META-ANALYSIS

# The Therapeutic Effect and Possible Harm of Puerarin for Treatment of Stage III Diabetic Nephropathy: A Meta-analysis

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## ABSTRACT

**Context** • Diabetic nephropathy (DN) is the main cause of end-stage kidney disease in developed countries. Current therapy can slow the rate of progression of DN, but eventually end-stage renal failure will occur in a proportion of patients. Identification of new strategies and additional complementary and alternative therapies for treating DN are important.

**Objective** • The research team wanted to assess the beneficial and harmful effects of using puerarin plus angiotensin converting enzyme inhibitor (ACEI) compared with using only ACEI for treatment of individuals with stage III DN.

**Design** • The research team performed a meta-analysis of randomized, controlled trials (RCTs) by searching the following electronic databases: (1) the Cochrane Database of Systematic Reviews, (2) the Cochrane Central Register of Controlled Trials (CENTRAL), (3) PubMed, (4) EMBASE (Elsevier), (5) the Allied and Complementary Medicine Database (AMED), (6) the Chinese Biomedicine Database (CBM), (7) the China National Knowledge Infrastructure (CNKI), and (8) the Chinese Biomedical Journals (VIP), with no language restrictions, as well as databases of clinical trials.

**Outcome Measures** • Measured outcomes included (1) urinary protein measured as urinary albumin excretion rate (UAER) (μg/min) and 24-h urine protein (24-h UP) (mg/24 h); (2) renal function measured as blood urea nitrogen (BUN) (mmol/L) and serum creatinine (SCr) (μmol/L); (3) $\alpha_1$-microglobulin ($\alpha_1$-MG) (mg/24 h) and endothelin-1 (ET-1) (ng/24 h); (4) end points (EPs); and (5) adverse events (AEs).

**Results** • Ten RCTs involving 669 participants were included. All trials were conducted and published in China. Treatment of DN with puerarin plus ACEI significantly decreased the UAER—$P < .0001$, MD = -23.43 (95% CI, -33.95 to -12.91), and had no effect on 24-h UP—$P = .09$, MD = -56.76 (95% CI, -122.65 to 9.12); BUN—$P = .17$, MD = -0.51 (95% CI, -1.24 to 0.21); and SCr—$P = .26$, MD = -4.43 (95% CI, -12.05 to 3.20). One trial reported abdominal discomfort and nausea (2 cases) in the treatment group.

**Conclusions** • Puerarin may be a beneficial therapy for treating DN; however, this hypothesis needs to be proven by additional high-quality studies using large samples and multicenter evidence. (*Altern Ther Health Med.* 2015;21(1):36-44.)


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Diabetic nephropathy (DN) is the main cause of end-stage kidney disease in developed countries. Worldwide, it is responsible for 25% to 50% of the need for patients to undergo dialysis treatment. Studies reveal its occurrence in 10% to 40% of individuals who have type 1 diabetes mellitus (T1DM) and 5% to 20% of those who have type 2 diabetes mellitus (T2DM). In general, the development of clinically manifested nephropathy is observed from 10 to 20 years after diagnosis of diabetes mellitus (DM).[1]

The characteristic features of DN are progressive structural alterations, such as glomerular and tubuloepithelial hypertrophy and thickened glomerular and tubular basement membranes, followed by hyperfiltration, albuminuria,

glomerulosclerosis, and tubulointerstitial fibrosis. These conditions lead eventually to end-stage kidney disease.[2] Many factors are involved in the pathogenesis of DN, and the data suggest that the following pathways are activated in the course of the disease: (1) a hemodynamic pathway, involving the renin–angiotensin–aldosterone and urotensin systems; (2) profibrotic and inflammatory cytokines, including transforming growth factor β (TGF-β) and tumor necrosis factor α (TNF-α); (3) various kinases such as the protein kinase C (PKC) and the Janus kinase pathways; and (4) oxidative stress mediators, such as nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase).[3]

Current therapy, which includes treatment for hyperglycemia, hypertension, and dyslipidemia, can slow the rate of progression of DN, but eventually end-stage renal failure will occur in a proportion of patients. Therefore, the effects of currently used treatments must be maximized, and identification of new strategies and additional complementary and alternative therapies for treating DN is important.[4]

Being the main isoflavone glycoside found in the radix of *Pueraria lobata* (wild)—a Chinese medicine known as *gegen*, puerarin (7-hydroxy-3-[4-hydroxyphenyl]-1-benzopyran-4-one8-[β-D-glucopyranoside]) has been used for various medicinal purposes in traditional Chinese medicine for thousands of years. Modern pharmacological research has demonstrated that puerarin exerts a protective effect against myocardial reperfusion injury and ischemic retinopathy. In China since the 1990s, puerarin has been used as a therapy for DM and its complications. Puerarin can improve insulin sensitivity, increase glucose utilization, and promote blood circulation. Besides these effects, it can also scavenge reactive oxygen species, increase superoxide dismutase activity, and inhibit protein nonenzymatic glycation.[5] In mainland China, it is used mainly in the form of an injection and has been approved by the State Food and Drug Administration (SFDA).[6]

Without intervention, patients with stage III DN have a much poorer prognosis, with a much higher risk of progression to overt renal disease during the following decade than patients in other stages. According to new morphometric studies, this finding is not surprising because microalbuminuria indicates that those patients are in a more advanced stage of glomerular disease than patients in other stages. Therefore, the research team restricted the participants included in the current study to those with stage III DN.[7]

Systematic reviews and meta-analyses are generally regarded as the most reliable tools for summarizing existing evidence. However, the evidence from systematic reviews on puerarin for DN is marginal. To determine whether puerarin is effective and safe as a complementary and alternative treatment for patients with DN, the research team performed a systematic review of all currently available data from randomized, controlled trials (RCTs) that used puerarin for patients with DN and conducted a quantitative meta-analysis of the results from those studies.

