Pharmaceutical Sciences Asia

Pharm Sci Asia 2023; 50(4), 371-380

DOI:10.29090/psa.2023.04.23.645

Research Article

Altenusin inhibits epithelial to mesenchymal transition via suppression of TGF-β/MAPK signaling pathway in human renal proximal tubular cells and unilateral ureteral obstruction mice

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ABSTRACT

Renal fibrosis is recognized as a key pathological feature of chronic kidney disease (CKD), which progresses toward end stage renal disease (ESRD). Transforming growth factor (TGF)-β-induced epithelial to mesenchymal transition (EMT) of renal epithelial tubular cells is the key mechanism of renal fibrosis. The aim of this study is to investigate the pharmacological effect of altenusin, an active compound derived from fungi, on TGF-β/mitogen-activated protein kinase (MAPK) signaling pathway-induced fibrosis in renal proximal tubular cells and in mouse unilateral ureteral obstruction (UUO) model. As a result, TGF-β1 induced EMT of RPTECT/TERT1 cells (an immortalized human renal proximal tubular cells) by concentration- and time-dependent manners. Incubating cells with 10 ng/ml TGF-β1 for 48 hours significantly upregulated MAPK signaling pathway by increase phosphorylated (p)-Jun N-terminal kinase (JNK), p-p38, and p-Extracellular signal-regulated kinase (ERK) 1/2. Treating the cells with altenusin (50 -100 μM) significantly attenuated TGF-β1-induced EMT. The inhibitory effect of altenusin on EMT was mediated by inhibition of p38 and ERK1/2 but not JNK. UUO in mice for 14 days dramatically increased p-JNK, p-p38, and p-ERK1/2, activation of these proteins by UUO were attenuated by co-treatment with altenusin 3 mg/kg. These results demonstrate the inhibitory effect of altenusin on TGF-β/MAPK signaling pathway-induced EMT in human renal proximal tubular cells and in animal model of renal fibrosis.

Keywords:
Altenusin, Epithelial to mesenchymal transition, Transforming growth factor β, MAPK, Unilateral ureteral obstruction

1. INTRODUCTION

Fibrosis in glomerulus and tubulointerstitium is a key pathological feature of chronic kidney disease (CKD) and progresses to end-stage renal disease (ESRD). Tubulointerstitial fibrosis is recognized as the best predictor of renal survival from kidney disease of any etiology[1-2]. Renal fibrosis is induced by numerous injurious tumuli such as hypertension, hyperglycemia, acute kidney injury (AKI), drugs (e.g. nonsteroidal anti-inflammatory drugs (NSAIDs), or chemotherapies), herbs, and toxins[3-4]. Epithelial to mesenchymal transition (EMT) is a mechanism by which transformation of tubular epithelial cells to fibroblasts leads to increase in extracellular matrix (ECM) producing cells and ECM accumulation[4], causing the development of renal fibrosis. Under induction of fibrosis by injurious stimuli, transforming growth factor (TGF-β) is secreted from the renal cells, which is the well-known inducer of EMT and fibrosis[5]. Upon TGF-β activation, it regulates transcription of genes involving in EMT and fibrosis via

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Smad signal pathway and mitogen-activated protein kinase (MAPK) signaling pathways[6]. TGF-β binds to TGF-β type 2 receptor (TβR2) and recruits TβR1, causing dimerization of TβRs. TβRs phosphorylate downstream of Smad (Smad2/3) and MAPK (JNK, p38, and ERK1/2) signaling pathways resulting in upregulation of genes involved in fibrosis such as fibronectin, α-SMA, vimentin, and collagens and downregulation of epithelial marker E-cadherin[6-7]. The phosphorylation of MAPK downstream signaling pathway (JNK, p38, and ERK) is upregulated in EMT of renal proximal tubular cells induced by TGF-β[8]. Previous study reported that ganoderic acid shows the protective effect against renal fibrosis in UUO mice by reducing p-Smad2/3, p-ERK, p-JNK, and p-p38[8].

Altenusin is an active compound from *Alternaria sp.* and *Penicillum sp.*[9-12], which has various biological properties. It shows inhibitory effects on several molecules such as myosin light chain kinase, specific neutral sphingomyelinase, nuclear factor-erythroid derived 2-like 2, and Tau fibrillization[9-10,13-16]. In addition, altenusin has an anti-diabetic properties by suppression of α-glucosidase and pancreatic lipase enzymes[17]. In 2017, altenusin was reported as a farnesoid X receptor (FXR) agonist, nonalcoholic fatty liver disease (NAFLD) disease progression in mice is attenuated by treatment with altenusin[18]. FXR is a member of nuclear super family, plays important role in bile acid, carbohydrates, and lipid metabolisms[19]. FXR is activated by bile acids (cholic acid, lithocholic acid, deoxycholic acid, chenodeoxycholic acid, and obeticholic acid) and synthetic FXR agonist (GW4064)[20-21]. In kidney, FXR expression is downregulated in diabetic nephropathy, a subtype of CKD[22]. FXR deficiency deleterious induces kidney injury and fibrosis in diabetic nephropathy[23]. FXR activation attenuates diabetic nephropathy and renal fibrosis in rodent models[22,24-25]. Interestingly, FXR alleviates liver and lung fibrosis by downregulated TGF-β expression[26-28]. Unfortunately, there is no report about the role of FXR in TGF-β regulation in renal proximal tubular cells. FXR activation by GW4064 reduces ERK1/2 phosphorylation resulting in suppression of esophageal squamous carcinoma cell proliferation and migration, inducing cell cycle arrest and apoptosis[29]. Therefore, it is interesting whether altenusin modulates MAPK signaling pathway in TGF-β-induced EMT in renal proximal tubular cells and UUO model. The present study investigated the pharmacological effect of altenusin on TGF-β/MAPK signaling pathway which participates in renal EMT and fibrosis.

2. MATERIALS AND METHODS

2.1. Chemicals

TGF-β1 (7754-BH-005) was purchased from R&D Systems (MN, USA.). Primary antibodies including E-cadherin (3195S), p-ERK1/2 (9102S), ERK1/2 (9101S), p38 (9212S), p-JNK (4668T), JNK (9252T), and GAPDH (2118S) were obtained from cell signaling (MA, USA.). Fibronectin (sc-8422), and p-p38 (sc-101759) primary antibodies were purchased from Santa Cruz Biotechnology (CA, USA.). Anti-NR1H4 (FXR) was purchased from Merck Millipore (Bangkok, Thailand). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was purchased from Invitrogen (Bangkok, Thailand). All other chemicals are analytical grade and purchased from commercial sources.

