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*Original Article*

# Acupuncture alleviates renal edema through upregulation of renal aquaporin expression

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**Abstract:** Objective: This study aimed to investigate the principles of acupoint selection and the prescription characteristics of acupuncture therapy for renal edema. Methods: Data mining technology was adopted to identify high-frequency acupoints and commonly used prescription combinations. Eight rats were selected as the control group without drug intervention, while the remaining rats were divided into the control group, model group, sham acupuncture group, and acupuncture group. Serum Ig-M and Ig-G expression levels were assessed by western blotting, and renal expression of AQP2 and AQP3 was examined using immunofluorescence. Results: In prescriptions for renal edema, the five most frequently selected acupoints were Shenshu (BL23), Shuishui (ST28), Guanyuan (CV4), Pishu (BL20), and Zusanli (ST36). The four most frequently involved meridians were the Bladder Meridian of Foot-Taiyang, the Conception Vessel, the Kidney Meridian of Foot-Shaoyin, and the Governor Vessel. Commonly used acupoints included Yinlingquan (SP9), Yongquan (KI1), Guanyuan (CV4), Pishu (BL20), Zusanli (ST36), Shenque (CV8), and Shuifen (CV9). Compared to the blank group, the model group exhibited significantly increased serum IgM and IgG expression, while these levels were reduced in the acupuncture group. In contrast, renal AQP2 and AQP3 expression was significantly decreased in the model group relative to the blank group ($P<0.05$). Conclusion: Acupuncture targeting the Bladder Meridian of Foot-Taiyang, the Conception Vessel, the Kidney Meridian of Foot-Shaoyin, and the Governor Vessel can alleviate renal edema by enhancing renal aquaporin expression and attenuating inflammatory response.

**Keywords:** Data mining, acupuncture, renal edema, law of acupoint selection

## Introduction

Renal edema is a common complication in renal diseases, and its pathogenesis involves complex physiologic and pathologic processes [1-4]. During disease progression, abnormal regulation of multiple hormones and cytokines disrupts renal water and sodium homeostasis [5-7]. In Western medicine, glucocorticoids, immunosuppressants, diuretics and other drugs are commonly used to control disease progression and relieve clinical symptoms. However, in patients with severely impaired renal function, these agents carry potential adverse effects such as increased risk of infection and osteoporosis. Therefore, the search for safe and effective therapeutic approaches has become a key clinical priority.

In traditional Chinese medicine (TCM), the pathogenesis of renal edema is considered multifactorial and is closely related to dysfunction of the spleen, lung and kidney, which together constitute the internal basis for edema formation [8]. Compared to pharmacological therapy, acupuncture avoids drug-related side effects and is generally well-accepted by patients. Furthermore, acupuncture can be combined with other TCM interventions to form comprehensive treatment regimens, thereby highlighting the holistic and individualized principles of TCM. Previous studies have shown that acupuncture can reduce edema severity and promote renal function recovery [9]. Despite its widespread clinical application, systematic analyses of the rules of acupoint selection patterns in the treatment of renal edema remain insufficient, which makes it difficult to

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fully reveal the scientific and regular nature of acupoint selection [10]. To address this gap, the present study collected a large body of clinical literature and case data on the acupuncture treatment of renal edema. Using data mining methods, we statistically analyzed acupoint utilization to identify commonly selected acupoints, frequent acupoint combinations, and their curative effects in the treatment of renal edema, with the aim of providing new insight and methods for clinical practice and future research.

## Materials and methods

### *Data mining*

#### *Data preparation*

*Data sources:* A comprehensive literature search was conducted on November 10th, 2024. The following databases were systematically searched: the China Academic Journals Full-text Database (CAJ), the Chinese Sci-tech Journal Database (VIP), the Chinese Academic Journal Database (Wanfang data) and the international medical literature database PubMed. The search period covered publications from January 1st, 1980, to November 10th, 2024. The inclusion criteria primarily consisted of randomized controlled trials (RCTs), cohort studies, and case-control studies that investigated acupuncture or moxibustion in the treatment of renal edema. Eligible studies were required to demonstrate a scientifically sound design, an adequate sample size, reliable measurement methods, and complete and accurate data reporting.

*Retrieval strategy:* The literature search was conducted up to November 10th, 2024. Search terms included disease-related keywords (e.g., renal edema, nephrogenic edema, renal failure-associated edema), intervention-related keywords (e.g., acupuncture, moxibustion, acupoint, acupoint application, warm acupuncture, electroacupuncture), as well as study design descriptors (e.g., clinical observation, clinical efficacy observation, controlled clinical observation). Both subject headings and free-text terms were applied, and Boolean operators were used to construct a comprehensive search expression combining disease names, treatment methods, and research type. An intelligent retrieval function was also employed to enhance both the sensitivity and specificity of the search, ensuring comprehensive coverage of clinical research literature on acupuncture and moxibustion in the treatment of renal edema.

*Literature extraction:* Inclusion criteria: Studies were included if they met the following criteria: (1) Clinical research on acupuncture or moxibustion for renal edema, published in peer-reviewed academic journals or academic thesis repositories. (2) Study populations diagnosed with renal edema according to established definitions in Nephrology. (3) Acupuncture or moxibustion used as the main intervention, including but not limited to filiform needle acupuncture, warm acupuncture, moxa stick moxibustion, barrier moxibustion and acupoint application. (4) Any efficacy evaluation criteria reported in the included literature were accepted to ensure comprehensiveness and objectivity of the study.

Exclusion criteria: Studies were excluded if they met any of the following conditions: (1) Review, lectures, animal experiments, experience summaries, meta-analyses, news reports, case reports, or monographs. (2) Studies without clear information on acupoint prescriptions. (3) Studies in which traditional Chinese medicine or Western medicine was the primary intervention. (4) Duplicate publications, conference abstracts, reviews, or theoretical discussions. (5) Full-text unavailable or studies with unclear or incomplete descriptions of acupuncture prescriptions and acupoints.

*Literature screening:* Initially, the titles of all retrieved articles were reviewed, and studies related to animal experiments, research progress reports, experience summaries, meta-analyses and commentaries were excluded to ensure adherence to the inclusion criteria. Subsequently, the full texts of the remaining documents were assessed in detail against the predefined inclusion and exclusion criteria. Finally, all eligible studies were cross-checked, and expert consultation was sought to ensure the accuracy and rigor of the screening process.

