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Original Article

Icariin attenuates renal interstitial fibrosis through G protein-coupled estrogen receptor in a UUO murine model

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Abstract: Background/Aims: Icariin plays an antifibrotic role in the unilateral ureteral obstruction (UUO) model; however, its primary mechanism has not been elucidated. G protein-coupled estrogen receptor (GPER) has been shown to be associated with fibrosis and mitochondrial biogenesis. In this study, we aimed to investigate the impact of GPER on renal fibrosis and whether icariin attenuates renal fibrosis dependent on GPER. Methods: In the in vivo study, 10-week-old mice were subjected to the UUO model followed by UUO with icariin, G-15 (a GPER antagonist), and icariin + G-15. GPER expression, renal fibrosis levels, and mitochondrial alterations were measured and analyzed. In an in vitro study, we examined the antifibrotic effect of icariin on rat renal fibroblasts (NRK-49F) via GPER. Results: Consistent with a previous study, icariin significantly attenuated fibrotic markers and protected the kidneys against mitochondrial injuries in the UUO model. However, G-15 exacerbated renal fibrosis and abolished the protective effect of icariin in the UUO model. Furthermore, antagonizing or knocking down GPER in NRK-49F significantly increased fibrotic markers and eliminated the antifibrotic effect of icariin. Conclusions: Our findings indicate that (1) GPER inhibition exacerbates renal fibrosis, and (2) icariin exerts antifibrotic effects against renal fibrosis through GPER.

Keywords: Icariin, G protein-coupled estrogen receptor, renal fibrosis, mitochondria

Introduction

Chronic kidney disease (CKD) is a prevalent disease that affects 10% of the global population. The pathology of CKD is characterized by renal fibrosis, resulting in irreversible progression to end-stage renal disease [1, 2]. Icariin (molecular weight: 676.67) is the main active ingredient isolated from Epimedium brevicornum Maxim [3]. We previously identified, using integrative analysis and from the decoctions published in recent journals, that Epimedium was the most frequently used Chinese herb in chronic renal failure treatment [4]. An in vivo study also showed that icariin could ameliorate fibrosis in IgA nephropathy [2] and diabetic kidney disease [5]. A previous study also demonstrated that icariin plays an antifibrotic and anti-inflammatory role in a unilateral ureteral obstruction model (UUO) [6]. However, the underlying mechanism remains to be elucidated.

G protein-coupled estrogen receptor (GPER), a novel estrogen receptor, exhibits high estradiol affinity and can mediate numerous estrogenic effects [7]. It is widely distributed in neural, breast, placental, heart, and hepatic tissues [8]. A previous study demonstrated that GPER deficiency exacerbates liver fibrosis [9]. GPER is also closely related to mitochondrial metabolism. Several studies have been conducted on the kidney area. GPER activation increases megalin expression in proximal convoluted tubules [10], inhibits mitochondrial permeability transition pore opening, and protects against ischemia reperfusion injury [11]. Estrogen also promotes mitochondrial regeneration through GPER [12]. Icariin, a phytoestrogen, has been

shown to bind and activate GPER in mesangial cells [13] and podocytes [14].

Taken together, we aimed to determine the role of GPER during the process of renal fibrosis and its association with icariin. The present study hypothesized that icariin might protect the kidney against fibrosis dependent on GPER.

Materials and methods

Animals and groups

Animal use and all experimental techniques at Yueyang Hospital were approved by the ethical committee (approval number: 18905). The National Research Council's Guidelines for the Care and Use of Laboratory Animals were followed. The animals were purchased from Shanghai Ling Chang Experimental Animal Co., Ltd. (certificate number: SCXK [Shanghai] 2018-0040). Ten-week-old male C57BL/6 mice weighing 20-22 g were kept in a room with a constant temperature (24±1°C), humidity, and ad libitum food and water. To determine whether icariin protects against renal fibrosis via GPER receptors, we included a GPER antagonist to inhibit GPER activation. The mice were randomly assigned to one of five groups (n=6): Control, UUO, UUO + icariin, UUO + GPER antagonist, UUO + GPER antagonist + icariin.

Experimental procedure

The mice underwent either a sham or UUO procedure, as previously reported [15]. Briefly, after a small incision was made in the left flank muscle, the left kidney and ureter were revealed. Then, a 4-0 silk suture was used to tie a surgical point around the left ureter toward the bottom of the left kidney. The control group mice were given normal saline. The mice were administered icariin (Meilun Biotech, Dalian, China), 250 mg/kg/d dissolved in DMSO and normal saline, through a gastric gavage once daily for one week prior to UUO modeling. Minipumps (Alzet, 1007D, Durect, CA, USA) infused with G-15 (Cayman, MI, USA) dissolved in DMSO and saline were subcutaneously implanted into mice at a rate of 200 μg/kg/d. The mice were sacrificed seven days after UUO modeling, and the obstructed kidney was removed, processed for histology, and stored at -80°C for western blot and RT-PCR analysis.

Cell culture

The American Type Culture Collection provided the NRK-49F cell line, which was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS). NRK-49F cells were grown to 70% confluence and starved for 48 h in DMEM supplemented with 0.5% FBS before being stimulated for 24 h with 1.0 μg/mL Wnt1 ligand. The cells were pretreated with or without G-15 (10 nM) and icariin (10^{-4} mol/L) 1 h before Wnt1 stimulation.

