OPEN ACCESS Freely available online

Journal of Cell Science & Therapy

Research Article

A Multi-Herb Botanical Hydrosol Attenuates Inflammation and Fibrosis While Enhancing Mitochondrial Function in Renal Cell Models

Yung-Kai Lin[1], Jian-Xin Wang[2], Yung-Hsiang Lin[3,4], Shu-Ting Chan[3], Yi-Wen Mao[3], Chi-Fu Chiang[3*]

[1]*Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan.*

[2]*Shanxi Agricultural Valley BaoRenTang Food Co., Ltd., Shanxi, China*

[3]*Research and Design Center, TCI CO., Ltd., Taipei, Taiwan*

[4]*Institute of Medical Genetics, TCI GENE INC, Taipei, Taiwan*

ABSTRACT

Chronic Kidney Disease (CKD) is a progressive condition characterized by inflammation, fibrosis, fluid imbalance, and mitochondrial dysfunction. While traditional medicine has long employed kidney-tonifying botanicals, their cellular mechanisms remain underexplored. This study evaluated a multi-herb botanical hydrosol formulation containing *Alpinia oxyphylla Miq.* and fermented extracts of *Polygonatum kingianum*, *Euryale ferox*, and *Lycium chinense*. Using renal epithelial cell models, we assessed its effects on inflammation, fibrosis, aquaporin regulation and mitochondrial gene activation. The hydrosol significantly inhibited Nitric Oxide (NO) production in LPS-stimulated macrophages. In *IL-1β*-treated HEK293 cells, it induced a time-dependent immunoregulatory response-enhancing anti-inflammatory cytokines (*IL-6*, *IL-8*, *IL-10*) at 6 hours, while suppressing pro-inflammatory cytokines (*IL-12A*, *IFN-γ*) at 24 hours. In *TGF-β1*-induced fibrotic models, it reduced extracellular matrix accumulation and preserved epithelial morphology. Although Aquaporin-3 (AQP3) expression showed a non-significant increase, a positive regulatory trend was observed. Notably, the formulation upregulated mitochondrial and proteostasis-related genes, including a >4-fold increase in Parkin and significant elevations in *Ubl5*, *NADSYN1*, *Atg8*, and chaperonin subunits *CCT2*, *CCT6A*, and *CCT8*. These findings suggest that the formulation exerts anti-inflammatory, antifibrotic, and mitochondrial-activating effects. Overall, this work provides mechanistic support for the potential application of hydrosol-based functional foods in promoting renal health and healthy aging.

Keywords: Botanical hydrosol; Chronic kidney disease; Inflammation; Mitochondrial activation; Functional food; Fermented herbal extract

INTRODUCTION

Chronic Kidney Disease (CKD) is an increasingly serious global health issue characterized by progressive loss of renal function, inflammation, and fibrosis, often exacerbated by lifestyle-related factors such as high-sodium diets and hyperglycaemia [1]. Concomitantly, fluid retention, cellular senescence, and impaired aquaporin-mediated water regulation further contribute to disease progression and diminished quality of life [2-4]. These pathological changes can accelerate glomerular hypertension, impair tubular reabsorption, disrupt electrolyte balance, and promote renal interstitial fibrosis-ultimately leading to End-Stage Renal Disease (ESRD) [5]. Although pharmacological interventions-such as Renin-Angiotensin System (RAS) inhibitors-are available, the global prevalence of CKD continues to rise, prompting growing interest in adjunctive nutritional strategies rooted in traditional medicine and functional food science. Epidemiological data show that high dietary sodium intake exacerbates hypertension and proteinuria in CKD patients, while chronic hyperglycemia is a major driver of CKD onset and progression, especially in type 2 diabetes [6,7].

Traditional Chinese Medicine (TCM) has long emphasized the dynamic relationship between kidney health, fluid metabolism, and systemic vitality. In this context, *Alpinia oxyphylla Miq.*, a medicinal and edible herb listed in the chinese pharmacopoeia and included

Correspondence to: Chi-Fu Chiang, Research and Design Center, TCI CO., Ltd., Taipei, Taiwan, E-mail: Jimmy.Chiang@tci-bio.com

Received: 05-Sep-2025, Manuscript No. JCEST-25-38670; **Editor assigned:** 08-Sep-2025, Pre QC No. JCEST-25-38670 (PQ); **Reviewed:** 22-Sep-2025, QC No. JCEST-25-38670; **Revised:** 29-Sep-2025, Manuscript No. JCEST-25-38670 (R); **Published:** 06-Oct-2025, DOI: 10.35248/2157-7013.25.15.492

Citation: Lin YK, Wang JX, Lin YH, Chan ST, Mao YW, Chiang CF (2025). A Multi-Herb Botanical Hydrosol Attenuates Inflammation and Fibrosis While Enhancing Mitochondrial Function in Renal Cell Models. J Cell Sci Therapy.15:492.

in classical formulas such as suoquan pill, is traditionally used to "warm the kidney," "secure essence," and relieve symptoms such as frequent urination, nocturnal emission, and coldness in the lower body. Modern phytochemical analyses have shown that *Alpinia oxyphylla Miq.* contains bioactive compounds including volatile oils (e.g., borneol and camphor), flavonoids, and sesquiterpenes, which exhibit antioxidant, anti-inflammatory, and neuroprotective effects [8,9]. Emerging pharmacological studies have started to elucidate the mechanisms underlying its traditional use. For instance, in LPS-stimulated macrophage and epithelial cell models, *Alpinia oxyphylla Miq.* extracts were found to suppress the production of proinflammatory mediators such as Nitric Oxide (NO), *IL-1β*, and TNF-α through the activation of the Nrf2/HO-1 signaling pathway, highlighting its immunomodulatory potential [10]. In renal contexts, *Alpinia oxyphylla Miq.* volatile oil has demonstrated nephroprotective activity by reducing oxidative stress and renal tissue damage in rodent models of cisplatin-induced acute kidney injury [11]. Furthermore, essential oil components such as camphor and linalool have been implicated in diuretic and smooth muscle-relaxing effects [12], potentially alleviating urinary retention and edema-symptoms commonly associated with early-stage kidney dysfunction [11]. These observations provide modern evidence supporting the traditional classification of *Alpinia oxyphylla Miq.* as a kidney-tonifying and fluid-regulating herb in Chinese medicine.

