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EVIDENCE-BASED INTEGRATIVE MEDICINE

Efficacy and Safety of *Flos Abelmoschus Manihot* (Malvaceae) on Type 2 Diabetic Nephropathy: A Systematic Review*

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ABSTRACT **Objective**: To evaluate the efficacy and safety of *Flos Abelmoschus manihot* (Malvaceae) on type 2 diabetic nephropathy (DN). **Methods**: The Cochrane Library, PubMed/MEDLINE, Excerpta Medical Database, Chinese electronic literature databases, and the references of relevant articles were searched in March 2012 for randomized controlled trials (RCTs) that reported the effects of *Flos A. manihot* on type 2 DN patients with overt but subnephrotic-range proteinuria (500–3,500 mg/24 h). The quality of trials was evaluated using the Cochrane-recommended method. The results were summarized as risk ratios (RRs) for dichotomous outcomes or mean differences (MDs) for continuous outcomes. **Results**: Seven trials (531 patients) were included. *Flos A. manihot* significantly decreased proteinuria [MD –317.32 mg/24 h, 95% confidence interval (CI) [–470.48, –164.17], *P*<0.01]. After excluding a trial that only included patients with well-preserved renal function, *Flos A. manihot* was associated with a significant decrease in serum creatinine (MD –11.99 μmol/L, 95% CI [–16.95, –7.04], *P*<0.01). Serious adverse events were not observed. The most common adverse event was mild to moderate gastrointestinal discomfort; however, patients receiving this herb did not have an increased risk for tolerated gastrointestinal discomfort (RR 1.48, 95% CI [0.39, 5.68], *P*=0.57). **Conclusions**: *Flos A. manihot* may be considered as an important adjunctive therapy with the first-line and indispensable therapeutic strategies for type 2 DN. High-quality RCTs are urgently needed to confirm the effect of *Flos A. manihot* on definite endpoints such as end-stage renal disease.

KEYWORDS *Flos Abelmoschus manihot*, Huangkui Capsule, Chinese medicine, type 2 diabetic nephropathy, systematic review, meta-analysis, randomized controlled trial

Type 2 diabetes is a public health concern.[1] A large proportion of patients with type 2 diabetes suffer from diabetic nephropathy (DN).[2,3] The magnitude of proteinuria is widely recognized as a predictor of end-stage renal disease in these patients.[4] More importantly, a reduction in proteinuria is invariably associated with improved renal and cardiovascular protection.[5,6] Despite decreases in proteinuria secondary to renin-angiotensin system inhibitors, DN continues to be the most common cause of end-stage renal disease.[7,8] Therefore, there is an urgent need to find new treatments for such patients.[9,10]

Many Chinese medicines have been used to treat chronic kidney disease, especially DN.[11-14] *Abelmoschus manihot* (Linn.) Medicus (family Malvaceae) is an annual herbal plant distributed widely in China. The flower of *A. manihot*, namely *Flos A. manihot*, has been used as a traditional Chinese herb to treat chronic glomerulonephritis in China for many centuries. Huangkui Capsule (黄葵胶囊, HKC), the extract of *Flos A. manihot*, gained regulatory approval from China's State Food and Drug Administration (SFDA) for the treatment of chronic nephritis in 1999.[15] Recently, HKC has been considered to be an important adjuvant therapy for Chinese patients with chronic kidney disease in current clinical practice.[16-18] HKC is often given in conjunction with other drugs, particularly angiotensin-

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converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs).[19-21] Recently, Chen, et al[22] has summarized the recent advances in the use of this herb as well as its potential mechanisms. HKC could ameliorate proteinuria and hematuria and improve renal function in patients with chronic kidney disease, including DN, IgA nephropathy, membranous nephropathy, and Henoch-Schonlein purpura nephritis. The above effects of HKC might be associated with the inhibition of immune reaction and inflammatory injury, the amelioration of renal interstitial fibrosis, and the protection of renal tubular epithelial cells.

Hundreds of thousands of Chinese patients with type 2 DN have been treated with HKC, mainly reported by non-randomized controlled trials (non-RCTs).[23-40] Few RCTs have also been performed in China.[41-47] However, the efficacy and safety of this herb have not been systematically reviewed. Thus, we performed a systematic review and meta-analysis of RCTs to synthesize the evidence for the efficacy and safety of HKC in type 2 DN patients with overt but subnephrotic-range proteinuria (500–3,500 mg/24 h). We anticipated that our results could help expedite the conduction of well-designed clinical trials of this herb for DN.

