Research Article

Huangqi-Danshen Decoction Ameliorates Adenine-Induced Chronic Kidney Disease by Modulating Mitochondrial Dynamics

Xinhui Liu [ID],[1] **Shiying Huang,**[2] **Fochang Wang,**[2] **Lin Zheng,**[2] **Jiandong Lu,**[1] **Jianping Chen** [ID],[2] **and Shunmin Li** [ID][1]

[1]*Department of Nephrology, Shenzhen Traditional Chinese Medicine Hospital, Guangzhou University of Chinese Medicine, Shenzhen, Guangdong, China*
[2]*Shenzhen Key Laboratory of Hospital Chinese Medicine Preparation, Shenzhen Traditional Chinese Medicine Hospital, Guangzhou University of Chinese Medicine, Shenzhen, Guangdong, China*

Correspondence should be addressed to Xinhui Liu; liuxinhui0317@163.com and Shunmin Li; zyylishunmin@163.com

Chronic kidney disease (CKD) is a leading public health problem with high morbidity and mortality. However, the therapies remain limited. Traditional Chinese medicine (TCM) has been used for treating kidney disease for thousands of years and is an effective alternative treatment for CKD patients in China and other Asian countries. In the present study, we aimed to investigate the effect and mechanism of Huangqi-Danshen decoction (HDD), a TCM herbal decoction, on treating CKD. CKD rat model was induced by adding 0.75% adenine to the diet for 4 weeks. HDD extract was administrated orally to CKD rats at the dose of 4.7 g/kg/d for consecutive 4 weeks in adenine-induced CKD rats. Kidney function was evaluated by the levels of serum creatinine (Scr) and blood urea nitrogen (BUN). The pathological changes of kidney tissues were observed by periodic acid-Schiff (PAS) and Masson's trichrome staining. The proteins expression of renal fibrosis and mitochondrial dynamics were determined and quantified by Western blot analysis. CKD rats showed obvious decline in renal function as evidenced by increased levels of Scr and BUN, which were blunted by HDD treatment. HDD could also improve tubular atrophy and interstitial fibrosis of CKD rats. Moreover, HDD downregulated fibronectin, type IV collagen, and α-smooth muscle actin expression in CKD rats. Furthermore, mitochondrial dynamics was disturbed in CKD rats, which manifested as increased mitochondrial fission and decreased mitochondrial fusion. HDD treatment restored mitochondrial dynamics in CKD rats by repressing dynamin-related protein 1 and Mid 49/51 expression, promoting mitofusin 2 expression, and suppressing optic atrophy 1 proteolysis. In conclusion, HDD could significantly retard CKD progression through modulating mitochondrial dynamics.

1. Introduction

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function persisting for at least 3 months, regardless of the underlying cause [1]. The prevalence of all stages of CKD varies between 7 and 12% in the different regions of the world [1]. CKD contributes 1.35% of the global burden of disability life years lost, growing at a rate of 1% per year [2]. Despite this, there are relatively few options for the treatment of CKD. The mainstay therapeutic approach to retard progressive CKD or renal fibrosis is based on rennin-angiotensin system (RAS) blockade as well as blood pressure and glycemic control [3–5]. These interventions could not

prevent the progression to end-stage renal disease (ESRD). In addition, the benefit of standard therapies varies across stages of CKD [6]. Therefore, mechanism investigation, discovering therapeutic targets, and searching effective medications are always necessary for CKD treatment.

Traditional Chinese medicine (TCM) is frequently used for treatment of CKD in China and many other Asian countries [7–10]. According to TCM theory, Qi deficiency and blood stasis (Qi-Xu-Xue-Yu) runs through the process of CKD development and progression. Therefore, replenishing Qi and activating blood (Yi-Qi-Huo-Xue) is the basic therapeutic principle of TCM in the treatment of CKD [11]. Huangqi-Danshen decoction (HDD) is composed of

Astragali Radix (Huang-qi) served as replenishing Qi and *Salviae Miltiorrhizae* Radix et Rhizoma (Dan-shen) served as activating blood. Both Huang-qi and Dan-shen were firstly recorded in "Shennong Bencao Jing" (The Devine Farmer's Materia Medica, 300-200 B.C., Warring States Period to Han Dynasty) and were the most frequently prescribed herbs for treating CKD in clinical practise [12–14]. Pharmacological studies showed that Huang-qi has antioxidant, anti-inflammatory, immuneregulatory, anticancer, hypolipidemic, antihyperglycemic, hepatoprotective, expectorant, and diuretic effects [15]. Dan-shen has been proven to have various pharmacological activities, such as cardiovascular and cerebrovascular effects, antioxidative, neuroprotective, antifibrotic, antiinflammatory, and antineoplastic activities [16]. According to TCM theory, combined used of Huang-qi and Dan-shen will benefit to retard CKD progression by conjugating replenishing Qi and activating blood. In the present study, we tested this theory in adenine-induced CKD rat model and investigated underlying mechanism.

Mitochondria are the main energy-producing organelles in mammalian cells, but they also have a central role in deciding cell injury and death [17]. Since kidney is a high energy demanded organ and rich in mitochondria, mitochondrial dysfunction plays a critical role in the pathogenesis of kidney diseases [18]. Mitochondria are a class of dynamic organelles that constantly undergo fission and fusion [19]. Emerging evidence has demonstrated the alterations of mitochondrial dynamics in renal ischemia–reperfusion injury, nephrotoxicity, and hyperglycemia-induced kidney injury [20]. However, very little is known about the effect of mitochondrial dynamics on CKD. Hence, in the present study, we investigated the alterations of mitochondrial dynamics in a CKD model. Moreover, we studied how HDD could blunt CKD progression by modulating mitochondrial dynamics.