In addition to *Alpinia oxyphylla Miq.*, botanicals such as *Polygonatum kingianum*, *Euryale ferox* and *Lycium chinense*-commonly recognized under the concept of Medicine-Food Homology (MFH)-have gained attention for their nephroprotective, antioxidant, and metabolic-regulatory properties. For instance, *P. kingianum* can restore mitochondrial membrane potential and activate GSK-3β/Fyn/Nrf2 signaling, while *E. ferox* and *L. chinense* exert anti-inflammatory, metabolic, and mitochondrial-supportive effects [13-15]. Recent studies show that fermentation-assisted hydrolysis enhances the bioactivity of these herbs, converting polysaccharides into low-molecular-weight oligosaccharides, peptides, and flavonoid glycosides, thereby improving cellular uptake and activating protective pathways such as Nrf2, AMPK, and mitophagy [16].

To harness the synergistic effects of these traditional botanicals, we developed a novel formulation consisting of *Alpinia oxyphylla Miq.* hydrosol combined with a fermented herbal complex derived from *P. kingianum*, *E. ferox*, and *L. chinense*. The hydrosol formulation preserves water-soluble and volatile constituents while offering a biocompatible, ingestion-friendly delivery system. Given the involvement of inflammatory signaling, aquaporin dysfunction, and cellular senescence in CKD progression, we hypothesized that this formulation may exert multi-pathway protective effects on renal cells.

We investigated its effects on inflammatory regulation by assessing NO production and cytokine gene expression in LPS and *IL-1β*-stimulated cells. Cytoprotective effects were evaluated under hyperosmotic stress using NaCl-treated Madin-Darby Canine Kidney (MDCK) cells. We further assessed the formulation's ability to restore Aquaporin-3 (AQP3) expression-an essential membrane protein involved in water transport and fluid balance. To examine anti-fibrotic potential, we applied the formulation to *TGF-β1*-induced fibrotic models. Finally, we investigated its influence on aging-related markers, including the expression of Chaperonin-Containing TCP1 complex (CCT) genes and mitochondrial biogenesis regulators. Therefore, this study aimed to systematically evaluate the *in vitro* efficacy of a multi-herb botanical hydrosol-composed of *Alpinia oxyphylla Miq.* and fermented MFH herbs-on renal inflammation, fibrosis, aquaporin regulation, and mitochondrial activation, using established epithelial cell models. To our knowledge, this is the first study to comprehensively characterize such a hydrosol-based formulation targeting CKD-associated mechanisms.

MATERIAL AND METHODS

Test sample and composition

The botanical hydrosol evaluated in this study is a multi-component formulation primarily composed of the hydrosol of *Alpinia oxyphylla Miq.*, combined with a fermented herbal complex derived from three traditional Medicine-Food Homology (MFH) plants: *Polygonatum kingianum* Coll. et Hemsl. (rhizome), *Euryale ferox* Salisb. (seed), and *Lycium chinense* Mill. (seed). These botanicals were selected based on their historical use in kidney-tonifying formulations in traditional Chinese medicine, as documented in the Chinese pharmacopoeia. The raw herbal materials were authenticated by TCI Co., Ltd. (Taipei, Taiwan) and fermented using a food-grade microbial fermentation process under controlled temperature and pH. The final formulation also contained plant-derived excipients, including concentrated juices of purple carrot (*Daucus carota*), mulberry (*Morus alba*), and white gourd (*Benincasa hispida*), raspberry (*Rubus idaeus*) powder, pectin, soy lecithin, gum acacia, zinc gluconate, citric acid, sucralose, and purified water. The product was manufactured by TCI Co., Ltd. and supplied by Shanxi Agricultural Valley BaoRenTang Food Co., Ltd. (Shanxi, China). For experiments, the hydrosol was freshly diluted in sterile water to the indicated concentrations immediately prior to use.

Cell culture

Three cell lines were used: RAW 264.7 murine macrophages (ATCC TIB-71, USA), Madin-Darby Canine Kidney (MDCK) cells (ATCC CCL-34, USA), and HEK293 human embryonic kidney cells (ATCC CRL-1573, USA). RAW 264.7 cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% Fetal Bovine Serum (FBS; Gibco, USA) and 1% penicillin-streptomycin. MDCK cells were maintained in Minimum Essential Medium (MEM; Gibco, USA) supplemented with 10% FBS, 1% penicillin-streptomycin, and 1 mM sodium pyruvate. HEK293 cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution (Gibco, USA). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Nitric Oxide (NO) production assay

Anti-inflammatory activity was assessed by measuring Nitric Oxide (NO) production in RAW 264.7 cells using a Griess assay. Cells were seeded at 1 × 10^4 cells/well in 96-well plates and allowed to adhere for 24 h. Cells were stimulated with lipopolysaccharide (LPS, 200 ng/mL; Sigma-Aldrich, USA) in serum-free DMEM with or without the hydrosol (0.0625% v/v) for 24 h. Supernatants were collected, mixed with freshly prepared Griess reagent (Invitrogen™ G-7921, Thermo Fisher Scientific, USA), incubated for 30 min at room temperature, and

absorbance was measured at 548 nm (Epoch™, BioTek, USA). NO production was expressed relative to the LPS-only group (set as 100%).

Cell viability and cytoprotection under hyperosmotic stress

Cytoprotective effects were assessed using MDCK cells exposed to hyperosmotic stress. Cells were seeded at 3 × 10^3 cells/well in 96-well plates, cultured overnight, and then treated with hyperosmotic MEM (500 mOsm/kg, adjusted with NaCl) with or without hydrosol (0.0625% v/v) for 48 h. Viability was assessed using an MTT assay: Cells were incubated with MTT solution (5 mg/mL; Sigma-Aldrich, USA) for 2-3 h, medium was removed, formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. Viability was expressed as a percentage of isotonic control cells.

Aquaporin-3 (AQP3) expression

To examine the regulation of renal water channels, AQP3 expression was measured by immunofluorescence in MDCK cells. Cells were seeded at 2 × 10^4 cells/well in 24-well plates, treated with or without hydrosol (0.125% v/v) for 24 h, fixed with 10% formaldehyde, permeabilized with 0.5% Triton X-100, and blocked with 1% Bovine Serum Albumin (BSA). Cells were incubated with primary anti-AQP3 antibody (1:1000, Boster, USA) overnight at 4°C, followed by Alexa Fluor 488-conjugated secondary antibody (1:2000, Thermo Fisher Scientific, USA) for 1 h at 37°C. Nuclei were counterstained with Hoechst 33342. Images were acquired with an inverted fluorescence microscope (Axio Vert.A1, ZEISS, Germany), and mean fluorescence intensity was quantified using ImageJ software.