*Literature extraction results:* According to the retrieval strategy, 53 records were identified from CNKI, 693 from the Chinese Sci-tech Journals Database (VIP), 325 from the Chinese

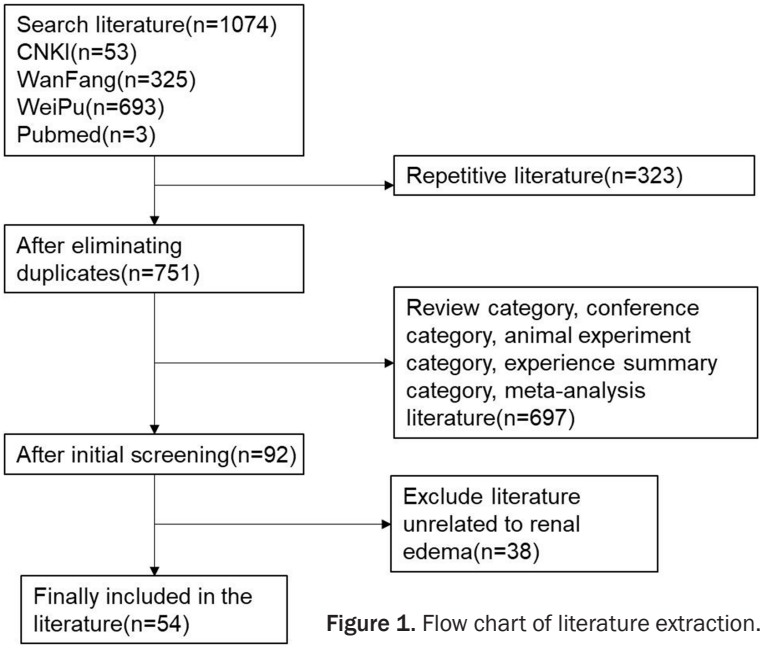


**Figure 1.** Flow chart of literature extraction.

Academic Journals Database (Wanfang data) and 3 from PubMed. After sequential screening of titles and full texts, 92 studies met the eligibility criteria and were included in the final analysis. The detailed screening process is shown in **Figure 1**.

*Data processing*

*Database establishment:* All eligible studies were organized into a literature database using Microsoft Excel. To facilitate management and referencing, each study was assigned a unique research ID in the format "Author + Publication Year". The database included the following information: serial number, research ID, study title, author(s), main acupoints, matching acupoints, intervention measures in the treatment/observation groups, blank group interventions, and related TCM syndromes. On this basis, a dedicated "database of acupuncture and moxibustion prescriptions for renal edema" was established in Microsoft Excel. This database contained detailed information on prescription records, including serial number, research ID and specific acupoint prescriptions. Following the principle of "one prescription = one main acupoint record + one acupoint matching record", a total of 54 prescription records were included.

*Database standardization:* To ensure accuracy and consistency, all extracted data were standardized. The terminology for acupoints and TCM syndromes was unified. Each acupoint name was carefully reviewed and standardized, resolving issues such as synonymous names, different acupoints with the same name, duplicate naming, and ambiguous descriptions. For example, "spleen fat" was standardized to "spleen Shu" and "kidney fat" to "Shenshu". The term "Ashi point", referring to a general category of acupuncture points, was retained without further subdivision.

*Animal experiments*

*Experimental materials*

*Experimental rats:* A total of 32 specific-pathogen-free (SPF) male Sprague-Dawley (SD) rats were purchased from Tianjin BioCisco Biomedical Technology. All rats were housed under controlled environmental conditions (temperature 20-24°C, humidity 60-80%) with free access to food and water.

All animal experiments were approved by the Ethics Committee of Shanghai Sixth People's Hospital (No: 2024-0549). All animals were euthanized using the carbon dioxide inhalation method, which causes death by asphyxiation in mice through excessive inhalation of carbon dioxide. This approach is relatively mild and suitable for the simultaneous handling of multiple mice.

Carbon dioxide inhalation method: (1) Equipment preparation: Utilize a sealed euthanasia chamber or IVC cage, ensuring that the animal density is maintained at an appropriate level. (2) Operating procedures: Place the mice in the chamber and firmly secure the lid. Connect a $CO_2$ tube to the water - bottle inlet, open the gas cylinder valve, and fill the chamber with $CO_2$ at a rate of 10%-30% of the chamber volume per minute. Once the mice lose consciousness and their locomotion stops, increase the gas flow (with a maximum limit of 0.5 KPa). Confirm that the mice show no movement, respiration, and have dilated pupils. Subsequently, terminate the $CO_2$ supply and

observe for an additional two minutes to confirm death.

*Renal edema model:* After adaptive feeding, 8 rats were selected as the blank group, while the remaining 24 rats were used to establish a renal edema model. On days 1 and 8 of the experiment, the model rats received intravenous injection of doxorubicin hydrochloride at doses of 4.0 mg/kg and 3.5 mg/kg, respectively. In addition, intraperitoneal hydrocortisone (3.75 mg/kg) was administered once daily for 14 consecutive days. The modeling criteria included clinical signs such as reduced activity, dull fur, and altered tolerance to cold and heat, as well as significant proteinuria compared to the blank group.

*Experimental groups*

Thirty-two SPF SD rats were divided into four groups (n = 8 per group): control group, model group, sham acupuncture group and acupuncture group. The acupuncture group received treatment based on the core acupoint prescription identified through data mining, so as to evaluate the therapeutic effect of acupuncture on 24-hour urinary protein levels in rats with renal edema. Acupuncture was performed at Shenshu (BL23), Zusanli (ST36) and Sanyinjiao (SP6). Acupoint locations, insertion angles, and needle depth were determined according to the Experimental Acupuncture (February 2014 edition), based on the bone measurement method used in humans. The sham acupuncture group received superficial needling at non-acupoint sites, while model rats without intervention served as the model group. The success of model establishment was further confirmed by evaluating urinary protein levels combined with histopathological examination of renal tissues using hematoxylin-eosin (H&E) staining.

*Detection of 24-hour urinary protein:* Urine was collected from all rats over a 24-hour period at weeks 1, 2, and 4 after model establishment. Samples were centrifuged to obtain the supernatant for subsequent analysis. Urinary protein concentration was determined using a protein assay kit (Bio-Rad Corporation, USA) according to the manufacturer's instructions and calculated based on a standard protein curve.

*Enzyme-linked immunosorbent assay (ELISA):* Blood samples were collected from experimental rats, and serum was obtained by centrifugation. Biochemical indicators, including blood urea nitrogen (BUN), total protein (TP), and albumin (ALB), as well as inflammatory markers, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-10 (IL-10), were measured using ELISA kits (Beyotime Biotechnology; Cat. No. 2024-1208), according to the manufacturer's instructions.

*Electrolyte detection:* Serum concentrations of sodium ($Na^+$), potassium ($K^+$) and chlorine ($Cl^-$) were detected using an automatic biochemical analyzer (Jiangsu Innova Company; DS-810 constant speed 800). Serum samples were loaded via the instrument's automatic sampling system. The analyzer automatically performed sample absorption, reagent mixing and electrolyte concentration determination.

