian qiao	连翘	1. *Forsythia suspensa* (Thunb.) Vahl	Forsythiae Fructus
Lian zi	莲子	1. *Nelumbo nucifera* Gaertn.	Nelumbinis Semen
Long gu	龙骨	1. Fossilised bone - various species	Fossilia Ossis Mastodi (Draconis Os)
Lu hui	芦荟	1. *Aloe barbadensis* Miller (syn. Aloe vera) 2. *Aloe ferox* miller	Aloe
Lu jiao	鹿角	1. *Cervus elaphus* Linnaeus 2. *Cervus nippon*Temminck	Cervi Cornu
Lu rong	鹿茸	1. *Cervus nippon* Temminck 2. *Cervus elaphus* Linnaeus	Cervi cornus pantotrichum
Mai dong (Mai meng dong)	麦冬（麦门冬）	1. *Ophiopogon japonicus* (L.f) Ker-Gawl.	Ophiopogonis Radix
Mang xiao	芒硝	1. Hydrated sodium sulfate	Natrii Sulfas
Mian bi xie	绵萆薢	1. *Dioscorea spongiosa* J. Q. Xi, M. Mizuno et W. L. Zhao 2. *Dioscorea futschauensis* Uline ex R. Kunth	Dioscoreae Spongiosae Rhizoma
Mu dan pi	牡丹皮	1. *Paeonia suffruticosa* Andr.	Moutan Cortex

Pin yin name	Simplified Chinese characters	Scientific name	Pharmaceutical name
Mu li	牡蛎	1. *Ostrea gigas* Thunberg 2. *Ostrea talienwhanensis* Crosse 3. *Ostrea rivularis* Gould	Ostreae Concha
Mu xiang	木香	1. *Aucklandia lappa* Decne.	Aucklandiae Radix
Niu bang zi	牛蒡子	1. *Arctium lappa* L.	Arctii Fructus
Niu xi	牛膝	1. *Achyranthes bidentata* Bl.	Achyranthis Bidentatae Radix
Nü zhen zi	女贞子	1. *Ligustrum lucidum* Ait.	Ligustri Lucidi Fructus
Pu huang	蒲黄	1. *Typha angustifolia* L. 2. *Typha orientalis* Presl	Typhae Pollen
Qian shi	芡实	1. *Euryale ferox* Salisb.	Euryales Semen
Qing dai	青黛	1. *Baphicacanthus cusia* (Nees) Bremek. 2. *Polygonum tinctorium* Ait. 3. *Isatis indigotica* Fort.	Indigo Naturalis
Qing feng teng	青风藤	1. *Sinomenium acutum* (Thunb.) Rehd. et Wils. 2. *Sinomenium acutum* (Thunb.) Rehd. et Wils. var. *cinereum* Rehd. et Wils	Sinomenii Caulis
Ren shen	人参	1. *Panax ginseng* C. A. Mey.	Ginseng Radix et Rhizoma
Rou cong rong	肉苁蓉	1. *Cistanche deserticola* Y. C. Ma 2. *Cistanche tubulosa* (Schrenk) Wight	Cistanches Herba
Rou gui	肉桂	1. *Cinnamomum cassia* Presl	Cinnamomi Cortex
Ru xiang	乳香	1. *Boswellia carterii* Birdw. 2. *Boswellia bhaw-dajiana* Birdw.	Olibanum
San qi (Tian qi)	三七（田七）	1. *Panax notoginseng* (Burk.) F.H.Chen	Notoginseng Radix et Rhizoma
Sang piao xiao	桑螵蛸	1. *Tenodera sinensis* Saussure 2. *Statilia maculata* (Thunberg) 3. *Hierodula patellifera* (Serville)	Mantidis Oötheca
Sha ren	砂仁	1. *Amomum villosum* Lour. 2. *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen 3. *Amomum longiligulare* T. L. Wu	Amomi Fructus
Shan yao	山药	1. *Dioscorea opposita* Thunb.	Dioscoreae Rhizoma