TGF-β1-Induced Fibrosis Assay

Antifibrotic effects were evaluated using MDCK cells stimulated with *TGF-β1*. Cells were seeded at 2 × 10^4 cells/well in 24-well plates and treated with *TGF-β1* (5 ng/mL; Sigma-Aldrich, USA) with or without hydrosol (0.0625% v/v) for 48 h. Cells were then fixed, permeabilized, and stained with ActinRed™ 555 (Thermo Fisher Scientific, USA) to visualize cytoskeletal structures, while nuclei were counterstained with Hoechst 33342. Fibrotic changes were qualitatively evaluated by fluorescence microscopy.

Quantitative PCR for cytokine and aging-related gene expression

Gene expression analysis was performed in HEK293 cells. For inflammatory markers, cells were treated with recombinant *IL-1β* (10 ng/mL; PeproTech, USA) with or without hydrosol (0.125% v/v) for 6 or 24 h. For aging-related markers, cells were treated with hydrosol (0.125% v/v) for 48 h. Total RNA was extracted (Geneaid, Taiwan), and 2000 ng was reverse-transcribed using SuperScript® III Reverse Transcriptase (Invitrogen, USA). qPCR was performed using KAPA SYBR® FAST qPCR Master Mix (KAPA Biosystems, USA) on a Step One Plus system (Applied Biosystems, USA). For inflammatory assays, target genes included *IL-6*, *IL-8*, *IL-10*, *IL-12A*, *IFN-γ* (main figures), and *IL-3*, *IL-18*, *IL-23* (supplementary). For aging-related assays, target genes included *CCT2*, CCT5, *CCT6A*, *CCT7*, *CCT8*, Parkin, *Atg8*, *NADSYN*, and *Ubl5*. *GAPDH* served as the internal reference. Primer sequences for all target genes are listed in Table 1. Primer sequences were designed and validated using Primer-BLAST (NCBI) and synthesized by genomics (New Taipei City, Taiwan). Relative gene expression was calculated using the 2^-ΔΔCt method.

Table 1: Primer sequences used for qPCR analysis.

Gene	Forward Primer	Reverse Primer
IL-3	CCTGCCGATCCAAACATG	CCCCATTGAGGTTGTTGAAGTC
IL-6	GGTACATCCTCGACGGCATCT	GTGCCTCTTTGCTGCTTTCAC
IL-8	ACTGAGAGTGATTGAGAGTGGAC	AACCCTCTGCACCCAGTTTTC
IL-10	TCAAGGCGCATGTGAACTCC	GATGTCAAACTCACTCATGGCT
IL-12A	GATGGCCCTGTGCCTTAGTAGTAT	GGGCCTGCATCAGCTCAT
IL-18	TCTTCATTGACCAAGGAAATCGG	TCCGGGGTGCATTATCTCTAC
IL-23	AGCAGCAATTAAGAACTGCCA	TACCAAAGCCGAGCTGTTGTT
IFN-γ	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
CCT2	AAGCCACGAAGGCTGCAA	TCATCGGAACCATGATCAACTG
CCT5	CGGATAAGTGCCCCACCTTA	TCCAGTGCGTCGGCAAA
CCT6A	TGGCCAGAACATCTCTTCGTACT	AGTCCACTACAGCCTCTGTTAAGACA
CCT7	GTGGCATGGACAAGCTTATTGTAG	CAGAATTGTGGCCCCATCA
CCT8	ACCCGGAGGTGGAGCAA	GGACATGTCTCTCCATATGATGTGA
Parkin	GCAGAGACCGTGGAGAAAAG	CTTTTCTCCACGGTCTCTGC
Atg8	TATCCAGACCGTGTGCCCGTC	GTGGATGCGCTTGCGAATGAGG
NADSYN	GCAAAATGTGCAGGCTCGAA	GCACTGGAGCAGTCGTACTT
Ubl-5	TCCTAGCGTTAACTGCGACC	CTAGCTGGAGCTCGAATCGC
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Statistical analysis

All results are expressed as mean ± Standard Deviation (SD). Group differences were analyzed using Student's t-test (Microsoft Excel, USA). A p-value <0.05 was considered statistically significant.

RESULTS

Nitric oxide inhibition by botanical hydrosol

To evaluate the anti-inflammatory potential of the botanical hydrosol, NO production was quantified in LPS-stimulated macrophages using the Griess assay. Stimulation with LPS significantly elevated NO levels compared to the unstimulated control (set as 100%) as shown in Figure 1. Treatment with the botanical hydrosol (0.0625%) markedly suppressed NO production to 85.7 ± 3.9% relative to the LPS group ($p<0.05$, $n=3$), indicating a statistically significant inhibition. Notably, NO levels in the hydrosol-treated group were even lower than those in the untreated control (87.9 ± 2.1%), suggesting a potent anti-inflammatory response.

Cytoprotective effect of botanical hydrosol under hyperosmotic stress

To assess the cytoprotective potential of the botanical hydrosol, MDCK cells were subjected to hyperosmotic stress induced by high-salt treatment (NaCl, final concentration: +50 mM) for 24 hours, with or without co-treatment with the botanical hydrosol (0.0625%). High-salt exposure significantly reduced cell viability to 46.5 ± 0.0% relative to the untreated control (100 ± 5.2%, $p<0.001$) as shown in Figure 2. Co-treatment with the botanical hydrosol partially restored viability to 58.1 ± 21.0%, although the difference compared to the high-salt group was not statistically significant ($p=0.393$). These data suggest a modest trend of protection against salt-induced cytotoxicity, albeit with variability across replicates.

Upregulation of Aquaporin-3 (AQP3) expression by botanical hydrosol

To evaluate the regulatory effect of the botanical hydrosol on water transport proteins, AQP3 expression was assessed in MDCK cells using immunofluorescence staining. After 24-hour treatment with the botanical hydrosol (0.125%), cells exhibited a modest increase in green fluorescence intensity corresponding to AQP3 expression as shown in Figure 3A. Quantitative analysis of relative Mean Fluorescence Intensity (MFI) revealed a 5.4% increase in the botanical hydrosol group compared to the control group (105.4 ± 7.0% *vs.* 100.0 ± 5.0%, $p=0.340$) as shown in Figure 3B. While the increase in AQP3 expression did not reach statistical significance, the observed trend may suggest a potential regulatory effect of the botanical hydrosol on water channel activity.

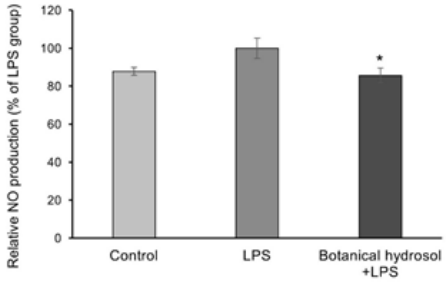

Figure 1: Inhibitory effect of the botanical hydrosol on nitric oxide production in LPS-stimulated macrophages. **Note:** RAW264.7 cells were treated with lipopolysaccharide (LPS, 1 μg/mL) in the absence or presence of the botanical hydrosol (0.0625%) for 24 hours. NO content in the culture supernatant was measured using the Griess assay and normalized to the LPS-only group (set as 100%). Data are expressed as mean ± SD (n=3). *p<0.05 compared to LPS group (Student's t-test).

