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Original Article

Ellagic acid ameliorates renal fibrogenesis by blocking epithelial-to-mesenchymal transition

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Abstract

Objectives: Ellagic acid (EA), a kind of polyphenol found in numerous fruits and vegetables, has anti-inflammatory, anti-apoptotic, anti-oxidant, and anti-fibrotic effects against a variety of diseases, but its role in mediating renal fibrogenesis remains unknown. **Materials and Methods:** We used an *in vivo* mouse unilateral ureteral obstruction (UUO) model and an *in vitro* model with HK-2 cell lines treated with EA and transforming growth factor β1 (TGF-β1). The expression of epithelial-to-mesenchymal transition (EMT)-related proteins of UUO mice was examined using immunohistochemical staining. Liver function and renal function were evaluated using biochemical testing. Western blot analysis was used to determine the proteins related to EMT, and MTT assay was used to determine cell viability. **Results:** In UUO mice fed EA, both microscopical examination with immunohistochemical staining and western blotting protein analysis showed reduced expression of fibrotic (α-SMA, fibronectin, and collagen I)- and EMT (vimentin and N-cadherin)-related proteins, compared with sham control. In HK-2 cells treated with TGF-β1, EA decreased motility as well as expression of α-SMA, collagen-I, fibronectin, N-cadherin, and vimentin. **Conclusion:** EA reduced the progression of the morphological transformations and concomitantly suppressed the expression of fibrotic- and EMT-related proteins *in vitro* and *in vivo*. These findings improved our understanding of the role of EA in suppressing renal fibrogenesis and demonstrated the promising role EA may play in the management of chronic kidney disease.

Keywords: *Ellagic acid, Epithelial-to-mesenchymal transition, Transforming growth factor β1, Unilateral ureteral obstruction*

Introduction

Chronic kidney disease (CKD) is a chronic condition with a high prevalence and low awareness. Comorbidities, such as diabetes mellitus, hypertension, and hyperlipidemia, increase the risk for CKD and increase its mortality rate [1,2]. Renal tubulointerstitial fibrosis and glomerulosclerosis, which gradually develop as a result of the loss of renal function, are a common pathological characteristic found in almost every form of CKD as it advances [3-5]. Treatment of underlying diseases, avoidance of nephrotoxic substances, a multidisciplinary care team [6,7], and more recently, the use of SGLT-2 inhibitors or aspartate aminotransferase (AST)-120 to relieve the diabetic-associated or indoxyl sulfate-induced renal tubulointerstitial fibrosis through slowing the epithelial-to-mesenchymal transition (EMT) process, are all strategies to slow the progression of CKD [8-10]. In addition, natural compounds have been demonstrated to have anti-fibrotic effects on this process [11,12]. However, the prevalence of CKD remains high, and there is still a need to find more effective adjuvant therapies to slow the progression of CKD.

Evidence indicates that chronic, relentless kidney damage would cause tubular epithelial cells to transdifferentiate, a process known as EMT, which eventually causes apoptosis and the deposition of fibrous tissues consisting of collagenous extracellular matrix (ECM) [13]. EMT is thought to be the process by which normal tubular cells lose their epithelial properties while simultaneously developing a mesenchymal phenotype. This process could lead to the development of renal fibrosis [14,15]. Studies have shown that substances, such as

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nimbolide or resveratrol, could alter the transdifferentiation and EMT of renal tubular cells [16,17]. The chemical structure of ellagic acid (EA) is shown in Figure 1, which is a naturally occurring bioactive and pharmacologically active polyphenolic compound and its potent anti-oxidant, anti-inflammatory, and anticarcinogenic properties [18]. In addition, recent studies have revealed that EA may be involved in regulating a variety of signaling pathways to exhibit protective effects against cardiovascular, hepatic, pulmonary, or renal disorders [19-23] as well as against metal toxicity and either pharmaceutical or natural toxins [24-27].

These studies identified EA as a promising agent for reducing the process of fibrogenesis in a number of organs, but its involvement in the development of renal fibrogenesis is still unclear. Therefore, we conducted this study to further clarify its potential impacts in preventing the progression of CKD.

Materials and methods

Chemical reagents and antibody

EA (E2250) was dissolved in DMSO solution to a final concentration at 100 mM. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (M2128), DMSO (D2650), and propidium iodide (PI; P4170) were purchased from Sigma (St. Louis, MO, USA). Western blotting antibodies against vimentin (#3634-100, BioVision, Inc.), N-cadherin antibody (IR46-143, iReal Biotechnology), α-SMA (sc-32251, Santa Cruz Biotechnology), collagen I antibody (#72026, Cell Signaling Technology), fibronectin (sc-8422, Santa Cruz Biotechnology), GAPDH (sc-32233, Santa Cruz Biotechnology), and second antibodies against goat anti-mouse immunoglobulin G (IgG)-HRP and goat anti-rabbit IgG-HRP antibodies were obtained from Santa Cruz Biotechnology. Transforming growth factor β1 (TGF-β1) (240-B-010; 3 months, −20 to −70°C under sterile conditions after reconstitution) was purchased from Bio-Techne Corporation (Minneapolis, USA).

