We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,300
Open access books available

130,000
International authors and editors

155M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

16

Knowledge-Based Discovery of Anti-Fibrotic and Pro-Fibrotic Activities from Chinese Materia Medica

Qihe Xu et al.*
King's College London, Department of Renal Medicine,
The Rayne Institute, London,
United Kingdom

1. Introduction

Fibrosis, also known as scarring, sclerosis or cirrhosis, is characterised by excessive accumulation of extracellular matrix (ECM) proteins leading to tissue contraction, disruption of tissue architecture and eventually chronic organ failure (Wynn, 2007; Xu et al., 2007). Research and development of anti-fibrotic drugs are generally based on two distinct but interactive strategies, with one based on mechanism studies and another based on exploring efficacy. In principle, the mechanism-based strategy begins with identification of molecular targets through mechanistic studies, and then development of inhibitors or enhancers targeting the molecules. On the other hand, efficacy-based strategy starts with screening drug candidates in disease models to identify activities and efficacy, with less reliance on analysis of mechanisms of action. There are certain limitations in both the mechanism-based strategy and the efficacy-based strategy, which largely account for the lack of success in development of anti-fibrotic drugs. The former is often associated with identification of multiple molecular targets impeding development of a single drug that tackles multiple targets, while the latter is often hampered by establishment of apt models ideal for efficacy-driven drug screens.

Efficacy-based strategy has been employed in development of both traditional and modern medicines. In the context of traditional medicine, the knowledge about efficacy of a given drug is largely derived from a trial-and-error process, namely by assessing patients' response upon treatment with natural drug candidates. However, in modern medicine, it is impossible to directly test any new drugs in patients. Solid scientific evidence on efficacy and safety of a given drug in experimental models is required prior to clinical trials. Understandably, quality of these models would determine the specificity and efficiency of the tested drug.

* Yuen Fei Wong[1], Shanshan Qu[1], Qingyang Kong[1], Xiu-Li Zhang[2], Xin-Miao Liang[2], Qin Hu[1], Mazhar Noor[1] and Bruce M. Hendry[1],
[1]*King's College London, Department of Renal Medicine, The Rayne Institute, London, United Kingdom*
[2] *Multi-Component TCM Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian PR China*

In vivo models are invaluable research tools but not without its limitation, especially in the development of drugs targeting fibrosis given the complex aetiologies and inflammatory processes involved in this pathological condition. In particular, drug leads displaying effectiveness in *in vivo* models of fibrosis may stem from (i) inhibition of the primary aetiological factors, (ii) inhibition of inflammation, a common inciting factor of fibrosis, (iii) inhibition of fibrosis *per se*, or (iv) a net effect of any combinations of the aforementioned.

For the initial development of drugs with specific anti-fibrotic effects independent of anti-inflammatory actions and any specific aetiological factors, *in vitro* models of fibrosis appear to be more appropriate. Our laboratory recently reported transforming growth factor-β1 (TGF-β1)-induced inflammation-free *in vitro* models of fibrosis in mesenchymal cells, featuring 2-dimentional (2D) ECM protein accumulation reflecting net collagen accumulation, and 3-dimentional (3D) nodular formation following ECM protein-induced disruption of cell monolayer, which can both be objectively quantified. By coating specific matrix on 96-well plates, which determines the 2D or 3D nature of the models, we are able to quantify readouts more reliably, permitting high-throughput screening of compounds with anti-fibrotic activities (Xu et al., 2007).

In contrast to the lack of "anti-fibrotics" in Western medicine clinics, there is accumulating evidence suggesting anti-fibrotic effects of Chinese materia medicas (CMMs), i.e., medicinal materials used in traditional Chinese medicine (TCM) (Hu et al., 2007). However, most of these conclusions were drawn from animal models or patients and hence it was not clear whether the reported efficacy of those CMMs was secondary to inhibition of aetiological factors or inflammation, or whether they exerted genuine anti-fibrotic activities (Hu et al., 2009). We hypothesised that at least some of the "anti-fibrotic" herbal derivatives reported in the literature are indeed anti-fibrotic by antagonising TGF-β1-specific pro-fibrotic pathways or common pathways of fibrosis. By employing the 2D *in vitro* model (Xu et al., 2007), we tested the anti-fibrotic activity of 21 CMM-derived compounds, 11 methanolic extracts of single CMMs and 27 formulae that contained two or more CMMs as mixtures, and found that five compounds, three single CMM extracts and 16 formulae had *in vitro* anti-fibrotic activities (Hu et al., 2009). Among the five CMM-derived compounds, three flavonoids (quercetin, baicalin and baicalein) showed similar dose-dependent *in vitro* anti-fibrotic activities while two non-flavonoids (salvianolic acid B and emodin) showed varied *in vitro* anti-fibrotic activities with poor dose dependency. Among the three CMM extracts showing significant *in vitro* anti-fibrotic activities, Huangqin (root of *Scutellaria baicalensis* Georgi) is rich in baicalin and baicalein, Danshen (root of *Salvia miltiorrhiza* Bunge) is rich in salvianolic acid B and Dahuang (root of *Rheum palmatum* L.) is rich in emodin. Among the 16 herbal formulae with *in vitro* anti-fibrotic effects, eight contained neither Huangqin, Danshen nor Dahuang, while the remaining eight contained at least one of the three CMMs (Hu et al., 2009).

Following successful identification of *in vitro* anti-fibrotic activities in herbal entities, we have extended our work to focus on TCM knowledge-based discovery of novel anti-fibrotic drug leads from natural sources, especially CMMs. In TCM, fibrosis is diagnosed as a kind of "Jie Zheng" which means "lump or clot". Based on this concept, two senior TCM practitioners were invited to choose a collection of 27 CMMs, including 26 medicinal plant parts and one medicinal fungus, which they would consider using in patients with fibrotic diseases. Based on the traditional categories of CMMs, the 27 CMMs fall into three functional subgroups, namely "Huo Xue Hua Yu" ("promoting the circulation and resolving

the clot"), "Hua Tan" ("resolving the sputum") and "Bu Xu" ("tonifying the deficiency"), where "clot" and "sputum" in TCM do not mean the same as the terms in Western medicine. In addition to the 27 CMMs, Chuanwutou, an unprocessed herb with well-known toxic effects, was also tested to serve as a control for cytotoxic effects, if any. In fact, owing to its strong "Qu Feng Shi" ("dispelling the wind and damp" or anti-rheumatic function) property, Chuanwutou is rather commonly prescribed to patients with diseases complicated by fibrosis, although only processed Chuanwutou is allowed for clinical use.

The aim of this project was to examine the *in vitro* anti-fibrotic and pro-fibrotic activities of these 28 CMMs (Table 1) and herein we report that eight CMMs (Baibeiyegen, Liedang, Gusuibu, Jixueteng, Lingzhi, Meiguiqie, Moyao and Shiliuhua) have *in vitro* anti-fibrotic activities while three (Chuanwutou, Dangshen and Yimucao) have pro-fibrotic activities.