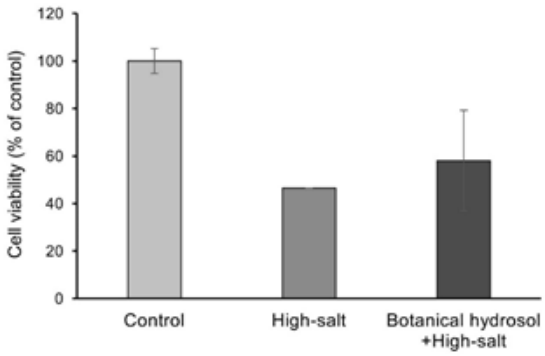

Figure 2: Cytoprotective effect of the botanical hydrosol against high-salt-induced cell stress. **Note:** MDCK cells were exposed to hyperosmotic conditions by supplementing the medium with NaCl (final concentration: +50 mM) for 24 hours, with or without co-treatment with the botanical hydrosol (0.0625%). Cell viability was assessed using the MTT assay and expressed as a percentage relative to the untreated control group (set as 100%). High-salt treatment significantly reduced cell viability, while co-treatment with the botanical hydrosol showed a partial, non-significant restoration. Data are presented as mean ± SD (n=3). Statistical comparisons were performed using Student's t-test.

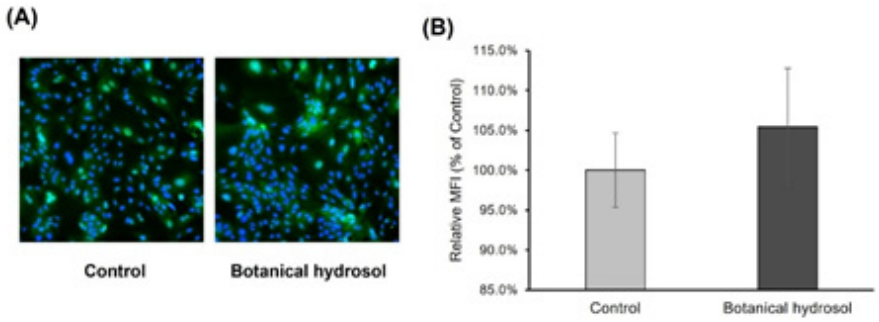

Figure 3: Effect of botanical hydrosol on aquaporin-3 expression in MDCK cells. **Note:** (A) Representative immunofluorescence images showing AQP3 expression (green) and nuclear staining (blue) after 24-hour treatment with or without the botanical hydrosol (0.125%). (B) Quantification of relative mean fluorescence intensity (MFI), expressed as % of the control group. Data are shown as mean ± SD (n=3). Statistical comparisons were performed using Student's t-test.

Attenuation of *TGF-β1*-induced fibrotic phenotype by botanical hydrosol

To assess the anti-fibrotic potential of the botanical hydrosol, MDCK cells were stimulated with *TGF-β1* (5 ng/mL) for 48 hours to induce fibrotic changes, with or without co-treatment with the botanical hydrosol (0.0625%). Immunofluorescence staining revealed increased extracellular matrix deposition and fiber-like structures (yellow signal) in the *TGF-β1*-treated group, indicative of a fibrotic phenotype, compared to untreated controls as shown in Figure 4. In contrast, co-treatment with the botanical hydrosol visibly reduced these fibrotic features, resulting in a more organized and less densely stained pattern resembling that of the control. These findings suggest that the botanical hydrosol may attenuate *TGF-β1*-induced fibrotic responses in renal epithelial cells.

Botanical hydrosol modulates inflammatory cytokine gene expression

To investigate the immunomodulatory effects of the botanical hydrosol, the expression of inflammation-related cytokines was assessed in *IL-1β*-stimulated HEK293 cells using quantitative RT-PCR. At the 6-hour time point, co-treatment with the botanical hydrosol (0.125%) significantly increased the expression of anti-inflammatory cytokines *IL-6* (1.79 ± 0.20, $p<0.01$), *IL-8* (1.50 ± 0.18, $p<0.05$), and *IL-10* (1.45 ± 0.32, $p<0.05$) as shown in Figure-5A compared to the *IL-1β*-only group, suggesting a compensatory anti-inflammatory response during early stimulation. At 24 hours, the expression of pro-inflammatory cytokines *IL-12A* and *IFN-γ* was significantly suppressed in the botanical hydrosol group (0.64 ± 0.06 and 0.61 ± 0.03, respectively; $p<0.05$) as shown in Figure 5B, indicating an attenuation of sustained inflammatory signalling. Additional genes including *IL-3*, *IL-18*, and *IL-23* also exhibited downregulation trends at 24 hours, though without statistical significance. These results suggest a broader immunoregulatory potential of the botanical hydrosol (Supplementary Figure 1).

Anti-aging gene expression: CCT family and mitochondrial activation

To explore the anti-aging potential of the botanical hydrosol, gene expression levels of the Chaperonin-Containing TCP1 complex (CCTs) family and mitochondrial activation-related markers were evaluated in HEK293 cells after 48-hour treatment. Hydrosol treatment significantly upregulated several CCT genes, including *CCT2* ($p<0.05$), *CCT6A* and *CCT8* ($p<0.01$), while CCT5 and *CCT7* showed non-significant increases as shown in Figure-6A. In addition, mitochondrial function-related genes also showed enhanced expression. Parkin expression increased by over fourfold ($p<0.01$), accompanied by significant upregulation of Ubl-5 ($p<0.001$), *NADSYN* ($p<0.01$), and *Atg8* ($p<0.05$) as shown in Figure 6B. These results suggest that the botanical hydrosol may support cellular rejuvenation and mitochondrial activity *via* modulation of aging-related gene networks.

