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# Electroacupuncture ameliorates diabetic renal injury by regulating the AMPK-autophagy signaling pathway

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**ABSTRACT**

***Objective(s):*** Diabetic nephropathy (DN) is a common complication of diabetes mellitus (DM) and the leading cause of end-stage renal disease worldwide. Despite evidence supporting the renoprotective effects of electroacupuncture (EA), the underpinning molecular mechanisms require further investigation. This study was designed to evaluate the therapeutic efficacy of EA in mice with DN and to elucidate its potential mechanisms of action.

***Materials and Methods:*** A DN model was established in male C57BL/6 mice by streptozotocin (STZ) injection and validated. Mice were randomly allocated into four groups: control, normal+electroacupuncture (EA), DN, and DN+EA. EA was applied at Yanglingquan and Zusanli acupoints in the treatment groups. Renal histology and ultrastructure were examined. AMPK and downstream autophagy-related molecule expression were analyzed by qRT-PCR and Western blot.

***Results:*** Renal tissues from DN mice exhibited suppressed autophagy, marked by decreased microtubule-associated protein 1 light chain 3-II (LC3-II) and increased Sequestosome-1 (p62) expression, along with reduced phosphorylation of AMP-activated protein kinase (AMPK) and Unc-51 Like Autophagy Activating Kinase 1(ULK1) and increased Phosphorylated mammalian target of rapamycin (p-mTOR) levels after 8 weeks, indicating impaired autophagy and renal injury. EA treatment reversed these alterations by enhancing AMPK and ULK1 phosphorylation, restoring autophagy, and mitigating renal damage.

***Conclusion:*** The AMPK signaling pathway represents a novel therapeutic target for DN. Our data indicate that EA facilitates renal protection by activating AMPK/ULK1-mediated autophagy, implicating EA as a viable strategy for targeting this pathway.

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## Introduction

Diabetes mellitus (DM) is one of the common epidemics in the world and is an important public health issue (1). Diabetic nephropathy (DN) is a prevalent complication of DM and is the leading cause of end-stage kidney disease worldwide (2, 3). Recent publications suggest that endoplasmic reticulum stress, hypoxia, and oxidative stress from reactive oxygen species (ROS) are the main contributors to DN (4-7). Therefore, further investigation is needed to clarify various aspects. Studying the development of DN is crucial for identifying potential new treatment targets (8-10).

Autophagy is an intracellular degradation process crucial for the cellular stress response (11, 12). Under normal conditions, cells maintain a low basal level of autophagic activity, primarily to recycle cellular components and ensure proper organ function, including that of the kidneys (13). Autophagy has been observed to protect the kidneys during acute kidney injury and aging (14, 15). However, the autophagic function of renal cells was compromised in patients with DN, making them more susceptible to damage (16, 17). Therefore, regulating autophagy might be a new strategy to reduce diabetic renal injury. Microtubule-associated protein 1 light chain 3 (LC3/Atg8) serves as a protein marker on autophagosome membranes and is crucial for their formation (18). Sequestosome-1 (p62) localizes to autophagosomes and is degraded via the phagosome-lysosome system. Reduced autophagy leads to decreased p62 degradation, making intracellular p62 levels indicative of cellular autophagic activity (19).

The signaling pathways regulating energy metabolism, such as mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK), also participate in DN (20), and AMPK and mammalian target of rapamycin complex 1 (mTORC1) can both induce autophagy through unc-51 like autophagy activating kinase 1 (ULK1)(21). Although mTORC1 inhibition can activate autophagy, excessive inhibition of mTORC1 leads to podocyte dysfunction (22), and the use of mTORC1 inhibitors remains controversial in the treatment of DN. AMPK is another kinase that regulates

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autophagic activity (23, 24). AMPK monitors intracellular energy status by sensing the AMP/ATP ratio (25). AMPK also induces autophagy by inhibiting mTORC1. AMPK is activated in the absence of energy and is inhibited in DN (26). Therefore, AMPK is not only an upstream energy sensor but also a downstream autophagy activator, and it might be closely related to the stability of autophagy in diabetic kidneys. The activation of AMPK might be a therapeutic target for restoring autophagy.

Electroacupuncture (EA), a modern adaptation of traditional acupuncture (a component of ancient Chinese medicine), is used globally to treat various illnesses (27, 28). EA is a relatively safe, nonpharmacological treatment (29). Research has shown that Acu-LFES, a form of low-frequency electrical stimulation, may reduce muscle atrophy resulting from chronic kidney disease (CKD)(30). Previous studies have indicated that EA slowed kidney disease progression in rats with 5/6 nephrectomy-induced kidney disease and in cases of toxin-induced acute kidney injury (31, 32). Nevertheless, the underlying molecular mechanisms of EA in DN have received limited in-depth research. Hence, this research aimed to investigate the effectiveness of EA in a DN mouse model and assess its possible underlying mechanisms, particularly focusing on autophagy. The results could provide innovative methods for the treatment of DN.

## Materials and Methods

### *Experimental animal models*

Male SPF C57BL/6 mice (6-8 weeks old, body weight 16-25 g) were sourced from Shanghai Slake Laboratory Animal Co., Ltd. and housed in the SPF facility at the Medical College of Lishui University, China. After one week of adaptive feeding, the mice were divided into four groups: normal control, normal+electroacupuncture (EA), diabetic nephropathy (DN), and DN+EA. During the experiment, an air conditioner malfunction caused an unexpected drop in room temperature (approximately 10-14 ˚C), resulting in cold stress. Some mice in the DN and DN+EA groups died due to this cold stress. After adjustment, each group ultimately comprised six mice. This incident was promptly reported to the Animal Ethics Committee, which conducted an onsite inspection and orally approved continuation of the experiment with the remaining animals, without requiring euthanasia.

The diabetic mouse model was induced by intraperitoneal injection of streptozotocin (180 mg/kg) dissolved in citrate buffer. Peripheral blood samples were collected from the tail on days 3, 4, 5, and 6 postinjection. From day 3 after STZ injection, the diabetes model was considered successfully established if fasting blood glucose levels consistently reached ≥16.7 mmol/l at three consecutive time points. Control group mice received an equivalent volume of citrate buffer. The Animal Care and Use Committee and the Ethics Committee of Lishui University Medical College approved the experimental procedure.

### *EA*

The mice in the normal+EA and DN+EA groups received EA treatment. Briefly, the mice were kept prone during EA. Yanglingquan and Zusanli acupuncture points were selected based on the WHO standard guidelines for acupuncture point locations. Disposable sterile acupuncture needles (0.25mm diameter) from Wujiang China Medical Hygienic Materials Co, Ltd. were connected to an SDZ-II electronic acupuncture device. The device delivered a constant pulse at 20 Hz and 1 mA for 15 min daily over 8 weeks.