2. Materials and methods

2.1 CMMs and extraction methods

Liedang was authenticated according to the criteria described in *Jilin Zhongcaoyao* (Changchun TCM College Revolutionary Committee (Ed.), (June 1970), *Jilin Chinese Herbal Medicine,* Jilin People's Press, Changchun, China) and all other CMMs were authenticated according to Chinese Pharmacopeia (2005 Edition). Voucher specimens were deposited at Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China. Individual CMM was grounded into fine powder, from which 85 g was precisely weighed out and added to 600 ml of 80% ethanol. The mixtures were boiled for 2 h and filtrated, after which the residual ethanol was evaporated. Ethanolic extracts were then concentrated and dried in an oven, and were stored at room temperature before use. For experimental purpose, ethanolic extracts were reconstituted in dimethyl sulfoxide (DMSO, Sigma-Aldrich Company Ltd., Dorset, UK) and were stored in freezer at -20 °C until use.

2.2 TGF-β1, Alk5 inhibitor and PPAR antagonists

Human TGF-β1 in lyophilised powder form (R&D Systems Europe Ltd., Abingdon, UK) was reconstituted in filter-sterilised buffer consisting of 1 mg/ml bovine serum albumin in 4 mM HCl, to a final concentration of 10 μg/ml and kept frozen at -80 ºC before experiments. IN-1130, a selective inhibitor of TGF-β type I receptor (Alk5), was a kind gift from Dr Dae Kee Kim, Ewha Women's University, Korea, and was used as a positive control for *in vitro* anti-fibrotic activity (Moon et al., 2006). Peroxisome proliferator-activated receptor (PPAR) antagonists, including PPARα antagonist GW6471 (Xu et al., 2002), PPARβ/δ antagonist GSK0660 (Shearer et al., 2008) and PPARγ antagonist T0070907 (Lee et al., 2002) were purchased from Sigma-Aldrich.

2.3 Cell culture, TGF-β1-induced *in vitro* models of fibrosis and related assays

A normal rat kidney fibroblast cell line (NRK-49F) was purchased from European Collection of Cell Cultures (ECACC, Health Protection Agency, Salisbury, UK). The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, PAA Laboratories Ltd., Somerset, UK) supplemented with 100 U/ml of penicillin G (PAA), 100 μg/ml of streptomycin (PAA), 0.25 μg/ml of amphotericin B (Invitrogen Ltd., Paisley, UK) and 5% foetal calf serum (FCS, Sigma-Aldrich), in Falcon tissue culture flasks (Marathon Laboratory supplies, London, UK), and incubated at 37 °C and 5% CO2. During routine maintenance,

Functional group	Pinyin name	Part, Latin name and authority	Authenticator	Extraction yield (%)	Voucher number
"Bu Xu"	Baibeiyegen	Dried roots of *Mallotus apelta* (Lour.) Muell.-Arg.	SJS*	21	B012081
	Liedang	Dried whole plants of *Orobanche coerulescens* Steph.	SJS	28.5	C013081
	Dangshen	Dried roots of *Codonopsis pilosula* (Franch.) Nannf.	SJS	58.7	D001081
	Gancao	Dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch.	SJS	81.7	G010081
	Roucongrong	Dried fleshy stem with scales of *Cistanche deserticola* Y. C. Ma	SJS	82.2	R002081
	Yinyanghuo	Dried rootless plants of *Epimedium brevicornum* Maxim.	SJS	42.1	Y006081
"Bu Xu" & "Hua Tan"	Lingzhi	Dried mushroom of *Ganoderma lucidum* (Leyss. Ex Fr.) Karst.	SJS	8.33	L003081
"Hua Tan"	Baiguo	Dried ripe seeds of *Ginkgo biloba* L.	HXR**	7.9	B013041
	Jiegeng	Dried roots of *Platycodon grandiflorum (Jacq.)* A. DC.	SJS	12.3	J009081
	Meiguiqie	Dried flowers of *Hibiseus sabdariffa* L.	SJS	37.6	M004081
	Tiannanxing	Dried tuberous roots of *Arisaema erubescens* (Wall.) Schott.	SJS	2.34	T001081
"Hua Tan" and "Huo Xue Hua Yu"	Yinxingye	Dried leaves of *Ginkgo bilobla* L.	SJS	65	Y009081
"Huo Xue Hua Yu" & "Qing Re Jie Du"***	Baihuasheshecao	Dried plants of *Oldenlandia diffusa* (Willd.) Roxb.	HXR	18	B010041
"Huo Xue Hua Yu"	Ezhu	Dried rhizomes of *Curcuma kwangsiensis* S. G. Lee et C. F. Liang	HXR	60.1	E001041
	Gusuibu	Dried rhizomes of *Drynaria fortunei* (Kunze) J. Sm.	SJS	27	G007081
	Jixueteng	Dried stem of *Spatholobus suberectus* Dunn	SJS	33.9	J001081
	Maqianzi	Dried ripe seeds of *Strychnos nux-vomica* L.	SJS	7.7	M003081
	Moyao	Dried resin of *Commiphora myrrha* Engl.	SJS	33.7	M002081
	Niuxi	Dried roots of *Achyranthes bidentata* Bl.	SJS	9.9	N002081

Functional group	Pinyin name	Part, Latin name and authority	Authenticator	Extraction yield (%)	Voucher number
	Ruxiang	Dried oleogum resin of *Boswellia carterii* Birdw.	SJS	60.6	R001081
	Sanleng	Dried tuberous roots of *Sparganium stoloniferum* Buch.–Ham.	SJS	25.1	S003081
	Sheputaogen	Dried roots of *Ampelopsis (Miq.)* W. T. Wang	SJS	14	S011081
	Shiliuhua	Dried flowers of *Punica granatum* L.	SJS	88	S012081
	Taoren	Dried ripe seeds of *Prunus persica (L.)* Batsch.	SJS	35.6	T004081
	Yanhusuo	Dried tuberous roots of *Corydalis yanhusuo* W. T. Wang	HXR	69.5	Y010041
	Yimucao	Dried rootless plants of *Leonurus japonicus* Houtt.	SJS	12.8	Y005081
	Zhenzhumei	Dried bark of the stems of *Sorbaria sorbifolia (L.)* A. Brown.	SJS	33.1	Z004081
"Qu Feng Shi"	Chuanwutou	Dried main roots of *Aconitum carmichaeli* Debx.	SJS	18.2	C005081

Table 1. Functional groups, species names and parts, authenticators, extraction yield and voucher numbers of the 28 test materials. * SJS: Shi Jian Sa, Tong De Chinese Materia Medica Co. Ltd., Anguo, Hebei, China; ** HXR: He Xi Rong, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China; *** "Qing Re Jie Du" means "clearing heat and detoxifying".