Unilateral ureteral obstruction model

Five-week-old male C57BL/6JNarl mice were purchased from the National Laboratory Animal Center (Taipei City, Taiwan). Male C57BL/6JNar mice weighing approximately 21 ± 2 g were acquired from the specific pathogen-free laboratory animal center of the Chung Shan Medical University and maintained according to the guidelines of the Institutional Animal Care and Use Committee at Chung Shan Medical University (IACUC number: 2503). The mice were randomly divided into five groups: (1) sham group (n = 3), (2) unilateral ureteral obstruction (UUO) group (n = 3), (3) UUO + 25 mg/kg EA (n = 3), (4) UUO + 50 mg/kg EA (n = 3), and (5) 50 mg/kg EA (n = 3). EA was administered orally every day for 7 days. The mice were then anesthetized by an intraperitoneal injection of pentobarbital sodium, and an incision was made in the left flank. The ureter was freed from the surrounding tissue, and UUO was performed by double-ligating the upper one-third of the left ureter using 4-0 silk, as previously described [28]. Seven days after UUO, all mice were sacrificed and the kidney tissues from the UUO and sham groups were collected for histological and protein analysis.

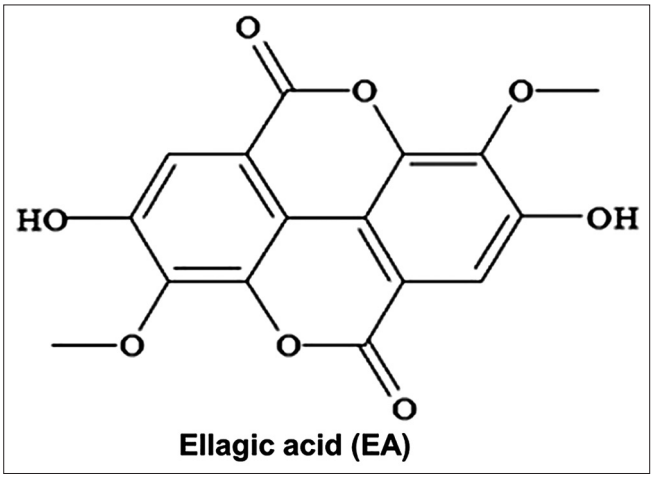

Figure 1: Chemical structure of EA. EA: Ellagic acid

Immunohistochemical stain assay and biochemical blood analysis

The sham and obstructed kidneys with and without EA treatment group were placed in 10% formalin and then embedded in paraffin after alcohol dehydration. Histological and immunohistochemical staining was performed on kidney sections with hematoxylin and eosin (H and E) to detect collagen I (1:100), α-SMA (1:100), and vimentin (1:50) expression. The stained tissue sections were observed under a light microscope (Nikon, NY, USA). The serum levels of alanine aminotransferase, AST, creatinine, and blood urea nitrogen (BUN) from each group were measured using an automatic analyzer at a commercial analytical service center (Lian-Ming Co., Taiwan, ROC).

Cell culture

HK-2 cells are a proximal tubular epithelial cell. They were maintained in Dulbecco's modified Eagle's medium and Ham's F12 medium (DMEM/F12, 1:1), to which $NaHCO_3$, penicillin/streptomycin solution, and 10% fetal bovine serum (FBS) were added. Then, the HK-2 cells were incubated at 37°C in a humidified atmosphere of 5% CO_2. In the experimental condition, HK-2 cells were grown in serum-free medium for 24 h prior to treatment with either TGF-β1 (20 ng/mL), treated with or without EA (25 μM) for 24 h.

Cell growth assay

HK-2 cells (5 × 10^3/well) were seeded into 24-well culture plates for 24 h. The next day, cells were treated with different concentrations of EA (0–30 μM) for 24 h. MTT reagent (0.5 mg/mL) in DMEM/F12 medium was then added for 4 h, and the formazan was dissolved in isopropanol (100%), which was then detected with a microplate reader (Helsinki, Finland) at 570 nm.

Western blotting

The total protein extraction of kidney tissues and cells was harvested and resuspended with lysate buffer (1% NP40 and proteinase/phosphatase inhibitor cocktails) for protein extraction and then measured using the Bradford assay for protein quantification. Twenty micrograms of

protein was loaded into 10% SDS-PAGE for 2 h at 100 V and then transferred into polyvinylidene difluoride (PVDF) membranes for 1.5 h at 4°C. Blocking buffer (5% nonfat milk in TBST buffer) was then added into the PVDF membrane at room temperature for 1 h. Samples were then incubated with the antibody against vimentin antibody (1:2000), N-cadherin antibody (1:1000), α-SMA (1:1000), collagen I antibody (1:1000), fibronectin (1:1000), and GAPDH (1:10,000) at 4°C overnight. The membrane was subsequently washed three times for 5 minutes with TBST buffer. then horseradish peroxidase (HRP)-linked anti-mouse or anti-rabbit antibodies (1:10,000 dilution) were applied. Antibodies were detected with Immunoblot Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA) and photographed with a LAS-4000 mini luminescent image analyzer.

Statistical analysis

All data are represented as mean ± standard deviation of at least three independent experiments. Data were analyzed using one-way analysis of variance, and *post hoc* analysis with Tukey's multiple-comparison test was used for evaluating parametric data. $*P < 0.05$ or $**P < 0.01$ was considered statistically significant.