METHODS

Identification of Trials

The Cochrane Library, PubMed/MEDLINE, Excerpta Medical Database (EMBASE), and the Chinese electronic literature databases [Chinese Biomedical Literature Database (CBM), China National Knowledge Infrastructure (CNKI), Wanfang, and Weipu Databases] were searched in March 2012 to identify RCTs using the following search terms: *Abelmoschus*, *Abelmoschus manihot*, *Flos Abelmoschus manihot*, *Abelmoschi corolla*, okra, ambrette, Huangkui, Huangshukui, Huangshukuihua, diabetes mellitus, diabetic nephropathies, diabetic nephropathy, diabetic glomerular, and [(diabetic or diabetes) and (kidney disease or renal disease)]. A manual search of the references of identified articles was performed to identify any additional trials. No language restriction was used. Trials were considered eligible for inclusion if they were conducted in adult type 2 DN with overt but subnephrotic-range proteinuria. Trials were excluded if any other Chinese medications, including single herbs, mixtures of herbs, extracts, raw materials, Chinese proprietary medicines, or practitioner-prescribed herbal formulae (individualized treatment), were used as comparators or co-interventions. Proteinuria and serum creatinine (SCr) levels after treatment were used to assess the efficacy profile, and adverse events (AEs) were used to assess the safety profile.

Trial Selection and Data Extraction

The titles and abstracts, and full texts if necessary, were screened by two authors (Chen YZ and Gao Q). Trial selection, data extraction, and quality assessment were analyzed by the same two authors. Disagreements were resolved by a third author (Chen XM). The extracted data included patient baseline characteristics (case number, age and sex), doses, routes, durations of *Flos A. manihot* treatment and co-interventions, proteinuria and SCr levels before and after treatment, AEs, and methodological characteristics.

Quality Assessment

The quality of trials was assessed using the Cochrane-recommended method. Six different aspects, including random sequence generation, allocation concealment, blinding of patients, personnel, and outcome assessors, incomplete outcome reporting, selective outcome reporting, and other potential bias, were judged with "low risk of bias", "high risk of bias", or "unclear risk of bias", respectively.[48]

Data Analysis

Dichotomous and continuous outcomes were expressed as risk ratio (RR) with 95% confidence interval (CI) and mean difference (MD) with 95% CI, respectively. The Cochrane Q test was used to determine heterogeneity between trials if the threshold P value was <0.10 and the I^2 test to further quantify the magnitude of heterogeneity.[48] The I^2 statistic was generated by the formula $[(Q–df)/Q] \times 100\%$, where Q was the chi-squared statistic and df was the degree of freedom. The I^2 statistic of 25%, 50%, and 75% was interpreted as indicating low, medium, and high levels of heterogeneity, respectively. A random-effects model was used for the analyses of both dichotomous and continuous data if there were medium or high levels of heterogeneity, whereas a fixed-effect model was used if there was a low level or the absence of heterogeneity. Sensitivity analysis and meta-regression was used to explore the possible sources

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of heterogeneity, such as baseline proteinuria, SCr, co-interventions, and total dosage of *Flos A. manihot*. Meta-analysis, including sensitivity analysis, was performed using Review Manager Software (version 5.1). Meta-regression was performed using STATA Software (version 11.2).

RESULTS

Literature Search Results and Trial Characteristics

A total of 337 publications were identified, of which 264 were excluded after screening titles and abstracts. Further full-text assessment excluded 66 publications: 35 were excluded because they were unrelated publications, reviews, or basic studies; another 31 clinical trials were excluded because they failed to fulfill the criteria of patients, interventions, controls, outcomes, or study design. Seven trials[41-47] with 531 patients were included (Figure 1), all of which were conducted in China.

