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Leonurine ameliorates kidney fibrosis via suppressing TGF-β and NF-κB signaling pathway in UUO mice

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ABSTRACT

Fibrosis is one of the characteristic features of chronic kidney disease (CKD). Inflammatory reactions and oxidative stress are implicated in the pathogenesis of fibrosis of CKD. Leonurine (LEO) is one of the active compounds from *Herba leonuri*. In this study, we further evaluated its renoprotective effect in a mouse unilateral urethral obstruction (UUO), featuring the renal tubulointerstitial fibrosis and inflammation. In this model, pretreat of LEO before ureteral obstruction abolished the expression of fibronectin, suppressed the expression of α-SMA and type I/III collagen and down-regulated vimentin. LEO also modified the cytokine expression of TGF-β, TNF-α, IL-6 and IL-1β and suppressed the phosphorylation of Smad3. Moreover, LEO blocked phosphorylation of NF-κB, and inactivated the signaling pathways associated with the progression of kidney inflammatory response. Our data support that LEO is a candidate renoprotective compound for renal fibrosis through targeting the TGF-β/Smad3 and NF-κB pathway.

1. Introduction

Chronic kidney disease (CKD), which is the major cause of end-stage renal disease (ESRD), is defined as a decreased glomerular filtration rate or increased urinary albumin excretion, or both. CKD is a major healthcare burden and a significant cause of death worldwide [1,2]. Fibrosis is one of the characteristic features in all forms of chronic kidney disease [3,4]. Currently, the aim of treating chronic kidney disease is to slow its progression to end-stage renal disease (ESRD). And it has a poor prognosis and unsatisfactory treatment regimens, and the therapies remain limited [5]. So, how to prevent chronic kidney disease from progressing to ESRD awaits even better treatment strategy.

Tubular cells not only play an important role in the regulation of kidney filtration and reabsorption but are also believed to play a role in various forms of renal pathogenesis [6]. The key pathologic features mediating the molecular and cellular bases of tubulointerstitial fibrosis and highlighting new insights, including fibroblast activation and tubular

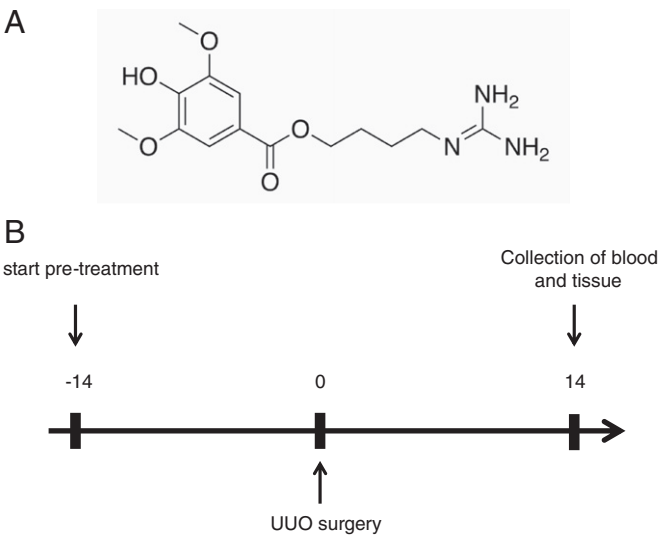

Fig. 1. A, the structure of LEO; B, schematic of the experimental was designed for pre-LEO treatment.

Abbreviations: CKD, chronic kidney disease; ESRD, end-stage renal disease; LEO, leonurine; LPO, lipid peroxides; GSH, reduced glutathione; ROS, reactive oxygen species; BSA, bovine serum albumin; DCFH-DA, 2′7′-dichlorodihydrofluorescein diacetate; DCF, 2′7′-dichlorofluorescein; MDA, malondialdehyde; TBA, thiobarbituric acid; TBARS, TBA reactive substances; EMT, epithelial-to-mesenchymal transition; UUO, unilateral urethral obstruction.

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cell that occurs in EMT, have been identified as the major avenues in renal fibrosis conditions and may lead to novel therapies [7,8]. UUO mouse models represent the renal injury which is characterized by progressive tubulointerstitial fibrosis and extensively renal damage [9–11]. In UUO model, tubular cells show fibrosis and EMT.