*Western blot analysis:* Blood samples were collected from rats, and serum was separated by centrifugation. Total protein concentration was determined using the Pierce BCA protein assay kit (Beyotime Biotechnology; Cat. No. 2024-0092). Protein samples were mixed with SDS loading buffer, denatured by heating, and separated by SDS-polyacrylamide gel electrophoresis. Proteins were then transferred onto PVDF or nitrocellulose membranes at 100 V for 1-2 hours. The internal reference protein in the Ig-M detection process is GAPDH (Beyotime Biotechnology; Cat. No. 2024-1053). Membranes were blocked with 5% milk or bovine serum albumin solution, followed by incubation with mouse anti-rat Ig-M monoclonal antibody (dilution 1:1000-1:5000; Beyotime Biotechnology; Cat. No. 2024-JU26). After washing, membranes were incubated with horseradish peroxidase-labeled secondary antibody (dilution 1:10,000-1:50,000; Beyotime Biotechnology; Cat. No. 2024-0052) for 1-2 hours. Finally, signal detection was carried out using enhanced chemiluminescence (ECL) reagent (Beyotime Biotechnology; Cat. No. 2024-1257), and signals were captured using photosensitive film or a digital imaging system.

*Immunofluorescence detection:* Rat kidneys were harvested and fixed in 4% formaldehyde for 24 hours. After xylene clearing, tissues were dehydrated and embedded in paraffin. Sections were mounted on glass slides, dewaxed, and hydrated through a series of xylene and ethanol. Antigen retrieval was performed using citric acid buffer, and non-specific binding sites were blocked with 5% bovine serum albumin.

**Table 1.** Statistical analysis of acupoint frequency

| Acupoint | Frequency | Acupoint | Frequency |
|---|---|---|---|
| Shenshu | 38 | Mingmen | 4 |
| Moisture content | 27 | Right collateral vein | 2 |
| Guanyuan | 20 | Left collateral vein | 2 |
| Pishu | 19 | Dazhui | 2 |
| Zusanli | 18 | Yangguan | 2 |
| Shenque | 18 | Yanglingquan | 2 |
| Qihai | 17 | be critically ill | 1 |
| Sanyinjiao | 15 | Xuanshu | 1 |
| Yongquan | 11 | Dachangshu | 1 |
| Shuidao | 11 | philtrum | 1 |
| Yinlingquan | 10 | Hegu | 1 |
| Fuliu | 8 | Tianshu | 1 |
| Sanjiaoshu | 7 | Qugu | 1 |
| Middle pole | 6 | Pangguangshu | 1 |
| Zhiyang | 5 | Taixi | 1 |
| Weiyang | 5 | Ashi point | 1 |
| Feishu | 4 | | |

Sections were incubated overnight at 4°C with the following primary antibodies: mouse anti-rat AQP2 monoclonal antibody (dilution 1:200-1:500; Beyotime Biotechnology; Cat. No. 2024-5687), mouse anti-rat AQP3 monoclonal antibody (dilution 1:200-1:500; Beyotime Biotechnology; Cat. No. 2024-7025), mouse anti-rat RIPK1 monoclonal antibody (dilution 1:200-1:500; Beyotime Biotechnology; Cat. No. 2024-0014), and mouse anti-rat MLKL monoclonal antibody (dilution at 1:200-1:500; Beyotime Biotechnology; Cat. No. 2024-1369). Subsequently, sections were incubated for 1 hour with a fluorescently labeled secondary antibody (TRITC, dilution 1:500; Beyotime Biotechnology; Cat. No. 2024-3618). After nuclear staining with DAPI, slides were mounted using an antifade medium and examined under a fluorescence microscope. Fluorescence intensity of the DPI-PolyP complex was measured under a fluorescence microscope for quantification, which could indirectly analyze the DNA content in the cell nucleus. The analysis involved: (1) Intensity calibration: a standard sample with known fluorescence (e.g., fluorescent microspheres) was used to generate a standard curve to correct for light source attenuation and camera gain; (2) Parameter setting: exposure time, gain, and objective magnification were kept constant to prevent saturation (pixel value reaching 255) or excessive noise (insufficient exposure); (3) Region selection: regions of interest (ROI) were defined and background fluorescence was subtracted (average gray value of the area without sample); (4) Data processing: average gray value or integral intensity (total gray value × pixel number) within the ROI was calculated using software such as Zen or ImageJ, and converted to absolute fluorescence intensity based on the standard curve.

*Selection of statistical tools*

All data were processed using SPSS 20.0 software (IBM, USA). Measured data were expressed as mean ± SD. For data comparison among multiple groups, One-way ANOVA was performed first. If significant differences were found between groups, a *post hoc* test (such as LSD-t test) was then used for pairwise comparisons. The counted data were expressed as percentages (%), with intergroup comparisons conducted using $\chi^2$ test. A *P*-value <0.05 was considered significant.

## Results

*Statistical analysis of acupoint frequency*

A total of 33 acupoints were identified across the included acupuncture prescriptions, with a cumulative application frequency of 263. The five most frequently used acupoints were Shenshu, Guanyuan, Pishu and Zusanli (**Table 1**).

*Statistical analysis of meridian distribution of acupoints*

A total of 19 acupoints were distributed across the twelve meridians, with 5 acupoints on the Renmai, 4 on the Dumai, 2 located on collateral veins, 1 Ashi point, and 7 others. The four most frequently represented meridians were the Bladder Meridian of Foot Taiyang, Ren Meridian, Kidney Meridian of Foot Shaoyin and Du Meridian (**Table 2**).

*Statistical analysis of acupoint distribution*

Among the included acupoints, 13 were located on the back and waist, representing the

**Table 2.** Statistical analysis of meridian distribution of acupoints

| Meridian | Number of acupoints | Total frequency | Acupoints (frequency) |
|---|---|---|---|
| Bladder Meridian of Foot Taiyang | 9 | 34 | Feishu (4), Dachangshu (1), Pangguangshu (1), be critically ill (1), Mingmen (4), Yangguan (2), Weiyang (5), Shuidao (11), Zhiyang (5) |
| Ren Meridian | 5 | 88 | Guanyuan (20), Qihai (17), Shenque (18), Middle pole (6), moisture content (27) |
| Kidney Meridian of Foot-Shaoyin | 4 | 58 | Shenshu (38), Fu Liu (8), Yongquan (11), Taixi(1) |
| Du Meridian | 3 | 4 | Dazhui (2), Xuanshu (1), philtrum (1) |
| Spleen Meridian of Foot Taiyin | 3 | 44 | Pishu (19), Sanyinjiao (15), Yinlingquan (10) |
| Other | 3 | 3 | Tianshu (1), Qugu (1), Ashi point (1) |
| Dai Meridian | 2 | 4 | Right collateral vein (2), Left collateral vein (2) |
| Stomach Meridian of Foot Yangming | 1 | 18 | Zusanli (18) |
| Gallbladder Meridian of Foot Shaoyang | 1 | 2 | Yanglingquan (2) |
| Hand Shaoyang Sanjiao Jing | 1 | 7 | Sanjiaoshu (7) |
| Large Intestine Meridian of Hand-yangming | 1 | 1 | Hegu (1) |