Pin yin name	Simplified Chinese characters	Scientific name	Pharmaceutical name
Shan zhu yu	山茱萸	1. *Cornus officinalis* Sieb. et Zucc.	Corni Fructus
She chuang zi	蛇床子	1. *Cnidium monnieri* (L.) Cuss.	Cnidii Fructus
Sheng di	生地	1. *Rehmannia glutinosa* Libosch.	Rehmanniae Radix
Shi gao	石膏	1. Hydrated calcium sulfate	Gypsum Fibrosum
Shi geng teng ye	匙羹藤叶	1. Gymnema sylvestre (Retz.) R.Br. ex Sm.	Gymnema latifolium
Shi hu	石斛	1. *Dendrobium nobile* Lindl. 2. *Dendrobium chysotoxum* Lindl. 3. *Dendrobium fimbriatum* Hook.	Dendrobii Caulis
Shu di huang	熟地黄	1. *Rehmannia glutinosa* Libosch.	Rehmanniae Radix Praeparata
Shui zhi	水蛭	1. *Whitmania pigra* Whitman 2. *Hirudo nipponica* Whitman 3. *Whitmania acranulata* Whitman	Hirudo
Tian dong	天冬	1. *Asparagus cochinchinensis* (Lour.) Merr.	Asparagi Radix
Tian hua fen (Gua lou gen)	天花粉（栝蒌根）	1. *Trichosanthes kirilowii* Maxim. 2. *Trichosanthes rosthornii* Harms	Trichosanthis Radix
Tu bie chong (Zhe chong)	土鳖虫（蜇虫）	1. *Eupolyphaga sinensis* Walker 2. *Steleophaga plancyi* (Boleny)	Eupolyphaga/ Steleophaga
Tu si zi	菟丝子	1. *Cuscuta australis* R. Br. 2. *Cuscuta chinensis* Lam.	Cuscutae Semen
Wei mao	卫矛	1. *Euonymus alatus* (Thunb.) Sieb	Euonymus alatus
Wu bei Zi	五倍子	1. *Rhus chinensis* Mill. 2. *Rhus potaninii* Maxim. 3. *Rhus punjabensis* Stew. var. sinica (Diels) Rehd. et Wils. 4. *Melaphis chinensis* (Bell) Baker	Galla Chinensis
Wu wei zi	五味子	1. *Schisandra chinensis* (Turcz.) Baill.	Schisandrae Chinensis Fructus
Xi yang shen	西洋参	1. *Panax quinquefolium* L.	Panacis Quinquefolii Radix
Xia ku cao	夏枯草	1. *Prunella vulgaris* L.	Prunellae Spica
Xiao hui xiang	小茴香	1. *Foeniculum vulgare* Mill.	Foeniculi Fructus
Xuan shen	玄参	1. *Scrophularia ningpoensis* Hemsl.	Scrophulariae Radix
Yi mu cao	益母草	1. *Leonurus japonicus* Houtt.	Leonuri herba

Pin yin name	Simplified Chinese characters	Scientific name	Pharmaceutical name
Yi zhi	益智	1. *Alpinia oxyphylla* Miq.	Alpiniae Oxyphyllae Fructus
Yin xing ye	银杏叶	1. *Ginkgo biloba* L.	Ginkgo Folium
Yin yang huo (Xian ling pi)	淫羊藿（仙灵脾）	1. *Epimedium brevicornum* Maxim. 2. *Epimedium sagittatum* (Sieb. et Zucc.) Maxim. 3. *Epimedium pubescens* Maxim. 4. *Epimedium koreanum* Nakai	Epimedii Folium
Yu gan zi	余甘子	1. *Phyllanthus emblica* L.	Phyllanthi Fructus
Yu yu liang	禹余粮	1. Basic ferric oxide	Limonitum
Yuan zhi	远志	1. *Polygala tenuifolia* Willd. 2. *Polygala sibirica* L.	Polygalae Radix
Ze xie	泽泻	1. *Alisma orientalis* (Sam.) Juzep.	Alismatis Rhizoma
Zhi mu	知母	1. *Anemarrhena asphodeloides* Bge.	Anemarrhenae Rhizoma
Zhi qiao (Zhi ke)	枳壳	1. *Citrus aurantium L.*	Aurantii Fructus
Zhi zi	栀子	1. *Gardenia jasminoides* Ellis	Gardeniae Fructus
Zhu sha	朱砂	1. Mercuric sulfide	Cinnabaris

Note: The use of some herbs may be restricted in some countries; readers are advised to comply with relevant regulations. *Qian dan* 铅丹, *jin bo* 金箔, *yin bo* 银箔, *tie fen* 铁粉, *bai shi zhi* 白石脂, *jian si* 茧丝, *hua cong rong* 花苁蓉 and *tu gua gen* 土瓜根 were cited in classical literature but are not indexed in contemporary pharmacopoeia, thus scrientific and pharmatheutical names can not be provided.

Appendix 2 Example search strategy of systematic review of CHM for DKD

Search block	Search terms
Intervention	Traditional Chinese Medicine OR Chinese Traditional Medicine OR Chinese Herbal Drugs OR Chinese Drugs, Plant OR Medicine, Traditional OR Ethnopharmacology OR Ethnomedicine OR Ethnobotany OR Medicine, Kampo OR Kanpo OR TCM OR Medicine, Ayurvedic OR Phytotherapy OR Herbology OR Plants, Medicinal OR Plant Preparation OR Plant Extract OR Plants, Medicine OR Materia Medica OR Single Prescription OR Chinese Medicine Herb OR Herbal Medicine OR Herbs
Condition	Diabetic Nephropathies OR Diabetic Nephropathy OR Diabetic Kidney Disease OR Diabetic Kidney Diseases OR Kimmelstiel Wilson Syndrome OR Kimmelstiel Wilson Disease OR Diabetic Glomerulosclerosis OR Nodular Glomerulosclerosis OR Intracapillary Glomerulosclerosis OR albuminuria OR Microalbuminuria OR proteinuria OR Glomerulosclerosis OR Glomerulonephritis OR Kimmelstiel wilson nephropathy OR diabetic nephrosclerosis
Study design	Systematic[sb] OR "randomized controlled trial"[pt] OR "controlled clinical trial"[pt] OR "randomized"[tiab] OR "placebo"[tiab] OR "drug therapy"[sh] OR "randomly"[tiab] OR "trial"[tiab] OR "groups"[tiab] OR "cohort studies"[mesh] OR "case-control studies"[mesh] OR "comparative study"[pt] OR "risk factors"[mesh] OR "cohort"[tw] OR "compared"[tw] OR "groups"[tw] OR "case control"[tw] OR "multivariate"[tw] OR "case series"[tw]