## METHODS

### Design

Reports in English or Chinese of RCTs that compared the use of puerarin plus angiotensin-converting enzyme inhibitor (ACEI) versus ACEI alone were reviewed in the current study. Only RCTs were included, both published and unpublished. No restrictions existed on the ethnicity, gender, age, or disease duration of the participants in the trials. The study excluded field reports, case series, case reports, studies without a control group, abstracts that preceded a full-length publication, translations of already published manuscripts, double publication of similar data, and internal reports. The research team also excluded RCTs that used any other active interventions for the treatment group, such as herbal medicines, acupuncture, and other pharmacological compounds. For obviously duplicated studies, the authors of the reports were contacted to clarify any uncertainty in the current research team's comparison of the 2 reports. If the author could not be contacted, the first published report was regarded as the original.

The current study reviewed RCTs that included patients with DM who met the diagnostic criteria established by the World Health Organization (WHO) (1999 and 1997) and the American Diabetes Association (1999 and 1997). Patients with stage III DN were included according to the Mogensen diagnostic criteria for DN (ie, a urinary albumin excretion rate [UAER] of 20-200 μg/min).[8] Studies that included patients with other chronic diseases were excluded, including those with patients suffering from schizophrenia; chronic pulmonary disease; liver disease such as autoimmune hepatitis, alcoholic liver injury, and drug-induced hepatitis; heart failure; myocardial infarction; fatal arrhythmia; autoimmune disease; infectious disease; malignant tumors; serious hypertension; and organ transplants.

The research team searched the following electronic databases from their inception to May 2013 for appropriate RCTs: (1) the Cochrane Database of Systematic Reviews, (2) the the Cochrane Central Register of Controlled Trials (CENTRAL), (3) PubMed, (4) EMBASE (Elsevier), (5) the Allied and Complementary Medicine Database (AMED), (6) the Chinese Biomedicine Database (CBM), (7) the Chinese National Knowledge Infrastructure (CNKI), and (8) Chinese Biomedical Journals (VIP). The team also searched the unpublished trials: (1) the Register of the Controlled Trials databases, http://www.controlled-trials.com; (2) the National Research Register for trials on complementary and alternative medicine, http://www.clinicaltrials.gov; and (3) the National Center for Complementary and Alternative Medicine, http://nccam.nih.gov. A hand-search of a list of Chinese and English journals was carried out to find the latest studies. The research team also referred to the reference lists of relevant papers to identify potential studies.

The research team used following keywords and medical subject headings to identify relevant articles in the electronic databases: *puerarin* or *gegensu* or *kudzu* or *gegen* AND *diabetic nephropathy* or *tangniaobing shenbing* or *diabetes* or

*kidney disease* AND *angiotensin-converting enzyme inhibitor* or *angiotensin receptor blocker*. No language restrictions were applied. All studies included were analyzed according to Cochrane handbook criteria.[9]

### Data Extraction

A standard method of data extraction was used independently by 2 members of the research team to record the following characteristics of each eligible study: (1) the study's design; (2) participants' characteristics (age, gender, baseline level of UAER and 24-h urine protein [24-h UP], presence of hypertension, and concomitant use of ACEI); (3) therapeutic intervention (ie, type of ACEI, dose, dose titration, background antidiabetic medication, and duration of treatment); (4) comparison groups; and (5) outcomes (UAER, 24-h UP, blood urea nitrogen [BUN], and serum creatinine [SCr]). Investigators of studies that fulfilled the current study's inclusion criteria, but for which the reported outcome of interest could not be assessed in a standardized manner from the relevant publication, were systematically contacted to provide additional information. Any disagreement on data extraction was resolved by discussion between the 2 members of the research team and by consultation with a third member.

### Quality Assessment

The quality of the eligible studies' methods was evaluated independently by 2 different members of the research team who prepared a risk-of-bias graph, as described in the *Cochrane Handbook for Systematic Reviews of Interventions*, version 5.1.0.[9] This evaluation included a description and a judgment on quality for each entry in a table that addressed a specific feature of every study: (1) random sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) completeness of outcome data, (6) selective reporting, and (7) other sources of bias. The judgment for each entry involved answering a question with (1) "yes," indicating a low risk of bias; (2) "no," indicating a high risk of bias; and (3) "unclear," indicating a lack of information or uncertainty about the possibility of bias. Disagreements between members of the research team were resolved by consensus. No study was excluded on the basis of the quality of its methods.

### Outcome Measures

The research team extracted data on the following outcomes: (1) primary outcomes—urinary protein measured as UAER (μg/min) and 24-h UP (mg/24 h); (2) secondary outcomes—renal function measured as BUN (mmol/L) and SCr (μmol/L); (3) other outcomes—$\alpha_1$-microglobulin ($\alpha_1$-MG) (mg/24 h) and endothelin-1 (ET-1) (ng/24 h); (4) end points (EPs)—end stage renal disease, all-cause mortality, fatal cardiovascular and cerebrovascular events, nonfatal cardiovascular and cerebrovascular events separately, defined as myocardial infarction, stroke, sudden death, or composites of these; and (5) adverse events (AEs), including elevated liver function tests and elevated creatinine kinase concentrations.

### Sensitivity Analyses

Sensitivity analyses were conducted by excluding low-quality studies based on descriptions of randomization, allocation concealment, blinded assessment of outcomes, and description/analyses of withdrawals and dropouts and on a comparison of the merger-analysis results for the fixed- and random-effects models.