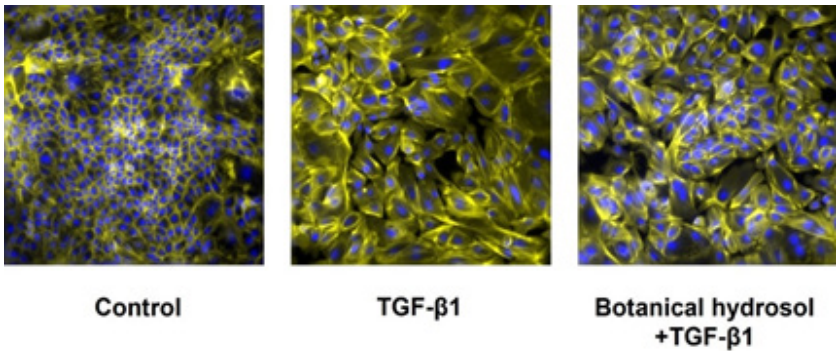

Figure 4: Anti-fibrotic effect of botanical hydrosol in a *TGF-β1*-induced renal fibrosis model. **Note:** MDCK cells were treated with *TGF-β1* (5 ng/mL) for 48 hours to induce fibrosis, with or without co-treatment with the botanical hydrosol (0.0625%). Fibrotic changes were assessed using immunofluorescence staining of extracellular matrix proteins (yellow), and nuclei were counterstained with DAPI (blue). Representative images show increased fibrotic structures in the *TGF-β1* group, while co-treatment with botanical hydrosol reduced matrix deposition and restored epithelial organization.

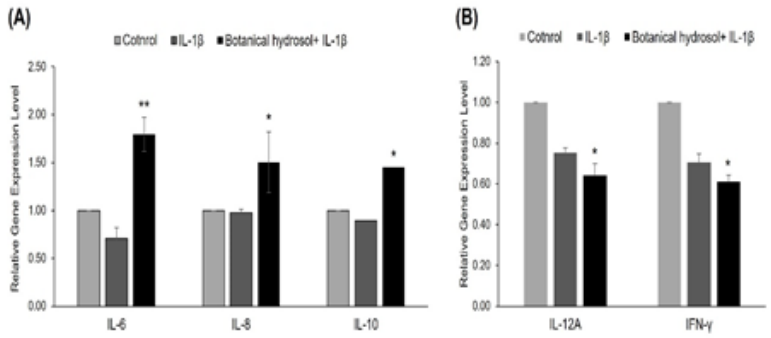

Figure 5: Modulation of inflammatory cytokine gene expression by botanical hydrosol in *IL-1β*-stimulated HEK293 cells. **Note:** (A) Relative mRNA expression levels of *IL-6*, *IL-8*, and *IL-10* after 6 hours of *IL-1β* stimulation with or without botanical hydrosol (0.125%). (B) Gene expression of *IL-12A* and *IFN-γ* measured at 24 hours under the same conditions. Gene expression was normalized to *GAPDH* and calculated using the 2^-ΔΔCt method. Values are expressed as fold changes relative to the *IL-1β*-only group (set as 1.0). Data are presented as mean ± SD (n=3). Statistical comparisons were performed using Student's t-test. *p<0.05, **p<0.01 *vs. IL-1β*-only group.

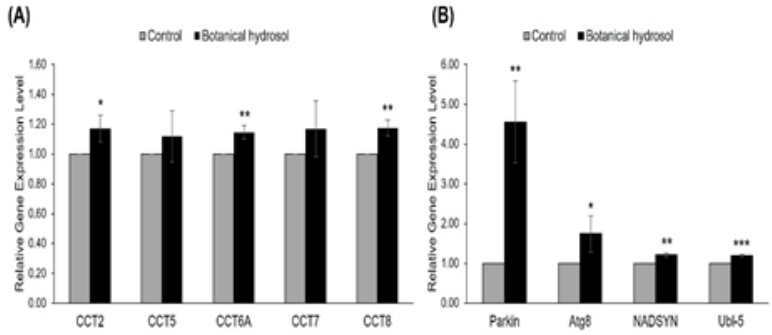

Figure 6: Effects of botanical hydrosol on anti-aging gene expression in HEK293 cells. **Note:** (A) Relative expression levels of CCT family genes (*CCT2*, CCT5, *CCT6A*, *CCT7*, *CCT8*) following 48-hour treatment. (B) Relative expression of mitochondrial activation markers, including Parkin, *Atg8*, *NADSYN*, and Ubl-5. Data are presented as mean ± SD (n=3), normalized to control group (set as 1.0). Statistical significance was evaluated using Student's t-test. * p<0.05, ** p<0.01, *** p<0.001 *vs.* control group..

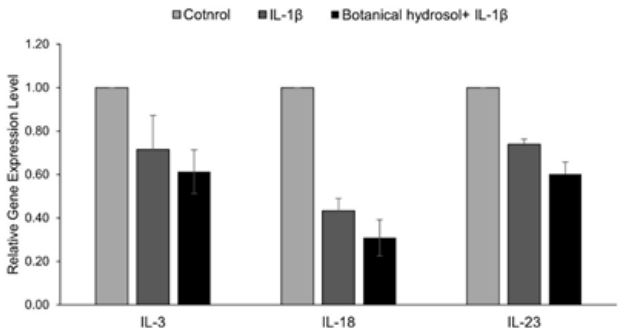

Figure S1: Expression of additional inflammatory cytokines in *IL-1β*-stimulated HEK293 cells. **Note:** Relative mRNA expression levels of *IL-3*, *IL-18*, and *IL-23* were analyzed 24 hours after *IL-1β* stimulation with or without botanical hydrosol (0.125%). Gene expression was normalized to *GAPDH* and expressed as fold change relative to the *IL-1β*-only group. Data are shown as mean ± SD (n=3). Statistical comparisons were performed using Student's t-test

DISCUSSION

CKD is a multifactorial condition involving immune dysregulation, fibrosis, impaired water transport, and mitochondrial dysfunction [17-19]. Our *in vitro* evaluation demonstrates that a multi-herb botanical hydrosol formulation-composed of *Alpinia oxyphylla Miq.* and fermented extracts-modulates multiple CKD-relevant pathways, including NO suppression, anti-inflammatory cytokine induction, fibrosis attenuation, AQP3 regulation, and mitochondrial gene activation. Botanical compounds derived from *Alpinia oxyphylla Miq.* have been traditionally used to "warm the kidney" and regulate fluid balance in Chinese medicine [8,20]. Modern research supports its anti-inflammatory potential *via* Nrf2/HO-1 activation, primarily attributed to volatile oils such as borneol and camphor-findings consistent with our observed inhibition of NO and upregulation of *IL-6/IL-10* [21-22]. In addition to *Alpinia oxyphylla Miq.*, the inclusion of fermented *P. kingianum* (yellow essence root), *E. ferox*

(gorgon fruit), and *L. chinense* (goji berry) may have contributed synergistically to the observed bioactivities. These three herbs are classified as MFH (medicine-food homology) botanicals in Chinese medicine, known for their integrative health benefits. Polygonatum species are representative of Medicine-Food Homology (MFH) herbs, traditionally used to tonify the kidney, reinforce essence, and improve vitality [23]. *P. kingianum* is rich in saponins, polysaccharides, and homoisoflavonoids, which exhibit antioxidant, mitochondrial-protective, and immunomodulatory effects [13,24]. *E. ferox* contains polyphenols and alkaloids with known anti-inflammatory and diuretic properties, while *L. chinense* has been reported to support mitochondrial biogenesis and cellular energy regulation, partly attributed to its active component betaine [25,26]. The biological activities of these MFH herbs may be further enhanced through fermentation-assisted hydrolysis, which converts macromolecular components into low-molecular-weight metabolites, such as bioactive peptides, flavonoid glycosides, and oligosaccharides [27,28]. These smaller compounds are more readily absorbed by cells and have been shown to activate cytoprotective signaling pathways, including Nrf2, AMPK, and mitophagy-related cascades, thereby potentially amplifying the effects of individual herbal components through a synergistic network response.

