Sedum sarmentosum Bunge extract exerts renal anti-fibrotic effects in vivo and in vitro

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A B S T R A C T

Aims: Sedum sarmentosum Bunge, a traditional Chinese herbal medicine, has a wide range of clinical effects, including anti-oxidation, anti-inflammation, and anti-cancer properties. In this study, we determined whether S. sarmentosum Bunge Extract (SSBE) has anti-fibrotic effects on renal tissues.

Main methods: We investigated the effects of SSBE on aristolochic acid (AA)-induced injury to renal tubular epithelial cells (RTECs) in vitro and unilateral ureteral obstruction (UUO)-induced renal fibrosis in vivo by evaluating epithelial-to-mesenchymal transition (EMT) and the accumulation of extracellular matrix (ECM) components. Furthermore, we examined the expression levels of TGF-β1 and its receptor.

Key findings: In cultured RTECs (NRK-52E), AA promoted renal EMT and ECM accumulation by up-regulating the expression of mesenchymal markers and ECM components and by down-regulating the expression of epithelial markers. In addition, AA induced an imbalance between MMP-2 and TIMP-2 and enhanced expression of TGF-β1 and its receptor. SSBE treatment significantly inhibited AA-induced TGF-β1 expression and prevented the induction of EMT and deposition of ECM. In the UUO rats, tubular injury and interstitial fibrosis were obviously increased. SSBE administration protected renal function, as indicated by reduced serum creatinine levels, and alleviated renal fibrosis. These anti-fibrotic effects were associated with a reduction in TGF-β1 expression and inhibition of EMT and ECM accumulation.

Significance: These findings suggest that SSBE may have therapeutic potential for fibrotic kidney diseases.

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Introduction

Renal tubulointerstitial fibrosis is the ultimate common pathway for many types of progressive chronic kidney diseases (Vilayur and Harris, 2009). Renal fibrosis is a dynamic and converging process, and it is characterized by the pathological deposition of extracellular matrix (ECM) in association with infiltration of inflammatory cells, loss of tubular epithelial cells (RTECs), and accumulation of fibroblasts (Liu, 2011). Alpha smooth muscle actin (α-SMA)-positive myofibroblasts are increasingly recognized as having crucial roles after injury in fibrosis. These cells are responsible for the accumulation of ECM components such as types I and III collagen (Meran and Steadman, 2011). Myofibroblasts are terminally differentiated cells that drive renal fibrogenesis. Although a role for myofibroblasts in fibrosis is widely accepted, their origins and activation process in the fibrotic kidney remain largely undefined and controversial. Pioneering studies indicated that a large proportion of myofibroblasts originate from differentiated epithelial cells via epithelial-to-mesenchymal transition (EMT) (Strutz et al., 2002; Liu, 2004, 2010).

EMT is a biologic process in which tubular cells lose their epithelial phenotype, including expression of E-cadherin and zonula occluden-1 (ZO-1), and acquire new characteristic features such as expression of mesenchymal proteins, including vimentin and α-SMA (Zeisberg and Neilson, 2009). EMT may be an adaptive response of epithelial cells after injury and is an integral part of renal fibrogenesis. The major driving force behind EMT during the fibrogenic phase appears to be various profibrotic growth factors, including transforming growth factor-β (TGF-β) (Strutz et al., 2002; Burns et al., 2006). Amelioration of EMT by treatment with Smad7, an antagonist of TGF-β signaling, or bone morphogenetic protein-7 (BMP-7) dramatically reduces fibrotic lesions after injury (Zeisberg et al., 2003; Saika et al., 2006). Therefore, novel therapeutic strategies that halt or perhaps reverse EMT to attenuate renal fibrosis are promising.
**Sedum sarmentosum**

Bunge is a perennial plant that is widely distributed on the mountain slopes of Asian countries and its extract (SSBE) is traditionally used for the treatment of some inflammatory diseases (Cao et al., 2006). SSBE contains multiple active chemical components, including quercetin, tricin-7-O-β-D-glucoside, isorhamnetin, and kaempferide (Oh et al., 2004; Morikawa et al., 2007; Ninomiya et al., 2007). These active components endow SSBE with various pharmacological activities, including anti-inflammatory and anti-proliferative action. Inflammation is a critical pathogenic factor in fibrogenesis, and EMT is a process of abnormal proliferation of epithelial cells. Thus, SSBE may have potential for anti-fibrotic activity in renal tissues.