2.2. Altenusin isolation

Altenusin was isolated from the culture of the endophytic fungus *Alternaria destruens* PobtRO-6. An analysis of ^{1}H NMR of the isolated altenusin revealed purity >97% (see supporting information).

2.3. Cell culture

Renal proximal tubular (RPTEC/TERT1) cell line (lot number: 58702509) was obtained from ATCC (VA, USA). The cells were cultured as previous[30]. The cells were grown until matured, then treated with various conditions.

2.4. Cell viability assay

The matured RPTEC/TERT1 cells grown on 96-well plate were treated with several concentrations of altenusin for 48 hours. At the end of incubation periods, the cells were incubated with 100 µl MTT reagent (0.5 mg/ml in serum free media) for 1 hour at 37°C. After removing of MTT reagent, 100 µl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. MTT-reducing activity of cell was determined using a BioTek 800TS microplate reader (Agilent Technologies, CA, USA) at 590 nm. Cell viability was calculated as the percent of control (vehicle-treated group).

2.5. Induction of EMT in renal proximal tubular cells by TGF-β1

Mature RPTEC/TERT1 cells were incubated with TGF-β1 at concentrations of 0-10 ng/ml for 0-72 hours. At the end of the incubation periods, morphology change was observed. The fibrotic protein markers including E-cadherin and fibronectin were examined by Western blot analysis.

2.6. Mouse UUO model

Male C57BL/6 mice (7 weeks, 20-25 g) were purchased from Nomura Siam International Co, Ltd (Bangkok, Thailand). The animal protocols used in this study was approved by the Faculty of Science, Mahidol University Anaiml Care and Use Committee (protocol no:

MUSC64-036-585). Mice were acclimatized and housed accordance with standard condition (12:12 h light-dark cycle, room temperature of 22-24°C, and relative humidity of 50-60%) of Central animal facility, Faculty of Science, Mahidol University. The mice were freely accessible to normal diet and water, they were randomly divided into sham-operated group (Sham), UUO-operated group (UUO), and UUO plus 3 mg/kg i.p. altenusin (UUO+ALT). The UUO surgery was performed as previous[31]. Briefly, the mice were anaesthetized with isoflurane, abdominal cavity was opened through the middle line, next, the left ureter was obstructed and ligated with silk sutures. Sham underwent the same procedure as UUO to see the left ureter, abdominal cavity was closed without obstructing the left ureter. Mice were treated for 14 days consecutively to collect kidneys for protein expression observation.

2.7. Western blot analysis

Proteins of cells and kidney samples were extracted by using RIPA buffer. Equal amounts of proteins were denatured at 95°C for 5 min, separated by 10% acrylamide SDS-PAGE, and then transferred into 0.45 micrometer nitrocellulose membranes (Bio-Rad, CA, USA.). Membranes were blocked by 5% blotting grade blocker (Bio-Rad, CA, USA.) for 1 h, then, incubated overnight with primary antibodies. After primary incubation period, the membranes were washed 3 times with Tris-buffered saline with 0.1% Tween 20 (TBST) and incubated with horseradish peroxidase-conjugated secondary antibody. The immune complexes were detected using the Electro-Chemi-Luminescence (ECL) system and visualized by Azure 600 Gel Imaging System (Azure Biosystem, Inc., CA, USA). The intensity of the bands was quantified using ImageJ software, where data were shown as the ratio of densitometry of interested proteins normalized by loading control protein (GAPDH).

2.8. Statistical analysis

All data are represented as mean±SD using GraphPad Prism for Window (CA, USA). The statistical significance between control and treatment groups was analyzed using one-way analysis of variance (ANOVA). $P<0.05$ considered a significant difference.

3. RESULTS

3.1. Effect of altenusin on cell viability in human renal proximal tubular cells

Firstly, the concentration-dependent effect of altenusin on cell viability of human renal proximal tubular cells was determined using MTT assay. RPTEC/TERT1 cells were treated with vehicle or various concentration (1-100 µM) of altenusin for 48 hours. Referring to Figure 1, altenusin at concentrations of 1-100 µM did not reduce cell viability compared with vehicle-treated cells.

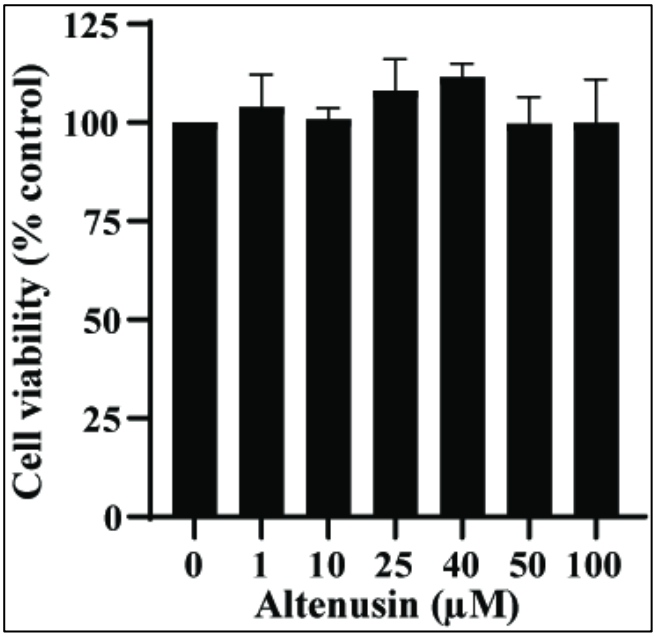

Figure 1. Effect of altenusin on cell viability in RPTEC/TERT1 cells. RPTEC/TERT1 cells were incubated with 0-100 µM altenusin for 48 hours. Data are shown as mean ± SD (n=3).

3.2. Effect of TGF-β1-induced EMT in renal proximal tubular cells

The concentration-and time-dependent effects of TGF-β1-induced EMT in RPTEC/TERT1 cells were examined. RPTEC/TERT1 cells were incubated with TGF-β1 (0-10 ng/ml) for 48 hours followed by measurement of E-cadherin and fibronectin protein expression. As shown in Figure 2, treatment the cell with TGF-β1 caused change in cell morphology of epithelium to a fibroblast-like structure. In addition, TGF-β1 reduced E-cadherin expression, a marker of epithelial cells, in concentration-dependent manner. In contrast to E-cadherin, TGF-β1 increased fibronectin expression, a marker of fibroblast, in concentration-dependent manner. TGF-β1 concentration of 10 ng/ml produced the strongest induction of EMT in this cell line, therefore, TGF-β1 concentration of 10 ng/ml was selected for the following experiments. Next, time-dependent effect of 10 ng/ml TGF-β1 on EMT was determined. Treatment of TGF-β1 concentration of 10 ng/ml for 24-72 hours significantly reduced E-cadherin expression whereas increased fibronectin expression at 48 and 72 hours (Figure 3).