### *Sample collection*

The mice were euthanized 8 weeks following the induction of the diabetes model (on the 3rd day post-STZ injection). The blood was taken from the eyeballs. The kidneys were removed and weighed. A part was immersed in 4% polyoxymethylene and prepared for Immunohistochemistry. A 1-4 mm section of renal cortex was fixed in 2.5% glutaraldehyde for transmission electron microscopy. The remaining renal tissue was sectioned, flash-frozen in liquid nitrogen, and prepared for western blot and qRT-PCR analyses.

### *Determination of urine protein/creatinine ratio*

Before sacrifice, the 8-hour urine was collected. Mice were kept in metabolic cages and fasted, with free access to water. The urine protein/creatinine ratio was determined according to the manufacturer's instructions (Beckman Coulter, USA). Blood creatinine was determined by an enzymatic method (Beckman Coulter, USA). Urine protein was determined by immunoturbidimetry (Beckman Coulter, USA).

### *Blood biochemical test*

Blood samples were centrifuged at 3000 rpm for 10 min post-coagulation to collect serum. BUN, SCR, GLU, and ALB levels were assessed using Beckman Coulter's commercial methods following the manufacturer's guidelines.

### *Histology*

After a 24-hour immersion in 4% polyoxymethylene solution, renal tissues were processed by dehydration, decolorization, paraffin embedding, dewaxing, and periodic acid-Schiff (PAS) staining. Pathological changes were examined under a light microscope (Olympus, Tokyo, Japan).

### *Immunohistochemistry*

Renal tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. After baking on a slide dryer, sections were deparaffinized, subjected to antigen retrieval, and treated with hydrogen peroxide to block endogenous peroxidase activity. Sections were then blocked with goat serum. Primary antibodies against p62 (1:100; 18420-1-AP; Proteintech), LC3 (1:100; 18725-1-AP; Proteintech), p-mTOR (1:100; 67778-1-Ig; Proteintech), and p-ULK1 (1:200; 80218-1-RR; Proteintech) were applied and incubated overnight at 4 ˚C. After washing with PBS (3×5 min), sections were incubated with HRP-conjugated secondary antibodies (1:500) at room temperature for 1.5 hr. Antigen-antibody complexes were visualized using the diaminobenzidine (DAB) substrate, and sections were counterstained with hematoxylin. Finally, sections were dehydrated, cleared, and mounted for microscopic examination.

### *Transmission electron microscopy*

Renal tissue specimens (1 mm3) were fixed in 2.5% glutaraldehyde, dehydrated, embedded, and cured. Ultra-

thin sections were obtained and double-stained with uranyl acetate and lead citrate. The ultrastructure was observed under transmission electron microscopy (Olympus, Tokyo, Japan). The renal glomerular basement membrane thickness was measured.

### *Real-time PCR*

Kidney samples were processed to extract total RNA using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and then converted to cDNA by reverse transcription according to the manufacturer's instructions. RT-PCR quantified target gene mRNA levels under the following conditions: an initial denaturation at 95 ˚C for 10 min, 40 cycles of 95 ˚C for 30 sec and 60 ˚C for 1 minute, followed by a final extension at 72 ˚C for 10 min. Using β-actin as the internal control, the relative expression levels of the target genes were calculated using the 2-△△Ct method, with primers detailed in Table 1.

### *Western blot*

Renal tissue proteins were isolated with the RIPA lysis buffer from Beyotime Institute of Biotechnology in Haimen, China. Protein levels were measured using the Bradford method, followed by separation of equal amounts of protein by SDS-PAGE and transfer to a PVDF membrane. Non-specific signals were inhibited using 5% BSA at room temperature for 2 hours. Antibodies against GAPDH, LC3, p62, AMPKα/P-AMPKα, mTOR/p-mTOR, and ULK1/p-ULK1 were diluted 1:1000 and incubated overnight at 4 ˚C. The AMPKα/P-AMPKα antibody was obtained from Cell Signaling Technology (CST), Danvers, Massachusetts, USA, while all other antibodies were from Abcam, Cambridge, United Kingdom. Secondary antibodies (1:5000) were incubated at room temperature for 2 hours, and protein bands were visualized using electrochemiluminescence (ECL) at Beyotime Institute of Biotechnology, Haimen, China. The grayscale values of the protein bands were analyzed using Adobe Photoshop CS5 (San Jose, California, USA). Internal reference GAPDH was utilized. The results were expressed as the grayscale ratio of the target protein to the internal reference.

### *Statistical analysis*

Statistical analysis was conducted using SPSS version 6.0 (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test was utilized to assess normality. Information following a standard distribution was presented as means with standard deviations and assessed through one-way ANOVA and the Bonferroni *post hoc* test. Statistically significant was defined as $P$-values<0.05.

## Results

### *Characteristics of the mice*

After eight weeks of experimental observation, the DN model mice exhibited typical pathological features of diabetic nephropathy. Compared with the normal control group, mice in the DN group showed significantly increased water intake and urine output, with obvious polydipsia and polyuria; concurrently, their body weight decreased significantly, reflecting systemic metabolic disturbances in disease. Further analysis revealed that renal function indicators, BUN, SCR, blood glucose levels, serum albumin, and the urinary protein/creatinine ratio were all significantly elevated in the DN group, suggesting impaired glomerular filtration function and the persistence of a hyperglycemic state.

Following the EA intervention, these abnormalities improved markedly. Compared with the DN group, mice in the EA intervention group exhibited significantly reduced water intake and urination, along with a substantial recovery in body weight, indicating improved overall metabolic status (Figure 1A-C). More importantly, EA treatment significantly decreased serum creatinine, blood urea nitrogen, blood glucose, and serum albumin levels in DN mice, and the urinary protein/creatinine ratio was also significantly reduced, suggesting effective amelioration of renal function and attenuation of glomerular injury (Figure 1D-H).

In conclusion, EA intervention demonstrated positive effects in ameliorating DN-related metabolic disorders, effectively alleviating symptoms of polydipsia and polyuria, promoting body weight recovery, lowering blood glucose levels, and exerting significant protective effects against renal function impairment. These findings suggest that EA may serve as a potential intervention to delay the progression of diabetic nephropathy.