Results

Ellagic acid alleviated renal fibrogenesis and downregulated the expression of fibrotic- and epithelial-to-mesenchymal transition-related proteins in unilateral ureteral obstruction mice

EA is a naturally bioactive polyphenol compound that is abundant in many taxonomically diverse plants with anti-oxidant, anti-inflammatory, antiproliferative properties [18]. The renal tissues from sham and UUO mice treated with varying concentrations of EA (0, 25, and 50 mg/kg for consecutive 10 days) were examined for morphological and histological characteristics using H and E and immunohistochemistry (IHC). In a dose-dependent way, EA downregulated the expression of α-SMA, collagen I, and vimentin by IHC stain and dramatically reduced the tubule-interstitial fibrotic area compared to sham control mice [Figure 2a]. BUN and creatinine of mice with obstructive nephropathy treated with EA improved compared to sham control [Figure 2d and e], but EA had no effects on liver function [Figure 2b and c]. According to these findings, functionally and anatomically, EA might be protective against the harmful and pro-fibrotic effects of UUO.

Ellagic acid alleviated the expression of fibrotic- and epithelial-to-mesenchymal transition-related markers in unilateral ureteral obstruction mice

We used western blot analysis to evaluate the fibrotic-related markers, such as collagen I, fibronectin, and α-SMA, as well as EMT-related markers, such as N-cadherin and vimentin to investigate the effects of EA on the process of renal fibrogenesis. Mice with UUO treated with EA demonstrated profoundly decreased, dose-dependent expression of fibrotic- and EMT-related proteins compared to the sham control group [Figure 3]. These findings suggest that EA could have beneficial effects on renal fibrogenesis through the modulation of EMT.

Ellagic acid inhibited the motility of transforming growth factor β1-treated HK-2 cells

Studies have demonstrated that TGF-β1 plays a prominent pathogenic role in driving the progression of renal fibrosis [29]. Initially, MTT assay confirmed that HK-2 cells incubated with increasing concentration of EA from 5 μM to 25 μM for 24 h had no significant cytotoxicity compared to control (0 μM) [Figure 4a]. Then, HK-2 cells pretreated with TGF-β1 for 2 h were incubated with or without 25 μM EA for 24 h. We found that HK2 cell motility was increased significantly after treatment with TGF-β1 (8.84-fold, compared with control), while cell motility was decreased after treatment with EA (4.12-fold, compared with TGF-β1 group) in the TGF-β1-treated group [Figure 4b].

Ellagic acid alleviated the expression of fibrotic- and epithelial-to-mesenchymal transition-related proteins of transforming growth factor β1-treated HK-2 cells

In addition, we applied western blot analysis to evaluate the fibrotic- and EMT-related proteins to investigate the effects of EA on renal fibrogenesis induced by TGF-β1 of HK-2 cells. TGF-β1-treated HK-2 cells showed significantly increased expression of α-SMA, collagen I, vimentin, N-cadherin, and fibronectin, which increased by 225.2%, 342.6%, 265.4%, 187.6%, and 281.6% in the TGF-β1-treated group, respectively, and decreased by 34.2%, 110.2%, 84.5%, 75.2%, and 122.7% in the TGF-β1 + EA-treated group, respectively [Figure 5, $**P < 0.01$, compared with control; $^{\#}P < 0.05$, compared with TGF-β1 group]. These findings suggest that EA could mitigate the EMT process in HK-2 cells induced with TGF-β1.

Discussion

The current study investigates the role of EA in renal fibrosis *in vivo* and *in vitro*. We found that (a) EA improved kidney function in UUO mice, as demonstrated by significant decreases in serum creatinine and BUN levels; (b) EA significantly improved the morphologic changes and fibrotic areas in obstructive nephropathy; and (c) EA reduced the expression of fibrotic- and EMT-related proteins in both the obstructive nephropathy and the TGF-β1-treated HK-2 cells [Figure 6].

A common mechanism originally believed to be responsible for the progression of CKD to end-stage renal disease was the aberrant activation of renal fibroblasts and the subsequent accumulation of ECM [13]. Renal tubular epithelial cells would have morphological alterations as a result of chronic, persistent kidney injury, with the hallmarks being increased expression of α-SMA and vimentin as well as dysregulated expression of E-cadherin and N-cadherin [13]. Furthermore, research indicated a link between interstitial myofibroblasts that were positive for α-SMA and a progressive decline in renal function in individuals with diabetic and membranous nephropathy [14,15]. Additional studies have shown that substances such as nimbolide or arecoline that might alleviate or promote transdifferentiation could modulate tubular epithelial cells to become myofibroblasts through

