Review Article
Therapeutic effect of YiQi HuoXue BuShen decoction combined with Western medicine on hypertensive nephropathy: a meta-analysis

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Abstract: Objective: To comprehensively evaluate therapeutic effect of YiQi HuoXue BuShen decoction combined with Western medicine on hypertensive nephropathy. Methods: The CNKI, WanFang, VIP, Chinese Biomedical Database (CBM), PubMed, Embase and Cochrane Library databases were searched to collect the randomized controlled trials (RCTs) of YiQi HuoXue BuShen decoction combined with Western medicine on hypertensive nephropathy, which were published as of March 10, 2023. Then, these articles were screened to extract and evaluate the data. Revman 5.3 was applied for data analysis. Results: Eight RCTs involving 732 patients were included after screening. Comparing with Western medicine, YiQi HuoXue BuShen decoction combined with Western medicine enhanced the clinical effect \([OR = 3.48, 95\% CI: 2.12\sim5.73, P < 0.00001]\); reduced 24-hour urine protein content \([OR = -0.60, 95\% CI: -0.92\sim-0.28, P = 0.0003]\), serum creatinine (Scr) \([OR = -39.11, 95\% CI: -44.72\sim-33.51, P < 0.00001]\), blood urea nitrogen (BUN) \([OR = -2.51, 95\% CI: -4.06\sim-0.95, P = 0.002]\), cystatin C (Cys-C) \([OR = -0.30, 95\% CI: -0.36\sim-0.25, P < 0.00001]\), Urine β2-microglobulin \([OR = -0.42, 95\% CI: -0.87\sim-0.02, P = 0.06]\); and enhanced creatinine clear rate (Ccr) \([OR = 3.24, 95\% CI: 1.85\sim4.64, P < 0.00001]\). In addition, the combination treatment didn’t increase the incidence of adverse reaction compared with western medicine \([OR = 1.55, 95\% CI: 0.61\sim3.95, P > 0.05]\). Conclusions: The combination of YiQi Huoxue Bushen decoction and western medicine can effectively improve the clinical symptoms and renal function of patients with hypertensive nephropathy and provide more theoretical basis for clinical application.

Keywords: YiQi HuoXue BuShen decoction, Western medicine, hypertensive nephropathy, effect

Introduction
Hypertensive nephropathy is a special type of renal disease that causes arteriosclerosis and glomerulosclerosis due to primary hypertension [1]. Patients experience arteriolar spasm and decreased blood perfusion at an early stage [2]. If not controlled in a timely manner, it may develop into nephron sclerosis, fibrosis, and even renal failure, posing a serious threat to a patient’s life. In the early stages of hypertension, renal artery spasm occurs, leading to decreased blood flow, followed by progressive glomerulosclerosis, renal interstitial fibrosis, renal tubular atrophy, and even renal failure [3]. Its clinical manifestations are mainly acid-base imbalance, electrolyte disorder, retention of toxic metabolites, and damage to major organs and systems of the whole body, which may further develop into irreversible end-stage renal failure [4]. However, due to the fact that the early clinical symptoms of the disease are insidious, many patients have already entered the stage of renal failure when the disease is discovered. At present, the incidence of hypertensive nephropathy in China is increasing annually. It ranked the third in the primary incidence of hemodialysis patients in China in 1999, accounting for the first cause of chronic renal failure in the elderly [5].

Currently, Western medicine is mainly used to treat renal failure caused by hypertensive nephropathy. Nifedipine is a commonly used antihypertensive drug in clinical practice, which can alleviate renal dysfunction by lowering
blood pressure in patients [6]. However, because Nifedipine does not directly affect the kidneys, its efficacy is slow, and it is prone to develop drug resistance, making it unsuitable for long-term treatment [7]. Irbesartan is an angiotensin II receptor antagonist, which can effectively inhibit the conversion of angiotensin I into angiotensin II, thereby inhibiting vasoconstriction and aldosterone release, reducing water and sodium retention, lowering blood pressure, and protecting target organ function [8]. This drug is particularly suitable for patients with hypertension and renal dysfunction. However, the efficacy of irbesartan alone in the treatment of hypertensive renal damage is not ideal. It can cooperate with methods such as blood purification and kidney transplantation [9]. Although these methods can effectively improve or treat clinical symptoms, blood purification requires long-term and regular treatment [10]. The cost is quite high, and there are strict requirements for professional personnel and medical equipment. Kidney transplantation is not only costly, but also subject to stringent transplant conditions.