NRK-49F cells were sub-cultured before they became confluent to prevent transformation. The 2D model was employed for experiments in this project. Cells were seeded in collagen type I-coated 96-well plates (BD Biosciences, Oxford, UK) at a density of 1×10^4 cells per well in 200 µl DMEM supplemented with 2.5% FCS and 2.5% Nu-Serum™ V serum replacements (NU, BD Biosciences). After three days, the medium was changed to serum-free DMEM supplemented with 1% insulin-transferrin-selenium liquid media supplement (ITS, Sigma-Aldrich) for four days, and then changed to fresh ITS-supplemented serum-free medium containing 5 ng/ml TGF-β1 in the presence of different concentrations of herbal extracts or a vehicle control (an equal volume of DMSO) for 48 h. In all the experiments, 1 µM IN-1130 was used as a positive control for anti-fibrotic activities. For initial screening, the concentrations of CMM extracts tested were 10, 20, 40, 80, 160 and 200 µg/ml, 3-6 wells per group, and the screening was repeated at least twice. For follow-up confirmation studies, four herbal extracts were selected based on the results from initial screening studies, and their effects were further confirmed using three different concentrations, 3-6 wells per group. Each follow-up experiment was performed four times.

2.4 Cell detachment index (CDI) and lactate dehydrogenase (LDH) release assay

For the screening studies, *in vitro* cytotoxicity at the end of 48 h treatment was assessed by phase-contrast microscopy using the same CDI criteria that we reported before (Hu et al.,

2009). In brief, CDI reflects cell monolayer disruption, including cell detachment from the adherent surface and disorganisation of cell monolayer. Scores of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 represented an area of 0, 5%, 10%, 20%, 30%, 40%, 60%, 80% and 100%, respectively, of the total adherent surface not covered by cells. This method was used to approximate cytotoxicity, as well as to ensure the reliability of subsequent picro-Sirius red (PSR) staining results, which required minimum cell (and matrix) detachment and disruption of cell monolayer. For follow-up studies, LDH release assay was used to assess *in vitro* cytotoxicity at the end of 48 h treatment. Fifty microlitre of supernatant from each well was collected and tested for LDH release according to the manufacturer's instructions (Promega, Southampton, UK). LDH release was measured using a Dynex Technologies MRX spectrophotometer (Prior Laboratory Supplies Ltd., East Sussex, UK), at optical density (OD) value of 490 nm.

2.5 Microscopic examination

Microscopic examination was performed on a Nikon Eclipse TE2000-S microscope (Nikon Instruments Europe B.V., The Netherlands). Bright-field and phase-contrast images were captured with a DXM1200F Nikon digital camera (Nikon UK Limited, Surrey, UK) and processed with Adobe Photoshop (Adobe System Europe Ltd., London, UK).

2.6 PSR staining and spectrophotometric analysis

Total collagen accumulation was assessed qualitatively by microscopic examination of PSR-stained cells and quantitatively by spectrophotometric analysis of PSR staining. After CDI determination and conditioned medium collected for LDH release assay, cell monolayer in 96-well plates were fixed in ice-cold methanol (200 μl per well) overnight at –20 °C. Cells were carefully washed twice (5 min each) with 1x phosphate buffered saline (PBS, 200 μl per well) and then stained with 0.1% w/v PSR solution (200 μl per well, Sigma-Aldrich) at room temperature for 4–6 h. The staining solution was then removed and excessive PSR stain was carefully washed off with 0.1% v/v acetic acid (200 μl per well, VWR International Ltd, Lutterworth, UK) three times (5 min each). The stained wells were left to air dried for 24–48 h. PSR stain was then observed under bright-field microscopy and microscopic pictures were taken. Finally, PSR stain was eluted in 0.1 N NaOH (200 μl per well) on a rocking platform at room temperature for 1 h. The plate was then subjected to spectrophotometric analysis of OD at 540 nm on a Dynex Technologies MRX spectrophotometer (Hu et al., 2009).

2.7 PPARα, PPARβ/δ and PPARγ agonistic activities of Shiliuhua extract and effect of Shiliuhua extract in the presence of PPAR antagonists

We commissioned Tebu-bio Laboratories, Le Perray en Yvelines, France, to perform a pilot test on Shiliuhua extract for its isotype-specific PPAR activation. In brief, the assay was carried out *in vitro* in three HeLa cell lines stably expressing a chimeric protein containing the yeast transactivator GAL4 DNA binding domain fused to ligand binding domain regions of human PPARα, PPARβ/δ or PPARγ, and a luciferase reporter gene driven by a pentamer of the GAL4 recognition sequence in front of the β-globin promoter (Seimandi et al., 2005). The PPAR reporter cell lines were seeded in a 96-well plate in triplicates, and treated with 40 μg/ml Shiliuhua extract, or an equal volume of DMSO as negative control, for 24 h. Luciferase activity was determined with a luminometer and relative light units (RLU) were recorded. Three independent experiments were performed and the fold changes of RLU were normalised to the mean of negative control.

The effect of PPAR antagonism on Shiliuhua extract treatment was assessed following the protocol as described in sections 2.3-2.6. Cells were treated with 40 μg/ml Shiliuhua extract with and without PPARα antagonist, GW6471 (0.01-10 μM), PPARβ/δ antagonist, GSK0660 (0.001-1 μM), or PPARγ antagonist, T0070907 (0.1-25 μM). Three independent biological experiments were performed, 3-6 wells per group.

2.8 Statistical analysis

Results of PSR and LDH OD values were expressed as mean ± SEM unless stated otherwise. Statistical differences were computed with Prism 4.0 (GraphPad Software, San Diego, CA, USA), by one-way analysis of variance and Dunnett post test for comparison between a control group and all other groups; for PPAR reporter activity, one-tail paired *t* test was performed on log-transformed fold changes. $p<0.05$ was regarded as statistically significant.

3. Results

3.1 Initial screening

The screening results of CMM extracts were summarised in Table 2. Anti-fibrotic and pro-fibrotic effects were defined as PSR OD values significantly lower and higher than that of TGF-β1-treated group, respectively; cytotoxicity was defined as CDI significantly higher than that of TGF-β1-treated group. Doses at which extracts exhibited reproducible effects were indicated in the table. The CMMs were categorised into four groups, i.e., eight in Group A showed *in vitro* anti-fibrotic activities; three in Group B showed pro-fibrotic activities; six in Group C showed prominent cytotoxicity; 11 in Group D did not have profound effects on total collagen accumulation nor integrity of cell monolayer. In Group A, Liedang, Meiguiqie and Gusuibu extracts were well-tolerated agents with anti-fibrotic activities noted within the range of 80-200 μg/ml; extracts of Shiliuhua, Baibeiyegen, Jixueteng, Moyao and Lingzhi showed anti-fibrotic effects at concentrations ranging from 10–80 μg/ml, beyond which cytotoxicity was noted. In Group B, Chuanwutou showed a pro-fibrotic effect at concentrations as low as 20 μg/ml whereas pro-fibrotic effect of Dangshen and Yimucao were observed only at higher concentrations (160–200 μg/ml).