### Assessment of Publication Biases

If sufficient studies were included in analyses, the research team investigated publication biases using funnel plots based on effective sample size.

### Data Analysis

The statistical package (RevMan Version 5.2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012), which is provided by the Cochrane Collaboration, was used to analyze collected data. Dichotomous data were presented as risk ratios (RRs), with 95% confidence intervals (CIs). Continuous outcomes were presented as mean difference (MD), with 95% CI. Analyses were performed by intention-to-treat where possible. Heterogeneity between the trials' results was tested, and heterogeneity was presented as significant when $I^2$ was more than 50% ($P < .1$). A random-effects model was used for the meta-analysis if significant heterogeneity existed, and a fixed-effect model was used when the heterogeneity was not significant.

## RESULTS

### Characteristics of Included Studies

Studies that included a total of 669 patients with stage III DN, who were between the ages of 30 and 80 years, were examined. The sample sizes of all studies were small (N = 18-54). All of the trials were conducted in China. The puerarin injections used in the studies complied with the quality standards of the SFDA of China. The treatment duration ranged from 14 days to 6 months. In all studies, 200 to 500 mg per day of puerarin was used throughout the period of the study.

Ten trials used 3 diagnostic criteria; that is, 8 trials[10,11,14-19] used the diagnostic criteria for DN proposed by the WHO (1999 edition); 1 trial[12] used the diagnostic criteria for DN proposed by the WHO (1997 edition); and 1 trial[13] used the diagnostic criteria for DN proposed by the ADA (1997 edition). Follow-up was not used in all studies. All 10 trials compared the results for treatment with puerarin plus ACEI with those for treatment with ACEI only (4-25 mg).[10-13,14-19] Four trials[11,12,14,17] enrolled hypertensive individuals and equalized blood pressure between the experimental and control groups by administration of additional antihypertensive drugs with no ACEI. Urine protein was measured using UAER in 9 studies[10,11,13,15-19] and 24-h UP in 2 studies.[12,17] Renal function was measured using BUN in 9 studies,[11-13,14-19] and SCr was used in 9 studies.[11-13,14-19] No trials reported EPs that included long-term renal outcomes, cardiac events, cerebral events, or mortality. The characteristics of the included studies are summarized in Table 1.