Note: The above search strategy was designed for MEDLINE. The three search blocks were connected with the Boolean operators “AND” to build the overall search terms.

Appendix 3 Subgroup analysis of primary outcomes in systematic review of CHM for DKD

Outcome or subgroup	No. of trials	Pts	Statistical method	Effect estimate (95%CI)	I^2	*p* value
Urinary albumin excretion						
Subgroup-CHM formulae						
Qiwei Granules	2	104	MD	-70.06 [-88.84, -51.28]	0%	*p* <0.0001
Arctiin Granules	2	595	Std. MD	-0.38 [-0.56, -0.20]	0%	*p* <0.0001
Tang shen ning Formulae group	2	330	MD	-48.16 [-55.12, -41.20]	95%	*p* <0.0001
Subgroup-Measurement Units						
CHM vs placebo-AER	1	186	MD	-149.48 [-362.79, 63.83]	NA	*p* =0.17
CHM vs placebo-ACR	2	124	MD	-30.53 [-76.59, 15.53]	66%	*p* =0.19
CHM vs placebo-UAE	5	711	MD	-60.91 [-76.82, -45.01]	53%	*p* <0.0001
CHM vs placebo + ACEi/ARB-AER	1	119	MD	-48.85 [-53.30, -44.40]	NA	*p* <0.0001
CHM vs placebo + ACEi/ARB-UAE	2	330	MD	-48.16 [-55.12, -41.20]	95%	*p* <0.0001
24-hour proteinuria						
Subgroup-baseline UP						
CHM vs placebo-baseline UP < 0.5g/d	2	453	MD	-378.34 [-649.90, -106.77]	63%	*p* =0.006
CHM vs placebo-baseline UP > 0.5g/d	2	246	Std. MD	-1.49 [-3.97, 0.99]	97%	*p* =0.24
CHM + ACEi/ARB vs placebo + ACEi/ARB -baseline UP < 0.5g/d	2	284	MD	-31.30 [-68.61, 6.02]	61%	*p* =0.10
CHM + ACEi/ARB vs placebo + ACEi/ARB -baseline UP > 0.5g/d	2	205	MD	0.11 [-0.67, 0.88]	74%	*p* =0.79
Subgroup-CHM formulae						
Qiwei Granules	2	104	Std. MD	-2.47 [-3.11, -1.83]	21%	*p* <0.0001
Arctiin Granules	2	595	MD	-407.65 [-732.24, -83.05]	45%	*p* =0.01
Tang shen fang group	2	205	MD	0.11 [-0.67, 0.88]	74%	*p* =0.79

Outcome or subgroup	No. of trials	Pts	Statistical method	Effect estimate (95%CI)	I^2	*p* value
Subgroup-Measurement Units	8					
CHM vs placebo-g/24h	1	60	MD	-0.93 [-1.13, -0.73]	NA	*p* <0.0001
CHM vs placebo-mg/24h	3	639	MD	-324.42 [-485.15, -163.69]	30%	*p* <0.0001
CHM + ACEi/ARB vs placebo + ACEi/ARB-g/24h	2	205	MD	0.11 [-0.67, 0.88]	74%	*p* =0.79
CHM + ACEi/ARB vs placebo + ACEi/ARB-mg/24h	2	284	MD	-31.30 [-68.61, 6.02]	61%	*p* =0.10
Serum creatinine level						
Subgroup-baseline Scr						
CHM + ACEi/ARB vs placebo + ACEi/ARB -baseline Scr normal	3	227	MD	-2.12 [-6.48, 2.23]	0%	*p* =0.34
CHM + ACEi/ARB vs placebo + ACEi/ARB -baseline Scr abnormal	2	368	MD	-9.99 [-17.71, -2.26]	0%	*p* =0.01
CHM vs placebo + ACEi/ARB-baseline Scr normal	3	434	MD	-4.07 [-6.13, -2.01]	0%	*p* =0.0001
CHM vs placebo + ACEi/ARB-baseline Scr abnormal	1	156	MD	-2.84 [-18.18, 12.50]	NA	*p* =0.72
Subgroup-CHM formulae						
Tang shen fang group	2	286	MD	-6.06 [-14.60, 2.47]	0%	*p* =0.16
Tang shen ning Formulae group	2	330	MD	-3.96 [-6.13, -1.78]	6%	*p* =0.0004
Glomerular filtration rate						
Subgroup-baseline GFR						
CHM + ACEi/ARB vs placebo + ACEi/ARB -baseline GFR>90	2	249	MD	9.38 [1.07, 17.70]	4%	*p* =0.03
CHM + ACEi/ARB vs placebo + ACEi/ARB -baseline GFR<90	2	286	MD	5.22 [0.69, 9.74]	0%	*p* =0.02
CHM vs placebo + ACEi/ARB-baseline GFR>90	1	90	MD	-9.99 [-13.62, -6.36]	NA	*p* <0.0001
CHM vs placebo + ACEi/ARB-baseline GFR<90	3	452	MD	4.48 [-1.32, 10.28]	70%	*p* =0.13
Subgroup-CHM formulae						
Tang shen fang group	2	286	MD	5.22 [0.69, 9.74]	0%	*p* =0.02