3.2 Follow-up studies

Based on the initial screening results, Shiliuhua (SLH), Liedang (LD), Meiguiqie (MGQ) and Chuanwutou (CWT) extracts were selected for follow-up studies in view of their minimum CDI changes indicating low cytotoxicity, and the results are shown in Fig. 1. In contrast to SLH, LD and MGQ extracts, which showed varying degrees of *in vitro* anti-fibrotic effects, CWT extract exhibited a marked pro-fibrotic effect. Representative effects of SLH, LD, MGQ and CWT extracts on PSR staining, relative PSR OD values and LDH release are shown in Fig. 2 and Fig. 3.

3.3 Activation of PPARs by SLH extract

As SLH extract is one of the CMM extracts with the most potent *in vitro* anti-fibrotic activity and it was previously reported to interfere with the PPAR signalling pathway (Li et al., 2008), we hypothesised that the anti-fibrotic effect of SLH extract was at least in part mediated by activation of one or more PPAR receptors. Indeed, SLH extract induced PPARα- and PPARγ-mediated reporter activity; it also marginally activates PPARβ/δ but the induction of reporter activity was just above the threshold of significance (p=0.066) (Fig. 4a). In order to

determine if the agonistic effect of SLH extract has an impact on TGF-β1-induced fibrogenesis, cells were treated with SLH extract and individual PPAR antagonists. PPARβ/δ antagonist, GSK0660 (IC_{50} 160 nM) (Shearer et al., 2008), did not affect the anti-fibrotic activity of SLH extract at all concentrations tested (up to 6.25-fold higher than its IC_{50}); PPARα antagonist, GW6471 (IC_{50} 240 nM) (Xu et al., 2002), also did not show any significant effect at concentrations up to 4.2-fold of its IC_{50}, but at 10 μM (42-fold higher than its IC_{50}), it did moderately suppress the anti-fibrotic activity of SLH extract; PPARγ antagonist, T0070907 (IC_{50} 1 nM in inhibiting rosiglitazone binding to PPARγ and 3.2-24.3 μM in inhibiting proliferation of different cancer cell lines) (Lee et al., 2002; Burton et al., 2007), did not show any significant effect at concentrations of 0.1, 1 and 10 μM, but further increased the anti-fibrotic effect of SLH extract at 25 μM (Fig. 4b).

Group	Pinyin name	Anti-fibrotic or pro-fibrotic doses (μg/ml)	Changes at optimum doses compared to TGF-β1 treated group	Cytotoxic doses (μg/ml)
A. Anti-fibrotic	Baibeiyegen	20-40	-81.4% (40 μg/ml) -63.3% (20 μg/ml)	≥80
	Liedang	80-160	-61.2% (160 μg/ml) -59.3% (80 μg/ml)	200
	Gusuibu	160-200	-36.8% (200 μg/ml) -30.0% (160 μg/ml)	—
	Jixueteng	10-40	-75.0% (20 μg/ml) -63.0% (10 μg/ml)	≥80
	Lingzhi	10-80	-34.9% (80 μg/ml) -47.4% (40 μg/ml)	≥160
	Meiguiqie	160-200	-28.0% (200 μg/ml) -21.0% (160 μg/ml)	—
	Moyao	10-40	-57.2% (40 μg/ml) -67.9% (20 μg/ml)	≥80
	Shiliuhua	10-40	-78.0% (40 μg/ml) -61.2% (20 μg/ml)	≥80
B. Pro-fibrotic	Chuanwutou	≥20	+90.2% (40 μg/ml) +68.5% (20 μg/ml)	—
	Dangshen	200	+32.4% (200 μg/ml)	—
	Yimucao	160-200	+65.7% (200 μg/ml) +53.9% (160 μg/ml)	—
C. Cytotoxic	Baihuasheshecao (≥80 μg/ml), Ruxiang (≥10 μg/ml), Sheputaogen (≥80 μg/ml), Tiannanxing (≥80 μg/ml), Yinxingye (≥20 μg/ml), Zhenzhumei (≥40 μg/ml).			
D. Inert	Baiguo, Ezhu, Gancao, Jiegeng, Maqianzi, Niuxi, Roucongrong, Sanleng, Taoren, Yanhusuo, Yinyanghuo.			

Table 2. Initial screening results of CMMs in TGF-β1-induced *in vitro* fibrogenesis. Minus (-) and plus (+) percentages represent relative reduction and increase in TGF-β1-induced PSR OD values, respectively. "—": Cytotoxicity, assessed from CDI, was not significantly different from TGF-β1-treated group at all concentrations tested (10-200 μg/ml).

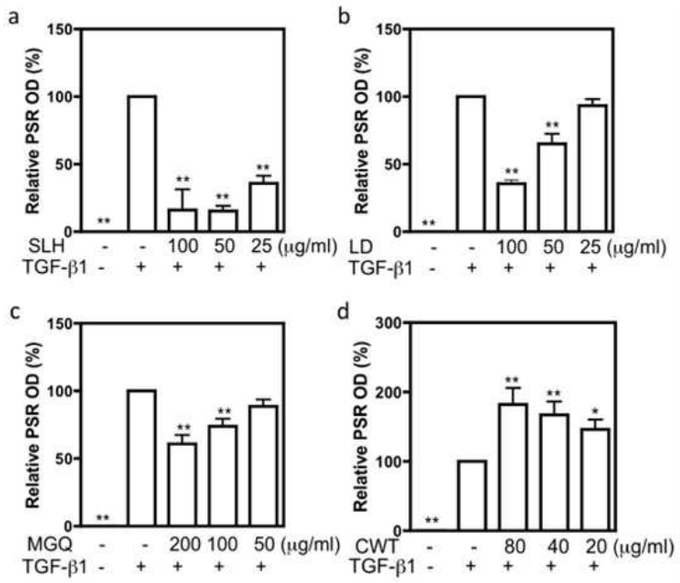

Fig. 1. *In vitro* anti-fibrotic and pro-fibrotic effects of four selected extracts, Shiliuhua (SLH) (a), Liedang (LD) (b), Meiguiqie (MGQ) (c) and Chuanwutou (CWT) (d) at three selected concentrations. Shown here are relative PSR OD changes of four independent experiments. The CDI changes were minimum and not shown. The average PSR OD values of negative control group and TGF-β1-treated group were normalised to 0 and 100%, respectively, and changes in percentage of herbal extract-treated groups were relative to the TGF-β1 only group. *, **: $p<0.05$ and $p<0.01$ vs TGF-β1 only group.