GPER siRNA transfection

Small interfering RNA (siRNA) duplexes targeting Gper were obtained from Shanghai Ruisai Biotechnology (China). Cells were grown to 30-50% confluence before being transfected with control siRNA or Gper siRNA using Lipofectamine 2000 reagent (Invitrogen, CA, USA) and cultured in Opti-MEM reduced serum media for 4 h. Next, the Opti-MEM reduced serum transfection medium was withdrawn from the plates and replaced with DMEM with 5% FBS for a further 24 h of incubation before the experiments. The cells were harvested following treatment with the indicated medicines.

Histological procedure

Half of the left renal tissue was fixed with 4% paraformaldehyde. Renal samples were cut into slices after being coated with paraffin. To assess histological alterations and renal fibrosis, the renal sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and Masson. Light microscopy was used to analyze the slices, which were then photographed. Inflammatory cell infiltration and interstitial dilation were scored 0-5 on HE staining. 0: normal; 1: <10% stained; 2: 10-25% of tubules injured area stained; 3: 25-50% stained; 4: 50-75% stained; 5: >75% stained. Ten non-overlapping tubular interstitial fields were randomly selected. The proportion of positive regions stained in the visual field was used to perform a semiquantitative analysis.

Immunofluorescence and immunohistochemistry staining

α-SMA staining and collagen type I staining were performed as previously described [15]. Briefly, 4-6 μm slices of paraffin-embedded

Table 1. List of primer sequences for real-time PCR

Gene	Forward primer	Reverse primer
Cpt1α (Mouse)	CTTCCCATTTGACACCTTTG	ATACGTGAGGCAGAACTTGC
Fn (Mouse)	ATGGTACAGCTGATCCTGCC	GCCCTGGTTTGTACCTGCTA
Pgc-1α (Mouse)	AGTCCCATACACAACCGCAG	CCCTTGGGGTCATTTGGTGA
Col1α1 (Mouse)	CCCAGCCGCAAAGAGTCTAC	AGCATACCTCGGGTTTCCAC
Tgf-β1 (Mouse)	GGGAAGCAGTGCCCGAACCC	TGGGGGTCAGCAGCCGGTTA
Hprt1 (Mouse)	TATGCCGAGGATTTGGAAAA	TCCCATCTCCTTCATGACATC
Fn (Rat)	AAGAGGCAGGCTCAGCAAAT	TAGCAGGTACAAACCAGGGC
Col1α1 (Rat)	CCCAGCGGTGGTTATGACTT	TCGATCCAGTACTCTCCGCT
Vim (Rat)	TGCGGCTGCGAGAAAAATTG	GGTCAAGACGTGCCAGAGAA
Gapdh (Rat)	TGCTGGTGCTGAGTATGTC	AGTTGTCATATTTCTCGTGG

renal tissues were blocked for 1 h with 5% normal serum/phosphate-buffered saline (PBS)/0.3% Triton. The sections were incubated at 4°C overnight with anti-rabbit α-SMA antibody (1:200, Abcam, Cambridge, UK) and anti-rabbit collagen type I antibody (1:200, Abcam) antibodies. After washing three times with PBS, the tissues were incubated with a secondary antibody for 1 h at room temperature before being examined under a fluorescence microscope. Renal tissues were subjected to immunohistochemical staining with an anti-GPER antibody (1:200, Abcam). The percentage of positively stained areas by immunohistochemistry was quantified using Image J software.

Real-time PCR

Total RNA was extracted from renal tissues or cells using TRIzol reagent (Invitrogen). According to the manufacturer's instructions, cDNA was generated using 2 μg of total RNA and real-time PCR reactions were performed using TB Green qPCR Master Mix (Takara, Shiga, Japan). The *Hprt1* gene was used as an internal control to standardize the relative gene expression levels of *fibronectin* (*Fn*), *Tgf-β1*, *Col1α1*, *vimentin*, carnitine palmitoyl-transferase 1α (*Cpt1α*), and peroxisome proliferator-activated receptor γ coactivator 1α (*Pgc1α*). **Table 1** shows the primer sequences.

Western blot

The kidney tissue samples were pulverized and suspended in lysis buffer for 30 min at 4°C. NRK-49F cells were rinsed twice with ice-cold PBS before being lysed in the same manner as the tissue. After removing the supernatant, the protein content was determined using a bicinchoninic acid assay protein assay kit (Thermo Fisher Scientific, MA, USA). SDS-PAGE was used to separate the total protein, which was then transferred to a PVDF membrane and blocked. The membranes were incubated at 4°C overnight with anti-Fn (1:500, Abcam), anti-GPER (1:200, Abcam), anti-tubulin (1:1000, Sigma-Aldrich, MO, USA), and anti-β-actin (1:1000, Sigma-Aldrich). The membrane was rinsed three times in PBS-Tween for 10 min prior to incubation with an anti-mouse/rabbit secondary antibody.

Statistical analyses

Data are presented as the mean ± SEM. Data were analyzed using one-way ANOVA followed by Tukey's test for multiple comparisons. Kruskal-Wallis tests were used for non-parametric analysis when data were non-normally distributed, with statistical significance set at $P<0.05$. All analyses were performed using GraphPad Prism software version 6.0 (GraphPad Software, Inc., CA, USA).

Results

GPER expression is decreased in the UUO model and elevated by icariin

To elucidate GPER expression alterations in the UUO model and whether icariin regulates GPER, we first quantified GPER expression as evaluated by immunohistochemical staining (**Figure 1A**). We observed that GPER expression was markedly decreased in the UUO group compared to that in the control group (**Figure 1B**), whereas GPER expression was significantly elevated with icariin intervention (**Figure 1B**). G-15 further suppressed GPER expression compared to the UUO model, and the GPER level was slightly restored by icariin in the icariin + G15 co-treated group (**Figure 1B**). These data suggest that GPER expression is decreased in the UUO model. Icariin treatment elevated GPER expression in contrast to G-15, which reduced GPER expression in renal tissues.