According to traditional Chinese medicine, hypertension can be divided into liver yang hyperactivity syndrome, yin deficiency yang hyperactivity syndrome, phlegm dampness obstruction syndrome, and qi stagnation and blood stasis syndrome according to symptoms and signs [11]. The syndrome of hyperactivity of liver yang and syndrome of qi stagnation and blood stasis are more common. Hypertension can cause pathological damage to the heart, brain, kidneys, blood vessels, and other organs [12]. Traditional Chinese medicine can effectively prevent and reduce damage to target organs, with a certain organ protection effect. With the vigorous development of traditional Chinese medicine therapy in China, certain achievements have been achieved in the treatment of hypertensive nephropathy [13]. Yiqi Huoxue BuShen decoction has the medicinal effects of supplementing qi, nourishing yin, tonifying the kidney, promoting blood circulation and resolving blood stasis. It can improve clinical efficacy by promoting renal function [14]. Studies confirmed that the combination treatment of Yiqi Huoxue BuShen decoction and Western medicine shows better efficacy, and the clinical symptoms and renal function have obvious improvement [15]. However, the quality of existing research is inconsistent, and each study has its own focus. In this study, we systematically evaluated the therapeutic effect of Yiqi Huoxue BuShen decoction combined with Western medicine on hypertensive nephropathy.

Methods

Literature retrieval strategy

The CNKI, WanFang, VIP, Chinese Biomedical Database (CBM), PubMed, Embase and Cochrane Library databases were searched to collect the randomized controlled trials (RCTs) of YiQi HuoXue BuShen decoction combined with Western medicine on hypertensive nephropathy, which were published as of March 10, 2023. The key words included YiQi HuoXue BuShen decoction, Western medicine, Nifedipine, Amlodipine besylate, enalapril maleate, Candesartan Cilexetil, hypertensive nephropathy. We use the search of PubMed as an example, the search formula was “(Yiqi Huoxue BuShen) and (Western medicine) or (Nifedipine) or (Amlodipine besylate) or (Enalapril maleate) or (Candesartan Cilexetil) and (“Hypertensive Nephropathy” [Supplementary Concept])”.

Inclusion and exclusion criteria

Inclusion criteria: (1) Randomized controlled trial (RCT) published in China or abroad; (2) Patients with hypertensive nephropathy; (3) The control group was treated with western medicine, and the experimental group was given the combination treatment of YiQi HuoXue BuShen decoction and western medicine; (4) Articles having at least one of the following indicators included: ① The total clinical effective rate; ② 24-hour urinary protein quantification; ③ Serum creatinine (Scr); ④ Blood urea nitrogen (BUN); ⑤ Cystatin C (Cys-C); ⑥ Urine β2-microglobulin; ⑦ Enhanced creatinine clearance rate (Ccr); ⑧ Adverse reaction.

Exclusion criteria: (1) Articles published repeatedly; (2) Articles that couldn’t provide relevant or specific data for extraction; (3) Review, conference papers, animal/cell experiment articles.

Literature screening and data extraction

After removing the duplicate documents, two researchers (Mingming Liu, Hong Wang) independently examined the document’s title and...
abstract and removed reviews, non-clinical studies, and other items that were blatantly irrelevant. Then the full text was read for further screening. Relevant information was extracted, including the number of cases, type of western medicine, course of treatment, and outcome indicators. Disputes were solved by consulting a third researcher (Ping Lv).