**Table 3.** Statistical analysis of acupoint distribution by body region

| Body region | Number of acupoints | Total frequency | Acupoints (frequency) |
|---|---|---|---|
| Back and waist | 13 | 90 | Feishu (4), Dachangshu (1), Pangguangshu (1), be critically ill (1), Mingmen (4), Yangguan (2), Weiyang (5), Sanjiaoshu (7), Pishu (19), Xuanshu (1), Zhiyang (5), Dazhui (2), Shenshu (38) |
| Lower limbs | 8 | 70 | Shuidao (11), Right collateral vein (2), Left collateral vein (2), Sanyinjiao (15), Yinling Spring (10), Tianshu (1), Zusanli (18), Yongquan (11) |
| Chest | 5 | 88 | Guanyuan (20), Qihai (17), Shenque (18), Middle pole (6), moisture content (27) |
| Upper limbs | 5 | 13 | Fu Liu (8), Taixi (1), Yanglingquan (2), Qugu (1), Hegu (1) |
| Head and face | 1 | 1 | Philtrum (1) |
| other | 1 | 1 | Ashi point (1) |

highest cumulative frequency (n = 90). This was followed by acupoints on the lower limbs (n = 70), chest (n = 88), and upper limbs (n = 13) (**Table 3**).

*Analysis of the application of specific acupoints*

The cumulative frequency of specific acupoint usage across the included prescriptions was 263. According to the frequency, the acupoints were classified and ranked as Intersection Point, Beishu Point, Five Shu Points, Mu Point, Other Point, Xiahe Point, Bahui Point and Yuanxue Point in turn (**Table 4**).

*Analysis of acupoint association rules*

A total of 20 strong association rules among acupoints were identified through association rule analysis. These comprised 3 two-point combinations and 17 three-point combinations (**Table 5**).

*Cluster analysis of high frequency acupoints*

Cluster analysis indicated that when the intergroup distance ranged from 20 to 80, the acupoints could be categorized into two main clusters. The first cluster included Yinlingquan, Yongquan, Guanyuan, Pishu, Zusanli, Shenque, and Moisture. The second cluster comprised Sanyinjiao, Qihai, Shenshu, Shuidao, Fuliu, Sanjiaoshu, Zhongji, Zhiyang, Weiyang, and Feishu (**Figure 2**).

*24 h urinary protein concentration*

During the first week after modeling, the 24-hour urinary protein levels in the model group and the sham acupuncture group were significantly higher than those in the control group ($P<0.01$). By the second and fourth weeks, the 24-hour urinary protein concentration in the acupuncture group was significantly lower than that of the model group ($P<0.01$) (**Figure 3**).

**Table 4.** Application analysis of specific acupoints

| Specific acupoint category | Number of acupoints | Total frequency | Acupoints (frequency) |
|---|---|---|---|
| Crossing point | 12 | 107 | Sanyinjiao (15), Guanyuan (20), Middle pole (6), Qugu (1), Dazhui (2), philtrum (1), Shenque (18), moisture content (27), Shuidao (11), Right collateral vein (2), Left collateral vein (2), Yangguan (2) |
| Back-shu point | 7 | 71 | Shenshu (38), Pishu (19), Sanjiaoshu (7), Feishu (4), Dachangshu (1), Pangguangshu (1), be critically ill (1) |
| Five shu points | 6 | 50 | Zusanli (18), Yinlingquan (10), Yongquan (11), Taixi (1), Fu Liu (8), Yanglingquan (2) |
| Front-mu point | 2 | 18 | Tianshu (1), Qihai (17) |
| Other | 3 | 7 | Xuanshu (1), Zhiyang (5), Ashi point (1) |
| Lower He-sea point | 1 | 5 | Weiyang (5) |
| The Eight Strategic Nerve Points | 1 | 4 | Mingmen (4) |
| Source point | 1 | 1 | Hegu (1) |

**Table 5.** Association rules of acupoints (Support ≥10%)

| Preceding paragraph | Consequent | Example | Support/% | Confidence (%) |
|---|---|---|---|---|
| Guanyuan, Qihai | Shenshu | 5 | 21.73 | 96.9 |
| Moisture content, Shenshu | Shuidao | 6 | 22.39 | 86.94 |
| Sanyinjiao, Zusanli | Fu Liu | 10 | 20.73 | 92.24 |
| Shenque | Sanjiaoshu | 8 | 22.3 | 98.7 |
| Taichong, Taixi | Qihai | 11 | 20.87 | 87.57 |
| Qihai, Hegu | Danzhong | 11 | 18.26 | 99.14 |
| Sanjiaoshu, Fu Liu | Shenque | 5 | 13.76 | 96.65 |
| Sanyinjiao | Zusanli | 9 | 22.02 | 89.75 |
| Pishu, Yinlingquan | Shenshu | 7 | 20.75 | 96.25 |
| Weiyang, Zhiyang | Taixi | 9 | 22.59 | 88.48 |
| Fenglong, Tianshu | Fu Liu | 14 | 17.51 | 87.92 |
| Zusanli, Qihai | Sanyinjiao | 5 | 18.23 | 88 |
| Shuidao, Mingmen | Guanyuan | 14 | 15.38 | 82.82 |
| Shenque, Qihai | Zusanli | 6 | 15.06 | 83.79 |
| Yanglingquan, Danzhong | Fenglong | 12 | 10.39 | 92.96 |
| Moisture content | Mingme | 5 | 22.88 | 80.01 |
| Sanjiaoshu, Taixi | Shuidao | 15 | 19.17 | 81.33 |
| Guanyuan, Mingme | Taichong | 8 | 13.18 | 84.33 |
| Fu Liu, Taichong | Tianshu | 7 | 20.75 | 98.38 |
| Tianshu, Pishu | Yanglingquan | 11 | 12.18 | 93.53 |

*Assessment of renal function*

The levels of BUN in model group and sham acupuncture group were higher than those in blank group ($P$<0.01), while the levels of TP and ALB in the model group and sham acupuncture group were lower than those in control group ($P$<0.01). However, the levels of BUN in acupuncture group were lower than those in model group ($P$<0.01) and TP and ALB were higher (**Figure 4**).