### *EA alleviates pathological changes in the renal tissues of DN mice*

PAS staining revealed glomerular hypertrophy, mesangial

**Table 1.** Primers used for RT-PCR

| Genes | Primer sequence (5'-3') |
|---|---|
| Microtubule-associated protein 1 light chain 3 alpha-forward (LC3A-F) | CAC CCC AGT AAG ATC CCG GT |
| Microtubule-associated protein 1 light chain 3 alpha-reverse (LC3A-R) | ATG TCA GCG ATG GGT GTG G |
| Microtubule-associated protein 1 light chain 3 beta-forward (LC3B-F) | TAA TCA GAC GGC GCT TGC AG |
| Microtubule-associated protein 1 light chain 3 beta-reverse (LC3B-R) | CGT ACA CTT CGG AGA TGG GAG |
| beta-actin-forward (β-actin-F) | GCT CCG GCA TGT GCA AAG |
| beta-actin-reverse (β-actin-R) | CCT TCT GAC CCA TTC CCA CC |
| Protein kinase AMP-activated catalytic subunit alpha 1-forward (PRKAA1-F) | ACT GTA GTG AAT CGT GTT GC |
| Protein kinase AMP-activated catalytic subunit alpha 1-reverse (PRKAA1-R) | CAA ACA GTA GTT GTA GGA GCT AAG |
| Unc-51 Like Autophagy Activating Kinase 1-forward (ULK1-F) | CGC CGA GCA AAC CCT AGT AA |
| Unc-51 Like Autophagy Activating Kinase 1-reverse (ULK1-R) | TCC ATA GGT CAG CCT TCC CA |

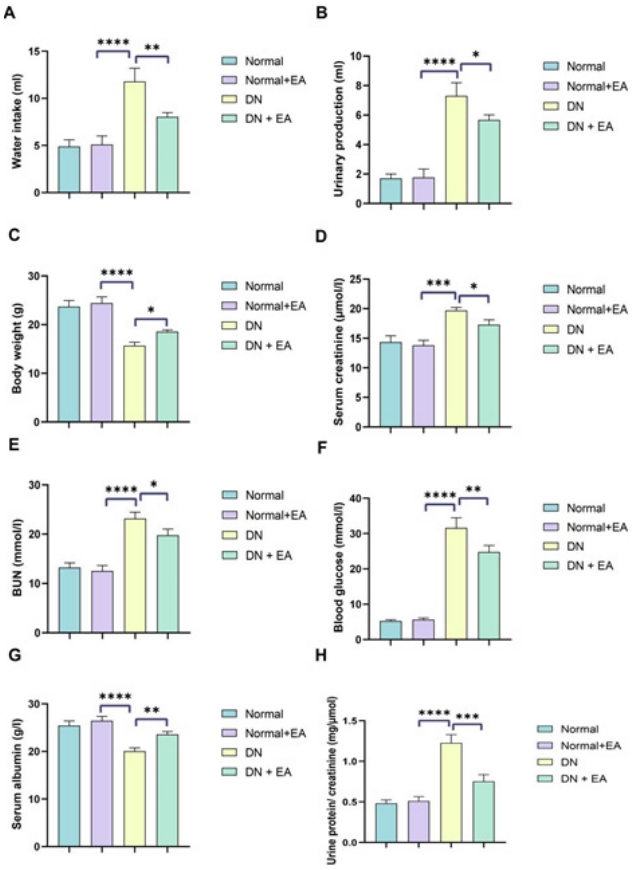


**Figure 1.** Electroacupuncture (EA) ameliorated renal function in mice
(A) Water intake in mice (n=6 per group). (B) Urine volume in mice(n=6 per group). (C) Body weight of mice (n=6 per group). (D) Serum creatinine level in mice(n=6 per group). (E) Blood urea nitrogen (BUN) level in mice (n=6 per group). (F) Blood glucose concentration in mice (n=6 per group). (G) Serum albumin level in mice (n=6 per group).(H) Urinary protein/creatinine ratio in mice(n=6 per group).Values are mean±SD; $^{*}P<0.05$, $^{**}P<0.01$. Data were analyzed using one-way analysis of variance (ANOVA)

cell proliferation, and increased mesangial matrix in the DN group compared to the normal control group, indicating DN in mice. The DN+EA group exhibited less glomerular hypertrophy than the DN group (Figure 2A).

Transmission electron microscopy revealed significant thickening of renal glomerular basement membranes, along with widened, flat, and fused foot processes and detached podocytes in the DN and DN+EA groups, confirming successful establishment of the DN model compared with the normal and normal+EA groups. Compared with DN, the thickness of GBM in the DN+EA group was thinner, and the difference was statistically significant (Table 2, Figure 2B).

### *EA increases autophagic activity in renal tissues induced by DN*

RT-qPCR analysis revealed a significant reduction in LC3A and LC3B mRNA levels in the DN group compared to the normal and normal+EA groups. In contrast, these levels were significantly increased in the DN+EA group relative to the DN group (Figure 3A-B). This suggests that DN reduces autophagosome numbers in renal cells, whereas EA treatment restores them. Comparable findings were noted in terms of protein expression (Figure 3C-D). The findings indicate a reduction in the number of autophagosomes in the kidney cells of diabetic mice. Still, EA increased the number of autophagosomes in the kidney tissues of diabetic+EA mice. LC3 expression was significantly elevated in renal tubular epithelial cells in the DN+EA group compared to the DN, normal, and normal+EA groups (Figure 3E-F).

**Table 2.** Changes in glomerular basement membrane (GBM) in each mouse group (n=6/group, mean±SD)

| Group | GBM |
|---|---|
| Normal group | 0.121083±0.0103276 |
| Normal + electroacupuncture (EA) group | 0.126293±0.0112841 |
| Diabetic nephropathy (DN) group | 0.172414±0.0181699* |
| DN + EA group | 0.145463±0.0192443*# |

$^{*}P<0.01$ vs normal group and the normal+EA group; $^{\#}P<0.05$ vs DN group

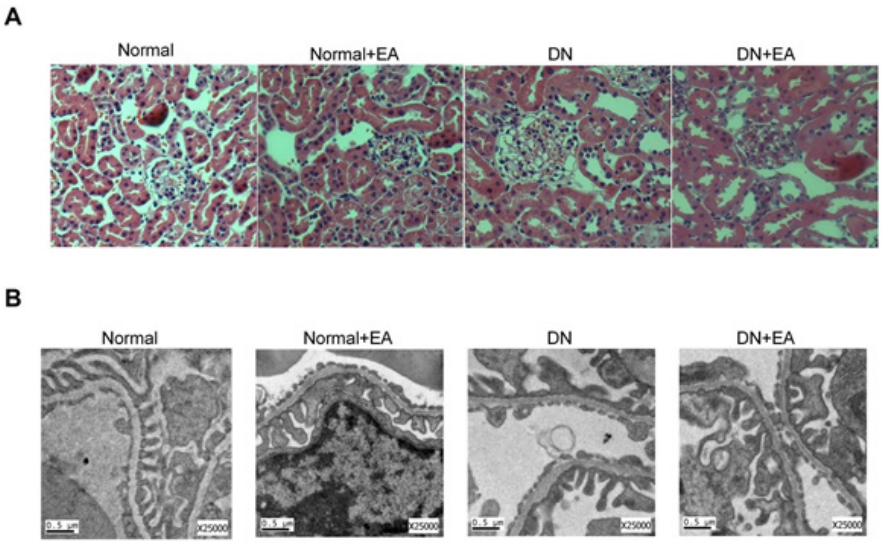


**Figure 2.** Improvement of diabetic renal (DN) injury in mice by electroacupuncture (EA)
(A) Comparison of glomerular size in the four groups by periodic acid-schiff (PAS) staining and light microscopy (×400 magnification) (n=6 per group). (B) Comparison of the renal glomerular basement membrane in each group under electron microscopy (×25,000 magnification)(n=6 per group). Values are mean±SD; $^{*}P<0.05$, $^{**}P<0.01$. Data were analyzed using one-way analysis of variance (ANOVA)