Outcome or subgroup	No. of trials	Pts	Statistical method	Effect estimate (95%CI)	I^2	*p* value
Tang shen ning Formulae group	2	330	MD	-0.89 [-18.62, 16.85]	99%	*p* =0.92
Subgroup-Measurements						
CHM + ACEi/ARB vs placebo + ACEi/ARB-Ccr	1	144	MD	5.80 [1.01, 10.59]	NA	*p* =0.02
CHM + ACEi/ARB vs placebo + ACEi/ARB-eGFR	3	391	MD	7.13 [-0.29, 14.56]	11%	*p* =0.06
CHM vs placebo + ACEi/ARB-Ccr	2	246	MD	-4.14 [-15.81, 7.53]	93%	*p* =0.49
CHM vs placebo + ACEi/ARB-eGFR	2	296	MD	5.25 [-4.65, 15.15]	46%	*p* =0.30

Abbreviations: ACEi: angiotensin-converting enzyme inhibitors; ACR: albuminuria-to-creatinine ratio; AER: albuminuria excretion rate; ARB: angiotensin receptor blockers; Ccr: creatinine clearance; CHM: Chinese herbal medicine; CI: confidence interval; GFR: glomerular filtration rate; MD: mean difference; NA: not applicable; Pts: patients; Std: standard.; Scr: serum creatinine concentration; UAE: urinary albuminuria excretion; UP: urinary proteinuria.

Appendix 4 Sensitivity analysis of primary outcomes in systematic review of CHM for DKD

Outcome	Studies	Participants	Statistical method	Effect estimate (95% CI)	I^2	*p* value
Urinary albumin excretion						
CHM vs placebo	4	798	Std. Mean Difference	-0.54 [-0.85, -0.22]	73%	*p* =0.0009
CHM+ACEi/ARB vs placebo+ACEi/ARB	3	330	Std. Mean Difference	-0.56 [-1.04, -0.08]	64%	*p* =0.02
24-hour proteinuria						
CHM vs placebo	2	595	Mean Difference	-407.65 [-732.24, -83.05]	45%	*p* =0.01
CHM+ACEi/ARB vs placebo+ACEi/ARB	3	429	Std. Mean Difference	-0.12 [-0.60, 0.37]	81%	*p* =0.63
CHM vs placebo+ACEi/ARB	2	260	Std. Mean Difference	0.00 [-0.32, 0.32]	26%	*p* =1.00
Serum creatinine level						
CHM vs placebo	1	41	Mean Difference	10.31 [-2.26, 22.88]	NA	*p* =0.11
CHM+ACEi/ARB vs placebo+ACEi/ARB	4	535	Mean Difference	-5.59 [-10.61, -0.58]	0%	*p* =0.03
CHM vs placebo+ACEi/ARB	2	260	Mean Difference	-6.23 [-19.51, 7.05]	0%	*p* =0.36
Glomerular filtration rate						
CHM+ACEi/ARB vs placebo+ACEi/ARB	4	535	Mean Difference	6.28 [2.42, 10.14]	0%	*p* =0.001
CHM vs placebo+ACEi/ARB	2	212	Mean Difference	1.50 [-3.08, 6.09]	0%	*p* =0.52

Abbreviations: ACEi: angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CHM: Chinese herbal medicine; CI: confidence interval; NA: not applicable; Std: standard.