However, no studies of the anti-fibrotic effect of SSBE have been performed thus far. Therefore, in the present study, we investigated the effects of SSBE on aristolochic acid (AA)-induced injury to RTECs in vitro and ureteral obstruction (UUO)-induced renal fibrosis in vivo. UUO in rodents is well-characterized experimental model resulting in interstitial fibrosis (Chevalier et al., 2009; Li et al., 2013). In kidneys after AA injury, tubulointerstitial fibrosis also is the most typical pathological characteristics (Yang et al., 2010; Fragiadaki et al., 2011). We hypothesized that SSBE exerts renal anti-fibrotic effects by decreasing TGF-β1 expression, inhibiting the induction of EMT and accumulation of ECM.

**Materials and methods**

**Cell culture and treatment**

The rat RTEC line (NRK-52E) was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). NRK-52E cells were maintained in Dulbecco's Modified Eagle Medium (Invitrogen, California, USA) supplemented with 5% fetal bovine serum (FBS, Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). The NRK-52E cells were seeded in the complete medium containing 5% FBS at approximately 70% confluency in six-well culture plates. After 24 h, the complete medium was replaced with serum-free medium for 24 h before the treatment with AA (Lot No. A5512, Sigma-Aldrich, St. Louis, MO, USA) or SSBE (Lot No. 20101017, Xuancheng Baicao, Anhui, China). The extraction protocol of *S. sarmentosum* Bunge is shown in Fig. 1; SSBE was dissolved in normal saline at a concentration of 100 mg/ml. NRK-52E cells were treated with either AA (5 μg/ml), AA with SSBE (10 μg/ml to 1000 μg/ml), and these drugs were added at the same time.

**Animal models**

Seventy-two male Sprague–Dawley rats that weighed approximately 180 to 200 g (6 to 8 weeks of age) were purchased from the Experimental Animal Center of Wenzhou Medical University (Wenzhou, China). The rats were housed in a temperature-, humidity- and light-controlled environment and fed with standard rat chow and water, except for 1 d fasting before the operation. The weight-matched rats were randomly assigned to three groups: Sham-operation (Sham, n = 24), vehicle (saline)-treated UUO (vehicle, n = 24), and SSBE (100 mg/kg · d)-treated UUO (SSBE, n = 24). This study was limited to less than 2 weeks, because if the obstruction time is too long, serious hydronephrosis results in disappearance of renal parenchyma. The UUO surgery was performed as previously described (Chevalier et al., 2009). The kidneys of the SSBE- or vehicle control-treated UUO rats were excised on days 3, 7 and 14, and samples were collected to examine the levels of serum creatinine and blood urea nitrogen using automatic biochemistry analyzers.

None of the animals in our study died, and the animal study protocols were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University, China.

**Renal histology and immunohistochemistry**

We stained the paraffin-embedded kidney sections using standard histology procedures, including hematoxylin and eosin (HE) and Masson’s trichrome staining. Immunohistochemical analysis was performed on 4-μm-thick kidney sections that had been dewaxed with xylene and rehydrated using sequential ethanol (100%, 95%, 85%, 70%, 50%, 30%, and water).}

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**Fig. 1.** Extraction protocol for *Sedum sarmentosum* Bunge.
and 75%) and distilled water. The endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Antigen retrieval was performed by heating the sections in 0.1% sodium citrate buffer (pH 6.0). Immunohistochemical analysis was performed using primary anti-TGF-β1 antibody (1:150, Biogot Technology, Shanghai, China). For immunofluorescence staining, the samples were processed with primary antibodies against type III collagen (1:100, Biogot Technology), α-SMA (1:200, Santa Cruz Biotechnology, California, USA), and E-cadherin (1:200, Abcam, Cambridge, MA, USA) and incubated with DyLight 488 (Green) or 594 (Red)–labeled secondary antibodies (Beyotime Biotechnology, Jiangsu, China). After being washed with PBS, a drop of an appropriate medium was applied to the sections, which were covered with a cover slip. The integrated optical density (IOD) was measured by image analysis. All samples were semi-quantitatively or quantitatively assessed by two independent investigators in a blinded manner.