3.3. Effect of altenusin on MAPK signaling pathway in TGF-β1-induced EMT in renal proximal tubular cells

There are evidences showing that TGF-β1 induces EMT and fibrosis in kidneys through TGF-β/MAPK

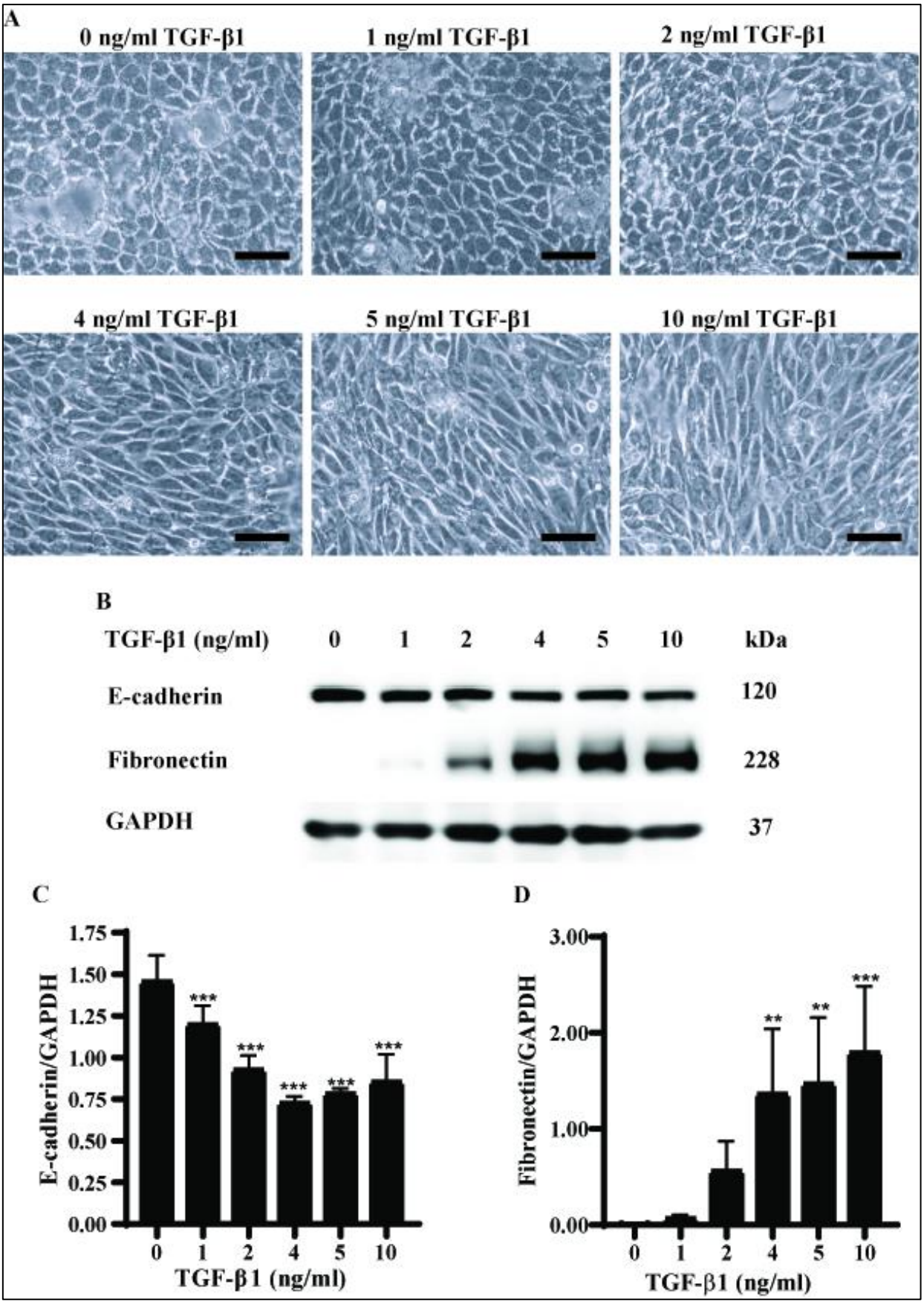

Figure 2. Concentration-dependent effect of TGF-β1-induced EMT in RPTEC/TERT1 cells.
(A) Morphology of RPTEC/TERT1 cells following 48 hours incubation of the control and TGF-β1 (1-10 ng/ml) (200X, scale bar=50 μm). (B) The representative immunoblotting of E-cadherin, fibronectin, and GAPDH proteins. (C-D) Densitometric analysis of bands are represented as graphs of indicated proteins in RPTEC/TERT1 cells. Data are shown as mean±SD of three experiments. $^{**}P<0.01$, and $^{***}P<0.001$ compared with 0 ng/ml TGF-β1.