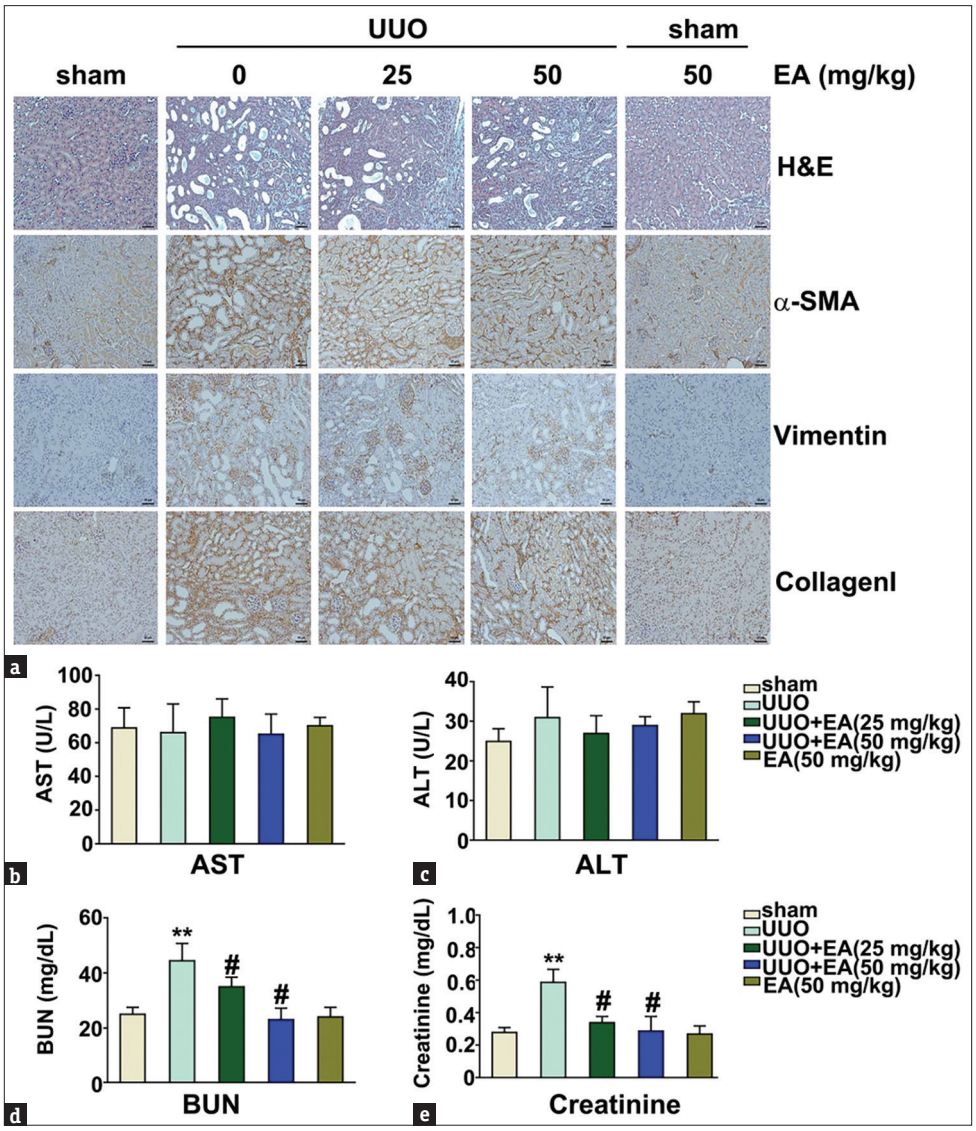

Figure 2: Effects of EA on the histological morphologic transformation and renal and liver function of mice with UUO. (a) Sham and UUO mice were treated with 0, 25 or 50 mg/kg EA for 10 sequential days and then sacrificed. Morphological features of renal tissues were visualized with H and E staining. Expression of collagen I, α-SMA, and vimentin was detected using immunohistochemical staining. The histogram represents the quantitative results of the changes in specific liver (b and c) and kidney function parameters (d and e). $^{**}P < 0.01$, compared with sham control. $^{\#}<0.05$, compared with UUO group. EA: Ellagic acid, UUO: Unilateral ureteral obstruction, H and E: Hematoxylin and eosin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen

the TGF-β pathway, which is known to be crucial for the emergence of interstitial fibrosis, with prominent expression of fibroblastic proteins such as collagen, vimentin, N-cadherin, and α-SMA [16,30]. Therefore, EMT is thought to lead to a vicious cycle of renal fibrosis, which in turn causes even more severe renal damage.

A well-known nephrotoxic substance known as arecoline was found to induce renal fibrogenesis in mice by increasing the expression of TGF-β and fibronectin [31]. Moreover, arecoline-treated HK-2 cells displayed markedly increased mesenchymal-like properties, characterized by decreased E-cadherin and increased N-cadherin, and vimentin, as well as increased α-SMA and collagen [30]. On the other hand, research has demonstrated that substances that inhibit the process of transdifferentiation by mediating renal tissue inflammation, oxidative stress, and apoptosis, such as resveratrol and ruxolitinib, can lessen the process of EMT-induced fibrogenesis [17,32]. We found that EA downregulated the expression of α-SMA, fibronectin, vimentin, collagen, and N-cadherin and showed that EA could regulate the phenotypic transformation of renal tubular epithelial cells to lessen the severity of renal fibrosis.

In addition to lifestyle modifications and multidisciplinary care, medications to block the renin–angiotensin system have been shown to be the main modality to slow the progression of CKD [4,6]. There is evidence that angiotensin II could mediate TGF-β1-dependent renal morphological transformation, with the downstream consequences of ECM deposition, inflammation, and apoptosis. Moreover, losartan, a frequently used angiotensin II receptor antagonist, could regulate the TGF-β1/Smad signaling pathway, resulting in the reduction of renal tubulointerstitial injury and fibrosis