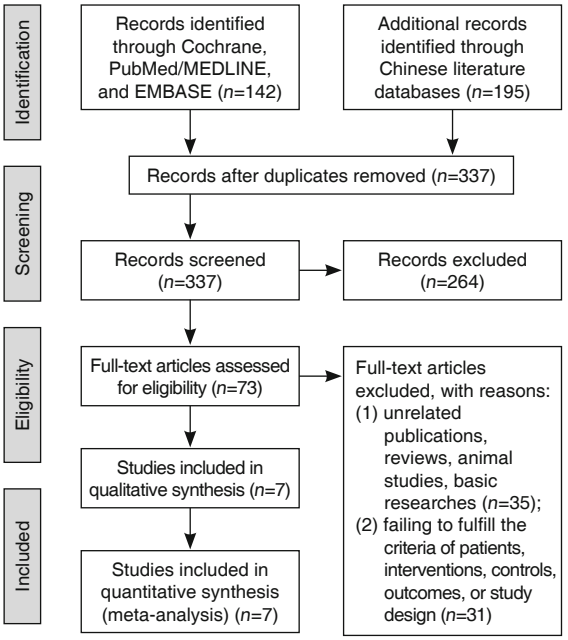

Figure 1. Flow Diagram of Trial Selection

The median sample size was 58 (40–124). The median baseline proteinuria was 1,430 (860–1,650) mg/24 h, and the median SCr was 120.31 (89.57–164.00) μmol/L. There were no significant differences in the key baseline characteristics, including proteinuria, SCr, blood glucose, glycosylated hemoglobin A1c (HbA1c), mean arterial pressure, and the percentage of hypertension between the treatment and control groups in each included trial. There were no other specific concomitant diseases in any of the included trials. Co-intervention therapies were used in all trials, of which five[41,44-47] used ACEIs or ARBs, one[43] used combined ACEIs and ARBs, and one[42] used alprostadil (Table 1). All of the co-intervention therapies were comparable between the treatment and control groups in all included trials. All other concomitant therapies, including insulin, oral antidiabetic drugs, antihypertensive drugs, antiplatelet drugs, and lipid-lowering drugs, were comparable between the treatment and control groups. The target systolic and diastolic pressures were 140 and 90 mm Hg, respectively. The fasting glucose and HbA1c goals were 7.0 mmol/L and 7.0%, respectively. Patients in the treatment groups

Table 1. Characteristics of the Interventions and Populations at Baseline in the Included Trials

First author (Year)	Groups	Sample size (Case)	Age (Year)	Male [Case (%)]	Proteinuria (mg/24 h)	SCr (μmol/L)
Yu (2011)[41]	*Flos A. manihot* plus candesartan	29	69 (42–82)	–	1570 ± 670	162.57 ± 25.61
	Candesartan	29		–	1490 ± 560	159.79 ± 33.92
Ding (2011)[42]	*Flos A. manihot* plus alprostadil	64	50 ± 13	44 (69)	1580 ± 540	114.82 ± 37.15
	Alprostadil	60	51 ± 13	36 (60)	1650 ± 720	110.56 ± 39.61
Li (2009)[43]	*Flos A. manihot* plus captopril and candesartan	30	54 (42–71)	–	885 ± 150	125.80 ± 14.30
	Captopril plus candesartan	31		–	876 ± 235	126.80 ± 13.00
Li (2009)[44]	*Flos A. manihot* plus fosinopril	40	52 (42–65)	26 (65)	1100 ± 300	164.00 ± 77.00
	Fosinopril	40		24 (60)	1200 ± 400	159.00 ± 69.00
Guan (2008)[45]	*Flos A. manihot* plus captopril/enalapril	40	45 ± 12	22 (55)	1460 ± 650	–
	Captopril/enalapril	40	46 ± 13	21 (53)	1410 ± 630	–
Li (2007)[46]	*Flos A. manihot* plus benazepril	30	41 ± 12	14 (47)	1480 ± 680	94.70 ± 18.92
	Benazepril	30	42 ± 12	16 (53)	1450 ± 690	89.57 ± 15.19
Yu (1995)[47]	*Flos A. manihot* plus captopril	35	55 (44–78)	23 (66)	890 ± 310	110.46 ± 29.35
	Captopril	33	54 (45–76)	21 (64)	860 ± 330	106.53 ± 31.74

received *Flos A. manihot* along with the same co-interventions and other concomitant therapies as the control groups. HKC 7.5 g/day was used in 6 trials. One trial was performed before the HKC was approved by the SFDA in 1999; therefore, the extract of *Flos A. manihot* 1.2 g/day was used in that trial.[47] The treatment duration was 8 weeks in 6 trials and 6 months in one trial.[41]

None of the trials provided details about random sequence generation and allocation concealment, nor did specify whether patients or investigators were blinded to the interventions (Figures 2 and 3). All of the trials included randomized patients in the data analysis. Proteinuria, SCr and AEs were reported in 7 (100%), 6 (86%), and 3 trials (43%), respectively.