*Electrolyte detection*

Compared to controls, the $Na^+$ level in the model group was significantly decreased ($P$< 0.05). The $CL^+$ levels in the other three groups were significantly higher than those in the control group. The trend of $K^+$ level changes remains unclear, but there were also significant differences among the three groups compared to the control group ($P$<0.05). The serum $Na^+$ level of rats in the acupuncture group was lower

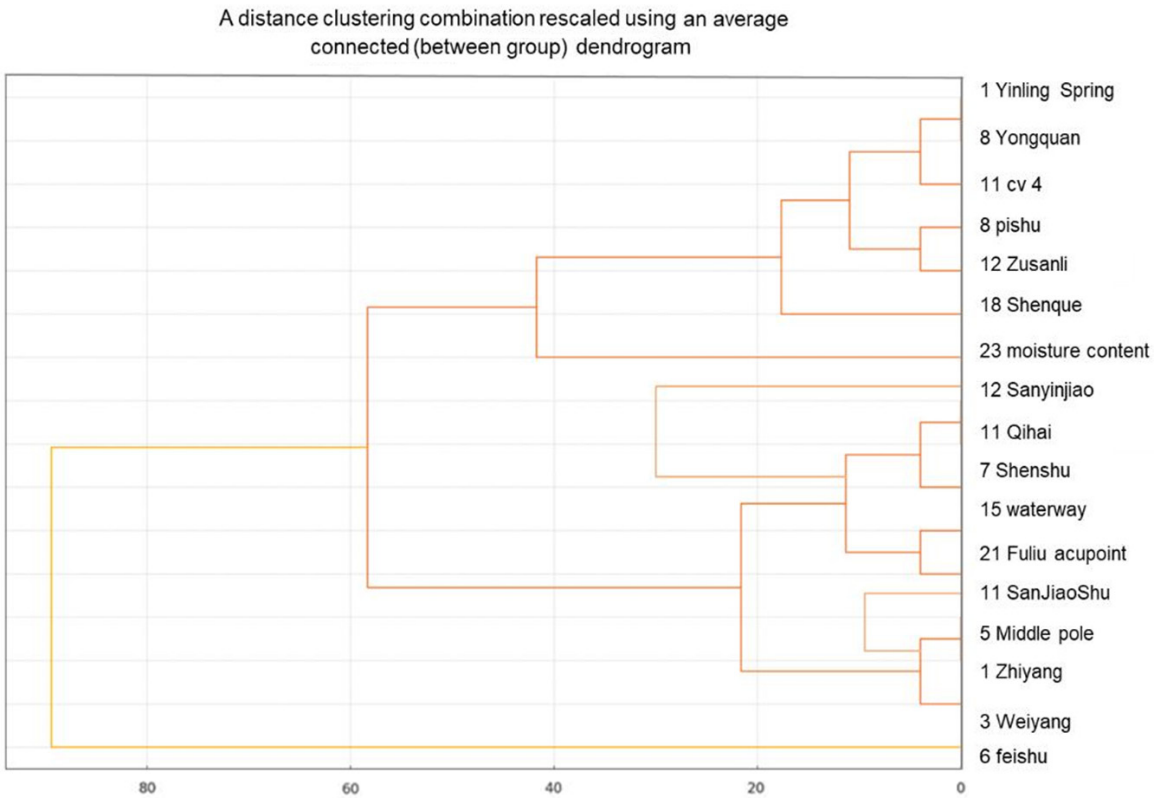


**Figure 2.** Dendrogram of high-frequency acupoint cluster analysis.

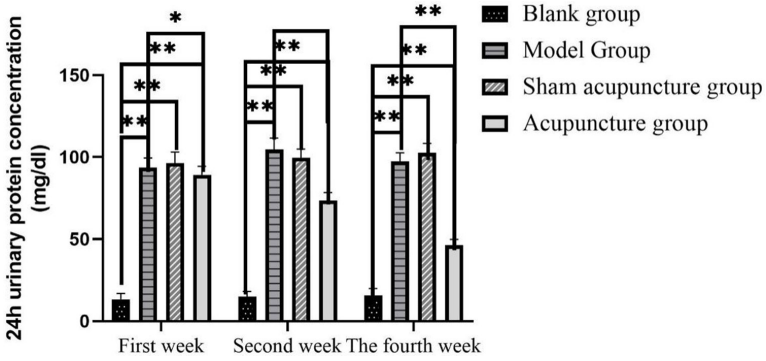


**Figure 3.** 24 h urinary protein concentration in rats. Note: ***P*<0.01.

than that in the model group but higher than that in the control group (*P*<0.05). The levels of serum $K^+$ and $CL^+$ in the acupuncture group were higher than those in the model group, but lower than those in the blank group and the blank control group (*P*<0.05) (**Figure 5**).

*Analysis of serum inflammatory factors*

Serum levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the model group were significantly higher than those in the control group (*P*<0.01), whereas interleukin-10 (IL-10) was significantly lower (*P*<0.05). In the acupuncture group, TNF-α and IL-1β levels were significantly reduced compared with the model group (*P*<0.01), while IL-10 levels were significantly increased (*P*<0.05) (**Figure 6**).

*Detection of immune function in rats*

The expression levels of Ig-M and Ig-G proteins in the model group were significantly higher than those in the control group (*P*<0.05), while acupuncture treatment significantly reduced Ig-M and Ig-G expression compared with the model group (*P*<0.05) (**Figure 7**).

*Expression analysis of AQP2 and AQP3 in the kidney*

The protein expression levels of AQP2 and AQP3 in the model group were significantly