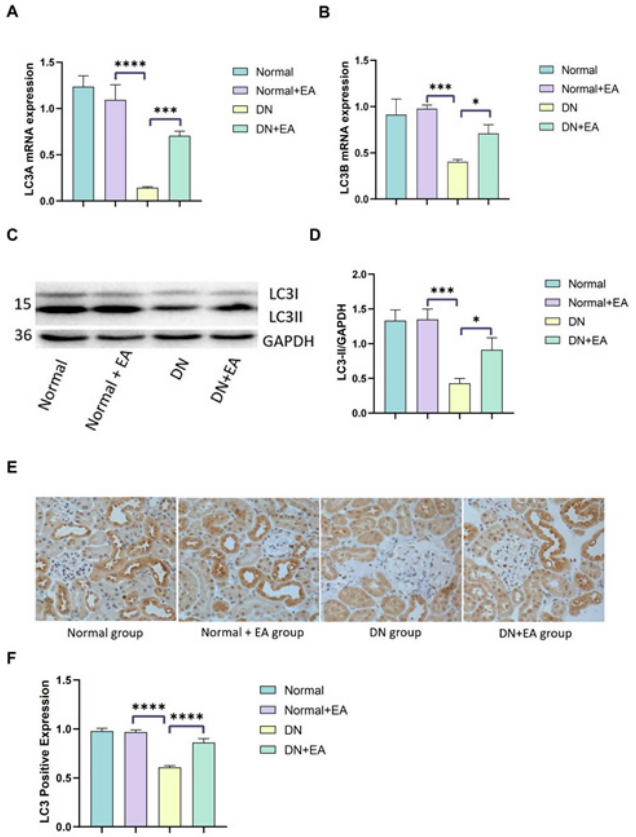


**Figure 3.** Restoration of diabetic renal (DN)-reduced autophagy in the renal of mice by electroacupuncture (EA)
(A-B) The mRNA expressions of microtubule°associated protein 1 light chain 3 alpha (LC3A) and microtubule°associated protein 1 light chain 3 beta (LC3B) in the different groups (n=6 per group). (C) Microtubule-associated protein 1 light chain 3-II (LC3-II) protein expression in the different groups(n=6 per group). (D) Semiquantitative analysis of LC3-II protein expression in the different groups(n=6 per group). (E-F) Immunohistochemistry of microtubule°associated protein 1 light chain 3 (LC3) in the different groups (n=6 per group). Values are mean±SD; $^{*}P<0.05$, $^{**}P<0.01$. Data were analyzed using one-way analysis of variance (ANOVA)

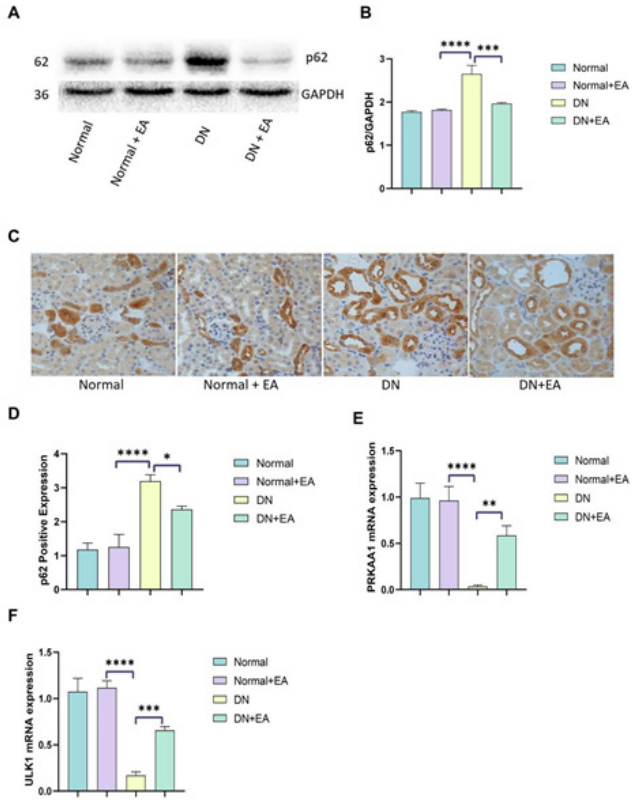


**Figure 4.** Restoration of diabetic renal (DN)-reduced autophagy in the renal of mice by electroacupuncture (EA)
The results from the changes of sequestosome 1 (p62) proteins involved in autophagy(n=6 per group): (A) p62 protein expression in the different groups(n=6 per group). (B) Semiquantitative analysis of p62 protein expression in the different groups(n=6 per group). (C-D) Immunohistochemistry analysis of p62 in the different groups (n=6 per group). (E) Protein kinase AMP°activated catalytic subunit alpha 1 (PRKAA1) mRNA expression in the different groups(n=6 per group). (F) Unc-51 Like Autophagy Activating Kinase 1 (ULK1) mRNA expression in the different groups(n=6 per group). Values are mean±SD; $^{*}P<0.05$, $^{**}P<0.01$. Data were analyzed using one-way analysis of variance (ANOVA)

### *EA improves the autophagic activity index (p62)*

p62 can mediate the transport of ubiquitin-tagged protein aggregates to lysosomes for degradation by binding to LC3. Therefore, the expression of p62 can reflect the function of autophagic degradation in cells. The findings indicated that the DN group had notably elevated p62 levels compared to the normal and normal+EA groups, whereas the DN+EA group showed a significant decrease in p62 levels (Figure 4A-B). The findings indicate reduced autophagic activity in diabetic kidney tissues, whereas EA increased it in the kidneys of diabetic mice (DN+EA group).

Immunohistochemistry revealed low p62 expression levels in renal tubular epithelial cells. The renal tubules in the DN group's kidney tissues exhibited a marked increase in p62 expression. (Figure 4C-D).