Chronic inflammation and fibrotic remodeling are critical contributors to the progression of CKD, often perpetuated by dysregulated cytokine signaling and sustained oxidative stress [29]. Our findings extend prior knowledge by demonstrating that the botanical hydrosol not only suppresses NO production but also exerts time-dependent immunomodulatory effects. At the early phase (6 h), co-treatment significantly enhanced *IL-6*, *IL-8*, and *IL-10* expression-cytokines known to mediate tissue repair and inflammation resolution [30-32]. At the late phase (24 h), downregulation of *IL-12A* and *IFN-γ* was observed, indicating a potential shift away from Th1-polarized immune activation [33]. These observations suggest that the formulation may influence both innate and adaptive immune dynamics, possibly through macrophage polarization or dendritic cell regulation [34]. In parallel, the botanical hydrosol effectively mitigated *TGF-β1*-induced fibrotic changes in MDCK renal epithelial cells. *TGF-β1* is a master profibrotic cytokine that activates SMAD2/3-dependent transcription of Extracellular Matrix (ECM) genes such as fibronectin and collagen type I [35]. The observed morphological protection and decreased ECM staining in the hydrosol-treated group suggest that the formulation may interfere with *TGF-β1* signaling, possibly through antioxidant pathways such as Nrf2-AKT-HO-1 axi or inhibition of Epithelial-to-Mesenchymal Transition (EMT) cascades [36,37]. These findings support a dual anti-inflammatory and anti-fibrotic role for the botanical hydrosol.

AQP3 is a membrane channel protein abundantly expressed in renal collecting ducts, playing a critical role in regulating water permeability and fluid balance [38]. Impaired AQP3 expression has been associated with reduced urine concentrating ability, interstitial edema, and impaired renal osmoregulation-features commonly observed in early-stage CKD and diabetic nephropathy [39]. In our study, treatment with the botanical hydrosol induced a mild increase in AQP3 expression in MDCK cells, suggesting a potential regulatory effect on water transport mechanisms. Although the observed increase in AQP3 expression did not reach statistical significance, the trend may reflect early membrane-stabilizing or transcriptional priming effects and warrants further exploration through dose- and time-dependent studies as well as protein-level validation. Notably, several phytochemicals present in the formulation-including polysaccharide derivatives and flavonoids from *L. chinense* and *P. kingianum*-have been reported, in analogous botanical models such as *Poria cocos*, to influence AQP3 regulation *via* cAMP-PKA or PI3K-Akt signaling pathways (as demonstrated in EPC-induced AQP3 upregulation in HaCaT cells) [40]. These pathways may promote trafficking or transcription of aquaporin genes in response to osmotic or inflammatory cues. Cellular senescence and mitochondrial dysfunction are now recognized as core drivers of renal aging and CKD [41]. In the present study, the botanical hydrosol significantly upregulated several members of the chaperonin-containing TCP1 complex (*CCT2*, *CCT6A*, *CCT8*), which are essential for maintaining proteostasis and cytoskeletal organization. Notably, *CCT2* has been shown to regulate aggrephagy, facilitating clearance of misfolded protein aggregates, while *CCT8* supports proteome stability during immune and stress responses [42,43]. Downregulation of CCT components is associated with aging-related decline and proteotoxic stress, whereas maintaining or restoring their expression may support cellular resilience and longevity [44,45]. CCT/TRiC is a well-characterized folding machinery for cytoskeletal proteins such as actin and tubulin, essential for maintaining cell structure and facilitating recovery during injury, including in renal epithelial cells [46]. In parallel, we observed robust activation of mitochondrial function-related genes, including a >4-fold increase in Parkin, a key E3 ubiquitin ligase in the PINK1-Parkin mitophagy pathway [47]. Activation of Parkin-mediated mitophagy has been shown in vivo to mitigate tubular injury, suppress NLRP3 inflammasome activation, and preserve renal function in mouse models of ischemia-reperfusion and cisplatin nephrotoxicity, highlighting its role in mitochondrial quality control and tissue repair [48,49]. The upregulation of *NADSYN1* (nicotinamide adenine dinucleotide synthetase), *Ubl5*, and *Atg8* further supports enhanced mitochondrial biosynthesis and autophagic flux, processes critical for energy metabolism and removal of damaged organelles [50]. Parkin functions downstream of the PINK1 Parkin axis, orchestrating the selective ubiquitination of damaged mitochondria and recruitment of mitophagy receptors such as p62 (SQSTM1) [51]. These findings suggest a coordinated rejuvenation response, possibly mediated by phytochemicals such as flavonoids or saponins present in *P. kingianum* and *L. chinense*.

Similarly, *Ubl5* upregulation observed in our study may indicate activation of the mitochondrial unfolded protein response (UPRmt). *Ubl5* is a conserved co-factor essential for UPRmt signaling, and its deficiency in mammalian models accelerates aging phenotypes and impairs mitochondrial proteostasis [52]. In the kidney, ATF5-*Ubl5*-driven UPRmt signaling has recently been implicated in modulating tubular injury in diabetic nephropathy, suggesting that reinforcement of this pathway may contribute to renal resilience [53].

CONCLUSION

In summary, this study demonstrates that a multi-herb botanical hydrosol formulation-comprising *Alpinia oxyphylla Miq.* and fermented MFH herbs-exerts multi-pathway protective effects in renal cell models. The formulation suppressed inflammatory mediators and promoted a time-dependent shift toward tissue-repairing cytokines, attenuated *TGF-β1*-induced fibrotic remodeling, and showed a trend toward aquaporin-3 regulation

relevant to fluid balance. At the molecular level, it significantly upregulated mitochondrial quality-control genes (Parkin, *Atg8*, *NADSYN1*), Unfolded protein response factors (*Ubl5*), and chaperonin subunits (*CCT2*, *CCT6A*, *CCT8*), suggesting enhanced mitophagy, UPRmt signaling, and proteostasis that may support cellular resilience against aging and stress. Together, these findings provide mechanistic support for the potential use of botanical hydrosols as functional formulations to promote kidney health and healthy aging. Nevertheless, the present results are limited to *in vitro* analyses, and future in vivo studies and protein-level validations will be critical to confirm physiological relevance and translational potential.