Appendix 5: Example search strategy in systematic review of *huang qi* for DKD

Step	Search block	Search terms
#1	Condition	Diabetic Nephropathies OR Diabetic Nephropathy OR Diabetic Kidney Disease OR Diabetic Kidney Diseases OR Kimmelstiel Wilson Syndrome OR Kimmelstiel Wilson Disease OR Diabetic Glomerulosclerosis OR Nodular Glomerulosclerosis OR Intracapillary Glomerulosclerosis OR albuminuria OR Microalbuminuria OR proteinuria OR Glomerulosclerosis OR Glomerulonephritis OR kimmelstiel wilson nephropathy OR diabetic nephrosclerosis
#2	Intervention	Astragal*[Title/Abstract] OR "A. membranaceus"[Title/Abstract] OR vetch [Title/Abstract] OR "Astragalus Plant"[Mesh:NoExp] OR "radix astragali"[Text Word] OR Milkvetch OR Astragalus OR Huangqi OR "huang qi" OR "Astragalus membranaceus"
#3	Condition combined with Intervention	#1 AND #2

Appendix 6: Subgroup analysis and of primary outcomes of syetematic review of *huang qi* for DKD

Outcome	Subgroups	Studies	Participants	Statistical method	Effect estimate [95% CI]	I2	p value
Albuminuria							
Duration	< 1 month	22	1551	Std. Mean Difference	-2.43 [-3.05, -1.82]	95%	*p* <0.0001
	1-3 months	13	877	Std. Mean Difference	-1.25 [-1.61, -0.89]	83%	*p* <0.0001
Baseline kidney function	Normal	8	511	Std. Mean Difference	-1.50 [-2.28, -0.71]	93%	*p* =0.0002
	Mild-moderate imparied	1	56	Std. Mean Difference	-1.22 [-1.79, -0.64]	NA	*p* <0.0001
Measurement	Albuminuria excretion rate (μg/min)	12	814	Mean Difference	-30.86 [-41.54, -20.17]	89%	*p* <0.0001
	24-hour albuminuria excretion (mg/24h)	17	1211	Mean Difference	-50.21 [-61.54, -38.87]	98%	*p* <0.0001
	Albuminuria-concentration (mg/L)	7	464	Mean Difference	-14.63 [-19.42, -9.85]	95%	*p* <0.0001
Astragalus Injection dosage	20 mL	5	312	Std. Mean Difference	-2.18 [-3.10, -1.27]	90%	*p* <0.0001
	30 mL	4	270	Std. Mean Difference	-1.18 [-1.80, -0.55]	82%	*p* =0.0002
	40 mL	13	950	Std. Mean Difference	-1.62 [-2.30, -0.94]	95%	*p* <0.0001
	50 mL	5	414	Std. Mean Difference	-2.80 [-4.36, -1.24]	97%	*p* =0.0004
	60 mL	5	307	Std. Mean Difference	-3.23 [-4.79, -1.67]	94%	*p* <0.0001
Proteinuria							
Duration	< 1 month	19	1312	Std. Mean Difference	-2.00 [-2.64, -1.35]	95%	*p* <0.0001
	1 - 3 months	8	526	Std. Mean Difference	-1.34 [-1.89, -0.79]	87%	*p* <0.0001
Baseline kidney function	Normal	1	43	Std. Mean Difference	-0.36 [-0.96, 0.24]	NA	*p* =0.24
	Mild-moderate impaired	4	263	Std. Mean Difference	-1.73 [-2.77, -0.68]	92%	*p* =0.001
Measurement	24-hour proteinuria excretion (g/24h)	20	1239	Mean Difference	-0.43 [-0.52, -0.34]	90%	*p* <0.0001
	24-hour proteinuria excretion (mg/24h)	7	599	Mean Difference	-59.64 [-74.32, -44.97]	96%	*p* <0.0001
	20 mL	13	866	Std. Mean Difference	-1.94 [-2.67, -1.21]	95%	*p* <0.0001

Outcome	Subgroups	Studies	Participants	Statistical method	Effect estimate [95% CI]	I2	p value
Astragalus Injection dosage	30 mL	4	290	Std. Mean Difference	-1.02 [-1.80, -0.25]	89%	*p* =0.009
	40 mL	8	512	Std. Mean Difference	-1.50 [-2.23, -0.78]	92%	*p* <0.0001
	50 mL	1	110	Std. Mean Difference	-4.97 [-5.74, -4.20]	NA	*p* <0.0001
	60 mL	1	60	Std. Mean Difference	-2.72 [-3.43, -2.00]	NA	*p* <0.0001
Serum Creatinine Concentration							
Duration	< 1 month	28	2284	Mean Difference	-14.50 [-19.02, -9.99]	97%	*p* <0.0001
	1 - 3 months	9	656	Mean Difference	-13.78 [-26.74, -0.83]	98%	*p* =0.04
Baseline kidney function	Normal	5	314	Mean Difference	-5.27 [-8.93, -1.61]	74%	*p* =0.005
	Mild-moderate impaired	3	213	Mean Difference	-53.98 [-105.61, -2.35]	99%	*p* =0.04
Astragalus dosage	20 mL	8	756	Mean Difference	-14.17 [-21.11, -7.23]	93%	*p* <0.0001
	30 mL	8	559	Mean Difference	-18.95 [-27.80, -10.10]	97%	*p* <0.0001
	40 mL	12	934	Mean Difference	-15.21 [-25.46, -4.96]	98%	*p* =0.004
	50 mL	5	444	Mean Difference	-7.65 [-17.05, 1.75]	95%	*p* =0.11
	60 mL	4	247	Mean Difference	-6.11 [-11.68, -0.55]	77%	*p* =0.03
Glomerulus Filtration Rate							
Duration	< 1 month	1	43	Mean Difference	-1.10 [-11.44, 9.24]	NA	*p* =0.83
	1 - 3 months	3	180	Mean Difference	-3.83 [-10.93, 3.28]	60%	*p* =0.29
Measurement	Cockcroft-Gault equation	3	171	Mean Difference	-0.22 [-5.02, 4.58]	0%	*p* =0.93
	Serum creatinine based estimation	1	52	Mean Difference	1.46 [-6.29, 9.21]	NA	*p* =0.71