**Figure 1.** Flow Diagram

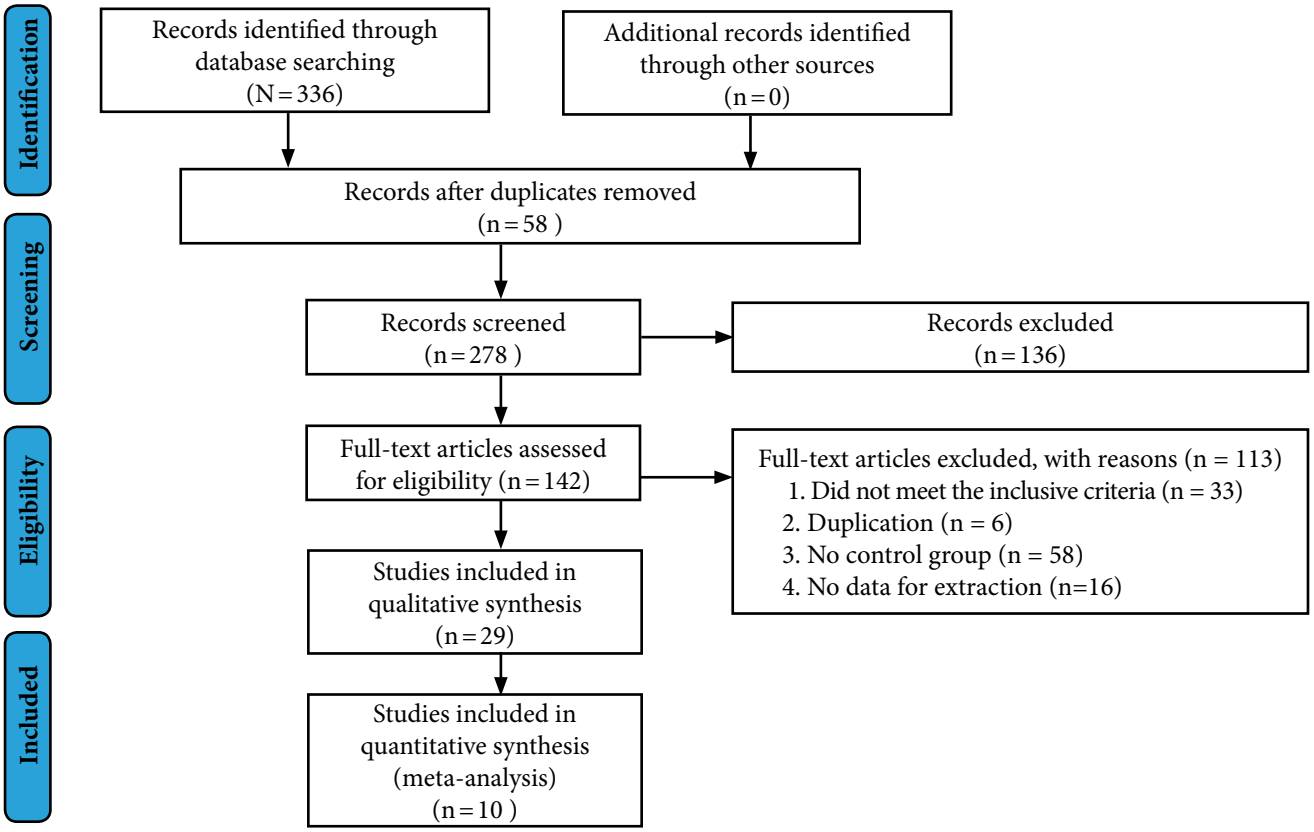



**Table 1.** Characteristics of Included Studies

| Included Studies | Diagnostic Criteria | n(T/C) | Interventions (T) | Interventions (C) | Duration (T/C) | Outcomes |
|---|---|---|---|---|---|---|
| Chen WS[10] (2009) | 1999 WHO | 34/34 | Puerarin + Control | 10 mg lisinopril | 4 wk/4 wk | UAER |
| Jiao FZ[11] (2011) | 1999 WHO | 54/42 | Puerarin + Control | 25 mg captopril | 30 d/30 d | UAER<br>BUN SCr |
| Li Q[12] (2003) | 1997 WHO | 31/31 | Puerarin + Control | 4 mg perindopril | 6 wk/6 wk | 24-h UP<br>BUN SCr<br>$\alpha_1$-MG |
| Liang F[13] (2006) | 1997 ADA | 25/20 | Puerarin + Control | 10 mg benazepril | 4 wk/4 wk | UAER<br>BUN SCr |
| Wei JL[14] (2007) | 1999 WHO | 26/22 | Puerarin + Control | 10 mg enalapril | 15 d/15 d | BUN<br>SCr |
| Wu YX[15] (2009) | 1999 WHO | 35/30 | Puerarin + Control | 10 mg enalapril | 2 wk/2 wk | UAER<br>BUN SCr |
| Xu JQ[16] (2011) | 1999 WHO | 22/18 | Puerarin + Control | 10 mg benazepril | 4 wk/4 wk | UAER<br>BUN SCr<br>AE |
| Yan AH[17] (2006) | 1999 WHO | 31/30 | Puerarin + Control | 10 mg fosinopril | 6 mo/6 mo | UAER<br>24-h UP<br>BUN SCr |
| Yuan DY[18] (2009) | 1999 WHO | 42/42 | Puerarin + Control | 10 mg benazepril | 2 wk/2 wk | UAER<br>BUN SCr |
| Zhang XW[19] (2005) | 1999 WHO | 42/38 | Puerarin + Control | 5 mg enalapril | 14 d/14 d | UAER<br>BUN SCr |

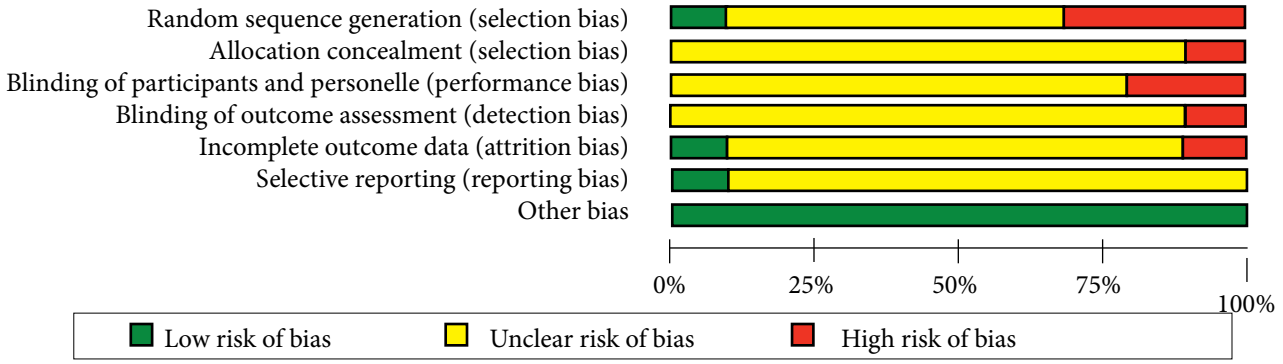
Abbreviations: T = treatment group; C = control group; UAER = urinary albumin excretion rate; 24-h UP = 24-hour urine protein; BUN = blood urea nitrogen; SCr = serum creatinine; $\alpha_1$-MG = $\alpha_1$-microglobulin; AE = adverse event.

**Figure 2.** Risk-of-Bias Summary

| | Chen WS[10] (2009) | Jiao FZ[11] (2011) | Liang F[13] (2006) | Li Q[12] (2013) | Wei JL[14] (2007) | Wu YX[15] (2009) | Xu JQ[16] (2011) | Yan AH[17] (2006) | Yuan DY[18] (2009) | Zhang XW[19] (2005) |
|---|---|---|---|---|---|---|---|---|---|---|
| Random sequence generation (selection bias) | ? | ? | - | - | ? | - | ? | ? | + | ? |
| Allocation concealment (selection bias) | ? | ? | - | ? | ? | ? | ? | ? | ? | ? |
| Blinding of participants and personelle (performance bias) | ? | ? | ? | - | - | ? | ? | ? | ? | ? |
| Blinding of outcome assessment (detection bias) | ? | ? | ? | ? | - | ? | ? | ? | ? | ? |
| Incomplete outcome data (attrition bias) | ? | ? | ? | ? | ? | - | ? | ? | + | ? |
| Selective reporting (reporting bias) | ? | ? | ? | ? | ? | ? | ? | ? | + | ? |
| Other bias | + | + | + | + | + | + | + | + | + | + |

+ Low risk of bias ? Unclear risk of bias - High risk of bias

**Figure 3.** Risk-of-Bias Graph




### Risk of Bias in Included Studies

The risk of bias in each study was assessed using the criteria recommended by the *Cochrane Handbook for Systematic Review of Interventions*.[9] Only 1 trial reported the method to generate the allocation sequence, a random number table in the paper.[18] One study reported incomplete outcome data.[15] No trial reported sample-size estimation and intention-to-treat analysis. No trial reported information on withdrawals/dropouts. All trials provided baseline data for comparability among groups. The methodological quality of each study is summarized in Figure 2 and Figure 3.