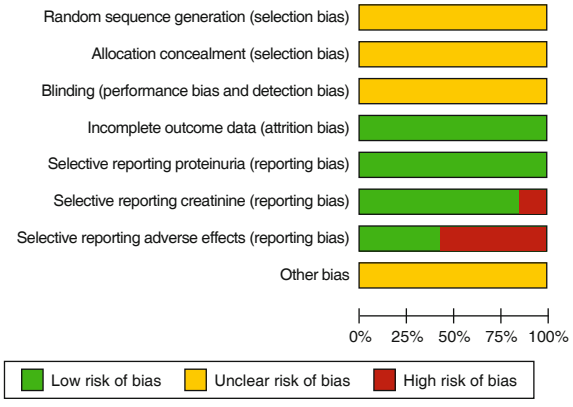

Figure 2. Risk of Bias Judgments about Each Risk of Bias Item Presented as Percentages across All Included RCTs

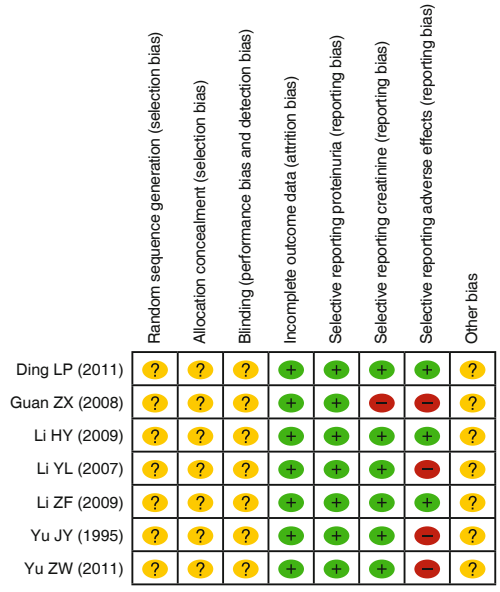

Figure 3. Risk of Bias Judgments about Each Risk of Bias Item for Each Included RCT

Summary Estimates of Outcomes

Flos A. manihot led to a significant reduction in proteinuria (MD –317.32 mg/24 h, 95% CI [–470.48, –164.17], $P<0.01$) with significant heterogeneity ($I^2=86\%$, $P<0.01$). One trial used alprostadil as the co-intervention;[42] another used both captopril and candesartan.[43] Sensitivity analysis, excluding these two trials, revealed a similar beneficial effect on proteinuria (MD –330.12 mg/24 h, 95% CI [–409.56, –250.68], $P<0.01$), but no evidence of heterogeneity ($I^2=0\%$, $P=0.96$, Figure 4). There was no significant difference in SCr (MD –7.30 μmol/L, 95% CI [–16.11, 1.51], $P=0.10$) between patients treated with and without *Flos A. manihot*. Significant heterogeneity existed between these trials ($I^2=78\%$, $P<0.01$). Sensitivity analysis, excluding 1 trial in which patients had well-preserved baseline renal function (89.57–94.70 μmol/L),[46] revealed that *Flos A. manihot* significantly decreased SCr (MD –11.99 μmol/L, 95% CI [–16.95, –7.04], $P<0.01$) without heterogeneity ($I^2=0\%$, $P=0.70$, Figure 5). Sensitivity analysis also confirmed the significant benefit of this herb in decreasing SCr in patients who had well-preserved renal function and received ACEIs/ARBs as background therapy (MD –12.72 μmol/L, 95% CI [–18.21, –7.24], $P<0.01$). Meta-regression indicated that there was no significant interaction between the effects of *Flos A. manihot* and baseline proteinuria or total dosage of *Flos A. manihot*.

No patients withdrew from treatment due to AEs caused by the herb in any of the included 7 trials. Mild to moderate gastrointestinal discomfort was the only reported AE, and there was no significant difference between the *Flos A. manihot* and control groups with respect to the incidence of gastrointestinal discomfort (RR 1.48, 95% CI [0.39, 5.68], $P=0.57$, heterogeneity $I^2=2\%$, $P=0.36$, Figure 6).