Abbreviations: CI: confidence interval; g: gram; L: litre; mg: milligram; mL: milliliter; min: minutes; NA: not applicable; Std: standard.

Appendix 7 Central indices of key targets of *huang qi*–DKD network

Entry	Gene symbol	Key target name	Degree centrality	Betweenness centrality	Closeness centrality	Eigenvector centrality
P00519	ABL1	Tyrosine-protein kinase ABL1	159	0.0046215	0.4910154	0.0426866
P60709	ACTB	Actin, cytoplasmic 1	147	0.0035973	0.4822454	0.0463436
P07550	ADRB2	Beta-2 adrenergic receptor	234	0.0160922	0.4789863	0.0409904
P31749	AKT1	RAC-alpha serine/threonine-protein kinase	209	0.0080394	0.4992296	0.0498464
P05067	APP	Amyloid-beta A4 protein	505	0.0815621	0.5506191	0.0749063
P10275	AR	Androgen receptor	207	0.005169	0.4887931	0.0501535
P49407	ARRB1	Beta-arrestin-1	151	0.0039702	0.4813243	0.045303
P32121	ARRB2	Beta-arrestin-2	185	0.0038177	0.4830671	0.0621951
O14965	AURKA	Aurora kinase A	146	0.0031807	0.4804067	0.0466104
P38398	BRCA1	Breast cancer type 1 susceptibility protein	234	0.0083851	0.4920807	0.0571401
Q9Y297	BTRC	F-box/WD repeat-containing protein 1A	144	0.004185	0.4721066	0.0344338
P0DP23	CALM1	Calmodulin-1	155	0.0028264	0.4824505	0.0429281
P0DP24	CALM2	Calmodulin-2	155	0.0028264	0.4824505	0.0429281
P0DP25	CALM3	Calmodulin-3	155	0.0028264	0.4824505	0.0429281
Q86VP6	CAND1	Cullin-associated NEDD8-dissociated protein 1	251	0.0044444	0.4817332	0.0847493
P22681	CBL	E3 ubiquitin-protein ligase CBL	145	0.0027306	0.4720083	0.0348361
Q9H0W5	CCDC8	Coiled-coil domain-containing protein 8	241	0.0060203	0.4967149	0.0709077
Q16543	CDC37	Hsp90 co-chaperone Cdc37	132	0.0031839	0.4770719	0.0338707
P06493	CDK1	Cyclin-dependent kinase 1	132	0.0026883	0.4749738	0.0369049
P38936	CDKN1A	Cyclin-dependent kinase inhibitor 1	129	0.001941	0.4758708	0.0391395

Entry	Gene symbol	Key target name	Degree centrality	Betweenness centrality	Closeness centrality	Eigenvector centrality
Q00610	CLTC	Clathrin heavy chain 1	144	0.0027881	0.479594	0.0468696
Q92905	COPS5	COP9 signalosome complex subunit 5	299	0.0082385	0.5072691	0.0957184
Q92793	CREBBP	CREB-binding protein	191	0.004252	0.4733876	0.0441258
P68400	CSNK2A1	Casein kinase II subunit alpha	165	0.0032998	0.4917606	0.0541033
Q8NEV1	CSNK2A3	Casein kinase II subunit alpha 3	165	0.0032998	0.4917606	0.0541033
P35222	CTNNB1	Catenin beta-1	204	0.0067945	0.5028825	0.052706
Q13617	CUL2	Cullin-2	198	0.0028157	0.474775	0.0701126
Q93034	CUL5	Cullin-5	182	0.0027184	0.4714197	0.065828
Q14999	CUL7	Cullin-7	303	0.0064753	0.5013263	0.0914254
O75530	EED	Polycomb protein EED	201	0.0030079	0.4672435	0.065134
P68104	EEF1A1	Elongation factor 1-alpha 1	206	0.0047365	0.491974	0.0752915
P00533	EGFR	Epidermal growth factor receptor	428	0.0339661	0.5346535	0.0871741
Q09472	EP300	Histone acetyltransferase p300	253	0.0079286	0.4981331	0.0642302
P03372	ESR1	Estrogen receptor	392	0.0183197	0.52102	0.1067129
Q01844	EWSR1	RNA-binding protein EWS	222	0.0072926	0.494333	0.0608807
Q9NRD1	FBXO6	F-box only protein 6	248	0.0081874	0.4846154	0.065012
P21333	FLNA	Filamin-A	150	0.0036073	0.4857571	0.0473432
P02751	FN1	Fibronectin	392	0.0255727	0.5246357	0.1081054
P35637	FUS	RNA-binding protein FUS	166	0.0025004	0.4855491	0.0610937
P04406	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	132	0.0020629	0.4891093	0.0473812
P62993	GRB2	Growth factor receptor-bound protein 2	284	0.0149739	0.511502	0.066884
P16104	H2AFX	Histone H2AX	142	0.0014854	0.4692737	0.0495455