Herbal remedies for the treatment of kidney disease are common practice in many parts of the world. *Leonurus cardiaca*, which is an herbaceous perennial plant in the mint family, has a long history in traditional medicine treating a variety of diseases in China and many other countries. Traditionally, extracts of the *L. cardiaca* have been

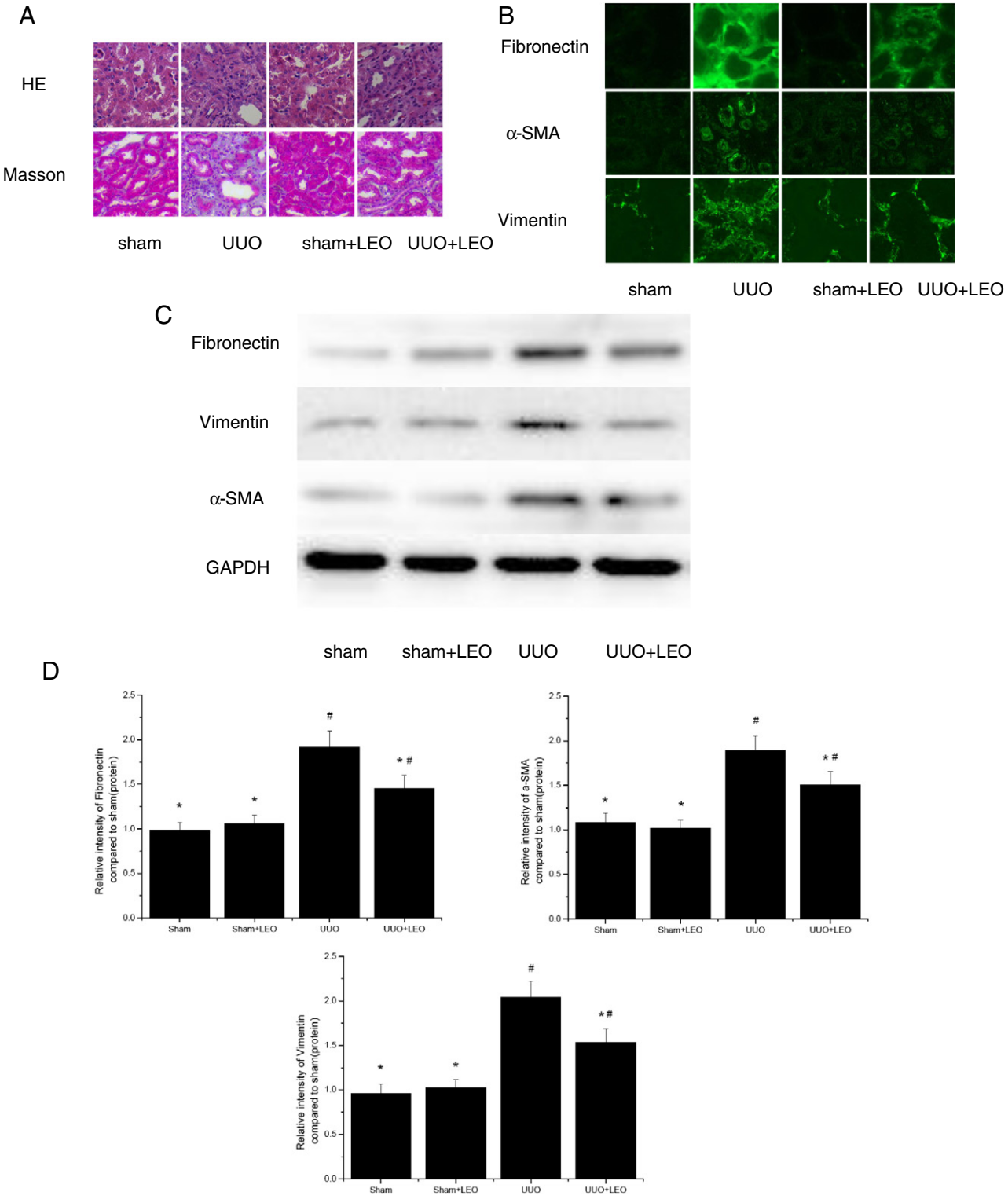

Fig. 2. Kidney fibrosis at 2 weeks after UUO in different groups of mice as indicated. A, kidney sections were subjected to HE and Masson-trichrome staining; B, kidney sections were subjected to immunofluorescence staining fibronectin, α-SMA and vimentin; C, the protein expression of fibronectin, α-SMA and vimentin in the obstructed kidneys in UUO mice; D, the relative protein expression levels of fibronectin, α-SMA and vimentin compared with the UUO group. UUO group is compared with normal control and LEO treatment groups. Values are statistically significant at $^{*}p < 0.05$; normal control is compared with UUO and LEO treatment groups. Values are statistically significant at $^{\#}p < 0.05$.