ACKNOWLEDGMENTS

We gratefully acknowledge the Cell Validation Laboratory, TCI GENE INC., for providing essential technical support and experimental resources that significantly contributed to the successful completion of this research.

AUTHORS' CONTRIBUTIONS

YK.L. contributed to study conception, experimental design, manuscript revision, and provided overall project supervision. JX.W. were responsible for experimental design, sample provision, project oversight, and data interpretation. YH.L. contributed to study design, provided conceptual input, and supervised the project. ST.C. and CF.C. provided experimental guidance, conducted data collection and analysis, and critically revised the manuscript. YW.M. supervised the study, performed statistical analyses, and was responsible for drafting and finalizing the manuscript.

FUNDING INFORMATION

This study was supported by the TCI Co., Ltd., Taiwan.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest related to this study.

REFERENCES

1. Wright JA, Cavanaugh KL. Dietary sodium in chronic kidney disease: A comprehensive approach. Semin Dial. 2010;23(4):415-421.
2. Tan H, Xu J, Liu Y. Ageing, cellular senescence and chronic kidney disease: Experimental evidence. Curr Opin Nephrol Hypertens. 2022;31(3):235-243.
3. Li C, Wang W, Knepper MA, Nielsen S, Frokiaer J. Downregulation of renal aquaporins in response to unilateral ureteral obstruction. Am J Physiol Renal Physiol. 2003;284(5):F1066-F1079.
4. Lei L, Wang W, Jia Y, Su L, Zhou H, Verkman AS, et al. Aquaporin-3 deletion in mice results in renal collecting duct abnormalities and worsens ischemia-reperfusion injury. Biochim Biophys Acta Mol Basis Dis. 2017;1863(6):1231-1241.
5. Borrelli S, Provenzano M, Gagliardi I, Michael A, Liberti ME, De Nicola L, et al. Sodium intake and chronic kidney disease. Int J Mol Sci. 2020;21(13):4744.
6. Swift SL, Drexler Y, Sotres-Alvarez D, Raij L, Llabre MM, Schneiderman N, et al. Associations of sodium and potassium intake with chronic kidney disease in a prospective cohort study: Findings from the Hispanic Community Health Study/Study of Latinos, 2008-2017. BMC Nephrol. 2022;23(1):133.
7. Koye DN, Magliano DJ, Nelson RG, Pavkov ME. The global epidemiology of diabetes and kidney disease. Adv Chronic Kidney Dis. 2018;25(2):121-132.
8. Wang X, Wang J, Bi H, Zhang Z, Zhang M, Wang M. Research progress in the extraction and purification, structural characteristics, pharmacological activities, structure-activity relationships, and applications from *Alpinia oxyphylla Miq*. polysaccharides. Int J Biol Macromol. 2025;315(Pt 1):144387
9. Wu B, Gan A, Wang R, Lin F, Yan T, Jia Y, et al. *Alpinia oxyphylla Miq*. volatile oil ameliorates depressive behaviors and inhibits neuroinflammation in CUMS-exposed mice by inhibiting the TLR4-medicated MyD88/NF-κB signaling pathway. J Chem Neuroanat. 2023;130:102270.
10. Qiu C, Mu L, Wang J, Tang R, Hou B, Hu W, et al. Sesquiterpenoids from the fruits of *Alpinia oxyphylla Miq*. and their neuroprotective effect. Phytochemistry. 2023;211:113680.
11. Mohamed ME, Abduldaium YS, Younis NS. Ameliorative effect of linalool in cisplatin-induced nephrotoxicity: The role of HMGB1/TLR4/NF-κB and Nrf2/HO1 pathways. Biomolecules. 2020;10(11):1488.
12. Lis-Balchin M, Hart S. A preliminary study of the effect of essential oils on skeletal and smooth muscle *in vitro*. J Ethnopharmacol. 1997;58(3):183-187.
13. Li W, Yu L, Fu B, Chu J, Chen C, Li X, et al. Protective effects of *Polygonatum kingianum* polysaccharides and aqueous extract on uranium-induced toxicity in human kidney (HK-2) cells. Int J Biol Macromol. 2022;202:68-79.
14. Lee SE, Ju EM, Kim JH. Antioxidant activity of extracts from *Euryale ferox* seed. Exp Mol Med. 2002;34(2):100-106.
15. Ren Y, Wang K, Wu Y, Li J, Ma J, Wang L, et al. Lycium barbarum polysaccharide mitigates high-fat-diet-induced skeletal muscle atrophy by promoting AMPK/PINK1/Parkin-mediated mitophagy. Int J Biol Macromol. 2025;301:140488.
16. Konig A, Sadova N, Dornmayr M, Schwarzinger B, Neuhauser C, Stadlbauer V, et al. Combined acid hydrolysis and fermentation improves bioactivity of citrus flavonoids *in vitro* and in vivo. Commun Biol. 2023;6(1):1083.
17. Ho HJ, Shirakawa H. Oxidative stress and mitochondrial dysfunction in chronic kidney disease. Cells. 2022;12(1):88.
18. Huang R, Fu P, Ma L. Kidney fibrosis: From mechanisms to therapeutic medicines. Signal Transduct Target Ther. 2023;8(1):129.
19. Stenvinkel P, Chertow GM, Devarajan P, Levin A, Andreoli SP, Bangalore S, et al. Chronic inflammation in chronic kidney disease progression: Role of Nrf2. Kidney Int Rep. 2021 ;6(7):1775-1787.
20. Chang YM, Chang HH, Tsai CC, Lin HJ, Ho TJ, Ye CX, et al. Alpinia oxyphylla Miq. fruit extract activates IGFR-PI3K/Akt signaling to induce Schwann cell proliferation and sciatic nerve regeneration. BMC Complement Altern Med. 2017;17(1):184.
21. Hur J, Pak SC, Koo BS, Jeon S. Borneol alleviates oxidative stress *via* upregulation of Nrf2 and Bcl-2 in SH-SY5Y cells. Pharm Biol. 2013;51(1):30-35.
22. Du G, Xiao M, Zhang X, Wen M, Pang C, Jiang S, et al. Alpinia oxyphylla Miq. extract changes miRNA expression profiles in db-/db- mouse kidney. Biol Res. 2017;50(1):9.
23. Ren Y, Sun Y, Liao YY, Wang S, Liu Q, Duan CY, et al. Mechanisms of action and applications of Polygonatum sibiricum polysaccharide at the intestinal mucosa barrier: A review. Front Pharmacol. 2024;15:1421607.
24. Xu X, Shan M, Chu C, Bie S, Wang H, Cai S, et al. Polysaccharides from *Polygonatum kingianum* Collett and Hemsl ameliorated fatigue

by regulating NRF2/HO-1/NQO1 and AMPK/PGC-1α/TFAM signaling pathways, and gut microbiota. Int J Biol Macromol. 2024;266:131440.