Entry	Gene symbol	Key target name	Degree centrality	Betweenness centrality	Closeness centrality	Eigenvector centrality
Q13547	HDAC1	Histone deacetylase 1	231	0.0058815	0.483479	0.0557269
Q92769	HDAC2	Histone deacetylase 2	142	0.0017343	0.4606947	0.042542
Q9UQL6	HDAC5	Histone deacetylase 5	233	0.0036348	0.4820404	0.0751394
Q9UBN7	HDAC6	Histone deacetylase 6	130	0.0021674	0.4756711	0.0406083
P68431	HIST1H3B HIST1H3G HIST1H3I HIST1H3H HIST1H3J HIST1H3D HIST1H3A HIST1H3E HIST1H3C	Histone H3.1	194	0.001495	0.470539	0.059863653
P09651	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	217	0.0054863	0.5023256	0.0759059
P61978	HNRNPK	Heterogeneous nuclear ribonucleoprotein K	142	0.0019441	0.4817332	0.0562167
Q00839	HNRNPU	Heterogeneous nuclear ribonucleoprotein U	214	0.0035583	0.4884773	0.0814565
P07900	HSP90AA1	Heat shock protein HSP 90-alpha	348	0.0203087	0.5271967	0.0899352
P08238	HSP90AB1	Heat shock protein HSP 90-beta	299	0.0135303	0.5120795	0.0807293
P34932	HSPA4	Heat shock 70 kDa protein 4	175	0.0036682	0.4957377	0.0577682
P11021	HSPA5	Endoplasmic reticulum chaperone BiP	229	0.0061049	0.5024369	0.0801503
P11142	HSPA8	Heat shock cognate 71 kDa protein	214	0.0054351	0.5034406	0.0703956
P38646	HSPA9	Stress-70 protein, mitochondrial	129	0.0014099	0.4870088	0.0494896

Entry	Gene symbol	Key target name	Degree centrality	Betweenness centrality	Closeness centrality	Eigenvector centrality
P04792	HSPB1	Heat shock protein beta-1	152	0.0046431	0.4802033	0.0453476
P10809	HSPD1	60 kDa heat shock protein, mitochondrial	119	0.002378	0.4849262	0.0477492
Q7Z6Z7	HUWE1	E3 ubiquitin-protein ligase HUWE1	253	0.0051529	0.4936874	0.0797692
Q14164	IKBKE	Inhibitor of nuclear factor kappa-B kinase subunit epsilon	179	0.0031282	0.4702467	0.0462451
Q9Y6K9	IKBKG	NF-kappa-B essential modulator	185	0.0048081	0.481938	0.048876
Q12906	ILF3	Interleukin enhancer-binding factor 3	152	0.0018507	0.4652308	0.0620917
P46940	IQGAP1	Ras GTPase-activating-like protein IQGAP1	122	0.0016852	0.474477	0.036837
P05412	JUN	Transcription factor AP-1	248	0.0116773	0.4936874	0.0467012
Q92993	KAT5	Histone acetyltransferase KAT5	117	0.0023257	0.4703443	0.0349824
Q13233	MAP3K1	Mitogen-activated protein kinase kinase kinase 1	135	0.0014275	0.4704418	0.0448479
P28482	MAPK1	Mitogen-activated protein kinase 1	155	0.0043458	0.4859653	0.0367602
P49736	MCM2	DNA replication licensing factor MCM2	392	0.0151963	0.5195876	0.1168254
Q00987	MDM2	E3 ubiquitin-protein ligase Mdm2	219	0.0064859	0.5002206	0.0614188
P01106	MYC	Myc proto-oncogene protein	245	0.007601	0.5001103	0.0626991
P35579	MYH9	Myosin-9	141	0.0028013	0.478481	0.042804
P19338	NCL	Nucleolin	151	0.0017594	0.4779768	0.0617038
P19838	NFKB1	Nuclear factor NF-kappa-B p105 subunit	132	0.0022238	0.4681115	0.0388613
P06748	NPM1	Nucleophosmin	285	0.0068373	0.5101215	0.1054719
P04150	NR3C1	Glucocorticoid receptor	145	0.0040541	0.4740803	0.0340242
P04629	NTRK1	High affinity nerve growth factor receptor	602	0.0479781	0.5546588	0.1348422
O75147	OBSL1	Obscurin-like protein 1	243	0.0045966	0.4873227	0.0759854
Q504Q3	PAN2	PAN2-PAN3 deadenylation complex catalytic subunit PAN2	192	0.0035701	0.4686919	0.0647358