**Immunocytochemical staining**

NRK-52E cells were cultured with AA with or without SSBE in the six-well plates containing glass slides and were then washed with PBS and fixed with 4% paraformaldehyde (Sigma-Aldrich) at 4 °C for 30 min. After permeabilization with 0.1% Triton X-100 for 10 min, the specimens were washed with PBS and then blocked with 10% PBS to eliminate the nonspecific fluorescence. Immunofluorescence staining was performed using anti-type III collagen (1:100), α-SMA (1:200), or E-cadherin (1:200) as the primary antibody, and the cell preparations were incubated with DyLight 488/594 labeled secondary antibodies. The immunocytochemical samples were semiquantitatively or quantitatively assessed by two independent investigators in a blinded manner.

**Quantitative reverse transcriptase-PCR (qRT-PCR)**

Total RNA was extracted from the NRK-52E cells using TRIzol reagent (Invitrogen), and reverse transcription into cDNA templates was performed using a ReverTra Ace qPCR RT Kit (Toyobo, Japan). qRT-PCR was performed using SYBR Green Realtime PCR Master Mix Plus (Toyobo). Quality was analyzed on agarose gels, and quantities were performed using a ReverTra Ace qPCR RT Kit (Toyobo, Japan). qRT-PCR agent (Invitrogen), and reverse transcription into cDNA templates was quantitatively assessed by two independent investigators in a blinded manner.

**ELISA assay**

Cells treated with AA and/or SSBE were cultured for 24 h, and the culture supernatant fluid was collected. An avidin–biotin–complex-enzyme-linked immunoabsorbent assay (ABC-ELISA) was used according to the manufacturer’s protocol to determine the TGF-β1 levels. ELISA kits were purchased from Xitang Biotechnology (Shanghai, China). All experiments were repeated at least three times.

**Statistical analysis**

All of the results are presented as the mean ± standard error of the mean. All statistical analyses were performed using the Statistical Package for the Social Sciences (version 16.0, SPSS Inc., Chicago, USA). Student’s t-test was used to analyze differences between two groups. ANOVA was used for comparisons of more than two groups. P < 0.05 was considered significant.

**Results**

**SSBE inhibits EMT and ECM accumulation in AA-treated NRK-52E cells**

First, we examined the expression of the ECM components type I and type III collagen after AA injury. As expected, low concentrations (1–10 μg/ml) of AA up-regulated the expression of type III collagen protein as indicated by immunofluorescence staining (Fig. 2A) and mRNA expression of Col1a1 and Col3a1 as demonstrated by qRT-PCR (Fig. 2C) in cultured NRK-52E cells. In addition, AA increased the expression of metalloproteinase-2 (MMP-2) mRNA and tissue inhibitor of metalloproteinase-2 (TIMP-2) mRNA (Fig. 2C), and the TIMP-2 inhibited the higher expression levels in AA-treated NRK-52E cells than MMP-2, suggesting that down-regulated expression ratio of MMP-2/TIMP-2. The AA-induced collagen accumulation and MMP-2/TIMP-2 reverse were inhibited by SSBE treatment. In the AA-treated RTECs, SSBE at concentrations of 10–100 μg/ml significantly decreased the expression of type III collagen protein (Fig. 2B), as well as the expression of MMP-2, TIMP-2, Col1a1 and Col3a1 mRNAs (Fig. 2C).

As a key process in fibrogenesis, EMT was also evaluated in cultured NRK-52E cells after AA injury. Immunofluorescence staining revealed that AA decreased the expression of the epithelial marker E-cadherin protein and increased expression of the mesenchymal marker α-SMA (Fig. 3A). Decreased expression of E-cadherin and ZO-1 and up-regulated expression of α-SMA and vimentin mRNAs were also observed in AA-treated cells (Fig. 3C). Moreover, AA down-regulated the mRNA expression of the EMT inhibitor BMP-7 (Fig. 3C). These findings support the concept that cellular phenotype trans-differentiation occurs during AA-induced RTEC injury. This EMT process can be attenuated or even prevented by SSBE treatment. In AA-treated NRK-52E cell cultures, SSBE significantly decreased the expression of α-SMA and vimentin and increased the expression of BMP-7, E-cadherin, and ZO-1 after AA injury (Figs. 3B and C). These changes in mRNA expression showed obvious concentration-dependent effects.