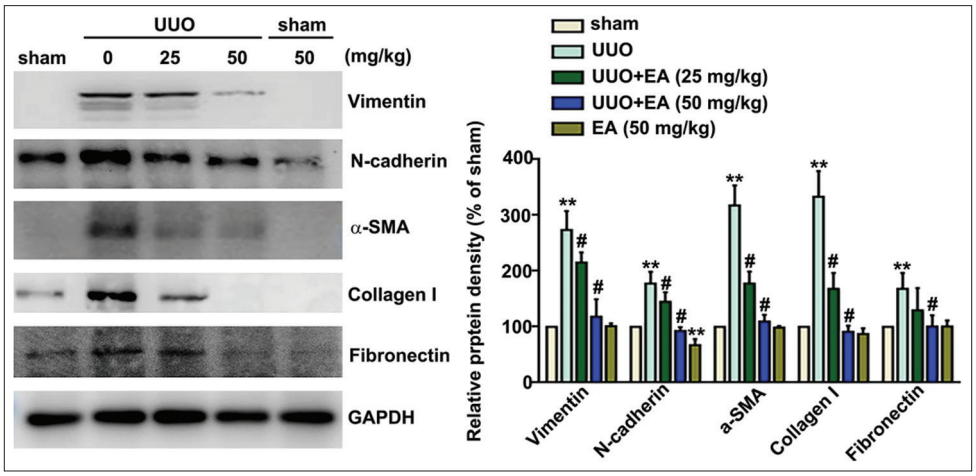

Figure 3: Effects of EA on the expression of fibrotic- and EMT-related markers. Sham mice and mice with UUO were treated with various concentrations (0, 25, and 50 mg/kg) of EA and then total cell lysates were analyzed with western blotting to determine the respective expression of fibrotic (collagen I, fibronectin, and α-SMA)- as well as EMT (vimentin and N-cadherin)-related proteins. GAPDH served as an internal control for protein equal loading (left panel). The histogram represents the quantitative results of the western blot analyses (right panel). Data are presented as the mean ± SD of at least three independent experiments. $^{**}P < 0.01$, compared with sham control. $^{\#}<0.05$, compared with UUO group. EA: Ellagic acid, EMT: Epithelial-to-mesenchymal transition, UUO: Unilateral ureteral obstruction, SD: Standard deviation

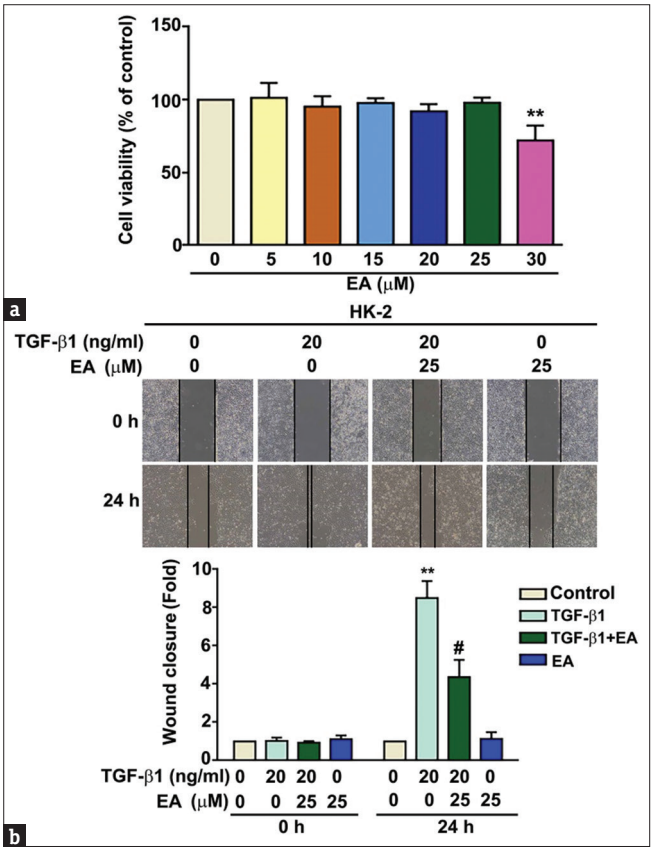

Figure 4: Effects of EA on cell viability and motility of HK-2 cell lines treated with TGF-β1. (a) HK-2 cells were incubated with various concentrations of EA for 24 h to determine cell viability using MTT assay. (b) HK-2 cells were incubated with TGF-β1 (20 ng/mL) for 2 h and then treated with either 0 or 25 μM EA for 24 h by wound healing assays and photographed under a light microscope at ×400. The histogram represents the densitometric analysis of the relative wound area (lower panel). Data are presented as the mean ± standard deviation of at least three independent experiments $^{**}P < 0.01$, compared with control. $^{\#}<0.05$, compared with TGF-β1-treated cells. EA: Ellagic acid, TGF-β1: Transforming growth factor β1

while also inhibiting the EMT process [33]. These drugs have also been shown to demonstrate positive effects on reducing TGF-β1 expression, tubulointerstitial fibrosis, and on reversing EMT by upregulating E-cadherin expression while also suppressing fibronectin, α-SMA, and vimentin expressions [8,34]. Since the prevalence and incidence of CKD are still increasing and the EMT process is essential for renal fibrogenesis, it is necessary to find more therapies to complement CKD treatments.

Numerous natural substances and phytochemicals have been demonstrated to have pharmacological effects and to reduce the process of renal fibrosis [11,12]. One of those was EA, a naturally occurring polyphenolic molecule that is bioactive and pharmacologically active and has been documented to have impacts on protecting the organs or reducing the toxicity of some substances [19-23]. By decreasing oxidative stress as well as inflammatory and apoptotic adverse effects, EA could lessen the hepatorenal damage *in vivo* caused by lead, an environmental toxin [19]. Similar to this, EA could reduce nephrotoxicity caused by chromium or arsenic. This has been previously demonstrated through improved serum biomarkers (BUN and creatinine), morphological injuries (tubular injury, glomerular hypertrophy, congestion, inflammatory, and necrosis), and oxidative stress, as evidenced by the recovery of anti-oxidant enzymes and mitochondrial ultrastructure [24,25]. In addition, EA showed both protective qualities and potential anti-fibrotic effects on a variety of organs [20-23,35]. Previous studies have also demonstrated the beneficial effects of EA against pulmonary fibrosis, both *in vivo* and *in vitro*, by inhibiting the Wnt signaling pathway, thus promoting the formation of myofibroblast autophagy [22]. Li *et al*. found that EA promoted the degradation of vesicle-associated membrane protein 2 through a proteasome-dependent route, which caused liver stellate cells to undergo ferroportin-dependent ferroptosis and hepatic fibrosis [21]. The results of a study utilizing TGF-β1-induced immortalized cardiac fibroblasts incubated with EA showed that EA could decrease the expression of pro-fibrotic proteins (collagen I, MMP2, and