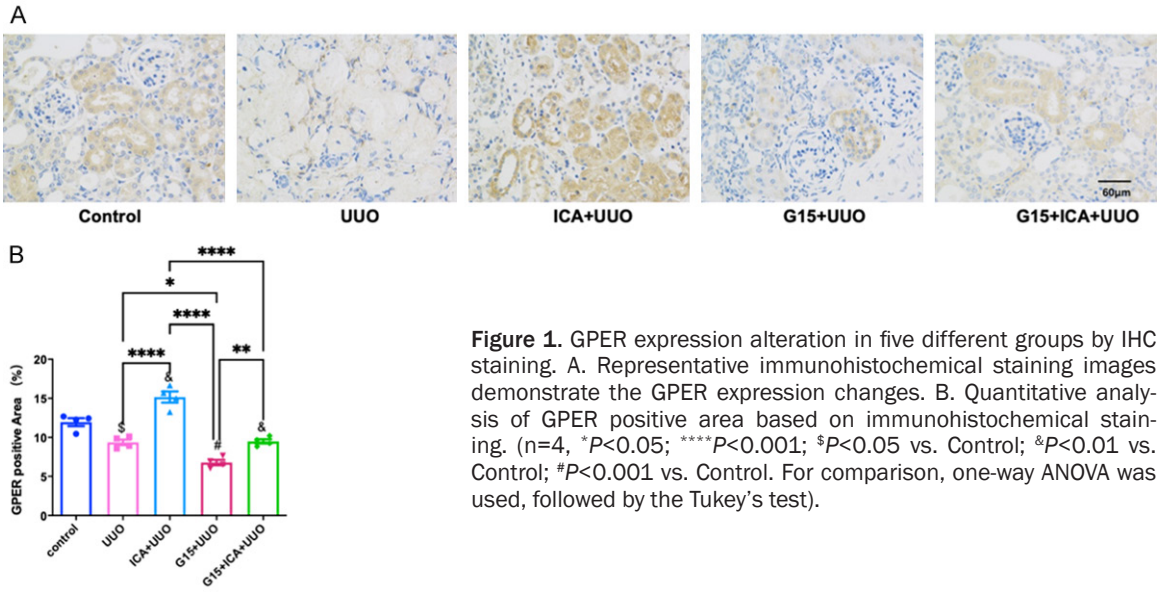

Figure 1. GPER expression alteration in five different groups by IHC staining. A. Representative immunohistochemical staining images demonstrate the GPER expression changes. B. Quantitative analysis of GPER positive area based on immunohistochemical staining. (n=4, $^{*}P<0.05$; $^{****}P<0.001$; $^{\$}P<0.05$ vs. Control; $^{\&}P<0.01$ vs. Control; $^{\#}P<0.001$ vs. Control. For comparison, one-way ANOVA was used, followed by the Tukey's test).

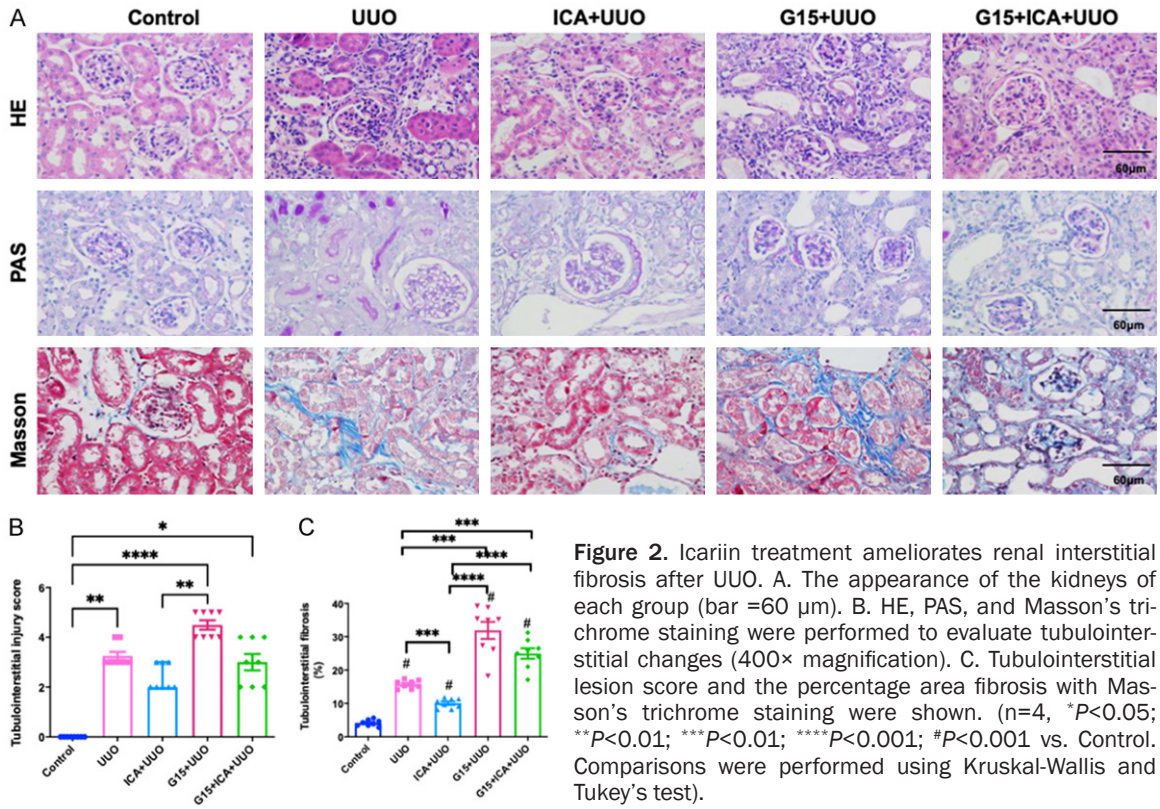

Figure 2. Icariin treatment ameliorates renal interstitial fibrosis after UUO. A. The appearance of the kidneys of each group (bar =60 μm). B. HE, PAS, and Masson's trichrome staining were performed to evaluate tubulointerstitial changes (400× magnification). C. Tubulointerstitial lesion score and the percentage area fibrosis with Masson's trichrome staining were shown. (n=4, $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.01$; $^{****}P<0.001$; $^{\#}P<0.001$ vs. Control. Comparisons were performed using Kruskal-Wallis and Tukey's test).

