

## Original Article

# Chana 1 exhibits a promising role in the inhibition of renal kidney fibrosis in rat model

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**Abstract:** In the present study, effect of chana 1, a potent member of chalcone series on the proliferation of kidney fibroblasts and in the prevention of unilateral ureter obstruction induced renal fibrosis in rat models was investigated. The fibroblasts were identified by the presence of vimentin and absence of desmin and keratin following incubation with mouse anti-vimentin, desmin and keratin antibodies. The results revealed a significant reduction in the proliferation rate of fibroblasts in a concentration and time dependent manner. Chana 1 treatment at 25  $\mu$ M dosage inhibited the expression of transforming growth factor (TGF)- $\beta$ 1 significantly compared to the control cultures. Furthermore, treatment of the rats with 25 mg/kg concentration of chana 1 for 10 days after unilateral ureter injury prevented accumulation of extracellular matrix on the renal tubules. The renal tissues from the control group showed expansion of tubules and formation of pustules. However, chana 1 treatment inhibited these morphological changes in the kidney tissues. Thus chana 1 can be of therapeutic value for treatment of kidney fibrosis patients.

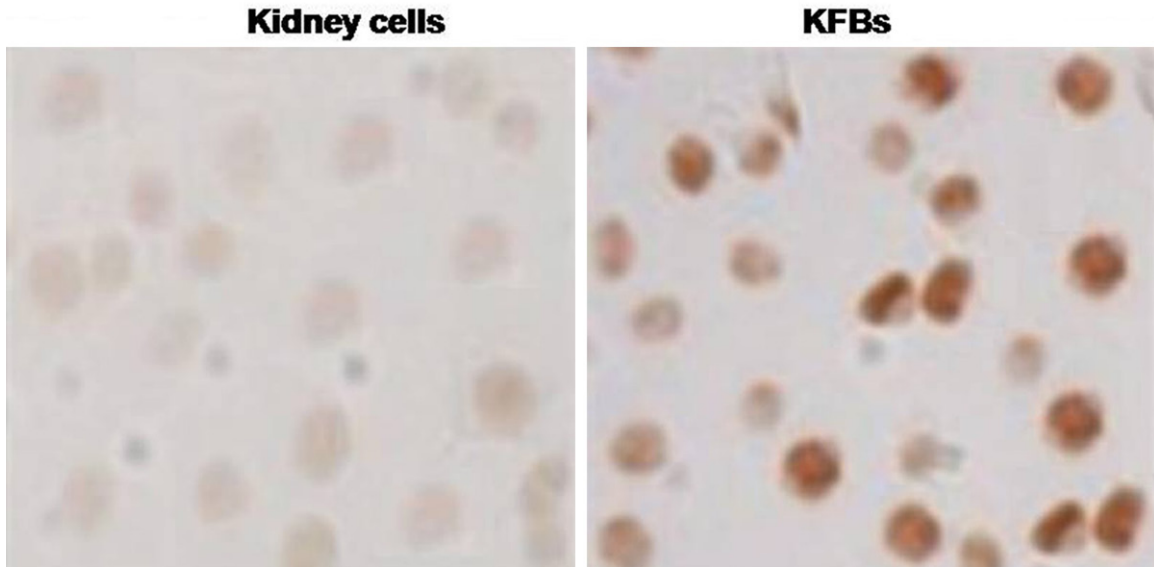
**Keywords:** Fibroblasts, chana 1, ureter, renal tubules, proliferation

## Introduction

In the structure of chalcone, two aromatic rings are attached to each other through a 3-carbon fragment containing  $\alpha,\beta$ -unsaturated carbonyl group. Chalcones are important structural fragments in many plant based natural products including, flavonoids and isoflavonoids. Biologically chalcones possess broad range of activities like anti-tumor [1], antibacterial [2], anti-inflammatory [3] and anti-invasive [4] properties. Analysis of the effect of chalcones has revealed induction of apoptosis in carcinoma cell lines on exposure to chalcones [5, 6]. Disturbance in the mitochondrial membrane potential has also been observed in the cancer cells treated with chalcones [7]. Investigation of the mechanism of action of chalcones has demonstrated that they exhibit anti-cancer effect without attaching themselves to the nucleic acidamine group [8].

Kidney is one of the most susceptible target organs of the inflammatory reactions and fibro-

sis development [9]. It is estimated that the rate of fibrosis in kidney injury patients is very high, and among such patients, rate of mortality is more than 65% [10, 11]. Kidney injury finally develops into tubule interstitial fibrosis, in which accumulation of extracellular matrix on the tubular walls in kidney takes place [12, 13]. Synthesis of ECM and its deposition on the walls of tubules is carried out by the kidney fibroblasts [14]. Renal injury in the kidney is accompanied by increase in expression of transforming growth factor (TGF)- $\beta$ 1 which plays a vital role in the fibrosis [15]. Several efforts have been made for the treatment of kidney fibrosis, however, the rate of prognosis is very poor. In the present study, effect of chana 1 (**Figure 1**), member of chalcone series, on the rate of proliferation of kidney fibroblasts and in the prevention of unilateral ureter obstruction induced renal fibrosis in rat models was investigated. The results demonstrated that chana 1 treatment caused a significant inhibition in the proliferation of fibroblasts through reduction in the expression of TGF- $\beta$ 1.