2. Materials and Methods

2.1. Preparation of HDD Water Extract. The herbal composition and proportion of HDD were summarized in Table 1. Raw herbs were purchased from Shenzhen Huahui Pharmaceutical Co., Ltd. (Shenzhen, China). The plant materials were authenticated by Jianping Chen based on their morphological characteristics. *Astragali Radix* (30 g) and *Salviae Miltiorrhizae* Radix et Rhizoma (15 g) were weighed and boiled twice in 8 times of ddH2O (w/v) for 1 hour per time. The HPLC profile of HDD extract was provided in Supplementary Figure 1. For the convenience of administration, the extract was dried by freeze dryer and stored at -80°C. Before the treatment, the freeze-dried powder was redissolved with ddH$_2$O to get HDD extract.

2.2. Animals and Experimental Treatment. All animal experiments were conducted with protocols approved by the Ethics Committee of Shenzhen Traditional Chinese Medicine Hospital, Guangzhou University of Chinese Medicine. Twenty-four male Spraque-Dawley (SD) rats weighting 180-220g were purchased from Guangdong Medical Laboratory Animal Center (Foshan, China, permission no. SCXK

(YUE) 2013-0002). The rats were maintained at a constant temperature (22°C–25°C) and humidity (40%–70%) with a 12-hour light/12-hour dark cycle. After one week of acclimatization, all rats were randomly divided into three groups: (1) control group (n=8), (2) CKD group (n=8), and (3) HDD-treated group with CKD (n=8). CKD was induced in rats by feeding adenine (Sigma-Aldrich, St Louis, MO, USA) in feed at a concentration of 0.75% w/w for 4 weeks [21]. Treatment group rats received 4.7 g/kg/d dose of HDD extract orally for 4 weeks with simultaneous adenine feeding. Control group rats received normal adenine-free feed for 4 weeks. During the experiment, 2 rats died in the CKD group, but no rats died in other groups. At the end of study, all rats were anesthetized, and blood samples were obtained by cardiac puncture. The rats were euthanized by cervical dislocation without regaining consciousness. Kidneys were removed and preserved for histological analysis and Western blot analysis.

2.3. Biochemical Analysis. Serum creatinine (Scr) and blood urea nitrogen (BUN) were measured by creatinine serum detection kit and BUN detection kit (StressMarq Biosciences, British Columbia, Canada), respectively, following the manufacturer's instructions.

2.4. Histological Examination. Rat kidney tissue was fixed with 4% buffered paraformaldehyde (pH 7.4) at 4°C overnight, dehydrated in graded alcohols, and embedded in paraffin. The paraffin-embedded kidneys were cut into 2 mm sections and stained with periodic acid-Schiff (PAS) and Masson's trichrome stains for the evaluation of pathological changes. The tubular atrophy score in PAS staining was performed as previously described [22]. Quantitative analysis of fibrotic area was conducted by Image J software (NIH, Bethesda, MD, USA). Tubular atrophy score and fibrotic area measurement were performed on at least 10 fields (200×) in each slide and six rats in each group by a colleague who was blinded to the study design.

2.5. Western Blot Analysis. The kidney cortexes were homogenized in lysis buffer and measured protein concentration as our previously described [23]. Equal amounts of kidney cortex lysates were loaded and electrophoresed through 7% or 10% SDS-PAGE gels and were then transferred to nitrocellulose membranes or polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). After being blocked in 5% nonfat milk for 1 hour at room temperature, the membranes were incubated with primary antibodies at 4°C overnight. Then, the membranes were incubated in horseradish peroxidase (HRP)-conjugated anti-mouse IgG or HRP-conjugated anti-rabbit IgG (Life Technologies, Carlsbad, CA, USA) for 1 hour at room temperature. HRP activity was visualized using Clarity Western ECL Substrate and a ChemiDoc MP Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). Image Lab software version 5.1 was used for densitometric analysis (Bio-Rad Laboratories, Hercules, CA, USA). The primary antibodies used in this study included dynamin-related protein 1 (Drp-1, 1:1000), mitofusin 2 (Mfn-2, 1:1000),
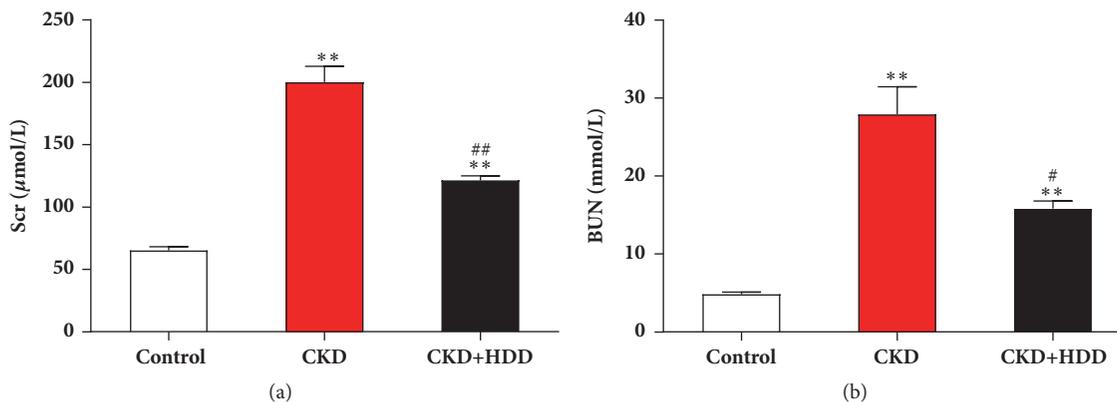
(a)

(b)

FIGURE 1: HDD improved renal function in CKD rats. The levels of Scr (a) and BUN (b) in different groups. Data are presented as the means ± SEM, n=6 rats per group ($^{**}P < 0.01$ compared with the control group; $^{#}P < 0.05$, $^{##}P < 0.01$ compared with the CKD group).