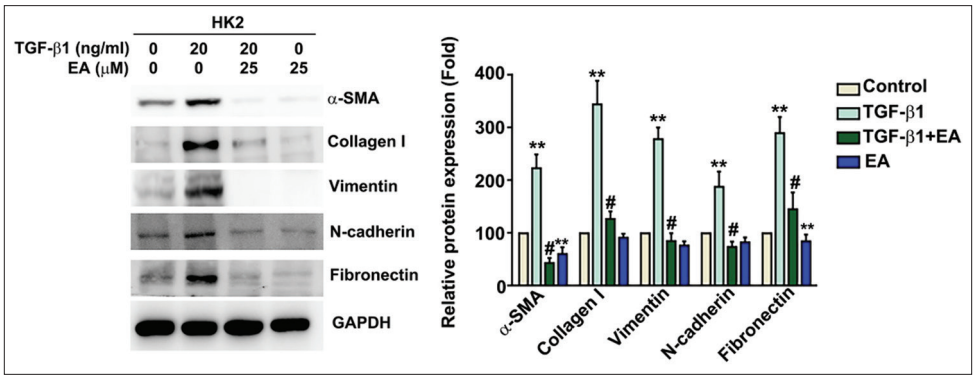

Figure 5: Effects of EA on the expression of fibrotic- and epithelial-to-mesenchymal transition (EMT)-related markers of HK-2 cell lines treated with TGF-β1. HK-2 cells were incubated with TGF-β1 (20 ng/mL) for 2 h and then treated with either 0 or 25 μM of EA for 24 h. Total cell lysates were analyzed with western blotting to determine the protein expression of fibrotic (collagen I, fibronectin, and α-SMA)- as well as EMT (vimentin and N-cadherin)-related proteins (left panel). GAPDH was used as an internal control for protein equal loading. The histogram represents the quantitative results of the western blot analyses. Data are presented as the mean ± standard deviation of at least three independent experiments. $^{**}P < 0.01$, compared with control. $^{\#}<0.05$, compared with TGF-β1-treated cells. TGF-β1: Transforming growth factor β1, EA: Ellagic acid

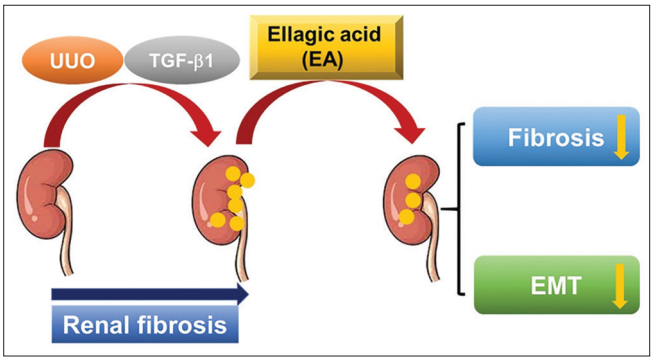

Figure 6: Schematic representation of the EA alleviated renal fibrosis via modulating the process of epithelial-to-mesenchymal transition both *in vitro* and *in vivo*. EA: Ellagic acid, TGF-β1: Transforming growth factor β1, EMT: Epithelial-to-mesenchymal transition, UUO: Unilateral ureteral obstruction

MMP9), inflammatory cytokines, and reactive oxygen species accumulation, which results in reducing collagen deposition by inhibiting the TGF-β1/Smad2/3, MMP2/9, and Wnt/β-catenin signaling pathways [23]. EA has also been demonstrated to inhibit oxidative stress and to have beneficial effects in alleviating chronic pancreatitis through decreased infiltration of inflammatory cells, activated α-SMA positive cells, reduced collagen content, and TGF-β1 expression [35]. These studies indicate that EA possesses anti-fibrotic properties that impact a variety of organs. Similar results were obtained in our investigation, where we discovered that mice with obstructive nephropathy given EA had improved renal function, decreased fibrotic- and EMT-related proteins, and reduced fibrosis within renal tissues.

As CKD could be induced by several mechanisms, EA was used in these models to examine the possible beneficial effects. Older rats fed EA exhibited significantly improved renal function, improved histopathological scores (inflammation, tubular atrophy, and tubulointerstitial fibrosis), and attenuated oxidative stress, as demonstrated by a notable elevation of anti-oxidant activities and a remarkable decrease in pro-oxidant activity when compared to older rats fed control diets [36]. In addition, studies of diabetic mice and renal proximal tubular cells incubated with high glucose demonstrated that EA exhibited renal protective effects, as evidenced by improved glycemic control, decreased serum levels of BUN and creatinine, suppressed oxidative stress and inflammatory markers, and reduced expressions of TGF-β1 and fibronectin in renal tissues [20,37]. Furthermore, feeding EA to 5/6 nephrectomy rats led to protective effects by reducing renal histological injuries, decreasing markers of oxidative stress and inflammation, and lowering the expression of TGF-β1, fibronectin, and apoptotic-related proteins in a dose-dependent manner [38]. Consistent with these studies, which were representative of multiple pathogenic CKD pathways, we found that EA might *in vivo* (obstructive nephropathy) and *in vitro* (TGF-β1 induced fibrogenesis) reduce the expression of fibrotic- and EMT-related proteins, mitigate the severity of renal fibrosis, and improve plasma levels of BUN and creatinine.