**Figure 1.** Characterization of KFBs using phase contrast microscopy. The cells were examined for the expression of vimentin, keratin and desmin cell culture devoid of primary antibody were used as the negative control.

## Materials and methods

### *Separation and culture of the KFBs*

The renal fibroblasts were obtained from kidney tissues of the rats. For this purpose, thin kidney sections of the rats were cultured in small plastic jars containing Dulbecco's modified Eagle's medium (DMEM; Gibco Corp., Carlsbad, CA, USA). At the time of formation of cell extrusions, tissue sections were removed to allow the cells grow. The fibroblasts were identified by the presence of vimentin and absence of desmin and keratin following incubation with mouse anti-vimentin, desmin and keratin (Sigma Aldrich, St. Louis, MO, USA) antibodies.

### *Animals and treatments*

Twenty 8-week old male Sprague-Dawley rats (200±20 g) were purchased from the Experimental Animal Center of Shandong Engineering Research Center for Natural Drugs (Yantai, China). The animals were provided vide certificate number 20014/034. The animals were maintained under laboratory conditions according to the Guidelines of Yantai University for the Care and Use of Laboratory Animals. The rats were housed on 12 h light and dark cycles with free access to food and water. The animals were assigned randomly into two groups of 10 each: Chana 1 and control group. The animals

in the chana 1 group were injected 25 mg/kg doses of chana 1 for 10 days following unilateral ureter injury. The control group of rats received an equal volume of normal saline at the same times.

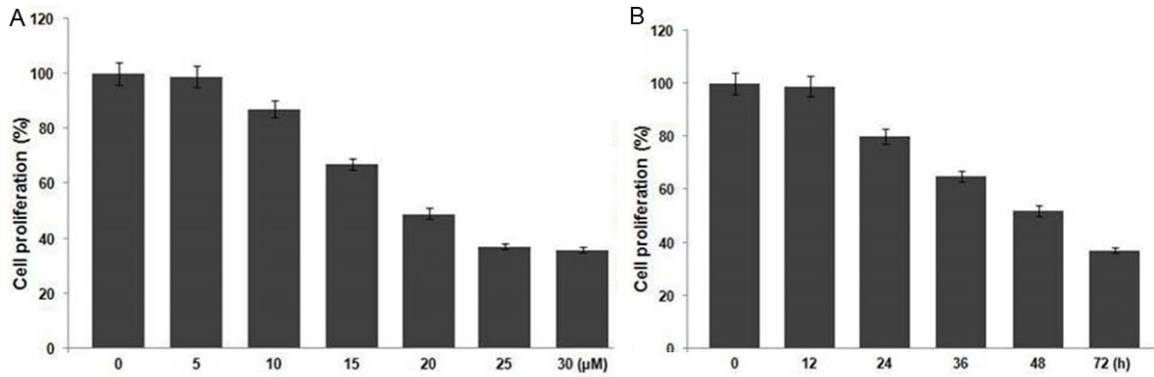
### *Analysis by immunocytochemical staining*

The cells distributed on cover slips were treated with chana 1 or left untreated as control. Following incubation, the cells were treated for 10 min with 4% paraformaldehyde and subsequently with 1% Triton X-100. The cells were then washed with PBS followed by 45 min treatment with 10% goat serum. After washing with PBS, the cells were incubated with primary antibodies overnight. Again, the cells were washed with PBS and then incubated with secondary antibodies for 1 h. For staining of the cells 3,3'-diaminobenzidine (DAB) was used whereas hematoxylin was used for nuclear staining. The anti-fade mounting media (Invitrogen, Carlsbad, CA, USA) was applied on the glass slides on which coverslips were fixed. The slides were examined under Zeiss LSM 710 laser confocal fluorescence microscope (Zeiss, Oberkochen, Germany) to take the images.