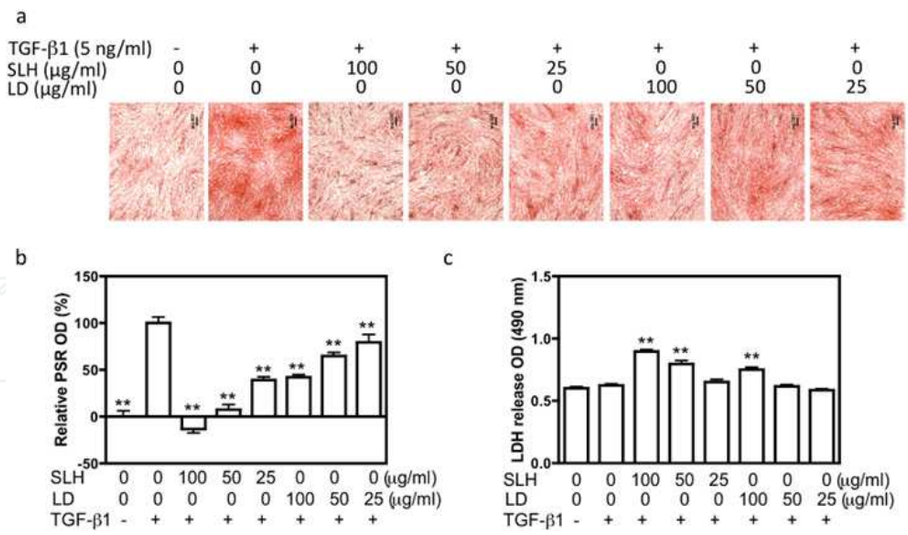

Fig. 2. Effects of Shiliuhua (SLH) and Liedang (LD) extracts on PSR staining (a), relative PSR OD values (b) and LDH release (c). Results in (b) and (c) shown here are in Mean ± SEM from one representative experiment, ** $p<0.01$ vs TGF-β1 only group, n=6 wells. The average PSR OD values of negative control group and TGF-β1 only group were normalised to 0 and 100%, respectively, and changes in percentage of herbal extract-treated groups were relative to the TGF-β1 only group.

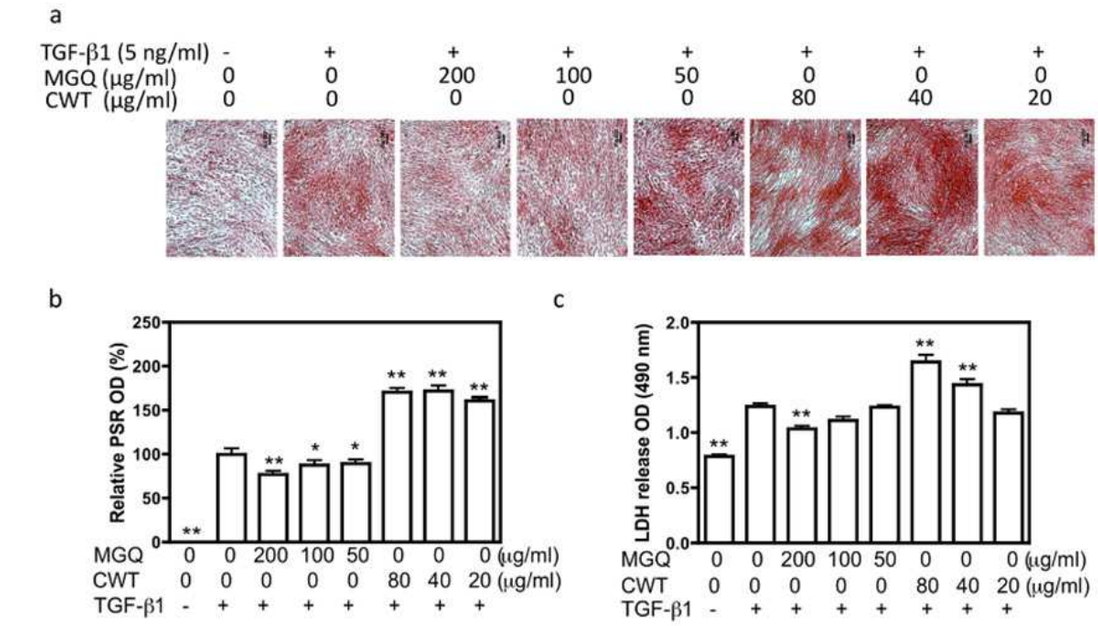

Fig. 3. Effects of Meiguiqie (MGQ) and Chuanwutou (CWT) extracts on PSR staining (a), relative PSR OD values (b) and LDH release (c). Results in (b) and (c) shown here are in Mean ± SEM from one representative experiment, *, ** $p<0.05$, $p<0.01$ vs TGF-β1 only group, n=6 wells. The average PSR OD values of negative control group and TGF-β1 only group were normalised to 0 and 100%, respectively, and changes in percentage of herbal extract-treated groups were relative to the TGF-β1 only group.

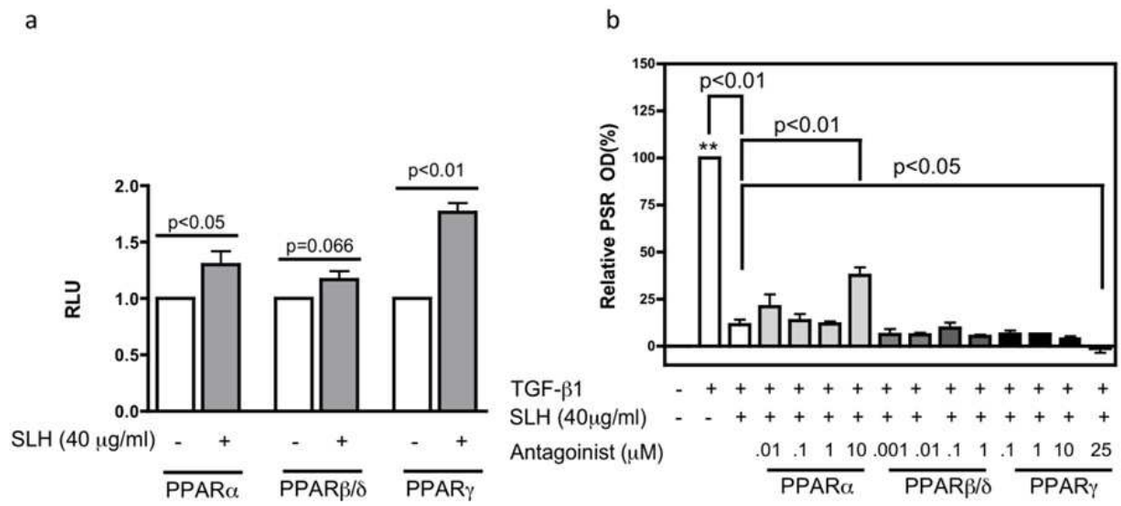

Fig. 4. Regulation of PPARα, PPARβ/δ and PPARγ activation by Shiliuhua (SLH) extract and effects of PPARα, PPARβ/δ and PPARγ antagonists on the anti-fibrotic effect of SLH extract. a. Effects of SLH extract on PPARα, PPARβ/δ and PPARγ activation, n=3 independent biological experiments; b. effects of increasing doses of PPARα, PPARβ/δ and PPARγ antagonists on the anti-fibrotic effect of SLH extract. ** $p<0.01$ vs control group, n=3 independent biological experiments. Other statistical results are as indicated in the figures.