There were several limitations of this study. Firstly, we hypothesized that EA's anti-oxidant and anti-inflammatory activities could alleviate obstructive nephropathy, but this study's relevant investigations were lacking. Secondly, while we have shown that EA could reduce EMT in both vitro and *in vivo*, further research is needed to explore the specific signaling mechanisms through which EA reduces renal fibrogenesis. This is an active area of research for the study authors.

Conclusion

The results of this study indicate that EA may be effective in preventing renal fibrogenesis both *in vivo* and *in vitro*. Figure 6 provides a schematic illustration of the beneficial properties of EA. We now have a better understanding of how EA inhibits renal fibrogenesis, and our study raises the possibility that EA could be a promising therapeutic option for delaying the progression of CKD. However, the downstream and upstream signaling pathways modulating the effects of EA on renal fibrogenesis require further study.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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This work was supported by grants from Buddhist Tzu Chi Medical Foundation (TCMF-CM1-111-02; TCMF-CP-110-02) and Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (DTCRD112-I-07).

Conflicts of interest

There are no conflicts of interest.

References

1. Hsu CC, Hwang SJ, Wen CP, Chang HY, Chen T, Shiu RS, et al. High prevalence and low awareness of CKD in Taiwan: A study on the relationship between serum creatinine and awareness from a nationally representative survey. Am J Kidney Dis 2006;48:727-38.
2. Wen CP, Cheng TY, Tsai MK, Chang YC, Chan HT, Tsai SP, et al. All-cause mortality attributable to chronic kidney disease: A prospective cohort study based on 462 293 adults in Taiwan. Lancet 2008;371:2173-82.
3. Liu Y. Renal fibrosis: New insights into the pathogenesis and therapeutics. Kidney Int 2006;69:213-7.
4. Ruggenenti P, Cravedi P, Remuzzi G. Mechanisms and treatment of CKD. J Am Soc Nephrol 2012;23:1917-28.
5. Corden B, Adami E, Sweeney M, Schafer S, Cook SA. IL-11 in cardiac and renal fibrosis: Late to the party but a central player. Br J Pharmacol 2020;177:1695-708.
6. Chen YR, Yang Y, Wang SC, Chiu PF, Chou WY, Lin CY, et al. Effectiveness of multidisciplinary care for chronic kidney disease in Taiwan: A 3-year prospective cohort study. Nephrol Dial Transplant 2013;28:671-82.
7. Hwang SJ, Tsai JC, Chen HC. Epidemiology, impact and preventive care of chronic kidney disease in Taiwan. Nephrology (Carlton) 2010; 15(Suppl 2): 3-9.
8. Huang F, Zhao Y, Wang Q, Hillebrands JL, van den Born J, Ji L, et al. Dapagliflozin attenuates renal tubulointerstitial fibrosis associated with type 1 diabetes by regulating STAT1/TGFβ1 signaling. Front Endocrinol (Lausanne) 2019;10:441.
9. Ndibalema AR, Kabuye D, Wen S, Li L, Li X, Fan Q. Empagliflozin protects against proximal renal tubular cell injury induced by high glucose via regulation of hypoxia-inducible factor 1-alpha. Diabetes Metab Syndr Obes 2020;13:1953-67.
10. Bolati D, Shimizu H, Niwa T. AST-120 ameliorates epithelial-to-mesenchymal transition and interstitial fibrosis in the kidneys of chronic kidney disease rats. J Ren Nutr 2012;22:176-80.
11. Hsieh YH, Hung TW, Chen YS, Huang YN, Chiou HL, Lee CC, et al. *In vitro* and *in vivo* antifibrotic effects of fraxetin on renal interstitial fibrosis via the ERK signaling pathway. Toxins (Basel) 2021;13:474.
12. Huang CF, Hung TW, Yang SF, Tsai YL, Yang JT, Lin CL, et al. Asiatic acid from *Centella asiatica* exert anti-invasive ability in human renal cancer cells by modulation of ERK/p38MAPK-mediated MMP15 expression. Phytomedicine 2022;100:154036.
13. Lovisa S, LeBleu VS, Tampe B, Sugimoto H, Vadnagara K, Carstens JL, et al. Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. Nat Med 2015;21:998-1009.
14. Essawy M, Soylemezoglu O, Muchaneta-Kubara EC, Shortland J, Brown CB, el Nahas AM. Myofibroblasts and the progression of diabetic nephropathy. Nephrol Dial Transplant 1997;12:43-50.
15. Roberts IS, Burrows C, Shanks JH, Venning M, McWilliam LJ. Interstitial myofibroblasts: Predictors of progression in membranous nephropathy. J Clin Pathol 1997;50:123-7.
16. Annaldas S, Saifi MA, Khurana A, Godugu C. Nimbolide ameliorates unilateral ureteral obstruction-induced renal fibrosis by inhibition of TGF-β and EMT/slug signalling. Mol Immunol 2019;112:247-55.