TABLE 1: The herbal composition and proportion of HDD.

Botanical name	Herbal name	Chinese name	Voucher number	Dosage
Astragalus membranaceus (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao	*Astragali Radix*	Huang-Qi	2010015Z	30 g
Salvia miltiorrhiza Bunge	*Salviae Miltiorrhizae* Radix et Rhizoma	Dan-Shen	2010006Z	15 g

α-tubulin (1:1000) (Cell Signaling Technology, Beverly, MA, USA), optic atrophy 1 (OPA-1, 1:2000) (BD Biosciences, San Jose, CA, USA), fibronectin (FN, 1:250), type IV collagen (Col-IV, 1:250) (abcam, Cambridge, MA, USA), α-smooth muscle actin (α-SMA, 1:1000) (Sigma-Aldrich, St Louis, MO, USA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:5000), Mid 49 (1:500), and Mid 51 (1:500) (Proteintech, Wuhan, China).

2.6. Immunofluorescence Analysis. The paraffin-embedded kidneys were cut into 6 μm sections, dewaxed, and rehydrated. After antigen retrieval in 10 mM sodium citrate (pH 6.0), the sections were incubated with block buffer (5% BSA in PBS) for 1 hour at room temperature. Then, the sections were stained with anti-Drp-1 (1:50) and anti-TOM 20 (1:100) at 4°C overnight followed by appropriate secondary antibodies. Nuclei were counterstained with the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI). In all cases, antibody negative controls were used to ensure the truth of positive staining. All images were captured by fluorescence microscope (Nikon, Japan).

2.7. Statistical Analysis. All data are presented as mean ± SEM. The statistical differences among groups were analyzed by one-way ANOVA followed by post hoc analysis with the Least Significant Difference (LSD) test or the Games-Howell test. The value of $P<0.05$ was considered statistically significant. Data were analyzed using SPSS 16.0 statistics software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. HDD Improved Renal Function in CKD Rats. Renal function was assessed by Scr and BUN levels. Compared with the control group, Scr and BUN levels in the CKD group were significantly elevated ($P<0.01$). Administration of HDD obviously reduced Scr ($P<0.01$) and BUN ($P<0.05$) levels in CKD rats (Figure 1). Moreover, HDD had no effect on liver function as estimated by the levels of aspartate transaminase (AST) and alanine transaminase (ALT), which means the treatment dosage of HDD in current study is safe (Supplementary Table 1). These data indicated that CKD model was established successfully, and HDD prevented renal function decline in CKD rats.

3.2. HDD Ameliorated Renal Pathological Injury in CKD Rats. The renal pathological injury in CKD is characterized by tubular atrophy and interstitial fibrosis. In PAS staining, CKD rats showed massive tubular atrophy, which could be improved by HDD treatment (Figures 2(a)–2(d)). Masson staining displayed obvious interstitial fibrosis in the CKD group, which was almost 4 times of the control group in quantitative analyses ($P<0.01$). HDD treatment reduced collagen deposition in Masson staining by 36% ($P<0.01$)
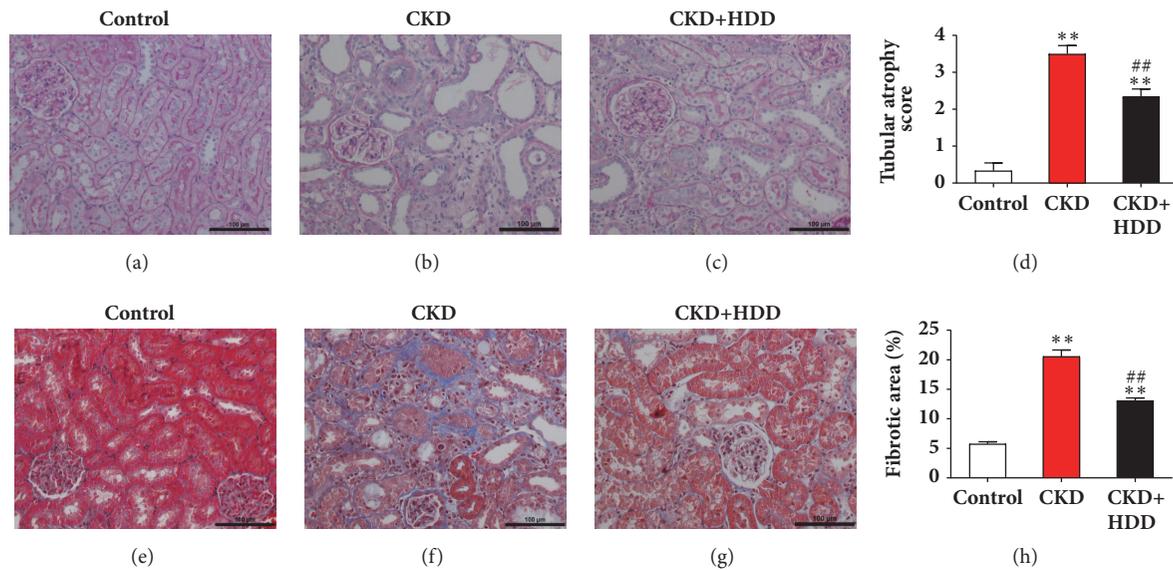
Figure 2: HDD ameliorated renal pathological injury in CKD rats. (a) Normal kidney structure in PAS staining of the control group. (b) Obvious tubular atrophy in the CKD group. (c) Reduced tubular atrophy in the CKD+HDD group. (d) Tubular atrophy score. (e) Normal kidney structure in Masson staining of the control group. (f) Massive collagen deposition in the CKD group. (g) Reduced collagen deposition in the CKD+HDD group. (h) Quantitative analysis of fibrotic area. All images are shown at identical magnification, ×200, scale bar=100μm. Data are presented as the means ± SEM, n=6 rats per group ($^{**}P < 0.01$ compared with the control group; $^{##}P < 0.01$ compared with the CKD group).