Entry	Gene symbol	Key target name	Degree centrality	Betweenness centrality	Closeness centrality	Eigenvector centrality
O60260	PARK2	E3 ubiquitin-protein ligase parkin	219	0.0050026	0.4944408	0.0626109
P09874	PARP1	Poly [ADP-ribose] polymerase 1	139	0.0022108	0.4691767	0.0484355
P12004	PCNA	Proliferating cell nuclear antigen	123	0.001906	0.4655172	0.0407847
P30153	PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	107	0.0016333	0.4684015	0.0373462
P78527	PRKDC	DNA-dependent protein kinase catalytic subunit	139	0.0018396	0.4842015	0.0526348
P25788	PSMA3	Proteasome subunit alpha type-3	128	0.0029856	0.4692737	0.0367463
P63244	RACK1	Receptor of activated protein C kinase 1	147	0.0029851	0.4823479	0.0542875
Q04206	RELA	Transcription factor p65	182	0.0049022	0.4829642	0.0483719
Q99496	RNF2	E3 ubiquitin-protein ligase RING2	271	0.0072138	0.4920807	0.0735229
P62979	RPS27A	Ubiquitin-40S ribosomal protein S27a	183	0.0038531	0.4911217	0.0627856
Q9UBS0	RPS6KB2	Ribosomal protein S6 kinase beta-2	141	0.0013986	0.46504	0.0473431
P29353	SHC1	SHC-transforming protein 1	163	0.0032561	0.4854452	0.0395685
Q96EB6	SIRT1	NAD-dependent protein deacetylase sirtuin-1	117	0.0020885	0.4628571	0.035949
Q9NRC8	SIRT7	NAD-dependent protein deacetylase sirtuin-7	226	0.0041181	0.4814265	0.0670389
Q15796	SMAD2	Mothers against decapentaplegic homolog 2	170	0.0044045	0.4791887	0.0389447
P84022	SMAD3	Mothers against decapentaplegic homolog 3	176	0.0064495	0.4898488	0.039211
P51532	SMARCA4	Transcription activator BRG1	129	0.0017867	0.4609756	0.0385377
Q9HCE7	SMURF1	E3 ubiquitin-protein ligase SMURF1	211	0.0052269	0.4881619	0.0595604
P08047	SP1	Transcription factor Sp1	124	0.0016153	0.4671473	0.0353158
Q13501	SQSTM1	Sequestosome-1	120	0.0016697	0.4729927	0.0338386
P12931	SRC	Proto-oncogene tyrosine-protein kinase Src	208	0.0099392	0.5003309	0.0435959
Q9UNE7	STUB1	E3 ubiquitin-protein ligase CHIP	129	0.0024094	0.4828614	0.0367776

Entry	Gene symbol	Key target name	Degree centrality	Betweenness centrality	Closeness centrality	Eigenvector centrality
Q13148	TARDBP	TAR DNA-binding protein 43	160	0.0015336	0.4740803	0.0626446
P04637	TP53	Cellular tumor antigen p53	428	0.0206154	0.5265846	0.1212566
Q9Y4K3	TRAF6	TNF receptor-associated factor 6	246	0.0075369	0.501992	0.0580968
Q71U36	TUBA1A	Tubulin alpha-1A chain	107	0.0015459	0.4708325	0.0357413
P07437	TUBB	Tubulin beta chain	142	0.0018779	0.4918673	0.0545581
P0CG48	UBC	Polyubiquitin-C [Cleaved into: Ubiquitin]	351	0.0164442	0.5227011	0.0918362
P63279	UBE2I	SUMO-conjugating enzyme UBC9	195	0.0061405	0.4878469	0.045404
P11441	UBL4A	Ubiquitin-like protein 4A	168	0.0022958	0.4700518	0.0609625
P55072	VCP	Transitional endoplasmic reticulum ATPase	264	0.0086621	0.507837	0.0786998
P40337	VHL	von Hippel-Lindau disease tumor suppressor	220	0.0069128	0.4941177	0.0633963
P08670	VIM	Vimentin	118	0.0021536	0.4793913	0.0342221
O14980	XPO1	Exportin-1	330	0.0174564	0.5137033	0.0807309
P12956	XRCC6	X-ray repair cross-complementing protein 6	143	0.002279	0.4772727	0.0523876
P31946	YWHAB	14-3-3 protein beta/alpha	145	0.0033159	0.4877419	0.0419516
P62258	YWHAE	14-3-3 protein epsilon	174	0.0035815	0.4859653	0.0565228
P61981	YWHAG	14-3-3 protein gamma	169	0.0040403	0.4974775	0.0542314
P27348	YWHAQ	14-3-3 protein theta	205	0.0043804	0.4893204	0.0606994
P63104	YWHAZ	14-3-3 protein zeta/delta	285	0.0094144	0.5131222	0.0887801