### Primary Outcomes

**Urinary Albumin Excretion Rate.** Eight trials investigated the UAER in a total of 539 participants.[10,11,13,15-19] Figure 4 presents the results of the random-effects analysis of the intervention using puerarin plus ACEI versus the control using ACEI only. The heterogeneity between studies was significant ($P < .00001$, $I^2 = 95\%$). Compared with the results for the groups using ACEI only, the UAER showed a statistically significant decline for the groups using puerarin plus ACEI ($P < .0001$, MD = -23.43 [95% CI, -33.95 to -12.91]).

**24-h Urine Protein.** Two trials with a total of 123 participants investigated the 24-h UP.[12, 17] Figure 5 presents the results of the random-effects analysis of the intervention using puerarin plus ACEI versus the control using ACEI only. Heterogeneity between studies was significant ($P < .004$, $I^2 = 88\%$). The results for the groups using puerarin plus ACEI were not statistically superior to those for the groups using ACEI only in reducing 24-h UP ($P = .09$, MD = -56.76 [95% CI, -122.65 to 9.12]).

### Secondary Outcomes

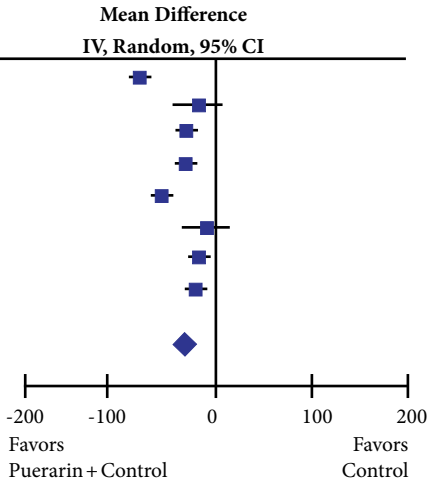
**Blood Urea Nitrogen.** Nine trials investigated the BUN for a total of 581 participants.[11-13,14-19] Figure 6 presents the result of the random-effects analysis of the intervention using puerarin plus ACEI versus the control using ACEI only. Heterogeneity between studies was significant ($P < .00001$, $I^2 = 80\%$). Compared with results for the groups using ACEI only, BUN did not decrease significantly in the groups using puerarin plus ACEI ($P = .17$, MD = -0.51 [95% CI, -1.24 to 0.21]).

**Serum Creatinine.** Nine trials with a total of 581 patients investigated the SCr.[11-13,14-19] Figure 7 presents the results of the random-effects analysis of the intervention using puerarin

**Figure 4.** UAER: Forest Plot of Comparison—Puerarin Plus ACEI Versus ACEI Only

| Study or Subgroup | Puerarin + Control Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Chen WS[10] (2009) | 52.41 | 17 | 34 | 114.5 | 25.3 | 34 | 12.3% | -62.09 (-72.34 to -51.84) |
| Jiao FZ[11] (2011) | 83.64 | 44.3 | 54 | 96.21 | 44.9 | 42 | 9.9% | -12.57 (-30.57 to 5.43) |
| Liang F[13] (2008) | 110 | 10.3 | 25 | 132.2 | 10.9 | 20 | 13.3% | -22.20 (-28.45 to -15.95) |
| Wu YX[15] (2009) | 110 | 10.3 | 35 | 132.2 | 10.9 | 30 | 13.5% | -22.20 (-27.38 to -17.02) |
| Xu JQ[16] (2011) | 32.4 | 10.9 | 22 | 71.9 | 9.8 | 18 | 13.3% | -39.50 (-45.92 to -33.08) |
| Yan AH[17] (2006) | 63 | 33 | 31 | 67 | 31 | 30 | 10.5% | -4.00 (-20.06 to 12.06) |
| Yuan DY[18] (2009) | 44.5 | 8.9 | 42 | 53.7 | 11.4 | 42 | 13.6% | -9.20 (-13.57 to -4.83) |
| Zhang XW[19] (2005) | 35.7 | 9.6 | 42 | 48.1 | 10.3 | 38 | 13.6% | -12.40 (-16.78 to -8.02) |
| **Total (95% CI)** | | | **285** | | | **254** | **100.0%** | **-23.43 (-33.95 to -12.91)** |

Heterogeneity: $\tau^2 = 206.71$; $\chi^2 = 140.87$; $df = 7$ ($P < .00001$); $I^2 = 95\%$.
Test for overall effect: $z = 4.36$ ($P < .0001$).

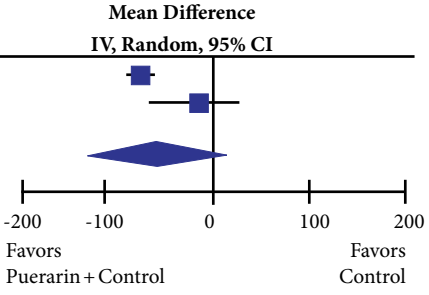



Abbreviations: UAER = urinary albumin excretion rate; ACEI = angiotensin-converting enzyme inhibitor; SD = standard deviation; CI = confidence interval.