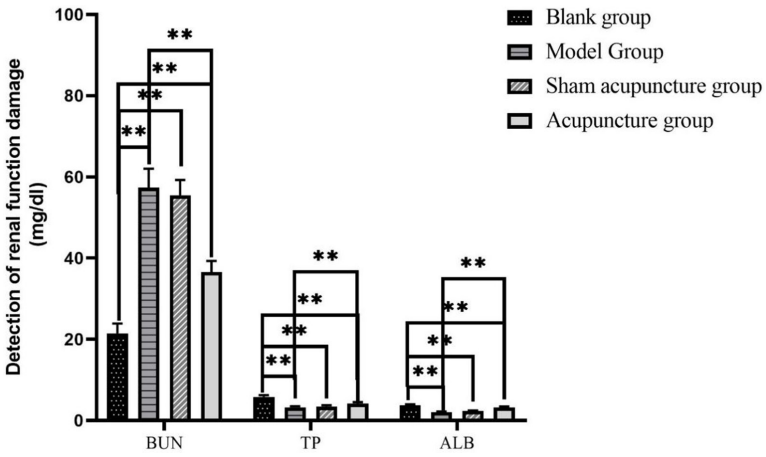


**Figure 4.** Detection of renal function damage (mg/dl). Note: ***P*<0.01.

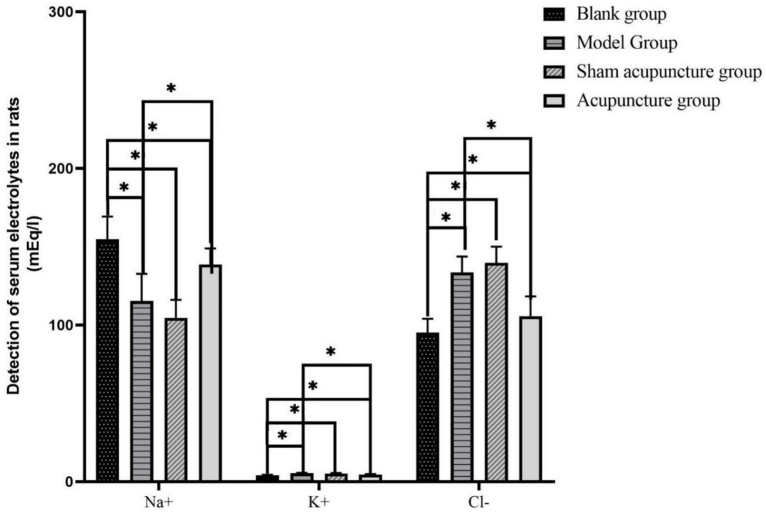


**Figure 5.** Serum electrolyte levels in rats (mEq/l). Note: **P*<0.05.

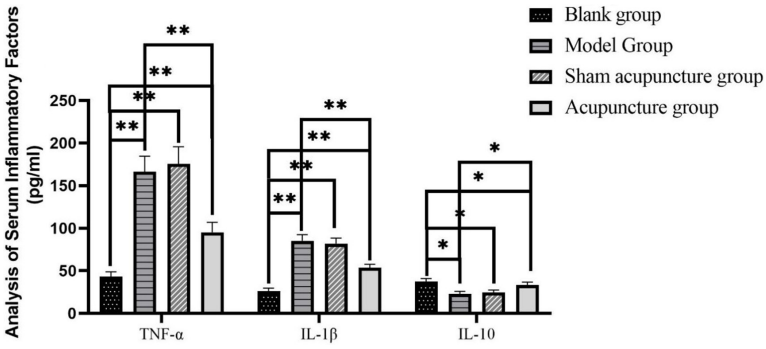


**Figure 6.** Serum inflammatory factor levels (pg/ml). Note: **P*<0.05; ***P*<0.01.

lower than those in the control group (*P*<0.05). In contrast, acupuncture treatment significantly up-regulated the expression of these aquaporins relative to the model group (*P*<0.05) (**Figure 8**).

## Discussion

Renal edema falls under the categories of "edema" and "water-gas disease" in traditional Chinese medicine, representing a syndrome of whole-body or localized fluid accumulation caused by abnormal water and liquid metabolism [11]. Shenshu, as the back Shu point of the kidney, is located on the waist and is considered a primary choice for treating renal edema. Acupuncture at the water point can directly affect water and liquid metabolism, its high application frequency further highlights its significance in managing renal edema. The Guanyuan point is frequently employed to warm and tonify the lower jiao yang qi, thereby promoting the metabolism and excretion of water and fluid. Similarly, Pishu point can enhance the spleen's transport function, reducing water and damp retention in the body, which contributes to alleviating edema symptoms. Zusanli point, another commonly used acupoint, supports the function of the spleen and stomach, promotes qi and blood generation and circulation, and provides sufficient nutrition and energy to facilitate edema regression.

In this study of acupuncture treatment for renal edema, the meridian affiliations of the selected acupoints were analyzed in depth. In this research on acupuncture and moxibustion treatment for renal edema, the use of specific points was analyzed [12]. The total frequency of specific point usage was 263 times, with the ranking in descending order as follows: Intersection

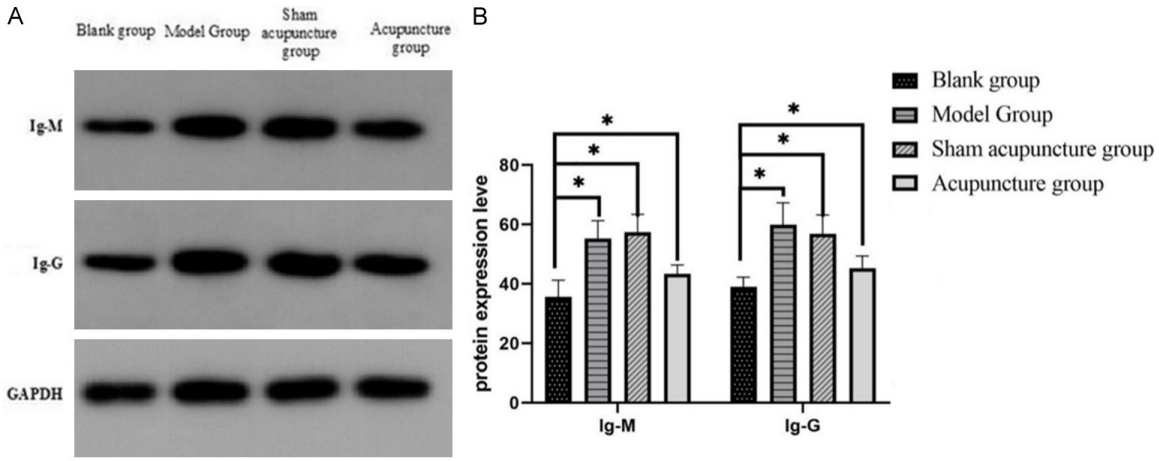


**Figure 7.** Western blot analysis of Ig-M and Ig-G expression. A. Western blotting band diagram of Ig-M and Ig-G expressed proteins; B. Bar chart of western blot analysis of Ig-M and Ig-G expressed proteins. Note: *P<0.05.