**Table 1**

qRT-PCR primers in this study for analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward sequence (5′ → 3′)</th>
<th>Reverse sequence (5′ → 3′)</th>
<th>Product size (bp)</th>
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<tr>
<td>Col3a1</td>
<td>AAAGGTCAGAAGAAATAG</td>
<td>AATGTCTAGAGGCTGATAA</td>
<td>147</td>
</tr>
<tr>
<td>MMP-2</td>
<td>GGGTATTGTCGCGCAAGCAG</td>
<td>TGGTACGTGTCGGGTTGAT</td>
<td>202</td>
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<tr>
<td>TIMP-2</td>
<td>GTGAGAAGGAGGTGAT</td>
<td>CTGGTGATGTTGACTGTTT</td>
<td>273</td>
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<tr>
<td>ZO-1</td>
<td>AACGAGGAGCAGGAGGTGCTCC</td>
<td>CAGGCGATGACGGTGACTCC</td>
<td>238</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>GTGACGAGGACAAACACT</td>
<td>GGGCAAGACCCCCTCAAT</td>
<td>195</td>
</tr>
<tr>
<td>α-SMA</td>
<td>CGGATAGGACGAGGACCAACT</td>
<td>CGGCGGATGACGAGAAGAAT</td>
<td>212</td>
</tr>
<tr>
<td>BMP-7</td>
<td>GTGACTCAAACCTGCGACCA</td>
<td>GGGCTGCTGAGCAGATT</td>
<td>215</td>
</tr>
<tr>
<td>Vimentin</td>
<td>TGACCCTGCTCCGCAACTAC</td>
<td>CGAATTCCTGCCATCCTCCTC</td>
<td>141</td>
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<tr>
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<td>AAGCGGTCAGCTCTGCTGCT</td>
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<tr>
<td>TGF-β3</td>
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<td>CTACGCTGTCGGATTGACTT</td>
<td>144</td>
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<tr>
<td>β-Actin</td>
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</table>
SSBE inhibits expression of TGF-β1 and its receptor in AA-treated NRK-52E cells

TGF-β1 is known to be the most important cytokine involved in EMT and fibrosis in a variety of animal models (Yang and Liu, 2001). Thus, we examined TGF-β1 expression in AA-treated NRK-52E cells. As shown in Fig. 4A, AA markedly increased the levels of the TGF-β1 protein as indicated by the ABC-ELISA results. In addition, AA also increased the expression of the mRNAs for TGF-β1 and its receptor, TGF-β1R (Fig. 4B). Up-regulation of TGF-β1 and TGF-β1R expression was suppressed by SSBE treatment, especially at concentrations of SSBE greater than 100 μg/ml. This inhibition may result in the prevention of the increased EMT and ECM deposition.

SSBE administration protects renal function in UUO rats

To assess whether similar anti-fibrotic effects occur in vivo, SSBE was administered continuously for 2 weeks to the rats subjected to the UUO. Table 2 shows the changes in these functional parameters in the experimental groups as a result of SSBE treatment. The vehicle-treated UUO group showed higher levels of serum creatinine and blood urea nitrogen than the Sham operation group. SSBE treatment did not affect the levels of blood urea nitrogen but reduced the levels of serum creatinine in the UUO rats. Serum creatinine and blood urea nitrogen are important indicators of renal function. However, serum creatinine is a more sensitive indicator of kidney function than blood urea nitrogen. Thus, the decreased levels of serum creatinine in the SSBE-treated rats indicate some degree of protection of kidney function.

SSBE alleviates interstitial fibrosis in kidney tissues of UUO rats

In the obstructed kidneys, hematoxylin and eosin (HE) staining revealed marked tubular dilation and atrophy associated with interstitial fibrosis (Fig. 5 top row). The deposition of total collagen determined by Masson trichrome staining was more severe with increasing time after the obstruction (Fig. 5 bottom row). SSBE administration significantly alleviated renal tubular injury and reduced total collagen deposition. Thus, SSBE inhibited ureteral obstruction-induced interstitial fibrosis in rats.