Icariin attenuates UUO-induced renal tubular injuries and fibrosis through GPER

To identify whether GPER protects against renal fibrosis and to elucidate its association with icariin, we measured and analyzed obstructed kidney morphological alterations. Consistent with the previous study, evaluation of renal lesions by light microscopy revealed loss of brush border, tubule dilation, and protein cast in the tubules with infiltration of a large number of inflammatory cells in the obstructed kidneys (**Figure 2A**). Masson trichrome staining also revealed that the UUO model exhibited severe extracellular matrix deposition (**Figure 2A**). Icariin intervention effectively improved

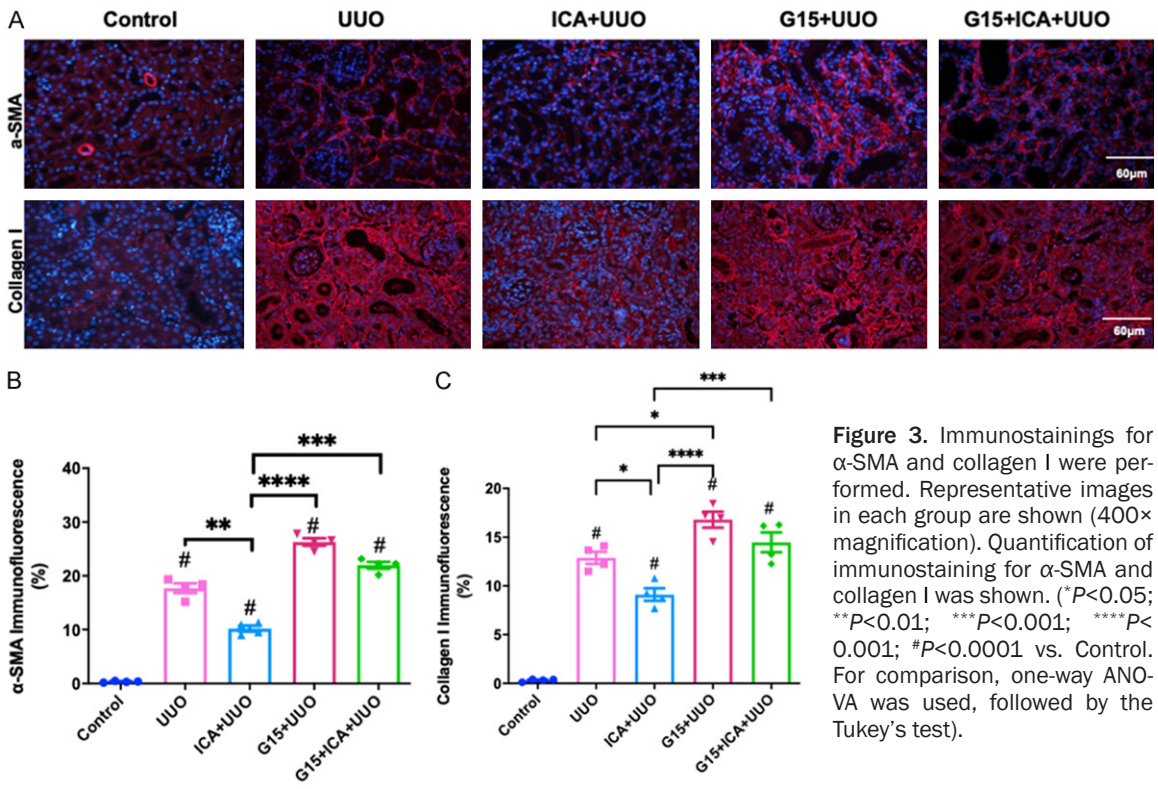

Figure 3. Immunostainings for α-SMA and collagen I were performed. Representative images in each group are shown (400× magnification). Quantification of immunostaining for α-SMA and collagen I was shown. ($^{*}P$<0.05; $^{**}P$<0.01; $^{***}P$<0.001; $^{****}P$<0.001; $^{\#}P$<0.0001 vs. Control. For comparison, one-way ANOVA was used, followed by the Tukey's test).

tubular injuries and reduced collagen deposition compared to that of the UUO group. Notably, G-15, the GPER antagonist, accelerated tubular injuries with increased deposition of extracellular matrix in the G-15-treated UUO model. Furthermore, the antifibrotic effect of icariin was abolished by G-15 in the icariin + G15-treated UUO model (**Figure 2B**, **2C**).

These findings were confirmed by immunostaining of α-SMA and Col1α1 (**Figure 3A**). Similarly, the UUO model exhibited pronounced positive staining of α-SMA and Col1α1. As quantified, the icariin-treated UUO model demonstrated a significant reduction in α-SMA and Col1α1 expression (**Figure 3B**, **3C**). α-SMA and Col1α1 positive staining was remarkably elevated in the G-15-treated UUO group (**Figure 3B**, **3C**). Icariin-attenuated renal fibrosis was abrogated by GPER suppression in the icariin + G15-co-treated UUO group (**Figure 3B**, **3C**). These data indicate that icariin ameliorates renal fibrosis via GPER in the UUO model.