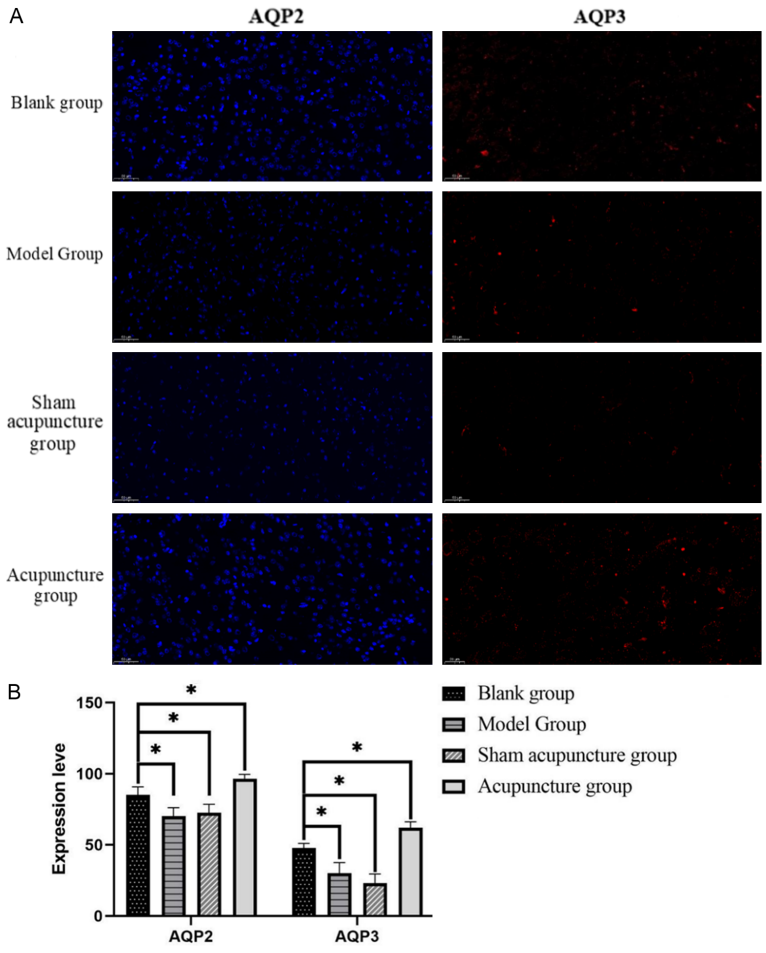


**Figure 8.** Detection of AQP2 and AQP3 expression. A. Fluorescence images of AQP2 and AQP3 expression; B. Quantified bar chart of AQP2 and AQP3 expression. Note: *P<0.05.

Point, Beishu Point, Five Shu Point, Mu Point, Other Point, Xiahe Point, Bahui Point and Original Point. Intersection Point, where two or more meridians meet, plays a key role in coordinating and integrating multiple meridians [13, 14]. In the treatment of renal edema, the Intersection Points were the most frequently used, reflecting their multi-channel connectivity. For example, Lieque, an intersection point of the Lung and Ren Meridians, can promote lung function and qi circulation, open water channels, and facilitate edema resolution. The use of the intersection point embodies the holistic principle of acupuncture and enhances the synergistic therapeutic effect [15]. Beishu points act directly on the viscera and regulate their function, while Five Shu points are primarily involved in regulating qi and blood and dredging meridians [16].

In this study, association rule analysis and cluster analysis were conducted, yielding 20 strong association rules for acupoints, including 3 two-point combinations and 17 three-point combinations. Cluster analysis of 24 acupoints with a usage frequency ≥5 showed that, at a grouping distance of 20-80, the acupoints

could be divided into two cluster groups. For two-point combinations, Shenque-Sanjiaoshu synergistically acts on the body's water-liquid metabolic system, enhancing renal gasification and promoting edema resolution. Sanyinjiao-Zusanli combination regulates qi and blood while invigorating the spleen and qi, producing a cooperative effect in treating renal edema. Water-Mingmen together act on the kidney, promoting its gasification function and accelerating edema dissipation. The combination of Shenque-Sanjiaoshu, Sanyinjiao-Zusanli and Shuiwu-Mingmen embodies the synergistic effect of acupoints in the theory of traditional Chinese medicine. Among the three combinations, Guanyuan-Qihai-Shenshu can comprehensively regulate renal function and water-liquid metabolism, achieving effective treatment of renal edema. The combination of water-Shenshu-waterway acts on the kidney and water-liquid metabolic system to promote edema regression. The Taichong and Taixi-Qihai combination can harmonize qi and blood, soothe the liver, regulate qi, and nourish the kidneys, contributing to synergistic treatment. The combination of Sanjiaoshu-Fuliu-Shenque can comprehensively regulate the body's water-liquid metabolism, promoting the dissipation of edema. Other combinations, such as Guanyuan-Qihai-Shenshu, Shuishui-Shenshu-Shuidao, Taichong, Taixi-Qihai, Sanjiaoshu-Fuliu-Shenque, further enhance the synergistic effects of acupoints, providing a more comprehensive therapeutic strategy for renal edema. Cluster analysis showed that, at a grouping distance of 20-80, acupoints can be divided into two cluster groups. The first cluster group - comprising Yinlingquan, Yongquan, Guanyuan, Pishu, Zusanli, Shenque, and Shuishui - primarily functions to induce diuresis, reduce swelling, warm yang, benefit qi, and invigorat the spleen, directly addressing the pathogenesis of renal edema. The second cluster, including Sanyinjiao, Qihai, Shenshu, Shuidao, Fuliu, Sanjiaoshu, Zhongji, Zhiyang, Weiyang, and Feishu, is primarily associated with harmonizing qi and blood circulation, preserving yang qi, dispersing lung qi to facilitate its flow, and regulating systemic functions, thereby indirectly promoting edema regression.

This study elucidates the principles underlying acupoint selection in the acupuncture treatment of renal edema. Shenshu, Pishu and other back-shu points, as specific acupoints of the back, directly act on the kidney and spleen, promoting the restoration of water-liquid metabolism and transport, thereby addressing renal edema at its source. Five Shu acupoints, such as Zusanli and Yinlingquan, promote edema regression by regulating qi and blood and dredging meridians. Acupoints such as Guanyuan and Shenque can harmonize the functions of zang-fu organs, further consolidating the therapeutic effects of acupuncture on renal edema. Data mining results indicate that acupoints act synergistically, forming the foundational therapeutic strategy for treating renal edema [17]. Association rule analysis identified several highly effective acupoint combinations, including Shenque-Sanjiaoshu, Sanyinjiao-Zusanli and Guanyuan-Qihai-Shenshu, Shuishui-Shenshu-Shuidao. For patients with severe edema and impaired spleen and kidney function, acupoints in the first cluster - such as Yinlingquan, Yongquan and Guanyuan - may be prioritized for key treatment. Conversely, for patients with mild edema and qi-blood disharmony, acupoints in the second cluster - such as Sanyinjiao, Qihai and Shenshu - can be selected to provide adjuvant treatment [18].