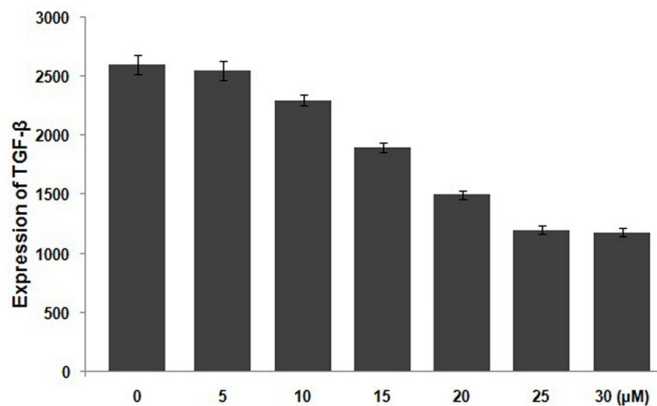
### *MTT assay*

Kidney fibroblast cells were distributed into 0.6% agarosecoated 96-well plates at a density

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**Figure 2.** Dose- and time dependent reduction in the proliferation of renal fibroblasts by chana 1. The cells were treated with 5, 10, 15, 20, 25 and 30 µM concentration of chana 1 for 12, 24, 48 and 72 h. Following incubation, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to examine cell proliferation. \*P<0.02 compared to control cells. OD, optical density.



**Figure 3.** Inhibition of TGF-β1 expression in the fibroblasts by chana 1. The cells were incubated with 25 µM concentration of chana 1 for 48 h and then expression of protein TGF-β1 was determined. \*P<0.01 compared to the control cells.

of  $2 \times 10^5$  cells per well. The cells were treated with various concentrations of chana 1 for 12, 24, 48 and 72 h or left untreated as the control. Following incubation at 37°C, the medium was removed and 5 mg/ml MTT solution was added to each of the well. The cells were incubated for 4 h more under the same conditions. The cells were then extracted using acidic isopropanol to measure the absorbance at 570 nm wavelength by an enzyme-labeling instrument (ELx800 Absorbance Microplate Reader type, BioTek Instruments, Inc., Winooski, VT, USA).

**TGF-β1 enzyme-linked immunosorbent assay (ELISA):** The cells distributed at a density of  $2 \times 10^5$  cells per ml were incubated with 25 µM concentration of chana 1 for 48 h. Following incubation, cell cultures were centrifuged to

obtain the supernatant. Expression of TGF-β1 was determined in the cell supernatant as per the manual protocol by using sandwich ELISA kit.

### *Preparation of unilateral kidney injury rat model and treatment strategy*

For the preparation of unilateral ureter animal model, a small incision was made in the abdominal region of each rat. Through the incision, the ureter in the left side of cavity was ligated. The role of chana 1 was examined in the treatment of fibrosis after induction of unilateral ureter injury. Chana 1 was given to the rats after induction of unilateral ureter injury at 25 mg/kg for 10 days. On the Day 20, kidneys from the

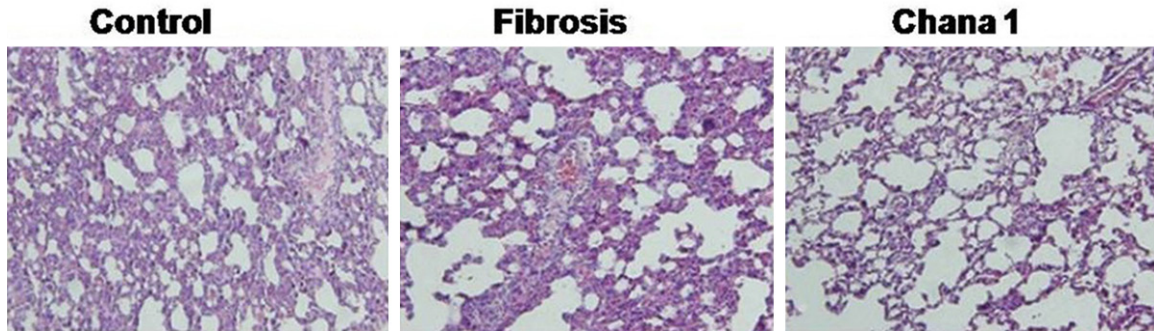
rats following induction of unilateral ureter injury were extracted after sacrifice.

### *Histological investigation*

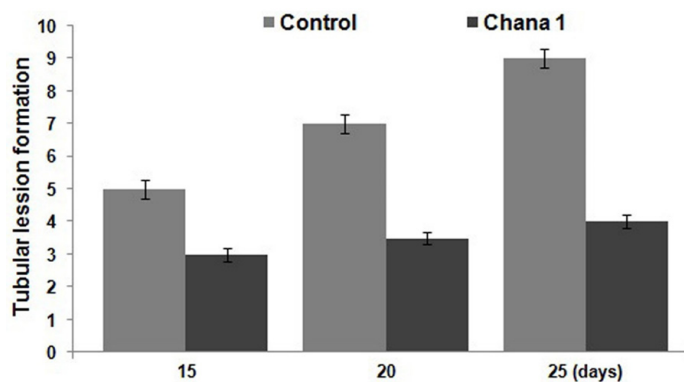
From the rats treated with chana 1 and control group kidneys were extracted following anesthesia with chloral hydrate (300 mg/kg, i.p.). The kidney tissues were stained with Masson's trichrome to analyze the alterations in the tissue morphology. For each of the animals three different tissue sections were analyzed by three different persons.