### *AMPK signaling pathway regulates autophagy in diabetic renal tissues by EA*

AMPK induces autophagy via the mTOR and ULK1 pathways and directly phosphorylates ULK1. RT-qPCR analysis revealed significantly lower mRNA expression levels of PRKAA1 and ULK1 in the DN group compared to the normal and normal+EA groups. The DN+EA group exhibited significantly higher levels of PRKAA1 and ULK1 than the DN group, indicating suppression of the AMPK pathway in DN and its activation in diabetic mice receiving EA treatment (Figure 4E-F).

Western blot analysis of mouse kidney samples revealed no significant difference in AMPKα expression between the DN group and the normal control or normal+EA groups. However, p-AMPKα expression was significantly lower in the DN group. Compared with the DN group, the DN+EA group showed a notable increase in p-AMPKα protein expression (Figure 5A-C), suggesting that AMPK signaling in diabetic renal tissues was suppressed; however, EA stimulated the AMPK pathway and promoted autophagy.

The expressions of mTOR and ULK1 in the kidneys of mice were assessed. In diabetic kidneys, p-ULK1 expression was reduced, whereas p-mTOR expression was elevated, compared to the normal control and normal+EA groups. EA reversed the effects in DN mice (Figure 5D-I), suggesting that EA activates the AMPK signaling pathway in diabetic kidney tissues by phosphorylating mTOR and ULK1, thereby initiating autophagy.

Immunohistochemistry revealed significantly reduced expression of p-ULK1 and p-mTOR in the DN group compared with the normal control and normal+EA groups. Compared with the DN group, the DN+EA groups showed a significant increase in p-ULK1 expression and a decrease in p-mTOR expression (Figure 6A-B).

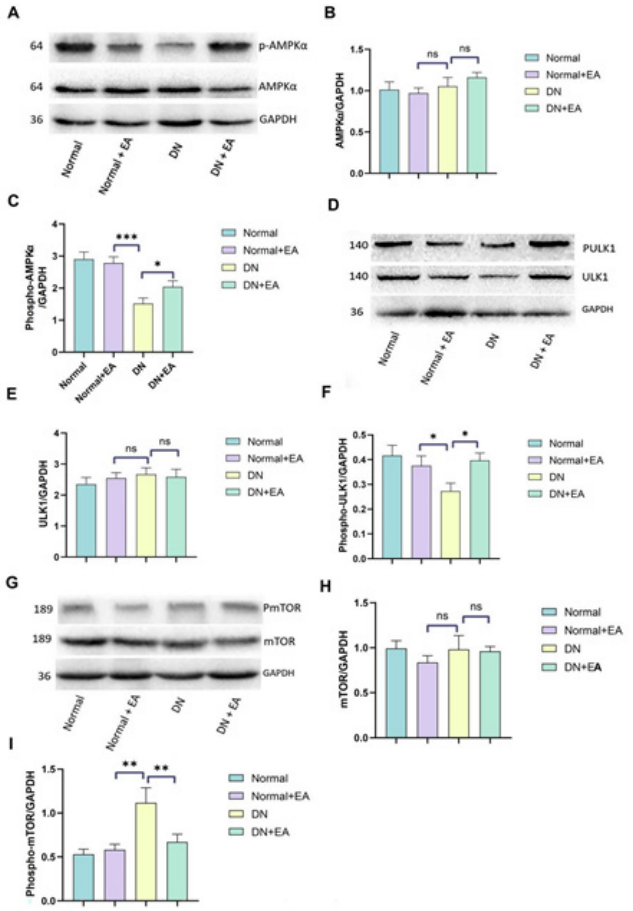


**Figure 5.** Protein expression of the AMP-activated protein kinase (AMPK) signaling pathway of kidney tissue in diabetic renal (DN) mice treated by electroacupuncture (EA)
(A) Protein expression of AMPactivated protein kinase alpha subunit (AMPKα) and phosphorylated AMPactivated protein kinase (p-AMPKα) in the different groups(n=6 per group). (B-C) Semiquantitative analysis of AMPKα and p-AMPKα in the different groups (n=6 per group). (D) Protein expression of phosphorylated unc-51 like autophagy activating kinase 1 (p-ULK1) and unc-51 like autophagy activating kinase 1 (ULK1) in the different groups(n=6 per group). (E-F) Semiquantitative analysis results of p-ULK1 and ULK1 in the different groups (n=6 per group). (G) Protein expression of phosphorylated mammalian target of rapamycin (p-mTOR) and mammalian target of rapamycin (mTOR) in the different groups(n=6 per group). (H-I) Semiquantitative analysis of p-mTOR and mTOR in the different groups (n=6 per group). Values are mean±SD; $^{*}P<0.05$, $^{**}P<0.01$. Data were analyzed using one-way analysis of variance (ANOVA)

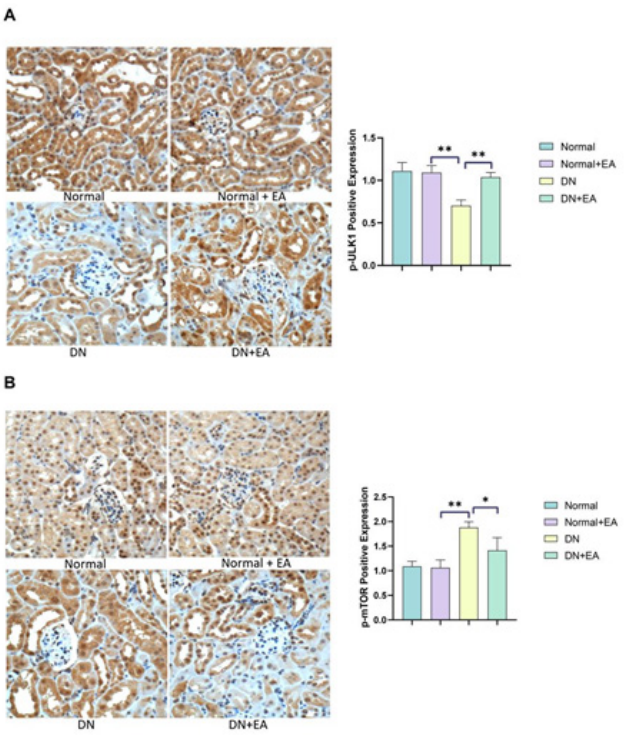


**Figure 6.** Protein expression of the phosphorylated unc-51 like autophagy activating kinase 1 (p-ULK1) and phosphorylated mammalian target of rapamycin (p-mTOR) of kidneys in diabetic renal (DN) mice treated by electroacupuncture (EA)
(A-B) Immunohistochemistry of p-ULK1 and p-mTOR proteins in the renal tissues of mice (n=6 per group). Values are mean±SD; $^{*}P<0.05$, $^{**}P<0.01$. Data were analyzed using one-way analysis of variance (ANOVA)