Acupuncture has been shown to improve renal blood supply, increasing both blood flow and flow velocity, thereby improving oxygen and nutrient supply to the kidney [19, 20]. Additionally, acupuncture modulates immune function, leading to a reduction in the production of inflammatory mediators, which are usually increased in nephropathy models and aggravate kidney damage [20-22]. By attenuating renal inflammation, acupuncture helps protect the kidney from further damage. In the model of renal edema, the expression of AQP2 and AQP3 is often inhibited, impairing water reabsorption and promoting the formation of edema [23, 24]. Acupuncture treatment has been demonstrated to upregulate the expression of these aquaporins, suggesting a mechanism by which it enhances renal water reabsorption capacity by promoting the expression of AQP2 and AQP3, thus effectively alleviating edema symptoms. This protective effect may be related to the anti-inflammatory and antioxidative effects of acupuncture, which help mitigate local inflammation and reduce cellular stress responses [25]. The causes of renal edema are complex and are often related to water and sodium retention and hypoproteinemia resulting from reduced renal function. Elevated SCr

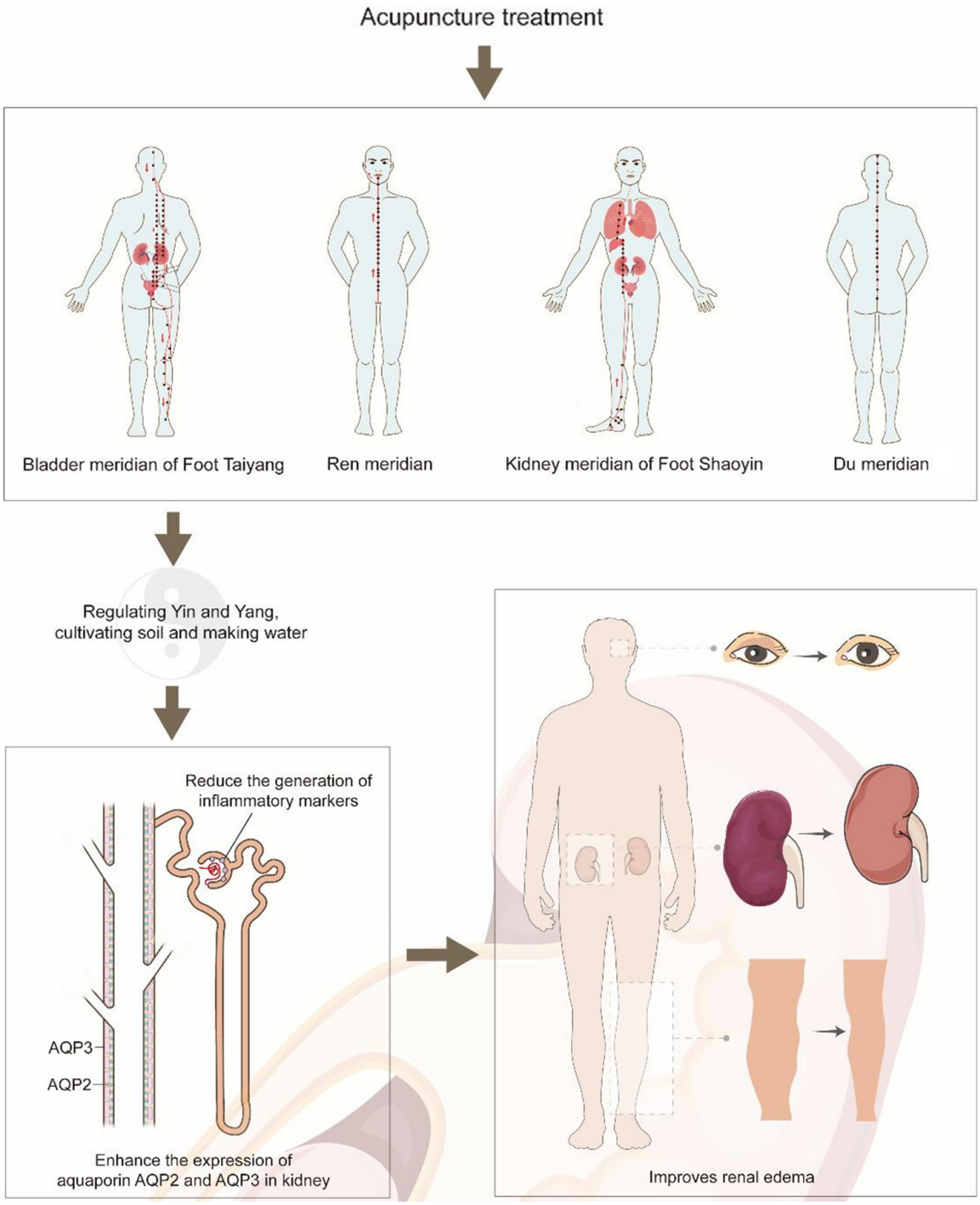


**Figure 9.** Schematic diagram of the proposed mechanism of acupuncture in treating renal edema.

levels may indicate impaired renal function, which in turn affects the pathologic process of edema. The lack of SCr data in the study may make it difficult to deeply analyze whether acupuncture treatment alleviates edema by improving renal function (such as enhancing glomerular filtration), thus limiting the depth of mechanism explanation.

The mechanisms through which inflammation suppression affects AQP expression are multifaceted. Under inflammatory conditions, pro-inflammatory factors can inhibit AQP gene transcription and protein synthesis. When inflammation is suppressed, these inhibitory signals weaken, intracellular signaling pathways achieve relative balance, and the normal expression

of AQP genes is restored, promoting increased protein synthesis. Upregulation of AQP is crucial for the relief of edema, as it enhances the function of water channels and accelerates transmembrane transport. This promotes efficient reabsorption of excess interstitial water into the bloodstream, reduces tissue fluid osmotic pressure, and diminishes fluid accumulation in the interstitial spaces, thereby effectively alleviating edema and maintaining systemic fluid balance. When we speculate that inflammatory factor expression is reduced, an upregulation of AQP can be observed, accompanied by improvement in edema. Conversely, increasing inflammatory factors leads to decreased AQP expression and worsened edema. These observations indicate a mechanistic chain of "inflammation suppression → AQP upregulation → edema relief" in the body.

However, the number of experimental animals in this study was relatively small, which may introduce some bias into the results. Further research with larger sample sizes and deeper mechanistic exploration will be necessary to validate and extend these findings.

To sum up, this study systematically reveals the application patterns of acupuncture and moxibustion in treating renal edema. The results indicate that acupuncture and moxibustion can alleviate renal edema, enhance the expression of renal aquaporin, reduce urine protein and blood urea nitrogen levels, and suppress the generation of inflammatory markers, thus effectively promoting the recovery of renal function. These findings highlight the clinical value of acupuncture and moxibustion in treating renal edema and are consistent with similar studies (**Figure 9**).

## Disclosure of conflict of interest

None.

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