Risk assessment of bias

The items of bias risk assessment include: (1) Generation of random sequence (selective bias); (2) Distribution concealment (selective bias); (3) Implementation of blind method for researchers and subjects (implementation bias); (4) Results Implementation of evaluator blind method (measurement bias); (5) Integrity of result data (wear bias); (6) Report bias; (7) Bias from other sources.

Statistical analysis

Software RevMan 5.3 was employed for the meta-analysis. For binary variables, the odds ratio (OR) was used for calculating the relationship between a variable and the likelihood of an event occurring, while the mean difference (MD), along with its 95% confidence interval (95% CI), was used for measuring the absolute difference between the mean value in two different groups. The Q test was used to examine the heterogeneity of the included research results. The fixed effect (FE) model was employed if the heterogeneity between the research outcomes was low ($P > 0.1$ and $I^2 < 50\%$); otherwise, the random effect (RE) model was applied. To determine whether there was publishing bias, a funnel map was created.

Results

Literature screening process and basic characteristics of inclusion research

At the time of the initial search, there were 325 relevant articles, as shown in Figure 1. Eight articles involving 732 hypertensive nephropathy patients were eventually included after a systematically review process. Table 1 lists the
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Bias risk assessment

The selection bias of 8 studies was “low risk” since they described the specific random classification method, in which 7 studies used a random number table method, and 1 study used the paper bag method. Two publications didn’t mention the allocation concealment and were therefore evaluated as “unclear”. Two studies didn’t explain the blinding of participants and personnel and was evaluated as “unclear”. In addition, one article didn’t meet the blind of outcome assessment, which was decided as “high risk”, and one article didn’t mention it, which was regarded as “unclear”. For the incomplete outcome data, there was one publication evaluated as “low risk”. For the selective reporting, one was “unclear”, the other was “high risk”. See Figures 2 and 3.

Meta-analysis results

The clinical effective rate: As shown in Figure 4, there were 6 studies which evaluated the clinical effective rate. The result obtained by FE model (I² = 0%) showed that the combination treatment of YiQi HuoXue BuShen decoction and western medicine enhanced the curative effect compared with western medicine alone [OR = 3.48, 95% CI: 2.12–5.73, P < 0.00001].

24-hour urinary protein quantification: There were 4 studies that evaluated the 24-hour urinary protein quantification. The results obtained by RE model (I² = 89%) showed that the combination treatment of YiQi HuoXue BuShen decoction and western medicine decreased more in the 24-hour urinary protein compared with western medicine alone (OR = -0.60, 95% CI: -0.92–-0.28, P = 0.0003) (Figure 5).

Table 1. The summarized information in each study

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Cases (n)</th>
<th>Kind of western medicine</th>
<th>Course of treatment (days)</th>
<th>Primary outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>He 2018 [16]</td>
<td>40/40</td>
<td>Nifedipine</td>
<td>3 months</td>
<td>(1) (2) (3) (4)</td>
</tr>
<tr>
<td>Hu 2018 [17]</td>
<td>55/55</td>
<td>Amlodipine besylate and enalapril maleate</td>
<td>12 weeks</td>
<td>(1) (2) (3) (4) (5) (8)</td>
</tr>
<tr>
<td>Lan 2012 [18]</td>
<td>80/48</td>
<td>Candesartan Cilexetil</td>
<td>30 days * 3 times</td>
<td>(1) (2) (3) (4)</td>
</tr>
<tr>
<td>Sun 2021 [20]</td>
<td>25/25</td>
<td>Enalapril maleate and amlodipine besylate</td>
<td>3 months</td>
<td>(2) (4) (5)</td>
</tr>
<tr>
<td>Wei 2018 [22]</td>
<td>50/50</td>
<td>Benazepril Tablets</td>
<td>3 months</td>
<td>(1) (4) (6) (7)</td>
</tr>
<tr>
<td>Yu 2017 [23]</td>
<td>30/30</td>
<td>Nifedipine</td>
<td>3 months</td>
<td>(1) (2) (3) (4) (5)</td>
</tr>
</tbody>
</table>

Note: (1) The total clinical effective rate; (2) 24-hour urinary protein quantification; (3) Serum creatinine (Scr); (4) Blood urea nitrogen (BUN); (5) Cystatin C (Cys-C); (6) Urine β2-microglobulin; (7) Creatinine clear rate (Ccr); (8) Adverse reaction.