**Figure 5.** 24-h UP: Forest Plot of Comparison—Puerarin Plus ACEI Versus ACEI Only

| Study or Subgroup | Puerarin + Control Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Li Q[12] (2003) | 113 | 27.8 | 31 | 200.5 | 36.6 | 31 | 54.5% | -87.50 (-103.68 to -71.32) |
| Yan AH[17] (2006) | 180 | 90 | 31 | 200 | 80 | 30 | 45.5% | -20.00 (-62.70 to 22.70) |
| **Total (95% CI)** | | | **62** | | | **61** | **100.0%** | **-56.76 (-122.65 to 9.12)** |

Heterogeneity: $\tau^2 = 2006.74$; $\chi^2 = 8.39$; $df = 1$ ($P = .004$); $I^2 = 88\%$.
Test for overall effect: $z = 1.69$ ($P = .09$).

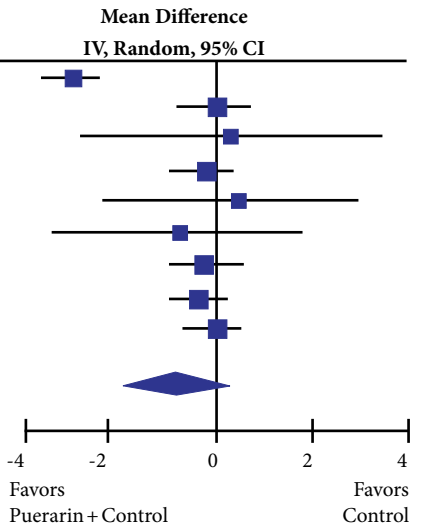



Abbreviations: 24-h UP = 24-hour urine protein; ACEI = angiotensin-converting enzyme inhibitor; SD = standard deviation; CI = confidence interval.

**Figure 6.** BUN: Forest Plot of Comparison—Puerarin Plus ACEI Versus ACEI Only

| Study or Subgroup | Puerarin + Control Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Jiao FZ[11] (2011) | 6.16 | 1.78 | 54 | 9.15 | 2.29 | 42 | 13.6% | -2.99 (-3.83 to -2.15) |
| Li Q[12] (2003) | 5.57 | 1.8 | 31 | 5.56 | 1.12 | 31 | 14.2% | 0.01 (-0.74 to 0.76) |
| Liang F[13] (2008) | 9.7 | 5 | 25 | 9.35 | 5.7 | 20 | 4.0% | 0.35 (-2.83 to 3.53) |
| Wei JL[14] (2007) | 5.43 | 1.29 | 26 | 5.63 | 1.32 | 22 | 14.2% | -0.20 (-0.94 to 0.54) |
| Wu YX[15] (2009) | 9.7 | 5 | 3 | 59.35 | 5.7 | 30 | 5.2% | 0.35 (-2.28 to 2.98) |
| Xu JQ[16] (2011) | 6.75 | 3.52 | 22 | 7.55 | 4.8 | 18 | 5.2% | -0.80 (-3.46 to 1.86) |
| Yan AH[17] (2006) | 5.6 | 1.6 | 31 | 5.8 | 1.5 | 30 | 14.0% | -0.20 (-0.98 to 0.58) |
| Yuan DY[18] (2009) | 6.24 | 1.42 | 42 | 6.56 | 1.42 | 42 | 14.9% | -0.32 (-0.93 to 0.29) |
| Zhang XW[19] (2005) | 6.61 | 1.48 | 42 | 6.58 | 1.45 | 38 | 14.7% | 0.03 (-0.61 to 0.67) |
| **Total (95% CI)** | | | **308** | | | **273** | **100.0%** | **-0.51 (-1.24 to 0.67)** |

Heterogeneity: $\tau^2 = 0.83$; $\chi^2 = 40.41$; $df = 8$ ($P < .00001$); $I^2 = 80\%$.
Test for overall effect: $z = 1.39$ ($P < .17$).

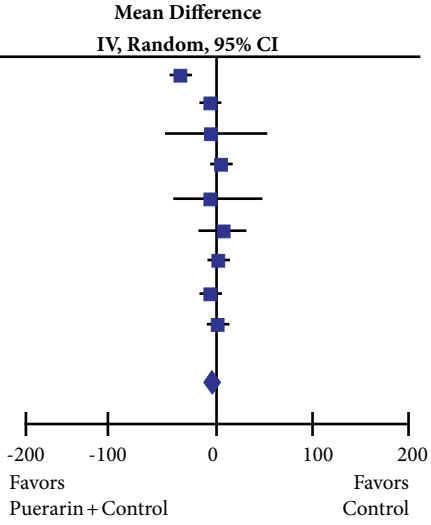



Abbreviations: BUN = blood urea nitrogen; ACEI = angiotensin-converting enzyme inhibitor; SD = standard deviation; CI = confidence interval.