DISCUSSION

This meta-analysis investigated the efficacy and safety of *Flos A. manihot* in the treatment of type 2 DN patients with overt but subnephrotic-range proteinuria. The results determined that *Flos A. manihot* could lower proteinuria with no increase in the risk of AEs. Although a trend toward decreased SCr was also observed, the decrease did not reach statistical significance until 1 trial that only included patients with well-preserved renal function at baseline was excluded. Heterogeneity was found to be statistically significant in proteinuria and SCr. Meta-regression excluded the possible

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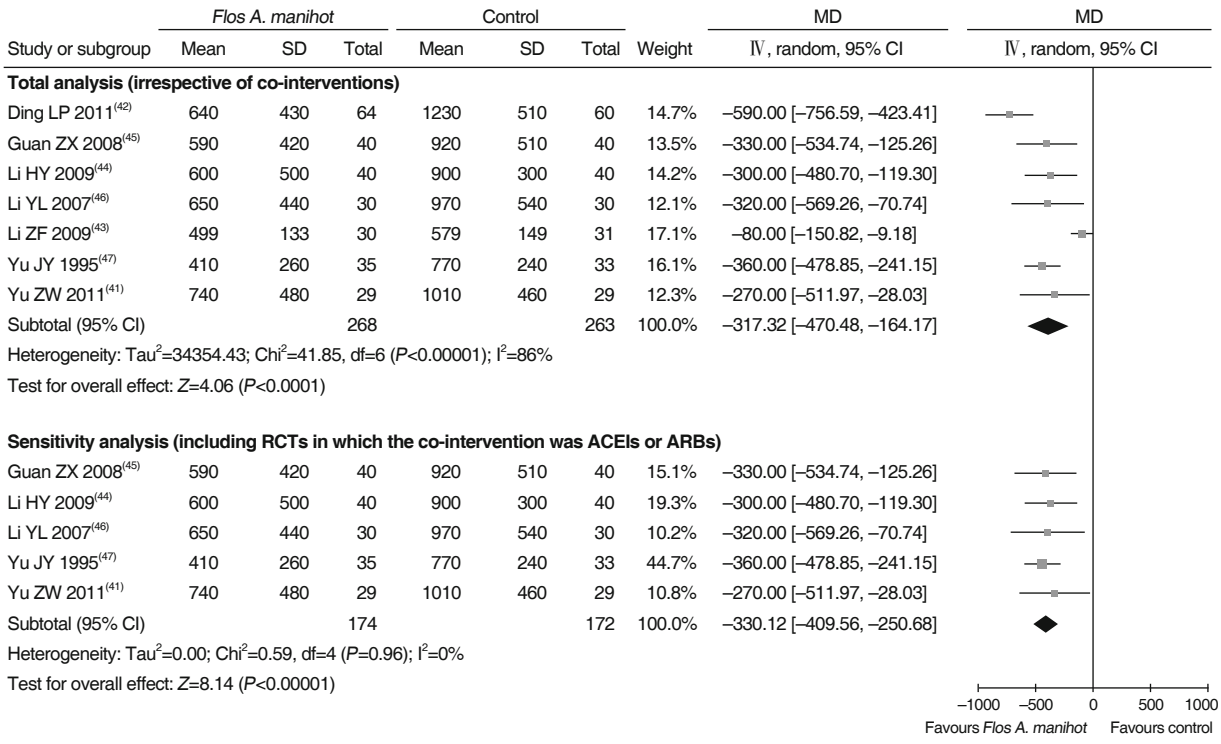

Figure 4. Effect of *Flos A. Manihot* on Proteinuria in Type 2 DN Patients with Overt but Subnephrotic-Range Proteinuria