Icariin ameliorates renal fibrotic marker expression via GPER in UUO model

We evaluated the mRNA level of fibrotic markers in the five groups using qPCR. The fibrotic markers (**Figure 4A-C**), including *Tgf-β1*, *Col1α1*, and *Fn*, were significantly upregulated in the UUO group. In the icariin-treated UUO model, icariin significantly reversed the change in mRNA level of fibrotic marker in the obstructed kidney. Similarly, fibrotic markers of the G-15-treated UUO model were significantly upregulated compared with those in the UUO model, and these fibrotic markers remained unchanged in the icariin + G-15-treated UUO model. We also measured Fn protein levels using western blotting (**Figure 5A**, **5B**). Consistent with the mRNA level, Fn levels were increased in the UUO model and reduced following icariin treatment. These results strongly confirmed that GPER inhibition exacerbated renal fibrosis at the mRNA and protein levels of fibrotic markers. Icariin attenuates renal fibrosis dependent on GPER in the UUO model.

Icariin protects mitochondria from injury in the UUO model through GPER

We evaluated the morphological alterations of mitochondria by transmission electron microscopy to determine whether icariin plays a protective role in mitochondrial structure and function through GPER. The UUO model presented enlarged and swollen mitochondria with

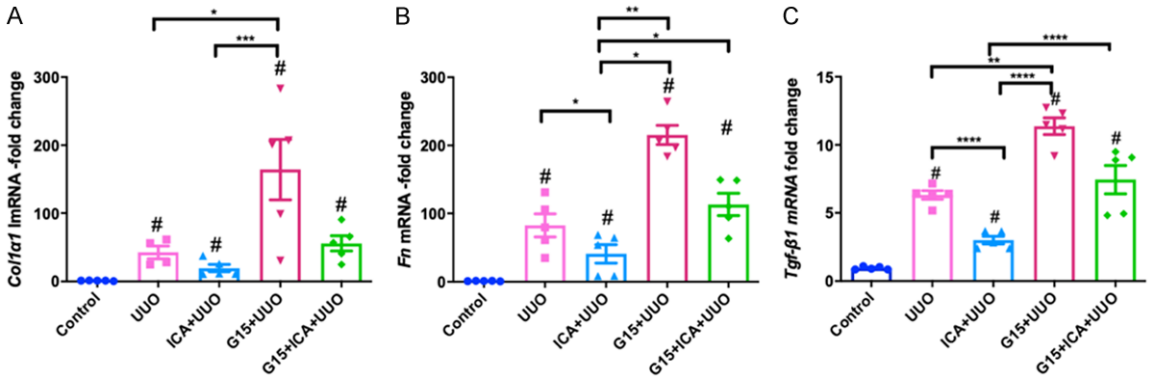

Figure 4. Icariin decreases the expression of fibrotic markers at 7 days after unilateral ureteral obstruction and G-15 aggravates UUO-induced renal interstitial fibrosis. Real-time polymerase chain reaction was performed for fibronectin (A), Type I (B), and TGF-β1 (C) expressions. (n=5, $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.001$; $^{\#}P<0.0001$ vs. Control. For comparison, one-way ANOVA was used, followed by the Tukey's test).

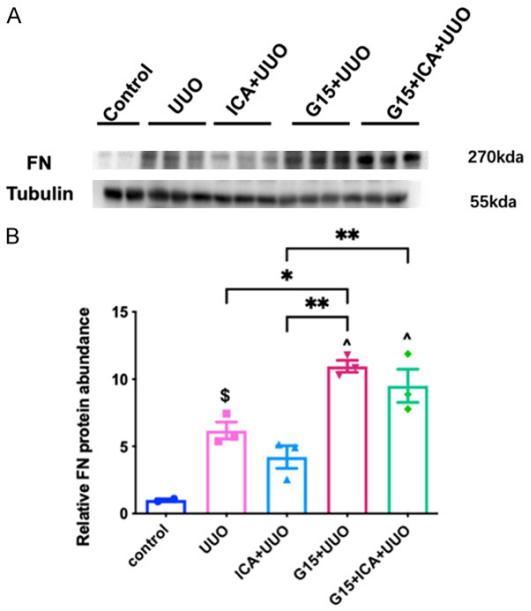

Figure 5. Icariin treatment increases fibronectin expression in UUO model and G-15 aggravates fibronectin expression. Representative western blot (A) is shown. (B) Western blot analyses presented FIBRONECTIN/kDa expression levels. (n=3, $^{*}P<0.05$; $^{**}P<0.01$; $^{\$}P<0.05$ vs. Control; $^{\wedge}P<0.01$ vs. Control. For comparison, one-way ANOVA was used, followed by the Tukey's test).

distorted cristae (**Figure 6A**), indicating severe mitochondrial injury. In contrast, icariin treatment protected mitochondrial structural integrity. Consistently, the G-15-treated UUO group demonstrated more severely damaged swollen mitochondria. The icariin + G-15-treated UUO model demonstrated injured mitochondria similar to that of the G-15-treated UUO group. These results confirmed that icariin protects tubules against injury through mitochondrial protection. G-15 abolished the protective effect of icariin on mitochondria, suggesting a GPER-dependent mechanism. CPT1α is a critical gene that regulates the transport of fatty acids into the mitochondria, and its decreased expression suggests a fatty acid transport disorder [16]. PGC1α has emerged as a critical mitochondrial biogenesis regulator. It plays critical roles in essential metabolic processes, such as fatty acid oxidation, oxidative phosphorylation, and reactive oxygen species detoxification [17]. Therefore, we measured the mRNA expression levels of *Cpt1α* and *Pgc1α*, and the results revealed a significant decrease in the UUO group. The mRNA expression of *Cpt1α* and *Pgc1α* in the icariin group was increased (**Figure 6B**, **6C**). G-15 abrogated the protective effect of icariin in the icariin + G-15 group.