SSBE inhibits renal EMT and ECM accumulation in UUO rats

Compared with Sham-operated rats, the expression of α-SMA and type III collagen proteins, determined by immunofluorescence staining, was significantly increased in the UUO rats. Furthermore, the expression of E-cadherin was decreased in renal cortical areas, especially around the renal tubules of the UUO rats (Fig. 6A, B, and C). In addition, ColIa1 and ColIa3 mRNA levels extracted from total kidney tissues were down-regulated (Fig. 6D). These results suggested that ureteral obstruction induced marked EMT and excessive deposition of ECM.
components in the kidney tissues of rats. Treatment with SSBE inhibited the up-regulated expression of α-SMA, Col1a1, and Col3a1. Additionally, SSBE prevented the UUO-induced changes in the expression levels of E-cadherin. Therefore, the induction of EMT and the accumulation of ECM in the UUO rats were ameliorated by SSBE administration, especially at 14 d after the ureteral obstruction.

**SSBE administration inhibits TGF-β1 expression in UUO rats**

As shown in Fig. 7A, TGF-β1 was mainly expressed around the renal tubules of the UUO rats as indicated by immunohistochemical staining. The expression levels of this protein were significantly increased in the UUO rats compared with Sham-operated rats, especially at 14 d after the ureteral obstruction. The increased expression of TGF-β1 mRNA was accompanied by a change in protein expression. Moreover, the TGF-β1 mRNA expression was elevated in the UUO rats after ureteral obstruction (Fig. 7B). SSBE treatment decreased the expression of TGF-β1 and TGF-β1R, but the effect was not time dependent.

**Discussion**

Previous pharmacological studies have revealed that SSBE possesses significant anti-tumor, anti-inflammation, and anti-viral infection bioactivities (Heo et al., 2007; Jung et al., 2008; Huang et al., 2010). In the present study, we identified the anti-fibrotic effects of SSBE in renal tissues in vivo and in vitro.

Interstitial fibrosis, associated with extensive accumulation of ECM components in the cortical interstitium, is directly correlated with the progression of renal disease (Meran and Steadman, 2011). In fibrotic kidneys, the widened interstitial spaces fill with fibrillar material.
consisting predominantly of type I and III collagens (Chen et al., 2001; Yang et al., 2010). In this study, the evidence showed that AA at certain concentrations increased the expression of type I and III collagens in the cultured RTECs, suggesting that AA induced ECM deposition in vitro. In vivo, ureteral obstruction induced marked tubular dilatation and atrophy in the kidneys of the rats, accompanied by tubulointerstitial fibrosis as indicated by the deposition of total collagen and type III collagen. Tubulointerstitial fibrosis became more severe as time after obstruction increased, especially at 14 d after ureteral obstruction. The excessive deposition of ECM disrupts the normal architecture of the kidney, leading to fibrosis and organ failure. However, the damage induced by AA in vitro or ureteral obstruction in vivo can be ameliorated by SSBE treatment. SSBE at concentrations of 10–100 μg/ml significantly decreased the expression of types I and III collagen after AA injury. In UUO rats, SSBE treatment exerted protective effects on kidney function as indicated by decreased levels of serum creatinine. Tubulointerstitial fibrosis was also reduced by SSBE administration as indicated by decreased expression of total collagen and types I and III collagen. Thus, these findings provided morphological and molecular evidence that SSBE exerts marked anti-fibrotic effects in renal tissue via suppression of the excessive production and deposition of ECM.

In addition, we observed that SSBE inhibited the up-regulated expression of MMP-2 and TIMP-2 in RTECs after AA injury. MMP-2, which is regulated by TIMP-2, is a member of the matrix metalloproteinase family that is involved in the breakdown of ECM in normal physiological processes, such as embryonic development and tissue remodeling (Ban and Twigg, 2008). In our study, AA-induced reverse of MMP-2/TIMP-2 expression may be responsible for a decrease in the degradation of ECM and increase in its synthesis, resulting in extensive accumulation of ECM constituents. Thus, drugs that restore the balance between MMP-2 and TIMP-2, including SSBE, can effectively inhibit synthesis and extensive accumulation of ECM (Peng et al., 2013).

Renal fibrogenesis is a dynamic process, and the transition of RTECs to ECM-producing cells is considered to be an important component of the generation of fibroblasts (Ducou and Erickson, 2004; Docherty et al., 2006). Compared with epithelial cells, ECM-producing cells (mesenchymal cells) possess a stronger ability to adapt to an injured micro-environment. This phenotypic transition may be an outcome of the abnormal proliferation of RTECs, resulting in excessive accumulation of ECM. In this study, up-regulated expression levels of mesenchymal genes and down-regulated expression levels of epithelial markers were observed in the AA-treated RTECs and in the UUO rats. In the kidneys of the UUO rats, increased expression of EMT-related molecules were expressed mainly in the renal cortical areas, especially around the renal tubules, which are rich in RTECs. Moreover, the expression levels of the EMT inhibitor BMP-7 were significantly decreased in RTECs after AA injury. These changes were reduced or even prevented by the treatment with SSBE both in vitro and in vivo, as indicated by the decreased expression of the mesenchymal proteins and increased levels of the epithelial markers. These data provide evidence that SSBE suppresses the EMT.