### *Statistical analysis*

All the data presented are the mean  $\pm$  SD. SPSS software, version 15.0 (SPSS, Inc., Chicago, IL, USA) was used for the analysis of obtained



**Figure 4.** Kidney tissues of the chana 1 treated and control group of rats were analyzed for ECM deposition using Masson trichrome staining. Accumulation of ECM was prevented in the kidney tissues by chana 1 treatment. Where; UUU and ECM stand for unilateral ureteral obstruction and extracellular matrix, respectively.



**Figure 5.** Chana 1 treatment at 25 mg/kg dosage for 10 days prevents formation of renal interstitial fibrosis in the rat.

data and one-way analysis of variance was also performed. The statistically significant differences were considered at  $P < 0.05$ .

## Results

### Characterization of KFBs

The KFBs were identified based on the criteria of presence of vimentin and absence of keratin and desmin using phase contrast microscopy (**Figure 1**).

**Effect of chana 1 on the cell proliferation in KFBs:** KFBs were allowed to reach confluence by culturing for 3 days and subsequently treated with various concentrations of chana 1. Examination of the cell cultures revealed a dose and time dependent inhibition in the proliferation of KFBs on treatment with chana 1 compared to control cells ( $P < 0.02$ ). The cells were treated with 5, 10, 15, 20, 25 and 30  $\mu\text{M}$  concentration of chana 1 for 12, 24, 48 and 72 h. Treatment with 25  $\mu\text{M}$  concentration of cha-

na 1 for 48 h reduced cell proliferation of 37% compared to 100% in the control (**Figure 2**).

**Chana 1 treatment inhibits TGF- $\beta$ 1 expression:** Chana 1 treatment caused reduction in the expression of TGF- $\beta$ 1 in dose dependent manner. Treatment of the KFBs with 25  $\mu\text{M}$  concentration of chana 1 for 48 h caused a significant ( $P < 0.01$ ) reduction in the expression of TGF- $\beta$ 1 (**Figure 3**). In the control cell cultures expression of TGF- $\beta$ 1 was markedly higher.

**Chana 1 prevents formation of renal fibrosis:** Masson trichrome staining in the rats with UUU injury and blocked ureter lead to the formation of pustules, expanded renal tubules and accumulation of the collagen on renal tubular walls. However, chana 1 treatment for 10 days at 25 mg/kg dosage prevented accumulation of collagen on renal tubular walls and formation of pustules in the rats (**Figure 4**).

Another, characteristic of the kidney injury in rats, tubulointerstitial lesion formation was inhibited on treatment with chana 1 for 10 days at 25 mg/kg dosage. In control group of rats, tubulointerstitial lesions were observed abundantly in the renal tubules following 20 days of UUU injury (**Figure 5**).

## Discussion

The present study was designed and performed to investigate the effect of chana 1 on proliferation of kidney fibroblasts and in the prevention of unilateral ureter obstruction induced renal



fibrosis in rat models. Kidney fibroblasts are considered to be the important target in the development therapeutic strategies for fibrosis [16]. Inhibition in the proliferation rate of fibroblasts by chemotherapeutic agents has a great impact in the fibrosis treatment. Our study demonstrated that chana 1 treatment induced dose and time reduction in the proliferation of fibroblasts with maximum reduction rate following 48 h at 25  $\mu$ M concentration.

For the regulation of kidney fibrosis and its progression, TGF- $\beta$ 1 produced in the fibroblasts play an important role [17]. The cellular processes responsible for formation of fibers and their deposition in the renal tubules are mediated by activated TGF- $\beta$ 1 [18]. Our results revealed that chana 1 treatment exhibited inhibitory effect on the expression of TGF- $\beta$ 1 in the fibroblasts.

The process of epithelial-myofibroblast trans-differentiation (EMT) causes conversion of the cell lining in the renal tubules into the myofibroblasts which then produce ECM [19, 20]. The resulting ECM on deposition to the tubular walls develops fibrosis. The results from our study revealed that treatment of the rats with chana 1 prevented accumulation of ECM on the tubular walls. There was no fiber formation in the kidney tissues of the rats treated with chana 1. The rats in the control group showed presence of interstitial cells and collagen which was consistent with the earlier reports [21]. However, treatment of the rats with chana 1 for 10 days prevented accumulation of interstitial cells and collagen deposition. There was no expansion in the tubules of rats treated with chana 1 for 10 days.

In conclusion, chana 1 causes reduction in the proliferation of KFB, inhibits expression of TGF- $\beta$ 1 and suppresses ECM accumulation in the kidney tissues. Thus chana 1 can be useful for the treatment of kidney injury.

### Disclosure of conflict of interest

None.

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