Icariin inhibits Wnt1 induced up-regulation of fibrotic markers in NRK-49F through GPER

Previous studies have demonstrated that Wnt, transforming growth factor-beta, platelet-derived growth factor-beta, and Notch are critical factors in renal fibrosis progression [18, 19]. Injured tubular epithelial cells release Wnt and stimulate fibroblasts to progress into myofibroblasts, contributing to interstitial fibrosis [20, 21]. To further explore whether icariin protects renal fibrosis through GPER, rat kidney fibroblasts (NRK-49F) were exposed to Wnt1 for 24 h with or without icariin or G-15. As expected, we observed increases in Col1α1,

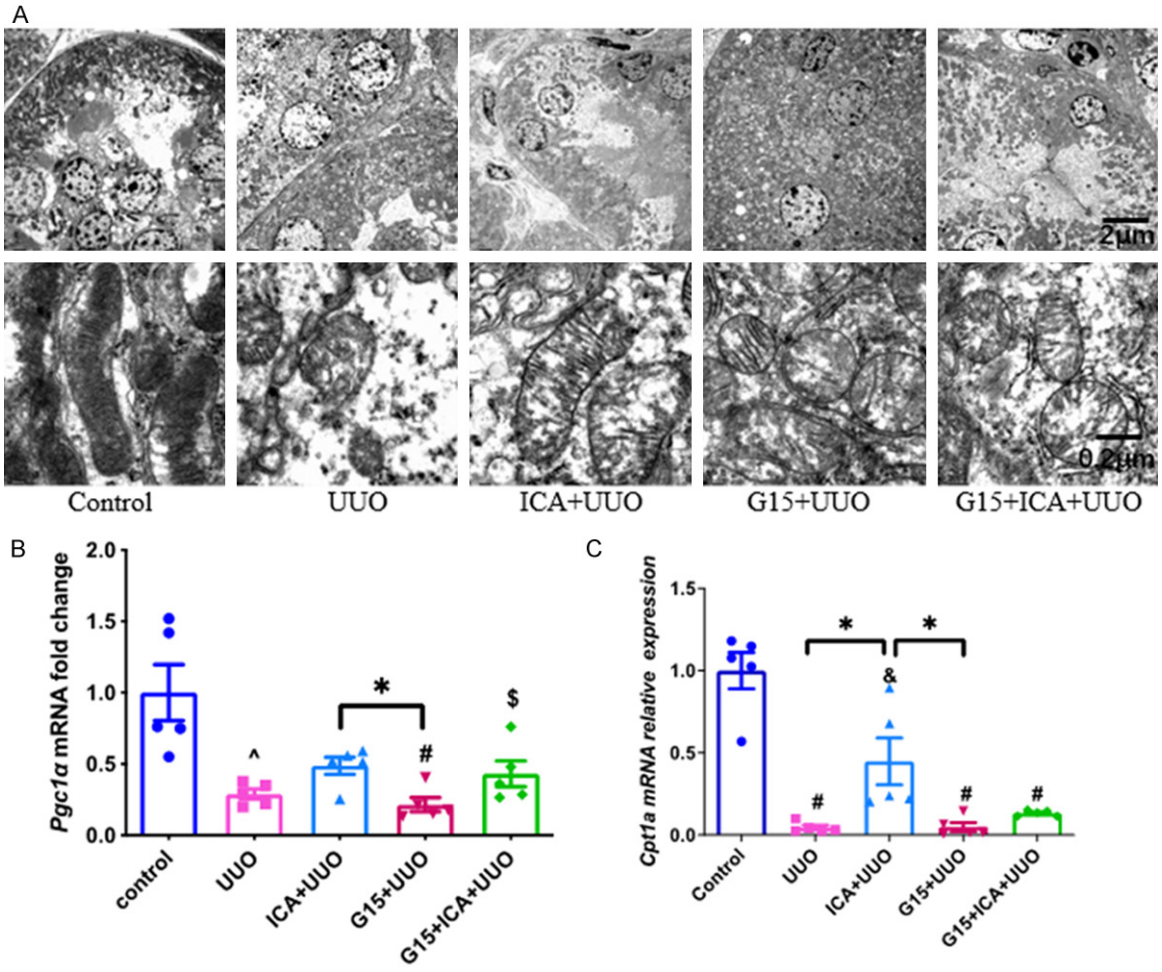

Figure 6. Icariin protects mitochondria from injury in the UUO model. A. Representative electron microscopy images in each group are shown (scale bar 2 μm-0.2 μm). B. The relative mRNA level of Cpt1α is presented. C. The relative mRNA level of Pgc-1α is presented. (n=5, $^{*}P<0.05$; $^{\$}P<0.05$ vs. Control; $^{\wedge}P<0.01$ vs. Control; $^{\&}P<0.001$ vs. Control; $^{\#}P<0.0001$ vs. Control. For comparison, one-way ANOVA was used, followed by the Tukey's test).