(Figures 2(e)–2(h)). These data indicated that HDD protected kidney structure in CKD rats.

3.3. HDD Inhibited Renal Fibrosis in CKD Rats. Renal fibrosis is the common and final pathway from CKD to ESRD. We further explored the effect of HDD on fibrosis by evaluation of fibrotic markers expression. As shown in Figure 3, the expression of FN, Col-IV, and α-SMA was all upregulated in the CKD group (*P*<0.01). In contrast, HDD treatment significantly restored these proteins expression. These data provided further evidence of the beneficial effect of HDD on kidney structure.

3.4. HDD Modulated Mitochondrial Dynamics in CKD Rats. The balance of mitochondrial fission and fusion maintains mitochondrial homeostasis and function. We further investigated the status of mitochondrial dynamics in CKD and the regulating effect of HDD. Western blotting revealed that the expression levels of Drp-1, Mid 51, and Mid 49, the main regulators of mitochondrial fission, were significantly upregulated in the CKD group (Figures 4(a) and 4(b)). Immunofluorescence analysis showed that more Drp-1 colocalized with TOM 20, a mitochondrial marker, in the CKD group, which indicated more Drp-1 translocated to mitochondria to perform fission in CKD rats (Figure 4(c)). HDD administration restored the protein abundance of Drp-1, Mid 51, and Mid 49 and reduced translocation of Drp-1 to mitochondria (Figures 4(a)–4(c)). We next examined the expression of core components for mitochondrial fusion. The results showed that Mfn-2 was downregulated in the CKD group and partially restored by HDD treatment (Figures 4(d)

and 4(e)). Two long isoforms (L1 and L2) and three short isoforms (S1, S2, and S3) of OPA-1 were detected in our study (Figure 4(d)). There was no significant difference in the expression of long isoforms of OPA-1 (L-OPA-1) among groups. But, there was a marked accumulation of short isoforms of OPA-1 (S-OPA-1) in the CKD group, indicative of OPA-1 proteolysis. Notably, the OPA-1 proteolysis was suppressed in the CKD+HDD group (Figures 4(d) and 4(e)). Excessive proteolysis leads to the inactivation of OPA-1 followed by the arrest of inner membrane fusion, contributing to inner membrane cleavage [24]. Therefore, these data collectively indicated that renal mitochondria were more prone to fission rather than fusion in our CKD model and HDD could improve this balance.

4. Discussion

Adenine-induced CKD rat model has been widely used for revealing the action mechanism of CKD and evaluating efficiency of TCM on CKD [25–30]. In the present study, HDD can improve renal function and attenuate renal pathological injury in the adenine-induced CKD rats. Moreover, HDD was found to modulate mitochondrial dynamics in the adenine-induced CKD rats manifested as inhibition of mitochondrial fission and promotion of mitochondrial fusion.

Mitochondria are highly dynamic organelles undergoing coordinated cycles of fission and fusion, referred as 'mitochondrial dynamics', in order to maintain their shape, function, inheritance and quality control [19]. Defects in mitochondrial dynamics have been associated with many biological processes such as apoptosis, autophagy,
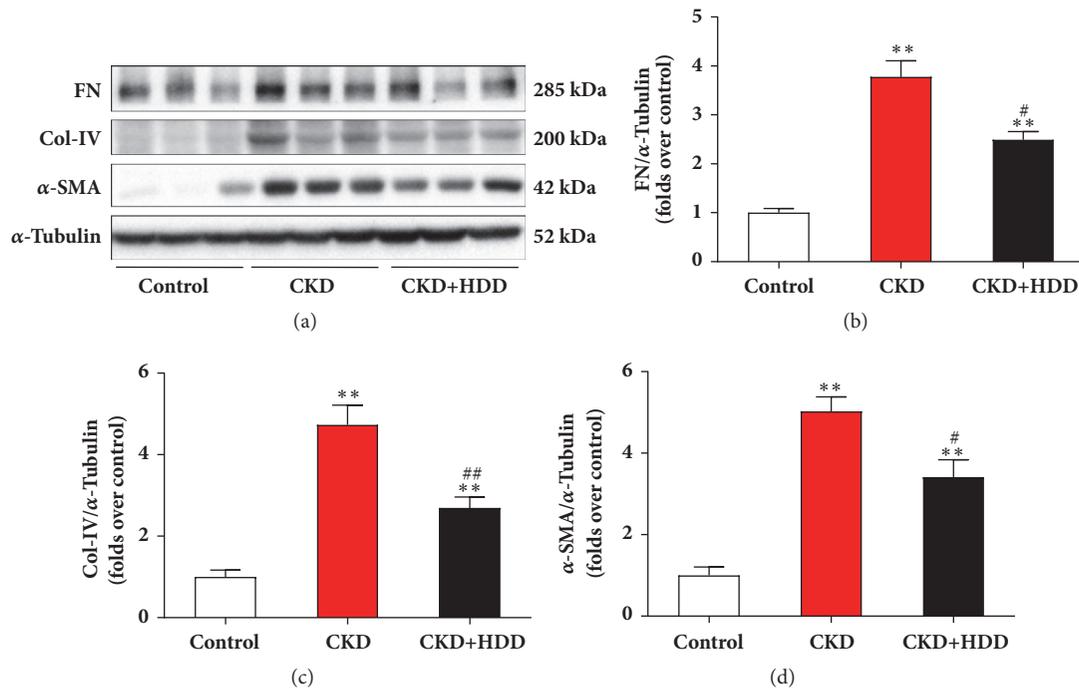
FIGURE 3: HDD inhibited fibrotic markers expression in CKD rats. (a) Representative Western blot images of FN, Col-IV, and α-SMA. (b-d) Densitometric analysis of FN, Col-IV, and α-SMA protein expression, respectively, normalized to α-Tubulin content. Data are presented as the means ± SEM, n=6 rats per group ($^{**}P < 0.01$ compared with the control group; $^{#}P < 0.05$, $^{##}P < 0.01$ compared with the CKD group).