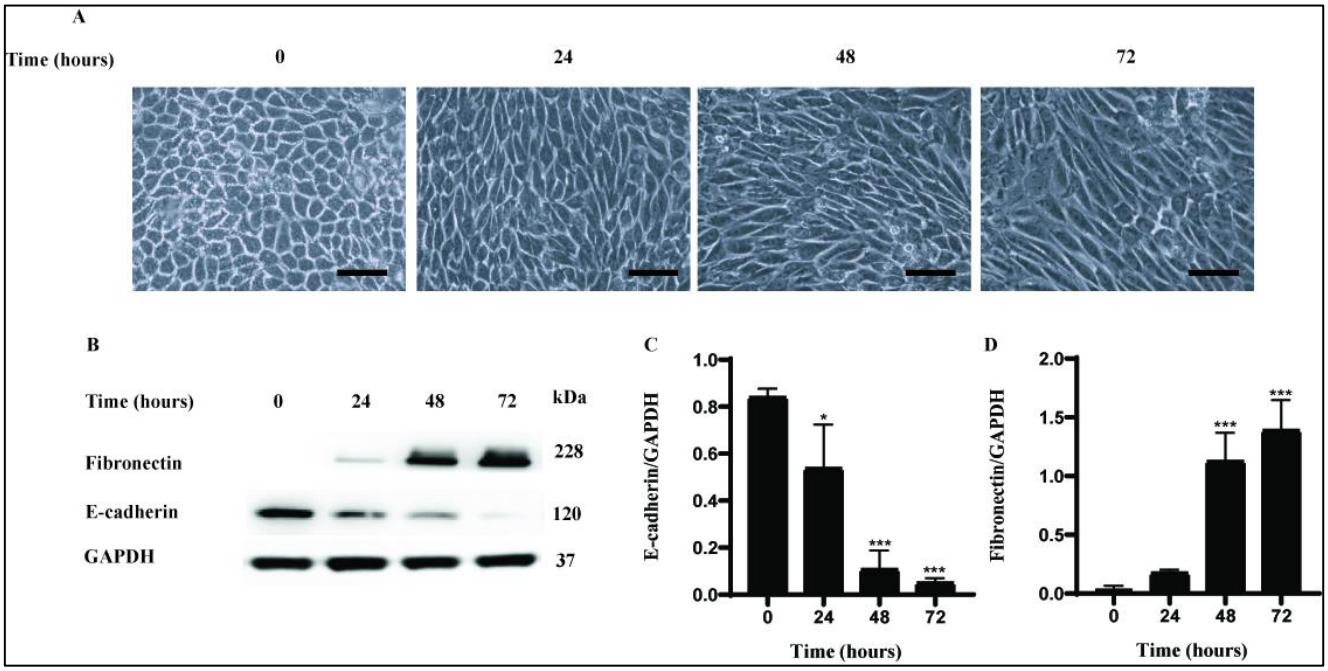

Figure 3. Time-dependent effect of TGF-β1 on expressions of E-cadherin and fibronectin in RPTEC/TERT1 cells.
(A) Morphology of RPTEC/TERT1 cells following 0-72 hours incubation of 10 ng/ml TGF-β1 (200X, scale bar=50 μm). (B) The representative immunoblots of E-cadherin, fibronectin, and GAPDH proteins. (C-D) Densitometric analysis of indicated proteins. Data are shown as mean± SD of three experiments. $^{*}P$<0.05, and $^{***}P$<0.001 compared with non-treated cells (0 hour).

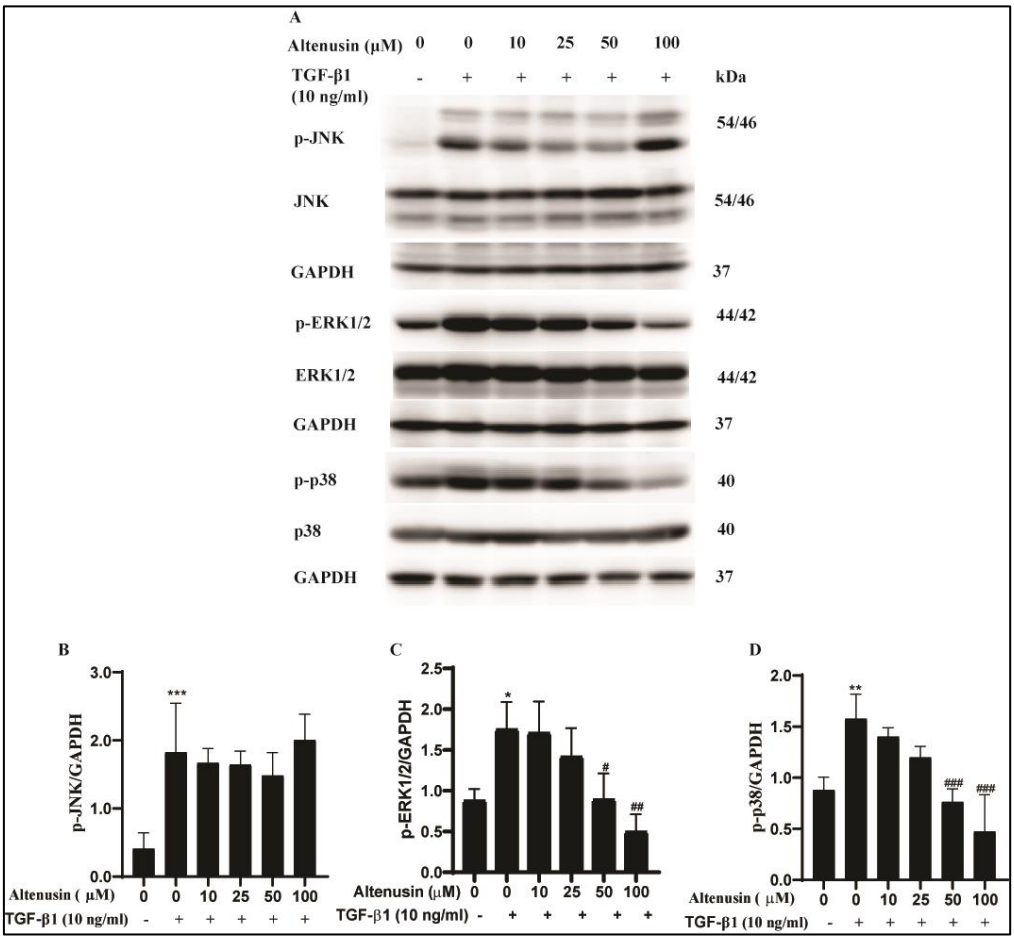

Figure 4. Effect of altenusin on MAPK signaling pathway in TGF-β-induced EMT in renal proximal tubular cells.
(A) The representative immunoblots of p-JNK, JNK, p-p38, p38, p-ERK1/2, ERK1/2, and GAPDH proteins following 48 hours incubation of control, 10 ng/ml TGF-β1, and 10 ng/ml TGF-β1 plus altenusin (10-100 μM). (B-D) Densitometric bands are represented as graphs of indicated proteins in RPTEC/TERT1 cells. Data are shown as mean±SD of 4-5 experiments. $^{*}P$<0.05, $^{**}P$<0.01, and $^{***}P$<0.001 vs control while $^{\#}P$<0.05, $^{\#\#}P$<0.01 and $^{\#\#\#}P$<0.001 vs 10 ng/ml TGF-β1.

signaling pathway[5-6]. Therefore, the effect of altenusin on MAPK signaling pathway in TGF-β1-induced EMT in renal proximal tubular cells was studied. As shown in Figure 4, treatment of 10 ng/ml TGF-β1 increased phosphorylation of ERK1/2, JNK, and p38. Interestingly, altenusin (50 and 100 μM) significantly reduced p-ERK1/2 and p-p38 levels induced by TGF-β1. In contrast to p-ERK1/2 and p-p38, altenusin did not significantly reduce p-JNK level. These results reveal that altenusin suppresses TGF-β1-induced EMT by modulating phosphorylation of ERK1/2 and p38 levels but not JNK in human renal proximal tubular cells.