17. Beshay ON, Ewees MG, Abdel-Bakky MS, Hafez SM, Abdelrehim AB, Bayoumi AM. Resveratrol reduces gentamicin-induced EMT in the kidney via inhibition of reactive oxygen species and involving TGF-β/Smad pathway. Life Sci 2020;258:118178.
18. Ríos JL, Giner RM, Marín M, Recio MC. A pharmacological update of ellagic acid. Planta Med 2018;84:1068-93.
19. Bidanchi RM, Lalrindika L, Khushboo M, Bhanushree B, Dinata R, Das M, et al. Antioxidative, anti-inflammatory and anti-apoptotic action of ellagic acid against lead acetate induced testicular and hepato-renal oxidative damages and pathophysiological changes in male long Evans rats. Environ Pollut 2022;302:119048.
20. Chao CY, Mong MC, Chan KC, Yin MC. Anti-glycative and anti-inflammatory effects of caffeic acid and ellagic acid in kidney of diabetic mice. Mol Nutr Food Res 2010;54:388-95.
21. Li L, Wang K, Jia R, Xie J, Ma L, Hao Z, et al. Ferroportin-dependent ferroptosis induced by ellagic acid retards liver fibrosis by impairing the SNARE complexes formation. Redox Biol 2022;56:102435.
22. Li X, Huang K, Liu X, Ruan H, Ma L, Liang J, et al. Ellagic acid attenuates BLM-induced pulmonary fibrosis via inhibiting Wnt signaling pathway. Front Pharmacol 2021;12:639574.
23. Mannino F, Imbesi C, Bitto A, Minutoli L, Squadrito F, D'Angelo T, et al. Anti-oxidant and anti-inflammatory effects of ellagic and punicic acid in an *in vitro* model of cardiac fibrosis. Biomed Pharmacother 2023;162:114666.
24. Cuevas-Magaña MY, Vega-García CC, León-Contreras JC, Hernández-Pando R, Zazueta C, García-Niño WR. Ellagic acid ameliorates hexavalent chromium-induced renal toxicity by attenuating oxidative stress, suppressing TNF-α and protecting mitochondria. Toxicol Appl Pharmacol 2022;454:116242.
25. Mehrzadi S, Fatemi I, Malayeri AR, Khodadadi A, Mohammadi F, Mansouri E, et al. Ellagic acid mitigates sodium arsenite-induced renal and hepatic toxicity in male Wistar rats. Pharmacol Rep 2018;70:712-9.
26. Rehman MU, Tahir M, Ali F, Qamar W, Lateef A, Khan R, et al. Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: The protective effect of ellagic acid. Mol Cell Biochem 2012;365:119-27.
27. Silfeler I, Alp H, Dorum BA, Nacar E, Arslan S, Uygur V. Protective effect of ellagic acid on paraquat-induced kidney Hazards in rats. Iran J Kidney Dis 2017;11:23-8.
28. Li X, Bu X, Yan F, Wang F, Wei D, Yuan J, et al. Deletion of discoidin domain receptor 2 attenuates renal interstitial fibrosis in a murine unilateral ureteral obstruction model. Ren Fail 2019;41:481-8.
29. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: The master regulator of fibrosis. Nat Rev Nephrol 2016;12:325-38.
30. Hsieh YH, Syu RJ, Lee CC, Lin SH, Lee CH, Cheng CW, et al. Arecoline induces epithelial mesenchymal transition in HK2 cells by upregulating the ERK-mediated signaling pathway. Environ Toxicol 2020;35:1007-14.
31. Lin SH, Chiou SJ, Ho WT, Chuang CT, Chuang LY, Guh JY. Arecoline-induced pro-fibrotic proteins in LLC-PK1 cells are dependent on c-Jun N-terminal kinase. Toxicology 2016;344-346:53-60.
32. Bai Y, Wang W, Yin P, Gao J, Na L, Sun Y, et al. Ruxolitinib alleviates renal interstitial fibrosis in UUO mice. Int J Biol Sci 2020;16:194-203.
33. Zou J, Zhou X, Ma Y, Yu R. Losartan ameliorates renal interstitial fibrosis through metabolic pathway and Smurfs-TGF-β/Smad. Biomed Pharmacother 2022;149:112931.
34. Das NA, Carpenter AJ, Belenchia A, Aroor AR, Noda M, Siebenlist U,

et al. Empagliflozin reduces high glucose-induced oxidative stress and miR-21-dependent TRAF3IP2 induction and RECK suppression, and inhibits human renal proximal tubular epithelial cell migration and epithelial-to-mesenchymal transition. Cell Signal 2020;68:109506.

35. Suzuki N, Masamune A, Kikuta K, Watanabe T, Satoh K, Shimosegawa T. Ellagic acid inhibits pancreatic fibrosis in male Wistar Bonn/Kobori rats. Dig Dis Sci 2009;54:802-10.
36. Naghibi N, Sadeghi A, Movahedinia S, Rahimi Naiini M, Rajizadeh MA, Bahri F, et al. Ellagic acid ameliorates aging-induced renal oxidative damage through upregulating SIRT1 and NRF2. BMC Complement Med Ther 2023;23:77.
37. Ahad A, Ganai AA, Mujeeb M, Siddiqui WA. Ellagic acid, an NF-κB inhibitor, ameliorates renal function in experimental diabetic nephropathy. Chem Biol Interact 2014;219:64-75.
38. Chen S, Zhou M, Ying X, Zhou C. Ellagic acid protects rats from chronic renal failure via MiR-182/FOXO3a axis. Mol Immunol 2021;138:150-60.