metabolism, development, and aging [31]. Mitochondrial dynamics in mammalian is governed by fission mediators (such as Drp-1, Mid 49/51, and mitochondrial fission factor) and fusion proteins (such as Mfn-1/2 and OPA-1) [32]. Emerging evidence suggested a pathogenic role of mitochondrial dynamics in renal diseases. Gall et al. investigated conditionally deleted MFN2 gene in the kidney of mice and isolated proximal tubular cells. They found these cells showed obvious mitochondrial fragmentation and were sensitive to mitochondrial outer membrane injury and apoptosis following metabolic stress by adenosine triphosphate depletion [33]. Currently, studies on the effect of mitochondrial dynamics on CKD mainly focus on diabetic nephropathy (DN), a leading cause of ESRD. Sun et al. demonstrated a markedly mitochondrial fragmentation along with cristae remodeling during tubular cell apoptosis in diabetic mouse kidneys and high glucose induced HK-2 cells [34]. Another study also found that hyperglycemia-induced mitochondrial fission by promoting Drp-1 recruitment to the mitochondria in DN mouse model [35]. Recently, Zhan et al. demonstrated that mitochondrial fragmentation was an important pathogenic feature of tubular cell injury in human DN [36]. In line with previous studies, our data demonstrated an alteration of mitochondrial dynamics shown as increased fission and decreased fusion in adenine-induced CKD rats. However, in an early 5/6 nephrectomized CKD model, Aparicio-Trejo et al. found that 5/6 nephrectomy shifted mitochondrial dynamics to fusion [37]. Apart from model-specific differences, the duration of CKD (4 weeks

versus 24 hours) maybe also associated with these distinct results.

Mounting evidence has demonstrated that TCM has been widely applied in clinic and established as an effective therapy for the treatment of CKD [38–42]. HDD, a Chinese herbal decoction, was established according to the TCM theory of replenishing Qi and activating blood, which is the basic therapeutic principle of TCM in the treatment of CKD. Since *Astragali Radix* and *Salviae Miltiorrhizae* Radix are representative herbs of Qi-regulating and blood-regulating respectively, our findings provide further evidence in CKD rats for previous patient-based study. The biological effects of *Astragali Radix* have been investigated in several animal models of kidney disease with the effects of anti-inflammation [43] and immune regulation [44]. *Salviae Miltiorrhizae* Radix and its active components could also attenuate kidney injury in streptozotocin-induced diabetic rats [45] and 5/6 nephrectomized rats [46]. Moreover, several studies have demonstrated salvianolic acid A exhibited a beneficial protective effect on glomerulus damage and renal fibrosis [47, 48]. Our study confirmed the renoprotective effect of *Astragali Radix* and *Salviae Miltiorrhizae* Radix by combining application of these two herbs as HDD. Furthermore, our data showed that HDD restored the balance between mitochondrial fission and fusion in CKD rats. Posttranslational modifications of the core components are the main regulatory mechanisms of mitochondrial dynamics, including phosphorylation, ubiquitination, sumoylation, S-nitrosylation, and proteolysis [49]. It is postulated that HDD
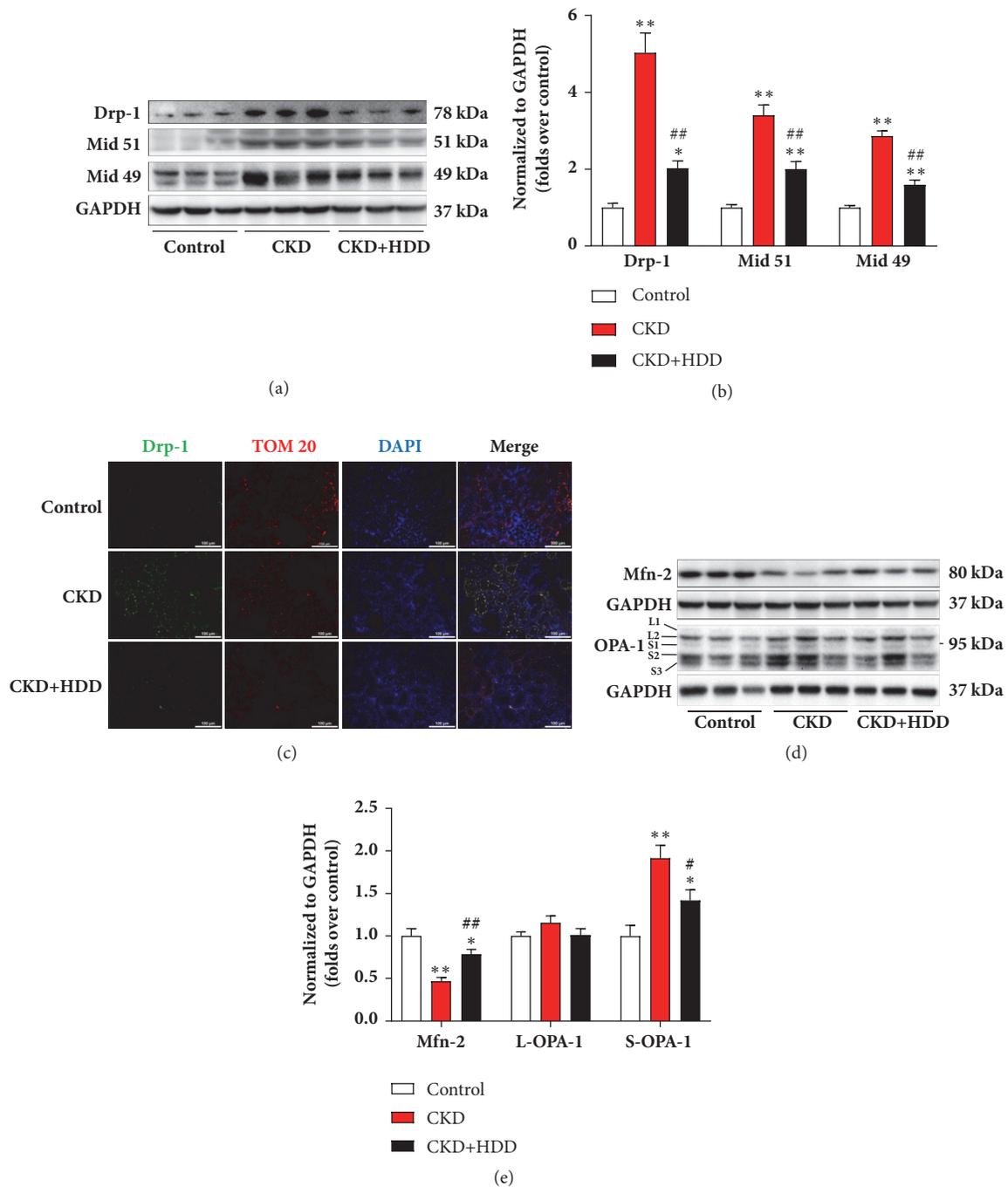
(a)