3.4. Effect of altenusin on MAPK signaling pathway in UUO mice

UUO is a model of renal fibrosis and TGF-β/MAPK signaling pathway is upregulated in UUO mice[8], we investigated the effect of altenusin on MAPK signaling pathway in UUO mice. UUO mice were treated with vehicle or altenusin 3 mg/kg/day via i.p. injection for 14 days. As shown in Figure 5, UUO treated with vehicle increased phosphorylation of JNK, p38, and ERK1/2 compared with sham-operated mice. Treatment UUO mice with altenusin significantly reduced p-JNK, p-p38, and p-ERK1/2 levels compared with vehicle-treated UUO mice.

3.5. Effect of altenusin on FXR protein expression

FXR protein has been reported as a protective factor in UUO mice[25]. Since altenusin is a FXR agonist, we investigated whether altenusin affected renal FXR protein expression in UUO mice. As expected, UUO dramatically decreased FXR protein expression compared to sham (Figure 6). Surprisingly, altenusin treatment did not atenuate the decreased FXR protein expression in kidney of UUO mice.

4. DISCUSSION

CKD is a serious health problem, causing morbidity and mortality worldwide[32], due to lack in specific drug treatment for CKD[33]. Kidney transplantation and dialysis are the last line therapies in CKD[3], thus searching for a specific regiment for CKD treatment is urgently needed. As renal EMT and fibrosis play a key role in CKD development, inhibition of these processes might be the potential targets for CKD treatment. Antagonizing TGF-β/MAPK signaling pathway has been proposed to protect against TGF-β-induced EMT of renal proximal tubular cell and renal fibrosis[8]. In this study, we reveal that altenusin inhibits TGF-β1-induced EMT via antagonizing the TGF-β/MAPK signaling pathway in human renal proximal tubular cells and mouse UUO model of renal fibrosis.

TGF-β1-induced EMT of renal proximal tubular cells was used to study anti-EMT effect of compounds in numerous studies[34]. In this study, we have developed an EMT model using RPTEC/TERT1 cells, an immortalized human renal proximal tubular cell line. TGF-β1, a key fibrotic inducer, showed concentration- and time-dependent effect on EMT as shown by decreased E-cadherin whereas increased fibronectin protein expression. We have demonstrated that altenusin attenuated TGF-β1-induced EMT in renal proximal tubular cells via suppression of Smad pathway (data presented in alongside manuscript). As activations of MAKPs including JNK, p38, and ERK1/2 are the downstream signaling for TGF-β1-induced EMT and fibrosis[8], we additionally explored whether altenusin also inhibited EMT in renal proximal tubular cells was mediated by suppression of MAPK pathway, a non-Samd pathway. Our results confirmed the involvement of MAPKs in TGF-β1-induced EMT in RPTEC/TERT1 cells and renal fibrosis of UUO mice. Altenusin reduced p-ERK1/2 and p-p38 in both human renal proximal tubular cells and kidney of UUO mice. Interestingly, the inhibitory effect of altenusin on activation of JNK was found only in UUO mice but not in human renal proximal tubular cells. The explanation for the differences is uncertain. The difference between human and murine, and the complex of *in vivo* might be the causes of the differences. There are possibilities of altenusin to reduce TGF-β-induced ERK1/2, p38, and JNK phosphorylation. Altenusin could induce TβR1 degradation leads to reduction of signaling pathway or it might directly inhibit or interfere ERK1/2, p38, and JNK phosphorylation[6]. This notion is supported by a study, Ovo-like transcriptional repressor 1 (OVOL1) prevents Smad7 degradation results in stabilization of Smad7 protein expression[35]. Smad7 stabilization influences TβR1 degradation and inhibits TGF-β-induced EMT, invasion and migration of breast cancer cells[35]. These contentions could be elucidated in further study.

Downregulation of FXR expression was presented in diabetic nephropathy, a form of CKD[22]. Moreover, suppression of FXR accelerated kidney injury and fibrosis in diabetic nephropathy[23]. Upregulation of FXR target genes coupled with positive regulation of FXR protein expression could attenuate fibrotic kidney in animal model of kidney disease. These data support the protective effect of FXR on renal fibrosis. Our study found that UUO downregulated FXR protein expression, nonetheless, treatment with altenusin did not increase FXR protein expression. Results from this study reveal that altenusin action on TGF-β/MAPK signaling pathway does not require increase in FXR protein expression. However, it is still ambiguous whether the effect of altenusin TGF-β-induced activation of MAPK in renal cells requires an increase in FXR activity.