4. Discussion

4.1 Knowledge-based discovery of anti-fibrotic activities from CMMs

Further to the evidence-based approach that we used in our earlier screens (Hu et al., 2009), in which we selected CMMs that had been reported to reduce fibrotic lesions *in vivo*, we used a knowledge-based approach in this study, namely, to screen candidate CMMs that were believed to be beneficial in treating fibrotic diseases based on the theory and practice of TCM. Of the 28 CMM extracts examined, eight (28.5%) showed reproducible *in vitro* anti-fibrotic activities, *i.e.*, Baibeiyegen, Liedang, Gusuibu, Jixueteng, Lingzhi, Meiguiqie, Moyao and Shiliuhua. Identification of these CMMs with anti-fibrotic activities *in vitro* underscores their inflammation-independent activities to inhibit TGF-β1-induced total collagen accumulation, perhaps through inhibiting TGF-β1-specific signalling or common pathways of fibrogenesis.

Among the eight CMM extracts with anti-fibrotic activities, Baibeiyegen (Zhao et al., 2002), Lingzhi (Lin et al., 2006; Wang et al., 2009; Wu et al., 2010), Meiguiqie (Liu et al., 2006), Moyao (Massoud et al., 2004) and Shiliuhua (Huang et al., 2005) have been reported anti-fibrotic in animal models of liver fibrosis or in hepatic stellate cells; Gusuibu was one of the weakest and its effect on fibrosis has never been previously reported either *in vivo* or *in vitro*; Liedang has not been reported on its effect on fibrosis either, but the ethanolic extract of its closely related family member *Boschniakia rossica* (Cham. & Schltdl.) B. Fedtsch. has been reported to mitigate liver fibrosis in a rat model of dimethylnitrosamine-induced liver fibrosis (Piao et al., 2005); Jixueteng is used in some herbal formulae, such as Huangqijixuetengtang (Li et al., 2006; Li et al., 2007) and Herbal Compound 861 (also known as Fufangdanshenheji) (Wang 2000; Wang et al., 2008), which were reported to be anti-fibrotic in patients and *in vitro*, but has never been reported as an individual anti-fibrotic herb.

Although only a quarter of the selected CMMs showed anti-fibrotic activities in our model, we do not exclude the possibilities that some of these CMMs might have a favourable effect in treating fibrotic diseases by interfering with other factors *e.g.* inflammation, TGF-β1 production and activation, molecules upstream of TGF-β1 signalling and TGF-β1-independent pro-fibrotic signalling pathways. Of note, CMMs which were identified as cytotoxic in the NRK-49F renal fibroblast model might be of therapeutic value in fibrotic diseases since loss of fibroblasts, the main producers of pathological matrices, may result in reduced ECM accumulation hence promoting regression of fibrosis. It is also worth reiterating that the results presented here were derived from extracts of CMMs in boiling 80% ethanol. Different extraction methods, including extraction solutions and efficiency, might have different impact on *in vitro* activities of the materials. Nevertheless, it would be of particular interest to isolate compounds contained in the eight ethanolic CMM extracts with *in vitro* anti-fibrotic activities, and to further test individual compounds for their *in vitro* anti-fibrotic activities.

4.2 *In vitro* anti-fibrotic activities and TCM categorisation of the CMMs

CMMs are traditionally characterised based on their function, nature, taste and channel tropism. By focusing on functional groups of the selected CMMs, we found that four out of 15 (27%) CMMs of "Huo Xue Hua Yu" group, two out of six (33%) CMMs of "Hua Tan" group and three out of seven (43%) CMMs of "Bu Xu" group, had *in vitro* anti-fibrotic activities. In view that "Hua Tan" and "Bu Xu"groups had rarely been the focus of previous

evidence-based studies of anti-fibrotic CMMs, we consider our findings significant in guiding future studies of anti-fibrotic herbal medicines and in selecting therapeutic options in the clinic. However, due to the small sample numbers involved in this study, we do not intend to conclude that CMMs of certain functional groups have a higher chance of being anti-fibrotic than another. For example, results of Danshen, a drug in the "Huo Xue Hua Yu" group, was excluded from this project as it has been tested and reported elsewhere (Hu et al., 2009). In fact, Danshen is the most used herb in the 16 herbal formulae that showed *in vitro* anti-fibrotic activities (Hu et al., 2009). On the other hand, our results do not negate the fact that other functional groups of CMMs may possess anti-fibrotic activities. For example, Dahuang (root of *Rheum palmatum* L.) and Huangqin (root of *Scutellaria baicalensis* Georgi), both not in these three functional groups, had been previously reported to be anti-fibrotic and their activities had been confirmed in our earlier studies (Hu et al., 2009).

4.3 Shiliuhua and PPAR

In this study, Shiliuhua extract was one of the most potent CMMs in reducing TGF-β1-induced matrix accumulation. Extracts of Shiliuhua had been previously reported to activate PPARα and induce PPARγ expression. We found that ethanolic extract of Shiliuhua significantly activated both PPARα and PPARγ, which might account for its anti-fibrotic activity since agonists of PPARα (Toyama et al., 2004; Iglarz et al., 2003) and PPARγ (Milam et al., 2008; Kawai et al., 2008; Iglarz et al., 2003) were previously reported to suppress fibrosis of liver, lung, heart and kidney. Contrary to our hypothesis, we found that the anti-fibrotic activities of Shiliuhua extract can only be partially blocked by GW6471, a PPARα antagonist, at a dose 42-fold of its reported IC_{50}. Since the dose of PPAR antagonists were selected based on their IC_{50} reported in other cell types, it is possible that the doses we employed were sub-optimal in blocking PPARs in NRK-49F renal fibroblast cells. More interestingly, T0070907, a PPARγ antagonist, further increased the anti-fibrotic effect of shiliuhua extract when used at high dose (25 μM). Thus, it appears that Shiliuhua extract can activate both PPARα and PPARγ, but this property could not explain the anti-fibrotic activity of Shiliuhua in full. Further studies are required to elucidate the involvement of PPAR signalling pathway in anti-fibrotic activities of shiliuhua, for example to establish if these two PPAR isotypes have opposing functions in regulating fibrogenesis in renal fibroblasts in view that they did have opposing effects on monocyte chemotaxis in endometriosis (Hornung et al., 2001).