Fn, and vimentin mRNA expression in response to Wnt1 (**Figure 7**). Moreover, icariin intervention attenuated Wnt1-induced upregulation of *Col1α1, Fn,* and *vimentin* mRNA. Conversely, GPER inactivation further exacerbated Wnt1-induced fibrosis. The inhibitory effect of icariin on *Col1α1*, *Fn*, and *vimentin* mRNA levels was reversed entirely by G-15. We further transfected NRK-49F with three Gper siRNAs to knock down GPER expression. siRNA2 effectively knocked down GPER expression in NRK-49F at both the mRNA and protein levels (**Figure 8A**, **8B**). We found that GPER deficiency resulted in increased Wnt1-induced *Col1α1* and *Fn* expression in NRK-49F cells. GPER silencing abolished the beneficial effect of icariin on *Col1α1* and *Fn* accumulation (**Figure 8C**, **8D**). Collectively, these in vitro results also confirmed that icariin reduced GPER-dependent fibrotic marker expression.

Discussion

It is widely accepted that the expected lifespan for women is longer than that for men. Besides lifestyle, an inclination to risk, and specific professions, we could not exclude the role of hormones in gender differences, particularly in cardiovascular and neurologic diseases.

Clinically, the male sex is considered a severe aggravating factor; in men, the incidence of end-stage renal failure is 10% higher than that in women [22]. A recent observational cohort study demonstrated that, compared to women, men had a higher rate of all-cause and cardio-

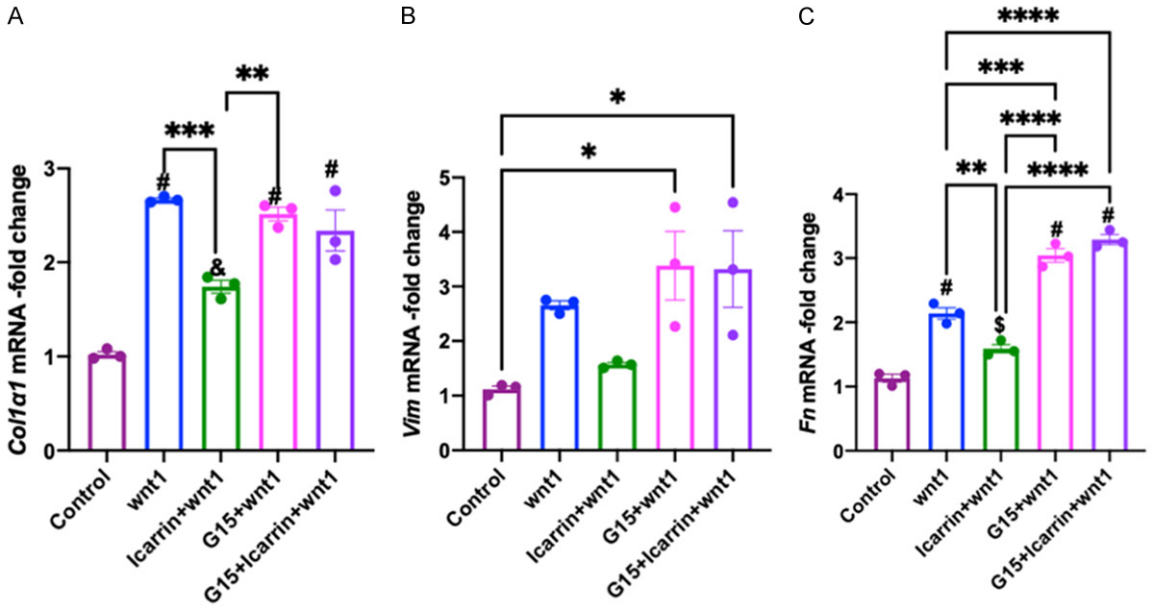

Figure 7. Icariin decreases fibrotic markers expression in NRK-49F via GPER. The relative mRNA levels of Col1α1 (A), Vim (B), Fn (C) are presented. (n=3, $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.001$; $^{\$}P<0.05$ vs. Control; $^{\&}P<0.001$ vs. Control; $^{\#}P<0.0001$ vs. Control. For comparison, one-way ANOVA was used, followed by the Tukey's test).