(b)

(c)

(d)

(e)

FIGURE 4: HDD decreased mitochondrial fission and increased mitochondrial fusion in CKD rats. (a) Representative Western blot images of Drp-1, Mid 51, and Mid 49. (b) Densitometric analysis of Drp-1, Mid 51, and Mid 49 protein expression, normalized to GAPDH content. (c) Representative immunofluorescence images indicating the colocalization of Drp-1 and TOM 20. Green corresponds to Drp-1, red corresponds to TOM 20, and blue corresponds to nuclear staining. All images are shown at identical magnification, ×200, scale bar=100 μm. (d) Representative Western blot images of Mfn-2 and OPA-1 spectrum. (e) Densitometric analysis of Mfn-2, L-OPA-1, and S-OPA-1 protein expression, normalized to GAPDH content. Data are presented as the means ± SEM, n=6 rats per group ($^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group; $^{#}P < 0.05$, $^{##}P < 0.01$ compared with the CKD group).

regulates mitochondrial dynamics by posttranscriptional modification of related proteins. However, the putative target and pathway of HDD need further detailed mechanistic studies.

5. Conclusions

In conclusion, HDD could significantly retard CKD progression, which might be associated with modulation of mitochondrial dynamics.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Xinhui Liu and Shiying Huang are co-first authors. The funders have no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

Supplementary Materials

Supplementary Figure 1. Typical LC-MS chromatogram of HDD. (a) Structures of chemical markers analyzed in HDD extract, including calycosin 7-O-β-glucoside (1), salvianolic acid B (2), astragaloside IV (3), protocatechualdehyde (4), caffeic acid (5), rosmarinic acid (6), and lithospermic acid (7). (b) The representative LC-MS chromatograms of mixed standards and HDD extract. **Supplementary Table 1**. The levels of aspartate transaminase (AST) and alanine transaminase (ALT) in different groups. *(Supplementary Materials)*

References

[1] A. C. Webster, E. V. Nagler, R. L. Morton, and P. Masson, "Chronic kidney disease," *The Lancet*, vol. 389, no. 10075, pp. 1238–1252, 2017.

[2] GBD 2015 DALYs and HALE Collaborators, "Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015," *The Lancet*, vol. 388, no. 10053, pp. 1603–1658, 2015.

[3] M. D. Breyer and K. Susztak, "Developing treatments for chronic kidney disease in the 21st century," *Seminars in Nephrology*, vol. 36, no. 6, pp. 436–447, 2016.

[4] L. Chen, D.-Q. Chen, M. Wang et al., "Role of RAS/Wnt/β-catenin axis activation in the pathogenesis of podocyte injury and tubulo-interstitial nephropathy," *Chemico-Biological Interactions*, vol. 273, pp. 56–72, 2017.

[5] M. Wang, D.-Q. Chen, L. Chen et al., "Novel RAS Inhibitors Poricoic Acid ZG and Poricoic Acid ZH Attenuate Renal Fibrosis via a Wnt/β-Catenin Pathway and Targeted Phosphorylation of smad3 Signaling," *Journal of Agricultural and Food Chemistry*, vol. 66, no. 8, pp. 1828–1842, 2018.

[6] J. E. Pena-Polanco and L. F. Fried, "Established and emerging strategies in the treatment of chronic kidney disease," *Seminars in Nephrology*, vol. 36, no. 4, pp. 331–342, 2016.

[7] D. Chen, Y. Feng, G. Cao, and Y. Zhao, "Natural Products as a Source for Antifibrosis Therapy," *Trends in Pharmacological Sciences*, vol. 39, no. 11, pp. 937–952, 2018.

[8] S. Lovisa, M. Zeisberg, and R. Kalluri, "Partial Epithelial-to-Mesenchymal Transition and Other New Mechanisms of Kidney Fibrosis," *Trends in Endocrinology & Metabolism*, vol. 27, no. 10, pp. 681–695, 2016.