## Discussion

Long-term clinical practice has demonstrated that Chinese medicine, including traditional Chinese medicine and acupuncture, shows beneficial effects on a variety of complex kidney diseases, including diabetic nephropathy (33-35). This study provides compelling evidence that EA significantly reduces renal injury in diabetic nephropathy mice. Key findings indicate that EA treatment significantly enhanced renal function in diabetic nephropathy mice by reducing the urine protein/creatinine ratio and blood urea nitrogen levels. Autophagy is a cellular process that metabolizes intracellular components to maintain energy homeostasis and reduce cell stress (36, 37). EA improved autophagic activity in renal tissues by up-regulating microtubule-associated protein 1 light chain 3-II protein expression while down-regulating Sequestosome-1 protein expression. EA stimulated the AMP-activated protein kinase signaling pathway by elevating the protein expression of phosphorylation of AMP-activated protein kinase and Unc-51 Like Autophagy Activating Kinase 1, accompanied by down-regulating the phosphorylation of mammalian target of rapamycin protein expression. Histological analysis revealed that EA-treated diabetic nephropathy mice exhibited less renal tissue damage compared to untreated diabetic nephropathy mice. These results suggest that EA provides renoprotective effects by modulating autophagy through the AMP-activated protein kinase signaling pathway (Figure 7).

The relationship between these critical outcomes and the existing literature underscores the novel contributions of this study. The improvement in renal function observed with EA aligns with previous research documenting acupuncture's beneficial effects on renal parameters across various pathological conditions (38). Inflammation and oxidative stress play central roles in chronic kidney disease, including diabetic nephropathy (7, 39-41). However, studies have found that acupuncture improves renal function and reduces oxidative stress in chronic kidney disease (42). This study extends that evidence by demonstrating that EA specifically enhances autophagic activity, a crucial cellular process for maintaining renal health. Previous studies indicate that autophagy alleviates diabetic nephropathy by preventing the buildup of damaged organelles and proteins, which otherwise worsen renal injury when autophagy is impaired (43, 44). By increasing microtubule-associated protein 1 light chain 3-II levels and decreasing Sequestosome-1 levels, EA appears to restore autophagic flux in mice with diabetic nephropathy, suggesting a mechanism for its protective effects. This finding is particularly significant because it highlights a potential therapeutic pathway that could be

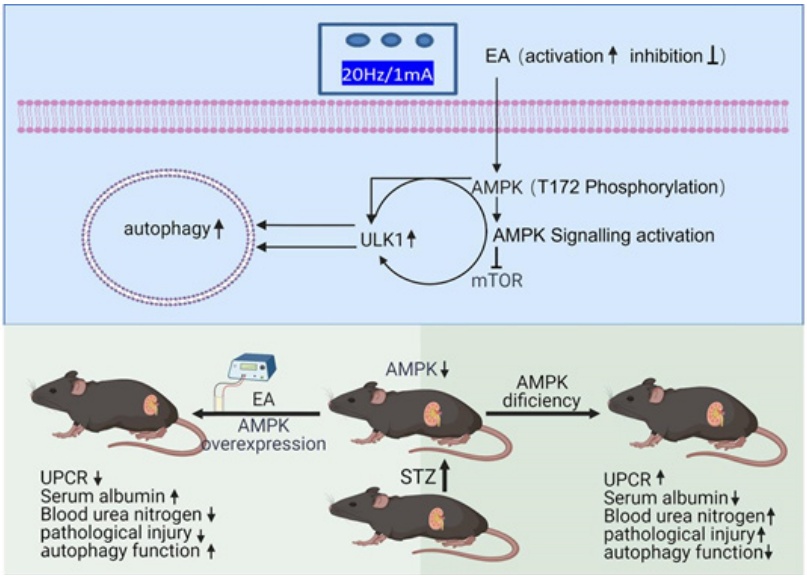


**Figure 7.** Schematic diagram of electroacupuncture (EA) protective effect in diabetic renal (DN)
Autophagy levels were decreased in the DN group, and this deficiency correlated with increased urine protein°to°creatinine ratio, blood urea nitrogen (BUN), and pathological injury. EA may protect against renal injury by inducing autophagy via activation of the AMP°activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR)/unc°51 like autophagy activating kinase 1 (ULK1) signaling pathway.

targeted to alleviate diabetic nephropathy and improve renal outcomes.

AMP-activated protein kinase is a crucial cellular energy sensor that regulates metabolism by phosphorylating many diverse substrates throughout the cells (45). Previous studies have demonstrated that natural compounds ameliorated acute kidney injury and chronic kidney disease, including diabetic nephropathy, by regulating the AMP-activated protein kinase pathway (46, 47). EA's activation of the AMP-activated protein kinase signaling pathway is crucial, as AMP-activated protein kinase plays a central role in cellular energy homeostasis and autophagy. Research has consistently shown that AMP-activated protein kinase activation promotes autophagy and protects against renal injury in diabetes and hypertensive nephropathy (48). This study corroborates these findings by demonstrating that EA increases phosphorylation of AMP-activated protein kinase and its downstream target, Unc-51-like Autophagy-Activating Kinase 1, thereby enhancing autophagic activity. The observed decrease in phosphorylated mammalian target of rapamycin phosphorylation further supports the involvement of the AMP-activated protein kinase pathway, as mammalian target of rapamycin is a well-known inhibitor of autophagy (49). These molecular changes suggest that EA may act by modulating key signaling pathways involved in cellular metabolism and stress responses, offering a potential therapeutic mechanism for addressing diabetic nephropathy.

The most important contributions of this study include identifying the AMP-activated protein kinase signaling pathway as a potential target for EA in treating diabetic nephropathy. This finding provides a mechanistic understanding of how EA alleviates renal injury. By elucidating the roles of autophagy and AMP-activated protein kinase in EA's renoprotective effects, this study offers a novel therapeutic approach for managing diabetic nephropathy. Additionally, the research highlights EA's potential as a non-pharmacological intervention, which could be particularly beneficial for patients who are unresponsive to conventional treatments or experience adverse effects. The integration of traditional acupuncture techniques with modern biomedical understanding underscores the relevance of interdisciplinary approaches in developing effective therapies. This study thus bridges the gap between traditional practice and contemporary science, paving the way for innovative treatment strategies that leverage both fields.

Several limitations of this study should be addressed in future research. The study used a mouse model, which offers valuable insights but may not capture the full complexity of human diabetic nephropathy. Clinical trials are essential to confirm the efficacy and safety of EA in patients with diabetic nephropathy. This study examined specific autophagy markers and the AMP-activated protein kinase signaling pathway, suggesting that additional molecular pathways and mechanisms may contribute to EA's renoprotective effects and require further exploration. The involvement of inflammatory cytokines and oxidative stress in EA-induced renal protection warrants investigation. The duration of the treatment was limited to 8 weeks. Further research is necessary to evaluate the long-term effects of EA on renal function and autophagy, as well as its ability to hinder the progression of diabetic nephropathy. Additionally, the precise parameters of EA, such as frequency, intensity, and duration, should be optimized to maximize therapeutic outcomes. Addressing these limitations in future research will enhance the understanding of EA's therapeutic potential and facilitate its integration into clinical practice for managing diabetic nephropathy.