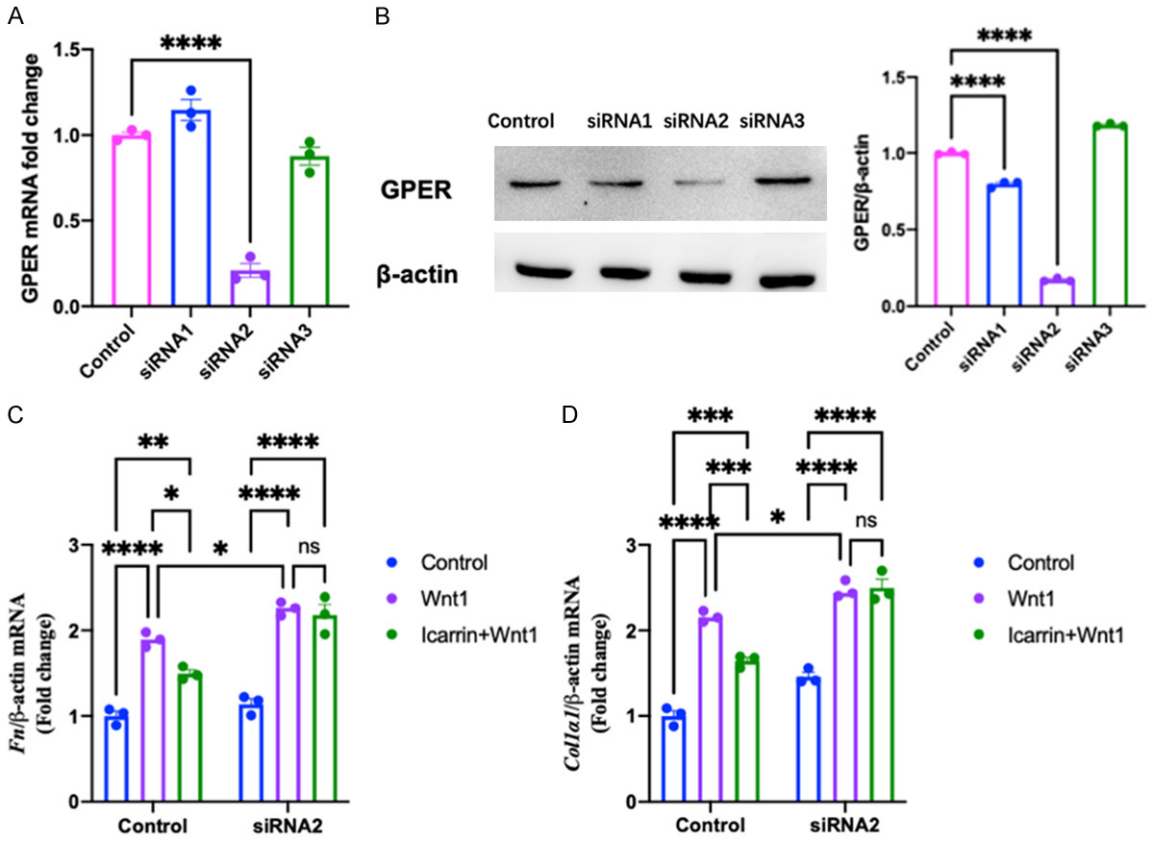

Figure 8. Icariin attenuates fibrotic markers expression in NRK-49F dependent on GPER. The relative mRNA level of Gper (A) is presented. Representative western blot (B) is shown. Western blot analyses presented GPER (42 kDa)/β-actin (42 kDa) expression levels. (n=3, $^{***}P<0.001$; $^{****}P<0.001$); The relative mRNA levels of *Col1α1* (C) and *Fn* (D) are presented. (n=3, $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.001$. For comparison, one-way ANOVA was used, followed by the Tukey's test).

vascular mortality, an increased risk of CKD progression, and a steeper decline in estimated glomerular filtration [23]. Numerous animal models, including the diabetic nephropathy model [24] and 5/6 nephrectomized models [25], showed that estrogen exerts an antifibrotic effect on the kidney. Thus, estrogen is believed to play a critical role in retarding CKD progression.

Estradiol effects are mediated by nuclear estrogen receptor alpha (ERα) and beta (ERβ), as well as GPER in various cell types. GPER initiates rapid nongenomic intracellular signaling cascades, such as adenylyl cyclase/cAMP and epidermal growth factor/mitogen-activated protein kinase (MAPK), whereas ERα and ERβ act as transcription factors [26]. Specifically, MAPK pathway activation regulates gene expression associated with mitochondrial biogenesis [27]. Rapid signaling in response to estradiol has been recognized as critical for overall estrogenic activity. Accumulating evidence shows that GPER plays a vital role in the action of estradiol and the regulation of metabolism, cardiovascular function, and cancer [28]. However, the association between GPER and renal fibrosis has not yet been elucidated.

Estrogen replacement treatment is not as effective as expected and is accompanied by multiple side effects. A broad array of xenoestrogens have been discovered and invented and shown to bind GPER to function as a GPER agonist.

We were interested in using icariin (the traditional Chinese medicine herb active ingredient) as an alternative to estrogen to observe its function in renal fibrosis and its association with GPER. We found that icariin had an antifibrotic effect on the UUO model, similar to reports from a previous study [6]. Furthermore, GPER inhibition using GPER antagonist G-15 substantially exacerbated renal fibrosis, consistent with a previous study which showed that GPER knockout aggravated liver fibrosis [9].

Since it is increasingly accepted that mitochondrial dysfunction is closely associated with the development and progression of renal fibrosis, we further evaluated the mitochondrial morphological alterations and fatty acid metabolism in these groups. Cpt1α is a crucial gene that regulates the transport of fatty acids into mitochondria [16]. Pgc1α is also an essential factor in mitochondrial biogenesis, dynamics, and mitophagy [17]. As in the UUO model, we found enlarged and swollen mitochondria with distorted cristae and decreased Cpt1α and Pgc1α mRNA expression, indicating mitochondrial injury and dysfunction. In contrast, icariin intervention protected mitochondrial structural integrity and restored mitochondrial function. Consistently, the GPER antagonist further exacerbated the mitochondrial structure and function. Icariin and GPER antagonists aggravated mitochondrial injuries. These results confirmed that icariin protects tubules against injury through mitochondrial protection.

Our study is the first to show that GPER plays an essential role in renal fibrosis. Additionally, the relationship between GPER and icariin requires further study. In vitro studies have shown that icariin attenuates Fn deposition in mesangial cells through GPER [13], and it could also modulate mitochondrial function and apoptosis in high glucose-induced glomerular cell podocytes through GPER [14]. Our study is the first in vivo study to demonstrate that icariin may elevate GPER expression. The protective role of icariin in the UUO model could be abrogated by GPER suppression. We also demonstrated that icariin attenuated fibrotic marker expression in rat kidney fibroblasts through GPER. In future studies, it is essential to generate GPER deficient mice to better understand the role of GPER in renal fibrosis and the relationship between icariin and GPER.

In conclusion, icariin should be considered as a potential drug target for anti-renal fibrosis therapy. The clinical implication of Chinese herbal medicines in fibrosis may be a novel therapeutic option for preventing and treating CKD.

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Disclosure of conflict of interest

None.

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