[9] M. Wang, D. Chen, L. Chen et al., "Novel inhibitors of the cellular renin-angiotensin system components, poricoic acids, target Smad3 phosphorylation and Wnt/β-catenin pathway against renal fibrosis," *British Journal of Pharmacology*, vol. 175, no. 13, pp. 2689–2708, 2018.

[10] H. Chen, T. Yang, M.-C. Wang, D.-Q. Chen, Y. Yang, and Y.-Y. Zhao, "Novel RAS inhibitor 25-O-methylalisol F attenuates epithelial-to-mesenchymal transition and tubulo-interstitial fibrosis by selectively inhibiting TGF-β-mediated Smad3 phosphorylation," *Phytomedicine*, vol. 42, pp. 207–218, 2018.

[11] Y. Zhong, M. C. Menon, Y. Deng, Y. Chen, and J. C. He, "Recent advances in traditional chinese medicine for kidney disease," *American Journal of Kidney Diseases*, vol. 66, no. 3, pp. 513–522, 2015.

[12] A. Gong, R. Duan, H. Wang et al., "Evaluation of the Pharmaceutical Properties and Value of Astragali Radix," *Medicines*, vol. 5, no. 2, p. 46, 2018.

[13] H. W. E. Zhang, Z. X. I. Lin, C. Xu, C. Leung, and L. S. U. Chan, "Astragalus (a traditional Chinese medicine) for treating chronic kidney disease," *Cochrane Database of Systematic Reviews*, vol. 10, Article ID CD008369, 2014.

[14] H. Pang, L. Wu, Y. Tang, G. Zhou, C. Qu, and J. Duan, "Chemical analysis of the herbal medicine salviae miltiorrhizae radix et rhizoma (Danshen)," *Molecules*, vol. 21, no. 1, article 51, 2016.

[15] P. Liu, H. Zhao, and Y. Luo, "Anti-aging implications of Astragalus Membranaceus (Huangqi): A well-known Chinese tonic," *Aging and Disease (A&D)*, vol. 8, no. 6, pp. 868–886, 2017.

[16] Q. Mahmood, G.-F. Wang, G. Wu et al., "Salvianolic acid A inhibits calpain activation and eNOS uncoupling during focal cerebral ischemia in mice," *Phytomedicine*, vol. 25, pp. 8–14, 2017.

[17] M. Picard, D. C. Wallace, and Y. Burelle, "The rise of mitochondria in medicine," *Mitochondrion*, vol. 30, pp. 105–116, 2016.

[18] R. Che, Y. Yuan, S. Huang, and A. Zhang, "Mitochondrial dysfunction in the pathophysiology of renal diseases," *American Journal of Physiology-Renal Physiology*, vol. 306, no. 4, pp. F367–F378, 2014.

[19] B. Westermann, "Mitochondrial fusion and fission in cell life and death," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 12, pp. 872–884, 2010.

[20] M. Zhan, C. Brooks, F. Liu, L. Sun, and Z. Dong, "Mitochondrial dynamics: regulatory mechanisms and emerging role in renal pathophysiology," *Kidney International*, vol. 83, no. 4, pp. 568–581, 2013.

[21] R. Thakur, A. Sharma, M. C. Lingaraju et al., "Ameliorative effect of ursolic acid on renal fibrosis in adenine-induced chronic kidney disease in rats," *Biomedicine & Pharmacotherapy*, vol. 101, pp. 972–980, 2018.

[22] J. Lu, X. Liu, Y. Liao et al., "Jian-Pi-Yi-Shen Formula Regulates Inflammatory Cytokines Production in 5/6 Nephrectomized Rats via Suppression of NF-kappaB Activation," *Evidence-Based Complementary and Alternative Medicine*, vol. 2018, Article ID 7203547, 7 pages, 2018.

[23] X. Liu, J. Wu, J. Wang et al., "Possible role of mitochondrial injury in Caulis Aristolochia manshuriensis-induced chronic aristolochic acid nephropathy," *Drug and Chemical Toxicology*, vol. 40, no. 1, pp. 115–124, 2017.

[24] R. Anand, T. Wai, M. J. Baker et al., "The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission," *The Journal of Cell Biology*, vol. 204, no. 6, pp. 919–929, 2014.

[25] S. Ma, Y. Shang, H. Zhang, W. Su, and Y. Zhao, "Action Mechanisms and Therapeutic Targets of Renal Fibrosis," *Journal of Nephrology Advances*, vol. 1, no. 2, pp. 4–14, 2016.

[26] Z.-H. Zhang, N. D. Vaziri, F. Wei, X.-L. Cheng, X. Bai, and Y.-Y. Zhao, "An integrated lipidomics and metabolomics reveal nephroprotective effect and biochemical mechanism of Rheum officinale in chronic renal failure," *Scientific Reports*, vol. 6, Article ID 22151, 2016.

[27] Z.-H. Zhang, F. Wei, N. D. Vaziri et al., "Metabolomics insights into chronic kidney disease and modulatory effect of rhubarb against tubulointerstitial fibrosis," *Scientific Reports*, vol. 5, no. 5, article 14472, 2015.

[28] Z. Zhang, M. Li, D. Liu et al., "Rhubarb Protect Against Tubulointerstitial Fibrosis by Inhibiting TGF-β/Smad Pathway and Improving Abnormal Metabolome in Chronic Kidney Disease," *Frontiers in Pharmacology*, vol. 9, no. 1029, 2018.