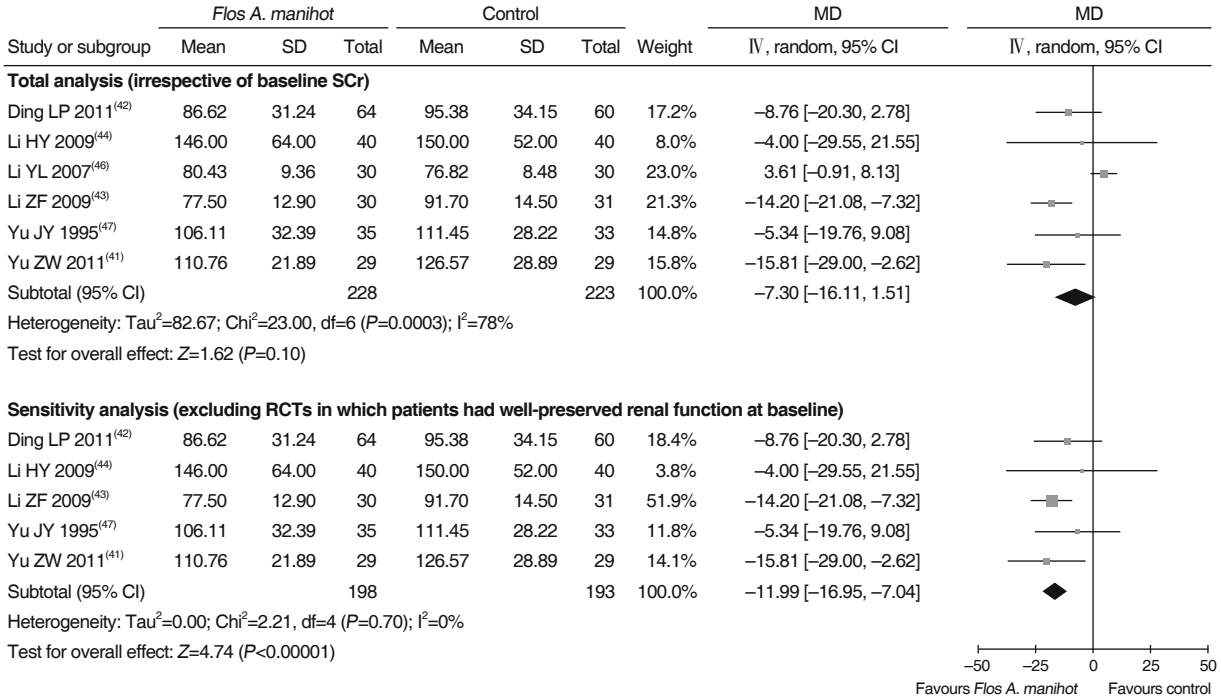

Figure 5. Effect of *Flos A. Manihot* on SCr in Type 2 DN Patients with Overt but Subnephrotic-Range Proteinuria

heterogeneity sources from the baseline proteinuria level or total dosage of *Flos A. manihot*. Sensitivity analyses indicated that different concomitant therapies and baseline SCr levels could explain the heterogeneity of proteinuria and SCr, respectively.

Priority should also be given to the safety profile of this herb. Current Chinese clinical practice

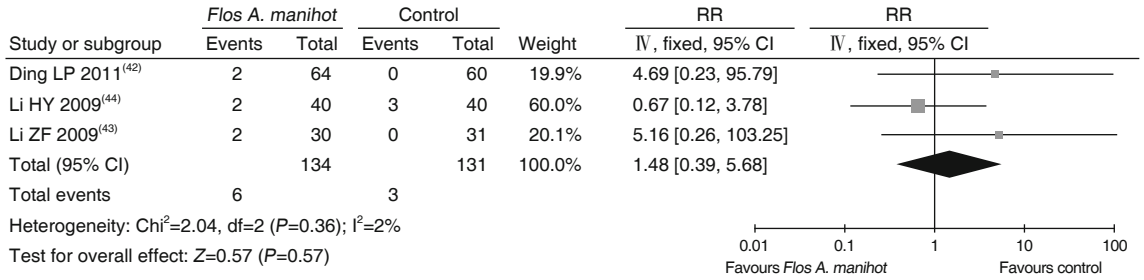

Figure 6. AEs of *Flos A. Manihot* in Type 2 DN Patients with Overt but Subnephrotic-Range Proteinuria

recommends that HKC should be taken at 7.5 g/day in 3 divided doses for at least 8 weeks. Overall, *Flos A. manihot* was well tolerated with minimal AEs. Since the HKC acquired national approval from SFDA in 1999, there have been no reports of severe AEs. The most common AE was mild to moderate gastrointestinal discomfort. Only this type of AE was reported by the included trials. Our meta-analysis demonstrated that the risk of well-tolerated gastrointestinal discomfort was not significantly different whether type 2 DN patients received this herb or not. We also published another meta-analysis of 23 clinical controlled trials and RCTs with 1,844 type 2 DN patients, irrespective of baseline proteinuria level.[49] Only three types of AEs (gastrointestinal discomfort, dizziness and dry mouth) were reported. There were no significant differences in these three types of AEs. Additionally, the latter two AEs were rarely reported.