4.4 *In vitro* pro-fibrotic activities observed in this project

The *in vitro* pro-fibrotic activities of Chuanwutou, Dangshen and Yimucao deserve special attention. It rationalises pharmacovigilant studies to establish clinical relevance of these effects. Before clinical conclusions are drawn, it might be wise to avoid un-necessary, large-dose and long-term use of these herbs, especially in patients prone to fibrotic diseases. This issue is important because Dangshen and Yimucao are commonly used in formulae for fibrotic diseases (Yao et al., 2003) and Chuanwutou is indicated for conditions such as osteoarthritis, muscular diseases and stroke that often are complicated by fibrosis as well. As CMMs are rarely used individually, it is important to explore if their pro-fibrotic activities could be antagonised or eliminated when used in formulae. Of note, Yimucao had been reported in animal models to induce nephrotoxicity, including renal fibrosis, but its toxicity was reduced when used in formulae (Sun et al., 2005a, 2005b).

Among the three pro-fibrotic herbs, Chuanwutou appeared to be the most potent. Chuanwutou has well-known toxic effects that are believed to be reduced through a special processing procedure known as "Paozhi" (Chan et al., 1994; Singhuber et al., 2009), but as far as we know, this is the first report linking Chuanwutou to potent pro-fibrotic activities. The Chuanwutou we examined in this project was a raw material that had not undergone any "Paozhi" before ethanolic extraction. It is important to establish if the potent pro-fibrotic effect of this toxic herb could be reduced or even eliminated through traditional "Paozhi" and if this newly identified adverse effect contributes to any known clinical toxicity and adverse effects, in view that only processed Chuanwutou is allowed to be used in TCM practice according to the Chinese Pharmacopeia.

Interestingly, while extract of Dangshen, the root of *Codonopsis pilosula* (Franch.) Nannf. was found pro-fibrotic in this study, pollen of the same plant had been previously reported to be effective in preventing carbon tetrachloride-induced liver damage including fibrosis (Xiao et al., 1989). Furthermore, Fuzi, the daughter roots of the same plant as Chuanwutou, is a CMM categorised into a different functional group. We examined Fuzi in our *in vitro* model but it did not show any pro-fibrotic, anti-fibrotic or apparent cytotoxicity (data not shown). Thus, different parts of the same plant might have different and even opposite effects in regulating fibrogenesis.

5. Conclusion

Among the 28 herbal and fungal materials tested, eight showed *in vitro* anti-fibrotic activities while another three, especially Chuanwutou, showed pro-fibrotic activities. These results warrant further prudent investigations of their potential translation into clinical efficacy and their adverse effects.

6. Acknowledgements

We thank Ms. Dan Jiang (Acu-herb Clinic Sheffield, Sheffield, UK) and Professor Dunxu Wu (Shanghai University of Traditional Chinese Medicine) for recommending CMMs screened in this project. We are grateful to Dr Dae Kee Kim (Ewha Women's University Korea) for his kind gift of IN-1130, a selective inhibitor of TGF-β type I receptor (Alk5). The project was funded by Innovation China UK, Kidney Research UK, as well as European Union's Framework Programme 7 Coordination Action grant, Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era.

7. References

Burton, J.D.; Castillo, M.E.; Goldenberg, D.M. & Blumenthal, R.D. (2007). Peroxisome proliferator-activated receptor-γ antagonists exhibit potent antiproliferative effects versus many hematopoietic and epithelial cancer cell lines. *Anticancer Drugs*, Vol. 18, No. 5, (June 2007), pp. 525-34, ISSN 0959-4973

Chan, T.Y.; Tomlinson, B.; Tse, L.K.; Chan, J.C.; Chan, W.W. & Critchley, J.A. (1994). Aconitine poisoning due to Chinese herbal medicines: a review. *Veterinary and Human Toxicology*, Vol. 36, No. 5, (October 1994), pp. 452-455, ISSN 0145-6296

Hornung, D.; Waite, L.L.; Ricke, E.A.; Bentzien, F.; Wallwiener, D. & Taylor, R.N. (2001). Nuclear peroxisome proliferator-activated receptors a and g have opposing effects

on monocyte chemotaxis in endometriosis. *The Journal of Clinical Endocrinology & Metabolism*, Vol. 86, No. 7, (July 2001), pp. 3108-3114, ISSN 0021-972X

Hu, Q.; Jiang, D. & Xu, Q. (2007). In vitro models of fibrosis: Anti-fibrotic herbal medicines revisited. In: *Elements of fibrosis and the prevention and treatment of fibrosis in traditional Chinese medicine*, J. Niu, (Ed.), pp678-pp682, People's Health Press, ISBN 978-7-117-09337-8, Beijing, China

Hu, Q.; Noor, M.; Wong, Y.F.; Hylands, P.; Simmonds, M.S.J.; Xu, Q.; Jiang, D.; Hendry, B.M. & Xu, Q. (2009). *In vitro* anti-fibrotic activities of herbal compounds and herbs. *Nephrology Dialysis & Transplantation,* Vol. 24, No. 10, (October 2009), pp. 3033-3041, ISSN 0931-0509

Huang, T.H.; Yang, Q.; Harada, M.; Li, G.Q.; Yamahara, J.; Roufogalis, B.D. & Li Y. (2005). Pomegranate flower extract diminishes cardiac fibrosis in Zucker diabetic fatty rats: modulation of cardiac endothelin-1 and nuclear factor-κB pathways. *Journal of Cardiovascular Pharmacology*, Vol. 46, No. 6, (December 2005), pp. 856-862, ISSN 0160-2446

Huang, W. & Sun, R. (2010). Pathological damage mechanism of rats' nephrotoxicity caused by alcohol extracted components of Herba Leonuri. *Chinese Journal of Experimental Traditional Medical Formulae*, Vol. 16, No. 9, pp. 111-114, ISSN 1005-9903

Iglarz, M.; Touyz, R.M.; Viel, E.C.; Paradis, P.; Amiri, F.; Diep, Q.N. & Schiffrin, E.L. (2003). Peroxisome proliferator-activated receptor-alpha and receptor-gamma activators prevent cardiac fibrosis in mineralocorticoid-dependent hypertension. *Hypertension*. Vol. 42, No. 4, (October 2003), pp. 737-43, ISSN 0914-91IX

Kawai, T.; Masaki, T.; Doi, S.; Arakawa, T.; Yokoyama, Y.; Doi, T.; Kohno, N. & Yorioka, N. (2009). PPAR-γ agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta. *Laboratory Investigation*, Vol. 89, No. 1, (January 2009), pp. 47-58, ISSN 0023-6837

Lee, G.; Elwood, F.; McNally, J.; Weiszmann, J.; Lindstrom, M.; Amaral, K.; Nakamura, M.; Miao, S.; Cao, P.; Learned, R.M.; Chen, J.L. & Li, Y. (2002). T0070907, a selective ligand for peroxisome proliferator-activated receptor γ, functions as an antagonist of biochemical and cellular activities. *Journal of Biological Chemistry*, Vol. 277, No. 22, (May 2002), pp. 19649-19657, ISSN 0021-9258

Li, R. & Zhao, M.Y. (2006). Effects of Huangqijixueteng decoction on TGF-β1, CTGF and PDGF-BB expression in experimental rat liver fibrosis. *Proceeding of Clinical Medicine*, Vol. 15, No. 9, (2006), pp. 659-661, ISSN 1671- 8631