In obstructed kidneys, numerous factors, including chemoattractants, growth factors, adhesion proteins, and matrix membrane proteins, are increased (Klahr and Morrissey, 2002). Among the various factors associated with this model, TGF-β1, an isoform of TGF-β, is regarded as critical in the development of renal fibrosis. TGF-β1, which is mainly expressed by tubular epithelial cells, is a crucial mediator of EMT during the fibrogenic phase of fibrosis (Docherty et al., 2006). In the present study, we further investigated the possible role of TGF-β1 in fibrosis and whether SSBE exerted its anti-fibrotic effects through the TGF-β1 signaling pathway. Our study revealed that the TGF-β1 expression levels were increased not only in the AA-treated RTECs but also in the UUO rats. Moreover, the expression levels of the TGF-β1 receptor TGF-β1R were significantly enhanced in vitro and in vivo. In the UUO rats, up-regulated TGF-β1 and TGF-β1R expression was associated with the time after establishing the obstruction. These changes in expression of TGF-β1 and TGF-β1R were suppressed by the treatment with SSBE. This inhibition decreased the expression of TGF-β1 and TGF-β1R and thus abolished the induction of EMT and reduced the deposition of ECM components in vitro and in vivo.

The dose/concentration of SSBE used in this study merits discussion. The optimal dose/concentration of SSBE for suppressing EMT and ECM...
accumulation had not previously been determined. In this study, in agreement with previous results of cell proliferation and apoptosis assays, SSBE treatment at high concentrations (greater than 1,000 μg/ml) could induce marked apoptosis and necrosis of RTECs. Thus, in vitro treatment with SSBE at concentrations less than 1,000 μg/ml was appropriate. In vivo, our previous experiments had shown that a lower dose of SSBE (30 mg/kg · d) did not show significant therapeutic effects for renal interstitial fibrosis, so a higher dose of SSBE (100 mg/kg · d) was selected for this study.

In summary, the renal anti-fibrotic effects of SSBE were demonstrated in vitro and in vivo. We speculated that the anti-inflammatory and anti-proliferative effect of SSBE may play an important role in

Fig. 5. SSBE alleviates interstitial fibrosis in the kidney tissues of UUO rats. (A) Renal tubular injury was assessed by HE staining. (B) The deposition of total fibrosis in kidney tissues of UUO rats was determined by Masson's trichrome staining. Blue (aniline blue) represents collagen fibers, red (acid fuchsin) represents muscle fibers. Bar = 50 μm.

Fig. 6. SSBE inhibits EMT and ECM accumulation in UUO rats. SSBE increased the expression of E-cadherin (A) and decreased the expression of α-SMA (B) and type III collagen (C) in kidney tissues of UUO rats compared with the vehicle groups, as determined by immunofluorescence staining. Bar = 50 μm. (D) qRT-PCR showed that expression of Col1a1 and Col3a1 mRNA was increased in UUO rats, but was decreased after SSBE treatment. *P < 0.05, **P < 0.01, compared with the control; *P < 0.05, **P < 0.01, compared with the AA10 group.
fibrogenesis (Mahmoud et al., 2013; Wang et al., 2013; Youn et al., 2013). The decrease in inflammatory factors released from epithelial cells may inhibit TGF-β1 expression and activation of proliferation-associated signaling pathways (Huang et al., 2013; Correa-Costa et al., 2014). Thus, SSBE inhibits abnormal proliferation of epithelial cells and ECM-producing cells, such as myofibroblasts. Further study we need to do is to identify the function of each component of SSBE, and to clarify the association of the release of inflammatory factors with renal EMT and ECM deposition.

Conclusion

Our in vitro and in vivo experiments identified the anti-fibrotic effects of SSBE in renal tissues via reductions in TGF-β1 expression and inhibition of EMT induction and ECM accumulation.

Conflict of interest statement

None.

Acknowledgements

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