Figure 2. The scale for assessing the quality of clinical trials.
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Serum creatinine: There were 5 articles that detected the Scr level. The heterogeneity was evaluated as $I^2 = 0\%$, so the FE model was performed. The result showed that there was a lower Scr level when the patients received combination treatment of YiQi Huoxue BuShen decoction and western medicine [$OR = -39.11$, 95% CI: $-44.72$ to $-33.51$, $P < 0.00001$] (Figure 6).

Blood urea nitrogen: There were 6 studies that investigated the BUN level. The result obtained by RE model ($I^2 = 88\%$) showed that the combination treatment of YiQi Huoxue BuShen decoction and western medicine resulted in a lower BUN level compared with western medicine alone [$OR = -2.51$, 95% CI: $-4.06$ to $-0.95$, $P = 0.002$] (Figure 7).

Cystatin C: There were 3 articles that detected the Cys-C level. The heterogeneity was evaluated as $I^2 = 48\%$, so the FE model was performed. The result showed that there was a lower Cys-C when the patients carrying on com-

Figure 3. Summary chart of the risk bias assessment.

Figure 4. The comparison of total effective rate between two groups.

Figure 5. The comparison of 24-hour urinary protein quantification between two groups.
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<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>Mean</td>
</tr>
<tr>
<td>He 2019</td>
<td>267.97</td>
<td>102.56</td>
<td>40</td>
<td>324.78</td>
</tr>
<tr>
<td>Hu 2018</td>
<td>190.41</td>
<td>15.38</td>
<td>55</td>
<td>216.77</td>
</tr>
<tr>
<td>Lan 2012</td>
<td>229.45</td>
<td>74.37</td>
<td>80</td>
<td>254.01</td>
</tr>
<tr>
<td>Sun 2021</td>
<td>180.43</td>
<td>15.37</td>
<td>25</td>
<td>216.78</td>
</tr>
<tr>
<td>Yu 2017</td>
<td>281.5</td>
<td>128.4</td>
<td>30</td>
<td>329.83</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>230</td>
<td>190</td>
<td>100.0%</td>
<td>-39.11 [-44.72, -33.51]</td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 1.46$, df = 4 ($P = 0.83$), $P = 0%$
Test for overall effect: $Z = 13.68$ ($P < 0.00001$)

**Figure 6.** The comparison of serum creatinine (Scr) between two groups.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>Mean</td>
</tr>
<tr>
<td>He 2018</td>
<td>12.56</td>
<td>4.24</td>
<td>40</td>
<td>15.76</td>
</tr>
<tr>
<td>Hu 2018</td>
<td>14.26</td>
<td>2.44</td>
<td>54</td>
<td>15.63</td>
</tr>
<tr>
<td>Lan 2012</td>
<td>10.31</td>
<td>1.31</td>
<td>80</td>
<td>10.61</td>
</tr>
<tr>
<td>Sun 2021</td>
<td>14.25</td>
<td>2.39</td>
<td>25</td>
<td>16.02</td>
</tr>
<tr>
<td>Wei 2018</td>
<td>12.56</td>
<td>4.24</td>
<td>50</td>
<td>19.12</td>
</tr>
<tr>
<td>Yu 2017</td>
<td>13.11</td>
<td>5.3</td>
<td>30</td>
<td>15.67</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>280</td>
<td>248</td>
<td>100.0%</td>
<td>-2.51 [-4.66, -0.95]</td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 3.07$, $df = 5$ ($P = 0.00001$), $P = 88%$
Test for overall effect: $Z = 3.16$ ($P = 0.002$)