**Figure 7.** SCr: Forest Plot of Comparison—Puerarin Plus ACEI Versus ACEI Only

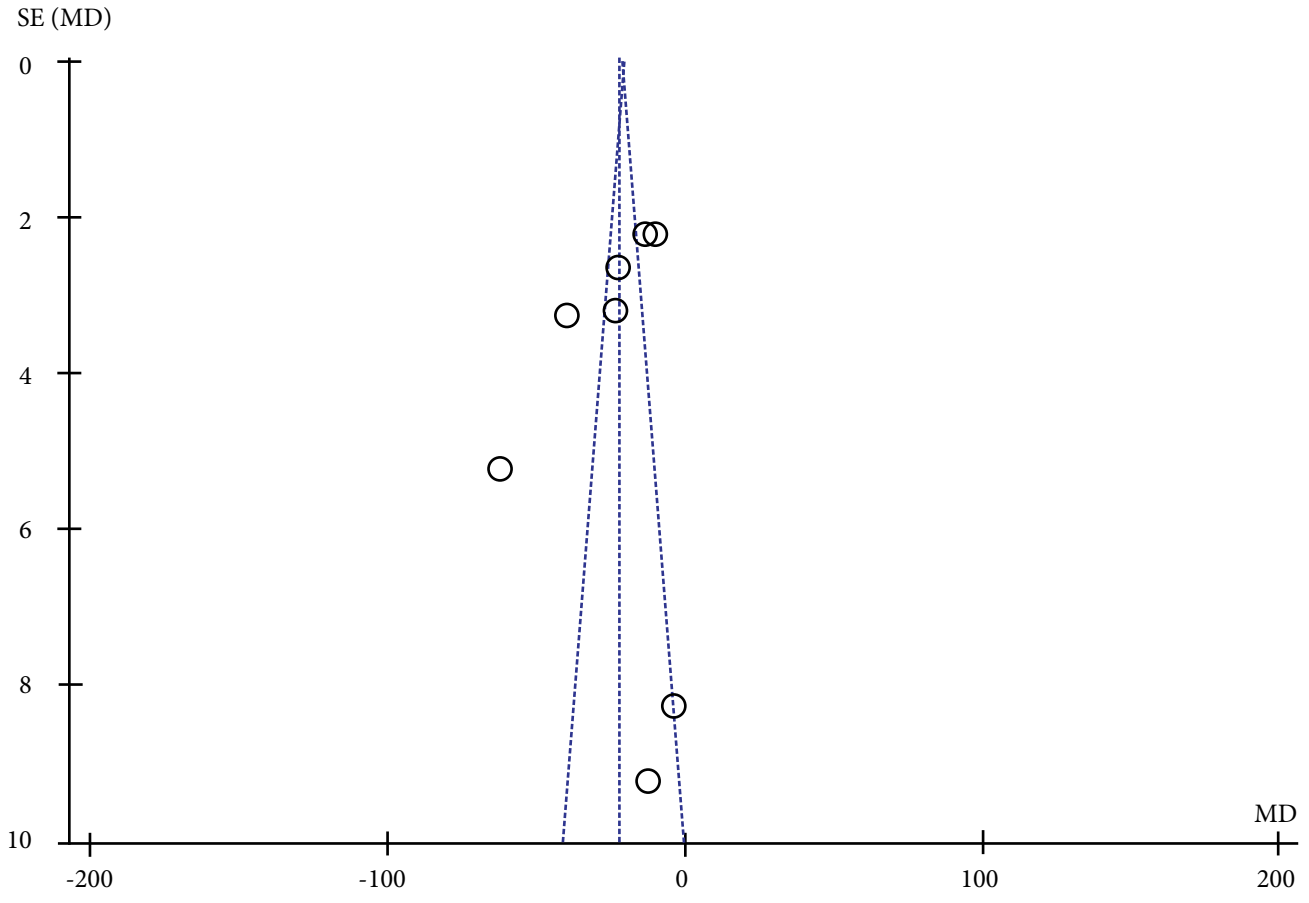
| Study or Subgroup | Puerarin + Control Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Jiao FZ[11] (2011) | 144.65 | 10.39 | 54 | 173.51 | 19.68 | 42 | 13.7% | -28.86 (-35.43 to -22.29) |
| Li Q[12] (2003) | 71.4 | 12.6 | 31 | 75.6 | 10.7 | 31 | 14.0% | -4.20 (-10.02 to 1.62) |
| Liang F[13] (2008) | 140.5 | 67.4 | 25 | 143 | 65.7 | 20 | 3.0% | -2.50 (-41.58 to 36.58) |
| Wei JL[14] (2007) | 74.31 | 3.96 | 26 | 72.29 | 4.36 | 22 | 15.0% | 2.02 (-0.35 to 4.39) |
| Wu YX[15] (2009) | 140.5 | 67.4 | 35 | 143 | 55.7 | 30 | 4.5% | -2.50 (-32.43 to 27.43) |
| Xu JQ[16] (2011) | 75.1 | 24.6 | 22 | 72.18 | 22.55 | 18 | 9.7% | 2.92 (-11.72 to 17.56) |
| Yan AH[17] (2006) | 75 | 11 | 31 | 75 | 13 | 30 | 13.9% | 2.92 (-11.72 to 17.56) |
| Yuan DY[18] (2009) | 80.8 | 16.8 | 42 | 84.6 | 16.6 | 42 | 13.4% | -3.80 (-10.94 to 3.34) |
| Zhang XW[19] (2005) | 110.9 | 19.8 | 42 | 109.2 | 19.5 | 38 | 12.7% | 1.70 (-6.92 to 10.32) |
| **Total (95% CI)** | | | **308** | | | **273** | **100.0%** | **-4.43 (-12.05 to 3.20)** |

Heterogeneity: $\tau^2 = 206.71$; $\chi^2 = 140.87$; $df = 7$ ($P < .00001$); $I^2 = 95\%$.
Test for overall effect: $z = 4.36$ ($P < .0001$).




Abbreviations: SCr = serum creatinine; ACEI = angiotensin-converting enzyme inhibitor; SD = standard deviation; CI = confidence interval.

**Figure 8.** UAER: Funnel Plot of Comparison




Abbreviation: UAER = urinary albumin excretion rates; SE = standard error; MD = median difference.

plus ACEI versus the control using ACEI only. Heterogeneity between studies was significant ($P < .00001$, $I^2 = 90\%$). Compared with the groups using ACEI only, SCr did not decrease significantly in the groups using puerarin plus ACEI ($P = .26$, MD = -4.43 [95% CI, -12.05 to 3.20]).