25. Kapoor S, Kaur A, Kaur R, Kumar V, Choudhary M. *Euryale ferox*, a prominent superfood: Nutritional, pharmaceutical, and its economical importance. J Food Biochem. 2022;46(12):e14435.

26. Ma J, Meng X, Kang SY, Zhang J, Jung HW, Park YK, et al. Regulatory effects of the fruit extract of *Lycium chinense* and its active compound, betaine, on muscle differentiation and mitochondrial biogenesis in C2C12 cells. Biomed Pharmacother. 2019;118:109297.

27. Gan Q, Wang X, Cao M, Zheng S, Ma Y, Huang Q, et al. NF-κB and AMPK-Nrf2 pathways support the protective effect of polysaccharides from Polygonatum cyrtonema Hua in lipopolysaccharide-induced acute lung injury. J Ethnopharmacol. 2022;291:115153.

28. Zikela L, Yu Z, Han J, Zhu H, Wang D, Wang X, et al. Protective effects of fermented Rosa roxburghii Tratt juice against ethanol-induced hepatocyte injury by regulating the NRF2-AMPK signaling pathway in AML-12 cells. Mol Med Rep. 2024;30(4):1-3.

29. Stenvinkel P, Chertow GM, Devarajan P, Levin A, Andreoli SP, Bangalore S, et al. Chronic inflammation in chronic kidney disease progression:Role of Nrf2. Kidney Int Rep. 2021;6(7):1775-1787.

30. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro-and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta. 2011;1813(5):878-888.

31. Moser B, Wolf M, Walz A, Loetscher P. Chemokines: multiple levels of leukocyte migration control. Trends Immunol. 2004;25(2):75-84.

32. Saraiva M, O'garra A. The regulation of *IL-10* production by immune cells. Nat Rev Immunol. 2010;10(3):170-181.

33. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol. 2003;3(2):133-146.

34. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity. 2016;44(3):450-462.

35. Ren LL, Miao H, Wang YN, Liu F, Li P, Zhao YY, et al. TGF-β as a master regulator of aging-associated tissue fibrosis. Aging Dis. 2023;14(5):1633-1650.

36. Potteti HR, Tamatam CR, Marreddy R, Reddy NM, Noel S, Rabb H, et al. Nrf2-AKT interactions regulate heme oxygenase 1 expression in kidney epithelia during hypoxia and hypoxia-reoxygenation. Am J Physiol Renal Physiol. 2016;311(5):F1025-F1034.

37. Grande MT, Lopez-Novoa JM. Fibroblast activation and myofibroblast generation in obstructive nephropathy. Nat Rev Nephrol. 2009;5(6):319-328.

38. Nielsen S, Frokiaer J, Marples D, Kwon TH, Agre P, Knepper MA. Aquaporins in the kidney: From molecules to medicine. Physiol Rev. 2002;82(1):205-244.

39. He J, Yang B. Aquaporins in renal diseases. Int J Mol Sci. 2019;20(2):366.

40. Park SG, Jo IJ, Park SA, Park MC, Mun YJ. Poria cocos extract from mushrooms stimulates aquaporin-3 *via* the PI3K/Akt/mTOR signaling pathway. Clin Cosmet Investig Dermatol. 2022;15:1919-1931.

41. Erbs E, Brasen CL, Lund AM, Rasmussen M. Adult patient diagnosed with *NADSYN1* associated congenital NAD deficiency and analysis of NAD levels to be published in: European Journal of Medical Genetics. Eur J Med Genet. 2023;66(3):104698.

42. Luo J, Feng ZS, Tang JX. The essential role of *CCT2* in the regulation of aggrephagy. Front Aging Neurosci. 2024;16:1491001.

43. Oftedal BE, Maio S, Handel AE, White MP, Howie D, Davis S, et al R. The chaperonin *CCT8* controls proteostasis essential for T cell maturation, selection, and function. Commun Biol. 2021;4(1):681.

44. Varte V, Rincon-Limas DE. Role of the Chaperonin TCP-1 Ring Complex (TRiC) in Protein Aggregation and Neurodegeneration. Front Mol Neurosci. 2025;18:1617771.

45. Zeng C, Han S, Pan Y, Huang Z, Zhang B, Zhang B, et al. Revisiting the chaperonin T-complex protein-1 ring complex in human health and disease: A proteostasis modulator and beyond. Clin Transl Med. 2024;14(2):e1592.

46. Yam AY, Xia Y, Lin HT, Burlingame A, Gerstein M, Frydman J, et al. Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. Nat Struct Mol Biol. 2008;15(12):1255-1262.

47. Narendra DP, Youle RJ. The role of PINK1-Parkin in mitochondrial quality control. Nat Cell Biol. 2024;26(10):1639-1651.

48. Tang C, Han H, Liu Z, Liu Y, Yin L, Cai J, et al. Activation of BNIP3-mediated mitophagy protects against renal ischemia-reperfusion injury. Cell Death Dis. 2019;10(9):677.

49. Wang Y, Tang C, Cai J, Chen G, Zhang D, Zhang Z, et al. PINK1/Parkin-mediated mitophagy is activated in cisplatin nephrotoxicity to protect against kidney injury. Cell Death Dis. 2018;9(11):1113.

50. Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ. 2013;20(1):31-42.

51. Silvian LF. PINK1/Parkin pathway activation for mitochondrial quality control-which is the best molecular target for therapy?. Front Aging Neurosci. 2022;14:890823.

52. Zhao Q, Wang J, Levichkin IV, Stasinopoulos S, Ryan MT, Hoogenraad NJ, et al. A mitochondrial specific stress response in mammalian cells. EMBO J. 2002; 21(17):4411-4419.

53. Liu Y, Zhang L, Zhang S, Liu J, Li X, Yang K, et al. ATF5 regulates tubulointerstitial injury in diabetic kidney disease *via* mitochondrial unfolded protein response. Mol Med. 2023;29(1):57.