**Figure 7.** The comparison of blood urea nitrogen (BUN) between two groups.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>Mean</td>
</tr>
<tr>
<td>Nian 2019</td>
<td>0.82</td>
<td>0.13</td>
<td>52</td>
<td>1.16</td>
</tr>
<tr>
<td>Wang 2021</td>
<td>1.44</td>
<td>0.31</td>
<td>50</td>
<td>1.63</td>
</tr>
<tr>
<td>Wei 2018</td>
<td>0.85</td>
<td>0.11</td>
<td>50</td>
<td>1.13</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>152</td>
<td>152</td>
<td>100.0%</td>
<td>-0.30 [-0.36, -0.25]</td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 3.65$, $df = 2$ ($P = 0.15$), $P = 48%$
Test for overall effect: $Z = 11.38$ ($P < 0.00001$)

**Figure 8.** The comparison of cystatin C (Cys-C) between two groups.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
<td>Mean</td>
</tr>
<tr>
<td>Nian 2019</td>
<td>2.41</td>
<td>0.33</td>
<td>52</td>
<td>3.02</td>
</tr>
<tr>
<td>Wang 2021</td>
<td>0.39</td>
<td>0.11</td>
<td>50</td>
<td>0.45</td>
</tr>
<tr>
<td>Wei 2018</td>
<td>1.78</td>
<td>0.34</td>
<td>50</td>
<td>2.41</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>152</td>
<td>152</td>
<td>100.0%</td>
<td>-0.42 [-0.87, -0.02]</td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 0.15$, $df = 2$ ($P < 0.00001$), $P = 96%$
Test for overall effect: $Z = 2.88$ ($P = 0.00005$)

**Figure 9.** The comparison of Urine β2-microglobulin between two groups.

Combination treatment of Yiqi Huoxue Bushen decoction and western medicine [$OR = -0.30$, 95% CI: $-0.36$ to $-0.25$, $P < 0.00001$] (Figure 8).

**Urine β2-microglobulin:** As shown in Figure 9, the detection of urine β2-microglobulin were included in 3 studies. The result obtained by RE model ($I^2 = 96\%$) showed that the combination treatment of Yiqi Huoxue Bushen decoction and western medicine decrease the level of Urine β2-microglobulin compared with western medicine alone [$OR = -0.42$, 95% CI: $-0.87$ to $-0.02$, $P = 0.06$].

Creatinine clear rate: There were 3 articles that detected the Ccr level. The heterogeneity was evaluated as $I^2 = 0\%$, so the FE model was performed. The result showed that there was a higher Ccr level when the patients carrying on combination treatment of Yiqi Huoxue Bushen decoction and western medicine [$OR = 3.24$, 95% CI: $1.85$ to $4.64$, $P < 0.00001$] (Figure 10).
Adverse reaction: In the analysis of adverse reaction, three publications were included. As shown in **Figure 11**, the heterogeneity was low ($I^2 = 0\%$), so the FE model was performed. The result showed that there was no obvious difference in the incidence of adverse reaction between two groups [OR = 1.55, 95% CI: 0.61–3.95, $P > 0.05$].

Publication bias

The funnel graphic of each outcome was created to assess publication bias. The dispersed distribution of included studies regarding each outcome index is asymmetrical, suggesting publication bias, which may be connected to the sample size of included research (**Figure 12**). In addition, the western medicines used in these 8 publications are not the same, including Nifedipine, Amlodipine besylate and enalapril maleate, Candesartan Cilexetil, Enalapril maleate tablets and amlodipine atorvastatin calcium tablets, Irbesartan, and Benazepril Tablets, which might also cause bias in conclusions to some extent.