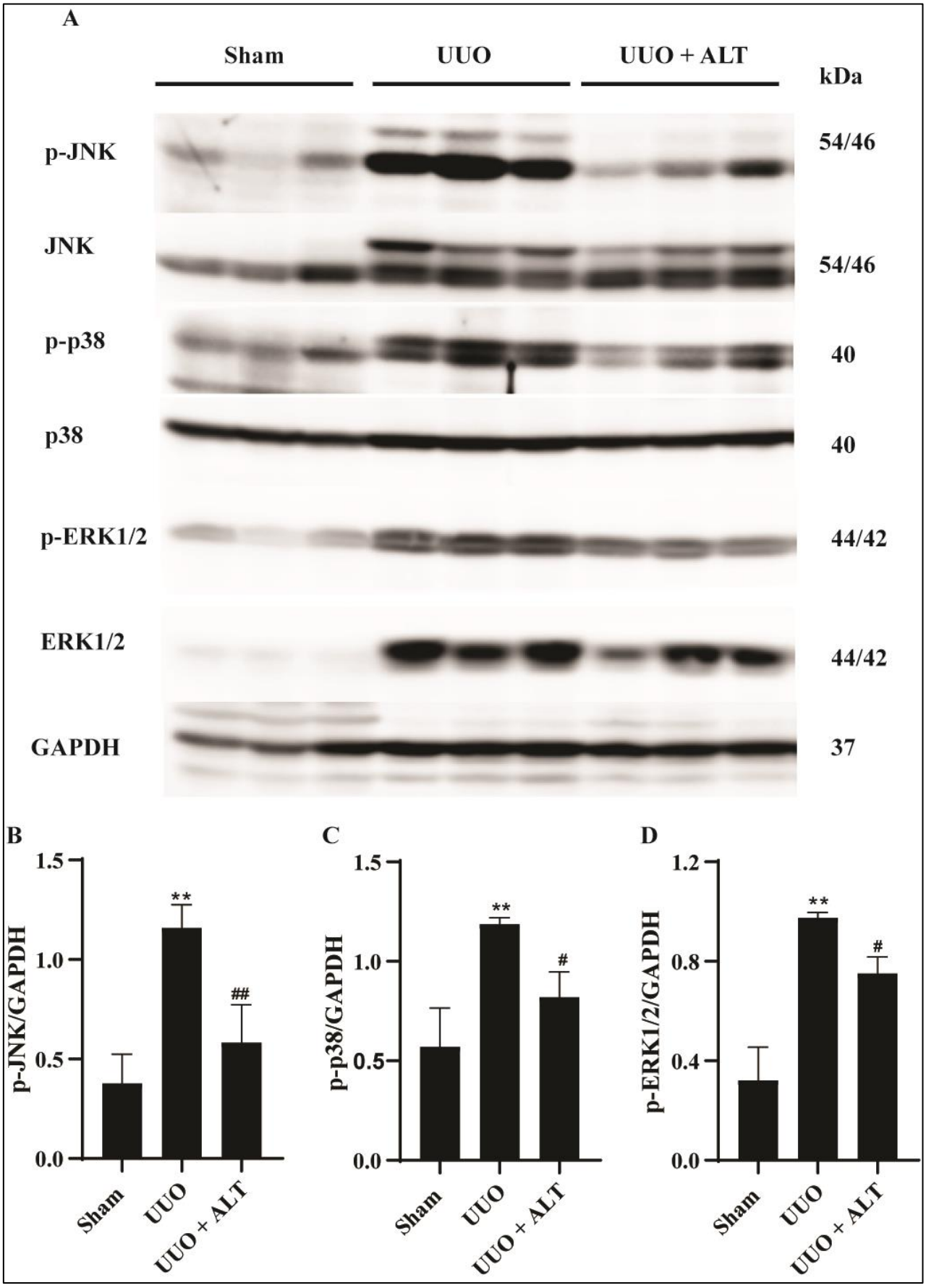

Figure 5. Effect of altenusin on MAPK signaling pathway in UUO mice.
(A) The representative immunoblotting of p-JNK, JNK, p-p38, p38, p-ERK1/2, ERK1/2, and GAPDH proteins of Sham, UUO, and UUO+ALT. (B-D) Densitometric bands of indicated proteins. Data are shown as mean±SD of three mice. Each lane represents each mouse. $^{**}P<0.01$ vs Sham, $^{\#}P<0.05$, and $^{\#\#}P<0.01$ vs UUO.

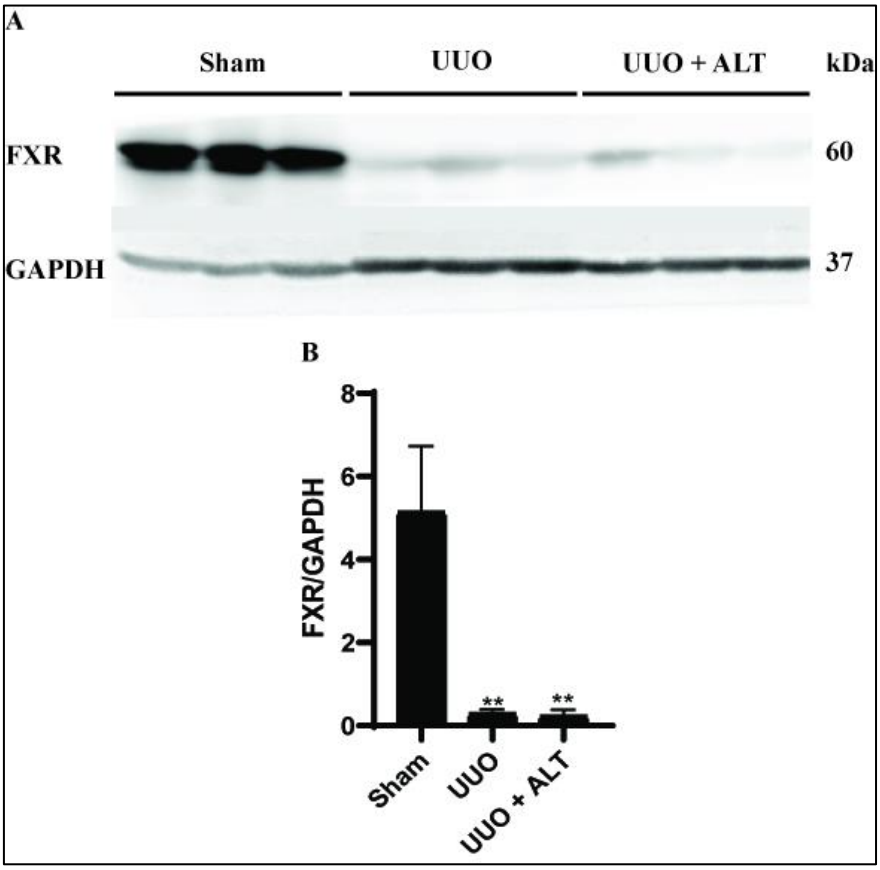

Figure 6. Effect of altenusin on FXR protein expression in UUO mice.
(A)The representative immunoblotting of FXR and GAPDH proteins of Sham, UUO, and UUO+ALT; (B) densitometric analysis of bands. Data are shown as mean±SD of three mice. Each lane represents each mouse. $^{**}P<0.01$ vs Sham.