## Conclusion

Our study provides strong evidence that EA alleviates renal injury in DN-mice by enhancing autophagy via the AMPK signaling pathway. These findings deepen understanding of the molecular mechanisms underlying EA's therapeutic effects and underscore its potential as a non-pharmacological treatment for DN. Future research should focus on translating these findings into clinical

practice, exploring additional molecular mechanisms, and optimizing EA parameters to improve its efficacy and applicability in managing DN. Addressing these areas will help establish EA as a viable treatment option and expand its use in clinical settings.

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## Data Availability

The data supporting the findings of this study are not publicly available due to institutional policy, but are available from the corresponding author upon reasonable request.

## Ethics Approval and Consent to Participate

This study was approved by the Animal Care and Use Committee and Ethics Committee at Medical College of Lishui University, China. All animal experiments meet the requirements of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No.8023). All methods were performed in accordance with the relevant guidelines and regulations.

## Authors' Contributions

Z Z, M X, L L, D P, L Y, M J, and J P designed the study; Z Z performed experiments and collected and processed data; M X, L L, and D P analyzed and interpreted the results; Z Z and L Y drafted the manuscript and prepared the visualization; M X, M J, and J P critically revised the article for important intellectual content; J P supervised the study, managed the project, and acquired funding. All authors approved the final version for publication.

## Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Declaration

We have not used any AI tools or technologies to prepare this manuscript.

## References

1. de Boer IH, Khunti K, Sadusky T, Tuttle KR, Neumiller JJ, Rhee CM, *et al.* Diabetes management in chronic kidney disease: A consensus report by the american diabetes association (ADA) and kidney disease: Improving global outcomes (KDIGO). Diabetes Care 2022; 45: 3075-3090.
2. GBD 2023 Breast Cancer Collaborators. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: A systematic analysis for the Global Burden of Disease Study 2021. Lancet 2023; 402: 203-234.
3. Glastras SJ, Pollock CA. Targeted identification of risk and treatment of diabetic kidney disease. Nat Rev Nephrol 2024; 20: 75-76.
4. Jung CY, Yoo TH. Pathophysiologic mechanisms and potential biomarkers in diabetic kidney disease. Diabetes Metab J 2022; 46: 181-197.
5. Li XJ, Shan QY, Wu X, Miao H, Zhao YY. Gut microbiota regulates oxidative stress and inflammation: A double-edged sword in renal fibrosis. Cell Mol Life Sci 2024; 81: 480.
6. Balakumar P. Unleashing the pathological role of epithelial-to-mesenchymal transition in diabetic nephropathy: The intricate connection with multifaceted mechanism. World J Nephrol 2024; 13: 95410.
7. Hou Y, Tan E, Shi H, Ren X, Wan X, Wu W, *et al.* Mitochondrial oxidative damage reprograms lipid metabolism of renal tubular epithelial cells in the diabetic kidney. Cell Mol Life Sci 2024; 81: 23.
8. Feng L, Feng YY, Ren Q, Fu P, Ma L. Mesangial cells in diabetic kidney disease: From mechanisms to therapeutic implications. Int J Biol Sci 2025; 21: 4762-4781.
9. Thompson AD, Hurtado KA, Janda J, Scholpa NE, Rohrer B, Schnellmann RG. MC16 promotes mitochondrial biogenesis and ameliorates acute and diabetic nephropathy. Br J Pharmacol 2025; 182: 1912-1929.
10. Zhao H, Guo J. Macrophages in focus: Key drivers and therapeutic opportunities in diabetic kidney disease. Int J Biol Sci 2025; 21: 4647-4662.
11. Ortega MA, Fraile-Martinez O, de Leon-Oliva D, Boaru DL, Lopez-Gonzalez L, García-Montero C, *et al.* Autophagy in its (proper) context: Molecular basis, biological relevance, pharmacological modulation, and lifestyle medicine. Int J Biol Sci 2024; 20: 2532-2554.
12. Florance I, Cordani M, Pashootan P, Moosavi MA, Zarrabi A, Chandrasekaran N. The impact of nanomaterials on autophagy across health and disease conditions. Cell Mol Life Sci 2024; 81: 184.
13. Wu MY, Li ZW, Lu JH. Molecular modulators and receptors of selective autophagy: Disease implication and identification strategies. Int J Biol Sci 2024; 20: 751-764.
14. Shi L, Song Z, Li C, Deng F, Xia Y, Huang J, *et al.* HDAC6 inhibition alleviates ischemia- and cisplatin-induced acute kidney injury by promoting autophagy. Cells 2022; 11: 3951.
15. Minami S, Yamamoto T, Yamamoto-Imoto H, Isaka Y, Hamasaki M. Autophagy and kidney aging. Prog Biophys Mol Biol 2023; 179: 10-15.
16. Erekat NS. Programmed cell death in diabetic nephropathy: A review of apoptosis, autophagy, and necroptosis. Med Sci Monit 2022; 28: e937766.
17. Zheng Z, Chen J, Xue X, Ma X, Zhang S, Wang M, *et al.* SESN2 inhibits tubular exosome secretion and diabetic kidney disease progression by restoring the autophagy–lysosome pathway. Int J Biol Sci 2025; 21: 4215-4230.
18. North BJ, Fracchiolla D, Ragusa MJ, Martens S, Shoemaker CJ. The rapidly expanding role of LC3-interacting regions in autophagy. J Cell Biol 2025; 224: e202504076.
19. Xiao S, Yu Y, Liao M, Song D, Xu X, Tian L, *et al.* Post-translational modification of p62: Roles and regulations in autophagy. Cells. 2025; 14: 1016.
20. Tang C, Deng X, Qu J, Miao Y, Tian L, Zhang M, *et al.* Fenofibrate attenuates renal tubular cell apoptosis by up-regulating MCAD in diabetic kidney disease. Drug Des Devel Ther 2023; 17: 1503-1514.
21. Juszczak F, Caron N, Mathew AV, Declèves AE. Critical role for AMPK in metabolic disease-induced chronic kidney disease. Int J Mol Sci 2020; 21: 7994.
22. Rogacka D, Audzeyenka I, Piwkowska A. Regulation of podocytes function by AMP-activated protein kinase. Arch Biochem Biophys 2020; 692: 108541.
23. Wen J, Zheng W, Zeng L, Wang B, Chen D, Chen Y, *et al.* LTF induces radioresistance by promoting autophagy and forms an AMPK/SP2/NEAT1/miR-214-5p feedback loop in lung squamous cell carcinoma. Int J Biol Sci 2023; 19: 1509-1527.
24. Li B, Wu GL, Dai W, Wang G, Su HY, Shen XP, *et al.* Aescin-induced reactive oxygen species play a pro-survival role in human cancer cells via ATM/AMPK/ULK1-mediated autophagy. Acta