### Other Outcomes

One trial[12] showed that $\alpha_1$-MG was reduced significantly after treatment for the intervention group compared with the control groups ($P < .05$).

### Adverse Events

One trial[16] reported abdominal discomfort and nausea (2 cases) in the intervention group treated with puerarin plus ACEI.

### Sensitivity Analysis

The quality of trials was too low, and the research team could not conduct the sensitivity analysis.

### Assessment of Publication Biases

In its analysis, the research team performed a funnel-plot test of UAER, and the test indicated that a publication bias existed in our meta-analysis (Figure 8).

## DISCUSSION

Proteinuria is the universal finding in progressive renal disease and is viewed as a measure of the severity and a determinant of the progression of DN. Proteinuria is a marker for early DN, is an independent predictor for mortality, and is associated with renal and cardiovascular risks.[20,21] The proteinuria that develops after induction of diabetes is mainly caused by an increased excretion of an array of proteins with a low molecular weight, where albumin constitutes a relatively low proportion of total urinary protein.[22] UAER and 24-h UP have been demonstrated to be good clinical predictors of renal lesions in DN.[21]

Studies with a total of 669 participants with stage III DN, between the ages of 30 and 80 years, were included in the current meta-analysis. The treatment duration ranged from 14 days to 6 months. Compared with ACEI only, UAER showed a statistically significant decline in the groups using puerarin plus ACEI. Use of puerarin plus ACEI, however, was not statistically superior to use of ACEI only for reducing 24-h UP. Compared with the groups using ACEI only, BUN and SCr did not decrease significantly for the groups using puerarin plus ACEI.

One trial showed that $\alpha_1$-MG was reduced significantly after treatment in the intervention group compared with the control group. However, administration of puerarin did not significantly decrease 24-h UP, BUN, and SCr in the DN patients. This result was possibly because of the short course of treatment in the included trial, mostly not more than 4 weeks.

The therapeutic mechanisms of puerarin on DN probably include the following processes: (1) puerarin-evoked inflammatory response; (2) impairment of the phosphoinositide 3-kinase-dependent, insulin-signaling pathway; (3) increased TNF-α and IL-6 overexpression and production; (4) modulated serine and tyrosine phosphorylation of insulin receptor substrate-1; and (5) improved insulin phosphoinositide 3-kinase signaling.[23] Puerarin provides a renal benefit in clinical stage III for patients with DN. It delays the progression to overt nephropathy in patients with microalbuminuria and reduces UAER in patients with microalbuminuria. The effect of puerarin plus ACEI on UAER in DN appears to be better than that of ACEI.

To the best of the research team's knowledge, the current meta-analysis is the first to evaluate RCTs exploring the effects of puerarin on DN. The main results agreed with another study on puerarin, but that study covered diabetic animals, not humans.[24] However, our analysis had some limitations. First, most of the trials evaluated were short-term, generally lasting no more than 6 months, which means that clinical EPs—such as all-cause death and the occurrence of cardiovascular events—may not reflect the AEs of puerarin therapy. Because the studies' periods were not long enough to evaluate slowly progressive DN, they assessed only proteinuria and not the true outcomes related to end-stage renal disease. Long-term follow-up studies are needed.[25]

Second, clinical heterogeneity in the RCTs was associated with the studies' varied settings. Considerable variation existed in the participants, in the timing of the puerarin treatment, in the outcome analyses (UAER or 24-h UP), in the level of reduction of urine protein, and in the durations of the studies. These differences may explain some of the heterogeneity observed. As a result, the values for the UAER, 24-h UP, BUN, and SCr were significantly different, with heterogeneity being statistically significant between the studies. The heterogeneity could be explained by the different baseline levels of UAER, 24-h UP, BUN, and SCr of the patients enrolled in these trials.

Third, the methodological quality of the included studies was generally poor. The randomization was not clear in most of the trials because of insufficient reporting of the generation methods of the allocation sequence and allocation concealment. Most trials stated only that patients were randomly assigned. Most of trials in this review did not introduce double blinding. Systematic reviews and meta-analyses such as the current one are often limited by the quality of the included studies. The quality of the present evidence is limited considering that most of the included studies provided an unclear risk of bias for the key methodological elements of adequate random sequence generation and allocation concealment. In addition to this issue, a high risk of bias that was associated with patient/outcome assessment blinding also may mean that the effect of puerarin was likely to have been estimated as higher than it actually was.[26] The current research team tried to avoid language bias and location bias, but it could not exclude potential dissemination bias.

The team had undertaken extensive searches for unpublished material; few of the trials identified qualified for inclusion, but at the same time, the team cannot disregard

the fact that trials with negative findings remain unpublished.[27] Also, most of the participants were recruited from Chinese populations, and this fact could have affected the applicability of the interventions for other populations. No long-term data on outcomes were reported in the included trials.[28]

Fourth, none of the included trials had a placebo control. All of them used an A + B versus B design in which patients were randomized to receive puerarin plus ACEI as the intervention versus ACEI drugs as the control, without a rigorous control for placebo effect. Thus, positive conclusions could have been made because of nonspecific placebo effects.[28]

Fifth, to verify efficacy and safety further to obtain the best evidence, populations outside of China should be included in high-quality, larger, multicenter, clinical studies with prolonged follow-up.

## CONCLUSIONS

The data from the reviewed studies demonstrated that puerarin decreased the UAER in DN patients with few AEs, suggesting that puerarin may be a beneficial therapy for treating DN.

### AUTHOR DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

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