Discussion

Hypertensive nephropathy is a common complication of hypertension. Currently, the pathogenesis of renal failure caused by hypertensive nephropathy has not been fully elucidated in the academic community, which may be related to metabolic product excretion disorders, acid-base imbalance, electrolyte disorders, and so on [24]. Currently, Western medicine can only alleviate some symptoms of renal failure caused by hypertensive nephropathy, with high treatment costs and many adverse reactions [25]. Enalapril maleate is a widely used angiotensin converting enzyme inhibitor in clinical practice, while amiodipine besylate is a widely used calcium antagonist. The combination of the two can effectively control the amount of urine protein in patients, help reduce blood pressure levels, and have certain advantages in improving quality of life [26]. However, after long-term use, its efficacy shows a progressive downward trend. Therefore, it is particularly important to seek effective treatment methods to control the development of the disease.

According to traditional Chinese medicine, renal failure caused by hypertensive nephropathy belongs to the category of “dizziness, edema, and depression”. The disease is located in the kidney, and can be induced by irregular lifestyle, deficiency of the body, and invasion of external pathogens [27]. The pathogenesis can be summarized as dampness, turbidness, poisoning, spleen and kidney deficiency, etc. Therefore, traditional Chinese medicine treatment should focus on tonifying kidney yin, dredging blood vessels, and strengthening spleen [28]. In this study, on the basis of Western medicine treatment, the effect of supplementing qi, activating blood circulation, and tonifying the kidney prescription is relatively
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Significant. In the prescription, orange peel regulates qi, strengthens spleen, and promotes the circulation of the five internal organs [29]; Achyranthes bidentata can remove blood stasis and dredge meridians, diuresis and drench; Pueraria root relieves external heat, elevates yang, and stops diarrhea; Fructus aurantii can regulate qi in a broad range [11]; diuresis and detumescence can tonify qi to anti perspiration; Salvia miltiorrhiza can promote blood circulation and remove blood stasis, clear the heart and remove irritation; Rehmannia glutinosa can nourish yin and promote fluid production, heat clearing and detoxification; Chinese wolfberry nourishes the liver and kidney, replenishes blood and calms the nerves; Epimedium can tonify the kidney and strengthen yang, dispel wind and remove dampness; Radix Pseudostellariae generates fluid to moisten the lungs, replenish qi and strengthen the spleen; Licorice can clear heat and detoxify, replenish spleen and replenish qi [31]. The combination of various drugs have the efficacy of removing dampness and blood stasis, nourishing the kidney and strengthening the spleen. Modern pharmacological studies have shown that Fructus Aurantii can effectively relax vascular smooth muscle, promote peripheral arteriolar relaxation, and help reduce peripheral blood flow resistance [12]; Salvia miltiorrhiza can play a good role in improving the internal environment of renal microcirculation and enhancing fibrinolytic activity in patients, helping to reduce blood viscosity, thereby reversing or delaying renal function damage; Rehmanna glutinosa can effectively enhance the patient’s immune system, increase blood perfusion, improve lipid metabolism and protein levels, and effectively alleviate glomerular oxygen free radical damage [32].

In this study, 8 RCTs involving 732 patients were included after screening. Comparing with Western medicine alone, YiQi HuoXue BuShen decoction combined with Western medicine enhanced the clinical effect, reduced 24-hour urine protein content, serum creatinine (Scr), blood urea nitrogen (BUN), cystatin C (Cys-C), Urine β2-microglobulin, and elevated creatinine clear rate (Ccr). It shows that Yiqi Huoxue Bushen Formula combined with western medicine can effectively delay and alleviate renal function damage while providing basic treat-
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Although this study has developed tight inclusion and exclusion criteria, it still has certain limitations: (1) There are currently few RCTs for the combination treatment of Yiqi Huoxue Bushen decoction on hypertensive nephropathy, and the sample size is small, which lowers the test efficiency; (2) The quality of methodology included in the study varies, and there is a certain bias in random method, blinding method, etc.; (3) There are few reports on the safety of Yiqi Huoxue Bushen decoction in the included studies; (4) Different Western medicines used in the included articles may lead to greater heterogeneity in the analysis. The findings need to be supported by additional research by gathering more studies. Confounding influences can be detected and controlled using a more stringent multi-center, randomized, double-blind trial procedure to raise the reliability of the study.

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Disclosure of conflict of interest

None.

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