Pharmacol Sin 2018; 39: 1874-1884.
25. Cho W, Oh H, Choi SW, Abd El-Aty AM, Birdal O, Jeong JH, *et al*. CTRP4 attenuates apoptosis and epithelial-mesenchymal transition markers in podocytes through an AMPK/autophagy-dependent pathway. Biochem Biophys Res Commun 2023; 682: 104-110.
26. Galluzzi L, Green DR. Autophagy-independent functions of the autophagy machinery. Cell 2019; 177: 1682-1699.
27. Xin YY, Wang JX, Xu AJ. Electroacupuncture ameliorates neuroinflammation in animal models. Acupunct Med 2022; 40: 474-483.
28. Liu S, Ge J, Liu W, Zhuang Z, Liu S. Involvement of the somatosensory-autonomic reflex and muscarinic receptors in exacerbation of allergic pulmonary inflammation by electroacupuncture. Br J Pharmacol 2025; 182: 2374-2391.
29. Han X, Gao Y, Yin X, Zhang Z, Lao L, Chen Q, *et al*. The mechanism of electroacupuncture for depression on basic research: A systematic review. Chin Med 2021; 16: 10.
30. Hu L, Klein JD, Hassounah F, Cai H, Zhang C, Xu P, *et al*. Low-frequency electrical stimulation attenuates muscle atrophy in CKD--a potential treatment strategy. J Am Soc Nephrol 2015; 26: 626-635.
31. Paterno JC, Freire AO, Soares MF, Franco MF, Schor N, Teixeira VP. Electroacupuncture and moxibustion attenuate the progression of renal disease in 5/6 nephrectomized rats. Kidney Blood Press Res 2008; 31: 367-373.
32. Harpin D, Simadibrata CL, Mihardja H, Barasila AC. Effect of electroacupuncture on urea and creatinine levels in the wistar sepsis model. Med Acupunct 2020; 32: 29-37.
33. Guo ZY, Wu X, Zhang SJ, Yang JH, Miao H, Zhao YY. Poria cocos: Traditional uses, triterpenoid components and their renoprotective pharmacology. Acta Pharmacol Sin 2025; 46: 836-851.
34. Feng HY, Wang YQ, Yang J, Miao H, Zhao YY, Li X. Anthraquinones from rheum officinale ameliorate renal fibrosis in acute kidney injury and chronic kidney disease. Drug Des Devel Ther 2025; 19: 5739-5760.
35. Wang YN, Wu X, Shan QY, Yang Q, Yu XY, Yang JH, *et al*. Acteoside-containing caffeic acid is bioactive functional group of antifibrotic effect by suppressing inflammation via inhibiting AHR nuclear translocation in chronic kidney disease. Acta Pharmacol Sin 2025; 46: 2975-2988.
36. Liang J, Sun J, Zhang W, Wang X, Xu Y, Peng Y, *et al*. Novel insights into the roles of N(6)-methyladenosine (m(6)A) modification and autophagy in human diseases. Int J Biol Sci 2023; 19: 705-720.
37. Huang Y, Zhang R, Lyu H, Xiao S, Guo D, Chen XZ, *et al*. LncRNAs as nodes for the cross-talk between autophagy and Wnt signaling in pancreatic cancer drug resistance. Int J Biol Sci 2024; 20: 2698-2726.
38. Xiong W, He FF, You RY, Xiong J, Wang YM, Zhang C, *et al*. Acupuncture application in chronic kidney disease and its potential mechanisms. Am J Chin Med 2018; 46: 1169-1185.
39. Wang YN, Miao H, Yu XY, Guo Y, Su W, Liu F, *et al*. Oxidative stress and inflammation are mediated via aryl hydrocarbon receptor signalling in idiopathic membranous nephropathy. Free Radic Biol Med 2023; 207: 89-106.
40. Liu Y, Qiao Y, Pan S, Chen J, Mao Z, Ren K, *et al*. Broadening horizons: The contribution of mitochondria-associated endoplasmic reticulum membrane (MAM) dysfunction in diabetic kidney disease. Int J Biol Sci 2023; 19: 4427-4441.
41. Wang YN, Li XJ, Wang WF, Zou L, Miao H, Zhao YY. Geniposidic acid attenuates chronic tubulointerstitial nephropathy through regulation of the NF-ƙB/Nrf2 pathway via aryl hydrocarbon receptor signaling. Phytother Res 2024; 38: 5441-5457.
42. Liu X, Wang X, Ma H, Zhang W. Mechanisms underlying acupuncture therapy in chronic kidney disease: A narrative overview of preclinical studies and clinical trials. Front Nephrol 2022; 2: 1006506.
43. Tseng CH, Shah KM, Chiu IJ, Hsiao LL. The role of autophagy in type 2 diabetic kidney disease management. Cells 2023; 12: 2691.
44. Huang SS, Ding DF, Chen S, Dong CL, Ye XL, Yuan YG, *et al*. Resveratrol protects podocytes against apoptosis via stimulation of autophagy in a mouse model of diabetic nephropathy. Sci Rep 2017; 7: 45692.
45. Yuan Y, Wang F, Liu X, Shuai B, Fan H. The role of AMPK signaling in ulcerative colitis. Drug Des Devel Ther 2023; 17: 3855-3875.
46. Sun MY, Ye HJ, Zheng C, Jin ZJ, Yuan Y, Weng HB. Astragalin ameliorates renal injury in diabetic mice by modulating mitochondrial quality control via AMPK-dependent PGC1α pathway. Acta Pharmacol Sin 2023; 44: 1676-1686.
47. Kuang BC, Wang ZH, Hou SH, Zhang J, Wang MQ, Zhang JS, *et al*. Methyl eugenol protects the kidney from oxidative damage in mice by blocking the Nrf2 nuclear export signal through activation of the AMPK/GSK3β axis. Acta Pharmacol Sin 2023; 44: 367-380.
48. Wang S, Li H, Yuan M, Fan H, Cai Z. Role of AMPK in autophagy. Front Physiol 2022; 13: 1015500.
49. Xu TT, Deng YY, Yu XY, Li M, Fu YY. Natural autophagy modulators in non-communicable diseases: From autophagy mechanisms to therapeutic potential. Acta Pharmacol Sin 2025; 46: 8-32.