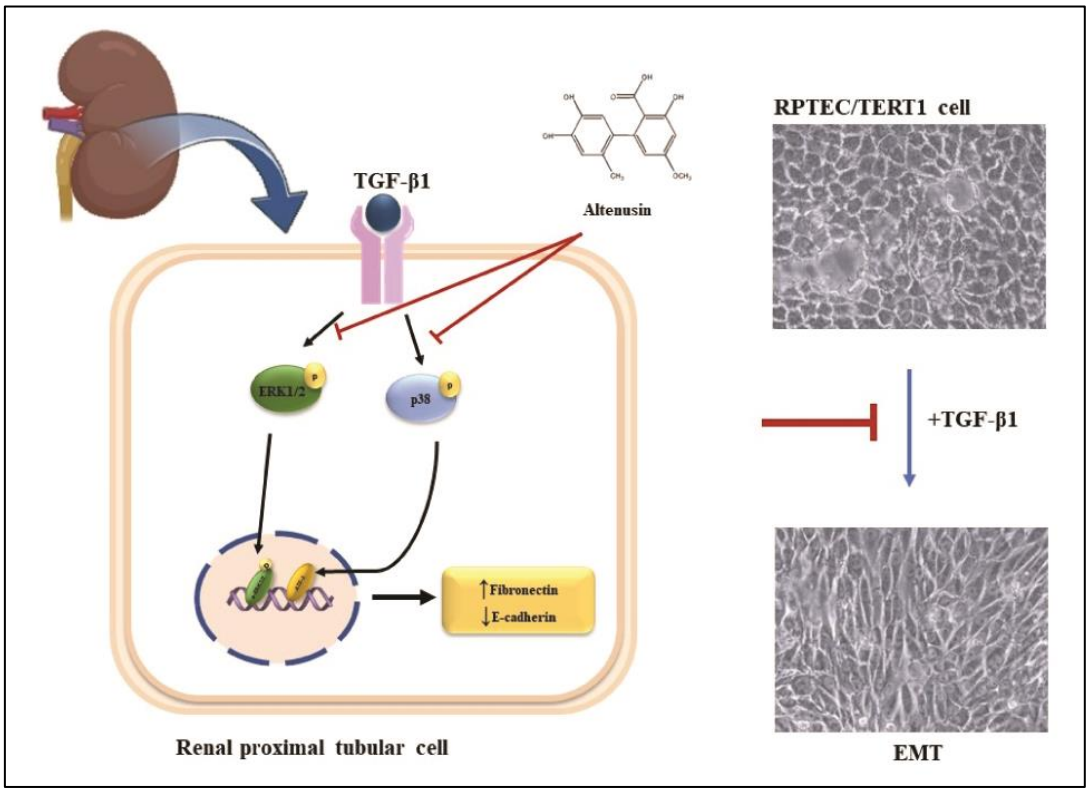

Figure 7. Proposed mechanism of altenusin prevents TGF-β1-induced EMT in renal proximal tubular cells.
TGF-β1 binds the receptor to stimulate phosphorylation of ERK1/2 and p38. Activated ERK1/2 and p-38, subsequently induce EMT in renal proximal tubular cells via induction of fibronectin expression whereas suppression of E-cadherin expression. Altenusin prevents EMT via inhibition of TGF-β1-induced phosphorylation of ERK1/2 and p38.

5. CONCLUSION

The present study reveals the protective effect of altenusin on TGF-β1-induced EMT in renal proximal tubular cells via suppression of ERK1/2 and p38 signaling pathway (Figure 7). Altenusin shows the potential compound for prevention of EMT and fibrosis in kidney. To be certain that altenusin could be a candidate compound for renal fibrosis treatment, more studies in other animal models of renal fibrosis such hypertension and diabetes are required.

ACKNOWLEDGEMENT

This research project is supported by Mahidol University (Fundamental Fund: fiscal year 2023 by National Science Research and Innovation Fund (NSRF)). N.T. is supported by the Science Achievement Scholarship of Thailand (SAST).

Conflict of interest

The authors declare no conflict of interest.

Funding

This research project is funded by Mahidol University (Fundamental Fund: fiscal year 2023 by National Science Research and Innovation Fund (NSRF)). N. Thipboonchoo is supported by the Science Achievement Scholarship of Thailand (SAST). S. Sureram is supported by Thailand Science Research and Innovation (TSRI, Chulabhorn Research Institute, Grant No. 36824/4274394).

Ethic approval

Animal use and care were approved by Faculty of Science, Mahidol University-Institutional Animal Care and Use Committee (MUSC-IACUC).

Article info:

Received June 19, 2023
Received in revised form October 19, 2023
Accepted October 19, 2023

Author contribution

N. Thipboonchoo and S. Soodvilai contributed to conception of experiments and designed the study. N. Thipboonchoo, S. Sureram, S. Sa-nguansak, C. Kesornpun, P. Kittakoop, and S. Soodvilai performed experiments. N. Thipboonchoo and S. Soodvilai wrote the manuscript.