[29] Y.-Y. Zhao, X.-L. Cheng, J.-H. Cui et al., "Effect of ergosta-4,6,8(14),22-tetraen-3-one (ergone) on adenine-induced chronic renal failure rat: A serum metabonomic study based on ultra performance liquid chromatography/high-sensitivity mass spectrometry coupled with MassLynx i-FIT algorithm," *Clinica Chimica Acta*, vol. 413, no. 19-20, pp. 1438–1445, 2012.

[30] Y.-Y. Zhao, X.-L. Cheng, F. Wei et al., "Intrarenal metabolomic investigation of chronic kidney disease and its TGF-β1 mechanism in induced-adenine rats using UPLC Q-TOF/HSMS/MS E," *Journal of Proteome Research*, vol. 12, no. 2, pp. 692–703, 2013.

[31] A. Y. Seo, A. M. Joseph, D. Dutta, J. C. Y. Hwang, J. P. Aris, and C. Leeuwenburgh, "New insights into the role of mitochondria in aging: mitochondrial dynamics and more," *Journal of Cell Science*, vol. 123, no. 15, pp. 2533–2542, 2010.

[32] R. A. Gottlieb and D. Bernstein, "Mitochondrial remodeling: rearranging, recycling, and reprogramming," *Cell Calcium*, vol. 60, no. 2, pp. 88–101, 2016.

[33] J. M. Gall, Z. Wang, M. Liesa et al., "Role of mitofusin 2 in the renal stress response," *PLoS ONE*, vol. 7, no. 1, Article ID e31074, 2012.

[34] L. Sun, P. Xie, J. Wada et al., "Rap1b GTPase ameliorates glucose-induced mitochondrial dysfunction," *Journal of the American Society of Nephrology*, vol. 19, no. 12, pp. 2293–2301, 2008.

[35] W. Wang, Y. Wang, J. Long et al., "Mitochondrial fission triggered by hyperglycemia is mediated by ROCK1 activation in podocytes and endothelial cells," *Cell Metabolism*, vol. 15, no. 2, pp. 186–200, 2012.

[36] M. Zhan, I. Usman, J. Yu et al., "Perturbations in mitochondrial dynamics by p66Shc lead to renal tubular oxidative injury in human diabetic nephropathy," *Clinical Science*, vol. 132, no. 12, pp. 1297–1314, 2018.

[37] O. E. Aparicio-Trejo, E. Tapia, E. Molina-Jijón et al., "Curcumin prevents mitochondrial dynamics disturbances in early 5/6 nephrectomy: Relation to oxidative stress and mitochondrial bioenergetics," *BioFactors*, vol. 43, no. 2, pp. 293–310, 2017.

[38] L. Chen, T. Yang, D. Lu et al., "Central role of dysregulation of TGF-β/Smad in CKD progression and potential targets of its treatment," *Biomedicine & Pharmacotherapy*, vol. 101, pp. 670–681, 2018.

[39] M. Wang, D.-Q. Chen, M.-C. Wang et al., "Poricoic acid ZA, a novel RAS inhibitor, attenuates tubulo-interstitial fibrosis and podocyte injury by inhibiting TGF-β/Smad signaling pathway," *Phytomedicine*, vol. 36, pp. 243–253, 2017.

[40] Y. Tu, L. Gu, D. Chen et al., "Rhein Inhibits Autophagy in Rat Renal Tubular Cells by Regulation of AMPK/mTOR Signaling," *Scientific Reports*, vol. 7, no. 1, Article ID 43790, 2017.

[41] J. Ren, J. Li, X. Liu et al., "Quercetin Inhibits Fibroblast Activation and Kidney Fibrosis Involving the Suppression of Mammalian Target of Rapamycin and β-catenin Signaling," *Scientific Reports*, vol. 6, article 23968, no. 1, 2016.

[42] H. Chen, G. Cao, D. Q. Chen et al., "Metabolomics insights into activated redox signaling and lipid metabolism dysfunction in chronic kidney disease progression," *Redox Biology*, vol. 10, pp. 168–178, 2016.

[43] M. Shahzad, D. M. Small, C. Morais, K. Wojcikowski, A. Shabbir, and G. C. Gobe, "Protection against oxidative stress-induced apoptosis in kidney epithelium by *Angelica* and *Astragalus*," *Journal of Ethnopharmacology*, vol. 179, pp. 412–419, 2016.

[44] Y. Xin, G. Li, H. Liu, and D. Ai, "AS-IV protects against kidney IRI through inhibition of NF-kappaB activity and PUMA upregulation," *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 10, pp. 18293–18301, 2015.

[45] D. Yin, J. Yin, Y. Yang, S. Chen, and X. Gao, "Renoprotection of Danshen Injection on streptozotocin-induced diabetic rats, associated with tubular function and structure," *Journal of Ethnopharmacology*, vol. 151, no. 1, pp. 667–674, 2014.

[46] H. Zhang, Y. Wang, C. Gao et al., "Salvianolic acid A attenuates kidney injury and inflammation by inhibiting NF-κB and p38 MAPK signaling pathways in 5/6 nephrectomized rats," *Acta Pharmacologica Sinica*, vol. 39, no. 12, pp. 1855–1864, 2018.

[47] D. Chen, H. Hu, Y. Wang, Y. Feng, G. Cao, and Y. Zhao, "Natural products for the prevention and treatment of kidney disease," *Phytomedicine*, vol. 50, pp. 50–60, 2018.

[48] H. Hu, D. Chen, Y. Wang et al., "New insights into TGF-β/Smad signaling in tissue fibrosis," *Chemico-Biological Interactions*, vol. 292, pp. 76–83, 2018.

[49] L. Tilokani, S. Nagashima, V. Paupe, and J. Prudent, "Mitochondrial dynamics: overview of molecular mechanisms," *Essays in Biochemistry*, vol. 62, no. 3, pp. 341–360, 2018.