Modern phytochemical studies recognized flavonoids as important bioactive compounds for *Flos A. manihot*.[50] Seven flavonoids, namely hibifolin, hyperoside, myricetin, quercetin, isoquercetin, quercetin-3'-O-glucoside, and quercetin-3-O-robinobioside, have been reported as the major active constituents. These constituents could be chosen as quality control markers for this herb and may be responsible for therapeutic efficacy in DN. Orally administered flavonoids could be rapidly absorbed into rat blood, quickly excreted into urine, and disappeared from the urine after 24 h. This *in vivo* pharmacokinetic study supported the clinical recommendations that the HKC should be taken 3 times a day.[51]

Metabolic profiles of these active components were critical for the understanding of their safety and efficacy. Hydrolysis, hydroxylation, and acetylation were the major metabolic pathways in human and rat intestinal bacteria,[52,53] whereas methylation and glucuronidation were the major metabolic pathways after the absorption of flavonoids in the rat gastrointestinal tract.[15] The most important metabolites in rat blood and urine were quercetin and gossypetin, mainly derived from hibifolin, hyperoside, isoquercetin, and quercetin-3'-O-glucoside.[15] Hyperoside, isoquercitrin, and quercetin monoglucuronide were also identified in rat kidney blood.[54] These potentially bioactive constituents *in vivo* could help clarify the efficacy and safety profiles of this herb as well as potential mechanism of its effects.

Oral administration of total flavonoids glycosides decreased proteinuria and glomerular cell apoptosis in the streptozotocin-induced DN rat model.[55] Orally administered *Flos A. manihot* also reduced proteinuria and plasma lipid peroxide and superoxide anion in patients with DN.[47] The scavenging of oxygen free radicals could retard the progression of DN. Hyperoside could reduce advanced glycation end product-induced podocyte apoptosis, which was mediated by the inhibition of caspase-3 and caspase-8 overexpression.[55] Podocyte detachment could also be alleviated by high-dose hyperoside. The amelioration of podocyte apoptosis and detachment might be a pivotal mechanism responsible for the antiproteinuric effect of *Flos A. manihot*. Zhao, et al[56] also demonstrated that the HKC significantly reduced proteinuria in the adriamycin-induced nephropathy rat model. This antiproteinuric effect was accompanied by the improvements in renal inflammatory injury *in vivo*, including ameliorated mesangial cell and matrix proliferation, decreased expression of α-smooth muscle actin and collagen type Ⅰ, and inhibition of ED1+ and ED3+ cell infiltration in glomeruli. The above effects of HKC might be attributed to the suppression of transforming growth factor-β1 generation and subsequent p38 mitogen-activated protein kinase pathway activation.

Tests for publication bias or other reporting

biases, such as small-study effects, should be used only when at least 10 studies were included in the meta-analysis.[48,57,58] Only 7 trials were included in our meta-analysis, therefore, the risk of reporting bias could not be statistically evaluated due to the small sample size. All trials were published in the Chinese language; therefore, language bias and publication bias should be carefully considered when interpreting our results. The suboptimal methodological quality of the included trials was another concern. The trials did not provide details related to randomization sequence generation, allocation concealment, or blinding. The objective outcomes, such as proteinuria, were not likely to be influenced by the lack of blinding; however, a lack of blinding could introduce potential biases in clinical trials.[48]

ACEIs/ARBs have been recognized as indispensable therapeutic strategies to reduce proteinuria for DN; however, there remains an urgent need for new antiproteinuric agents, especially in those patients who do not respond to the conventional therapy or who exhibit a more progressive renal disease. Thus, *Flos A. manihot* could be considered an important adjunctive therapy. However, the long-term effect of this herb on proteinuria and SCr has never been investigated. Animal studies suggested that this herb may prevent podocyte detachment.[55] However, it remains unclear whether and how long the renoprotective effects could be maintained after withdrawal of this herb in clinical trials. High-quality RCTs are urgently required to confirm the effect of this herb on definite endpoints, such as all-cause mortality and end-stage renal disease.

Conflict of Interest

None.

Author Contributions

Chen YZ, Gong ZX, and Gao Q selected studies, extracted data from studies, and entered data into RevMan. Chen YZ carried out the analysis. Chen YZ, Cai GY, Chen XM, Gao Q, Tang L, Wei RB, and Zhou JH interpreted the analysis and drafted the final review. Chen XM resolved the disagreement.

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