REFERENCES

1. Hewitson TD. Renal tubulointerstitial fibrosis: common but never simple. Am J Physiol Renal Physiology. 2009; 296(6):F1239-44.
2. Gewin L, Zent R. How does TGF-β mediate tubulointer-stitial fibrosis?. Semin Nephrol. 2012;33(3):228-35.
3. Chen TK, Knicely DH, Grams ME. Chronic kidney disease diagnosis and management: A review. Jama. 2019;322(13):1294-304.
4. Liu Y. Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol. 2011;7(12):684-96.
5. Efstratiadis G, Divani M, Katsioulis E, Vergoulas G. Renal fibrosis. Hippokratia. 2009;13(4):224.
6. Sureshbabu A, Muhsin SA, Choi ME. TGF-β signaling in the kidney: Profibrotic and protective effects. Am J Physiol Renal Physiol. 2016;310(7):F596-606.
7. Gu YY, Liu XS, Huang XR, Yu XQ, Lan HY. Diverse role of TGF-β in kidney disease. Frontn Cell Dev Biol. 2020;8:123.
8. Geng XQ, Ma A, He JZ, Wang L, Jia YL, Shao GY, et al. Ganoderic acid hinders renal fibrosis via suppressing the TGF-β/Smad and MAPK signaling pathways. Acta Pharmacol Sin. 2020;41(5): 670-7.
9. Nakanishi S, Toki S, Saitoh Y, Tsukuda E, Kawahara K, Ando K, et al. Isolation of myosin light chain kinase inhibitors from microorganisms: dehydroaltenusin, altenusin, atrovenetinone, and cyclooctasulfur. Biosci Biotechnol Biochem. 1995;59(7):1333-5.
10. Uchida R, Tomoda H, Dong Y, Omura S. Alutenusin, a specific neutral sphingomyelinase inhibitor, produced by *Penidltium* sp. FO-7436. J Antibiot. 1999;52(6):572-4.
11. Andersen B, Smedsgaard J, Jørring I, Skouboe P, Pedersen LH. Real-time PCR quantification of the AM-toxin gene and HPLC qualification of toxigenic metabolites from *Alternaria* species from apples. Int J Food Microbiol. 2006;111(2):105-11.
12. Cota BB, Rosa LH, Caligiorne RB, Rabello ALT, Almeida Alves TM, Rosa CA, et al. Altenusin, a biphenyl isolated from the endophytic fungus *Alternaria* sp., inhibits trypanothione reductase from Trypanosoma cruzi. FEMS Microbiol Lett. 2008;285(2): 177-82.
13. Singh SB, Jayasuriya H, Dewey R, Polishook JD, Dombrowski AW, Zink DL, et al. Isolation, structure, and HIV-1-integrase inhibitory activity of structurally diverse fungal metabolites. J Ind Microbiol Biotechnol. 2003;30(12):721-31.
14. Komai SI, Hosoe T, Itabashi T, Nozawa K, Yaguchi T, Fukushima K, et al. New penicillide derivatives isolated from Penicillium simplicissimum. J Nat Med. 2006;60(3):185-90.
15. Chua SW, Cornejo A, Van Eersel J, Stevens CH, Vaca I, Cueto M, et al. The polyphenol altenusin inhibits *in vitro* fibrillization of tau and reduces induced tau pathology in primary neurons. ACS Chem Neurosci. 2017;8(4):743-51.
16. Hou Y, Li J, Wu JC, Wu QX, Fang J. Activation of cellular antioxidant defense system by naturally occurring dibenzopyrone derivatives confers neuroprotection against oxidative insults. ACS Chem Neurosci. 2021;12(15):2798-809.
17. Elbermawi A, Ali AR, Amen Y, Ashour A, Ahmad KF, Mansour ESS, et al. Anti-diabetic activities of phenolic compounds of *Alternaria* sp., an endophyte isolated from the leaves of desert plants growing in Egypt. RSC Adv. 2022;12(38):24935-45.
18. Zheng Z, Zhao Z, Li S, Lu X, Jiang M, Lin J, et al. Altenusin, a nonsteroidal microbial metabolite, attenuates nonalcoholic fatty liver disease by activating the farnesoid X receptor. Mol Pharmacol. 2017;92(4):425-36.
19. Claudel T, Staels B, Kuipers F. The Farnesoid X receptor: A molecular link between bile acid and lipid and glucose metabolism. Arterioscler Thromb Vasc Biol. 2005;25(10):2020-30.
20. Herman-Edelstein M, Weinstein T, Levi M. Bile acid receptors and the kidney. Curr Opin Nephrol Hypertens. 2018;27(1):56-62.
21. Jiao Y, Lu Y, Li XY. Farnesoid X receptor: a master regulator of hepatic triglyceride and glucose homeostasis. Acta Pharmacol Sin. 2015;36(1):44-50.
22. Wang XX, Wang D, Luo Y, Myakala K, Dobrinskikh E, Rosenberg AZ, et al. FXR/TGR5 dual agonist prevents progression of nephropathy in diabetes and obesity. J Am Soc Nephrol. 2018;29 (1):118-37.
23. Wang XX, Jiang T, Shen Y, Caldas Y, Miyazaki-Anzai S, Santamaria H, et al. Diabetic nephropathy is accelerated by farnesoid X receptor deficiency and inhibited by farnesoid X receptor activation in a type 1 diabetes model. Diabetes. 2010;59(11):2916-27.
24. Marquardt A, Al-Dabet MM, Ghosh S, Kohli S, Manoharan J, ElWakiel A, et al. Farnesoid X receptor agonism protects against

diabetic tubulopathy: potential add-on therapy for diabetic nephropathy. J Am Soc Nephrol. 2017;28(11):3182-9.
25. Li S, Ghoshal S, Sojoodi M, Arora G, Masia R, Erstad DJ, et al. The farnesoid X receptor agonist EDP-305 reduces interstitial renal fibrosis in a mouse model of unilateral ureteral obstruction. FASEB J. 2019;33(6):7103-12.
26. Úbeda M, Lario M, Muñoz L, Borrero MJ, Rodríguez-Serrano M, Sánchez-Díaz AM, et al. Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. J Hepatol. 2016;64(5):1049-57.
27. Schwabl P, Hambruch E, Seeland BA, Hayden H, Wagner M, Garnys L, et al. The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction. J Hepatol. 2017;66(4):724-33.
28. Comeglio P, Filippi S, Sarchielli E, Morelli A, Cellai I, Corcetto F, et al. Anti-fibrotic effects of chronic treatment with the selective FXR agonist obeticholic acid in the bleomycin-induced rat model of pulmonary fibrosis. J Steroid Biochem Mol Biol. 2017;168: 26-37.
29. Feng Q, Zhang H, Yao D, Zhang X, Chen WD, Wang YD. Activation of FXR suppresses esophageal squamous cell carcinoma through antagonizing ERK1/2 signaling pathway. Cancer Manag Res. 2021;13:5907-18.
30. Worakajit N, Thipboonchoo N, Chaturongakul S, Jutabha P, Soontornniyomkij V, Tuchinda P, et al. Nephroprotective potential of Panduratin A against colistin-induced renal injury via attenuating mitochondrial dysfunction and cell apoptosis. Biomed Pharmacother. 2022;148:112732.
31. Ling L, Yang M, Ding W, Gu Y. Ghrelin attenuates UUO-induced renal fibrosis via attenuation of Nlrp3 inflammasome and endoplasmic reticulum stress. Am J Transl Res. 2019;11(1):131.
32. Kovesdy CP. Epidemiology of chronic kidney disease: An update 2022. Kidney Int Suppl (2011). 2022;12(1):7-11.
33. Ruiz-Ortega M, Rayego-Mateos S, Lamas S, Ortiz A, Rodrigues-Diez RR. Targeting the progression of chronic kidney disease. Nat Rev Nephrol. 2020;16(5):269-88.
34. Slyne J, Slattery C, McMorrow T, Ryan MP. New developments concerning the proximal tubule in diabetic nephropathy: *in vitro* models and mechanisms. Nephrol Dial Transplant. 2015;30 (suppl_4): iv60-7.
35. Fan C, Wang Q, van der Zon G, Ren J, Agaser C, Slieker RC, et al. OVOL1 inhibits breast cancer cell invasion by enhancing the degradation of TGF-β type I receptor. Sig Transduct Target Ther. 2022;7(1):126.