Li, R.; Lu W. & Zhao, M.Y. (2007). Preventive effect and mechanism of Huangqijixueteng decoction on hepatic fibrosis in rats. *Chinese Journal of Integrated Traditional and Western Medicine on Digestion*, Vol. 15, No. 2, (2007), pp. 99-103, ISSN 1671-038X

Li, Y.; Qi, Y.; Huang, T.H.; Yamahara, J. & Roufogalis, B.D. (2008). Pomegranate flower: a unique traditional antidiabetic medicine with dual PPAR-α/-γ activator properties. *Diabetes, Obesity and Metabolism* Vol. 10, No. 1, (January 2008), pp. 10-17, ISSN 1463-1326

Lin, W.C. & Lin, W.L. (2006). Ameliorative effect of Ganoderma lucidum on carbon tetrachloride-induced liver fibrosis in rats. *World Journal of Gastroenterology*, Vol. 12, No. 2, (January 2006), pp. 265-270, ISSN 1007-9327

Liu, J.Y.; Chen, C.C.; Wang, W.H.; Hsu, J.D.; Yang, M.Y. & Wang, C.J. (2006). The protective effects of Hibiscus sabdariffa extract on CCl4-induced liver fibrosis in rats. *Food and Chemical Toxicology*, Vol. 44, No. 3, (March 2006), pp. 336-343, ISSN 0278-6915

Massoud, A.M.; El Ebiary, F.H. & Abd El Salam, N.F. (2004). Effect of myrrh extract on the liver of normal and bilharzially infected mice. An ultrastructural study. *Journal of the Egyptian Society of Parasitology*, Vol. 34, No. 1, (April 2004), pp. 1-21, ISSN 1110-0583

Milam, J.E., Keshamouni, V.G.; Phan, S.H.; Hu, B.; Gangireddy, S.R.; Hogaboam, C.M.; Standiford, T.J.; Thannickal, V.J. & Reddy, R.C. (2008). PPAR-γ agonists inhibit profibrotic phenotypes in human lung fibroblasts and bleomycin-induced pulmonary fibrosis. *American Journal of Physiology - Lung Cellullar and Molecular Physiology*, Vol. 294, No. 5, (May 2008), pp. L891-901, ISSN 1040-0605

Moon, J.A.; Kim, H.T., Cho, I.S.; Sheen, Y.Y. & Kim, D.K. (2006). IN-1130, a novel transforming growth factor-β type I receptor kinase (ALK5) inhibitor, suppresses renal fibrosis in obstructive nephropathy. *Kidney International*, Vol. 70, No. 7, (October 2006), pp. 1234-1243, ISSN 0085-2538

Piao, X.-X.; Huang, H.-G. & Piao, D.M. (2005). Therapeutic role of ethanolic extract of Boschniakia Rossica in dimethylnitrosamine-induced liver fibrosis in rats. *World Chinese Journal of Digestology,* Vol. 13, No. 18, (September 2005), pp. 2205-2209, ISSN 1009-3079

Seimandi, M.; Lemaire, G.; Pillon, A.; Perrin, A.; Carlavan, I.; Voegel, J.J.; Vignon, F.; Nicolas, J.C. & Balaguer, P. (2005). Differential responses of PPARα, PPARδ, and PPARγ reporter cell lines to selective PPAR synthetic ligands. *Analytical Biochemistry,* Vol. 344, No. 1, *(*September 2005), pp. 8-15, ISSN 0003-2697

Shearer, B.G.; Steger, D.J.; Way, J.M.; Stanley, T.B.; Lobe, D.C.; Grillot, D.A.; Iannone, M.A.; Lazar, M.A.; Willson, T.M. & Billin, A.N. (2008). Identification and characterization of a selective peroxisome proliferator-activated receptor β/δ (NR1C2) antagonist. *Molecular Endocrinology*, Vol. 22, No. 2, (February 2008), pp. 523-529, ISSN 0888-8809

Singhuber, J.; Zhu, M.; Prinz, S. & Kopp B. Aconitum in traditional Chinese medicine: a valuable drug or an unpredictable risk? *Journal of Ethnopharmacology*, Vol. 126, No. 1, (October 2009), pp. 18-30, ISSN 0378-8741

Sun, R.; Wu, X.; Liu, J.; Sun, L. & Lv, L. (2005a). Experimental study of the rat renal toxicity of Tripterygium wilfordii, Caulis aristolochiae and Leonurus. *Pharmacology and Clinics of Chinese Materia Medica*, No. 2, 2005, pp. 26-28, ISSN 1001-859X

Sun, R.; Sun, L.; Wu, X. & Lv, L. (2005b). Formulation reduces the renal toxicity of Yimucao. *Chinese Journal of Pharmacovigilance*, No. 3, (June 2005), pp. 144-147, ISSN 1672—8629

Toyama, T.; Nakamura, H.; Harano, Y.; Yamauchi, N.; Morita, A.; Kirishima, T.; Minami, M.; Itoh, Y. & Okanoue, T. (2004). PPARα ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats. *Biochemical and Biophysical Research Communications. Vol. 324, No. 2,* (November 2004), pp. 697-704, ISSN 0006-291X

Wang, B.E. (2000). Treatment of chronic liver diseases with traditional Chinese medicine. *Journal of Gastroenterology & Hepatology*, No. 15 Suppl, (May 2000), pp. E67-70, ISSN 0815-9319

Wang, L.; Wang, B.E.; Wang, J.; Xiao, P.G. & Tan, X.H. (2008). Herbal compound 861 regulates mRNA expression of collagen synthesis- and degradation-related genes in human hepatic stellate cells. *World Journal of Gastroenterology*, Vol. 14, No. 11, (March 2008), pp. 1790-1794, ISSN 1007-9327

Wang, G.J.; Huang, Y.J.; Chen, D.H.; Lin, Y.L. (2009). Ganoderma lucidum extract attenuates the proliferation of hepatic stellate cells by blocking the PDGF receptor. *Phytotherapy Research*, Vol. 23, No. 6, (June 2009), pp. 833-939, ISSN 0951-418X

Wu, Y.W.; Fang, H.L. & Lin, W.C. (2010). Post-treatment of Ganoderma lucidum reduced liver fibrosis induced by thioacetamide in mice. *Phytotherapy Research*, Vol. 24, No. 4, (April 2010), pp. 494-499, ISSN 0951-418X

Wynn, T.A. (2007). Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *Journal of Clinical Investigation,* Vol. 117, No. 3, (March 2007), pp. 524-529, ISSN 0021-9738

Xiao, J.C.; Liu, H.J.; Han, D.; Li, Z.; Jiang, J.X. & Qing, C. (1989). Protective effects of the pollen of Codonopsis pilosula (Franch.) Nannf. on liver lesions at the ultrastructural level. *Zhongguo Zhong Yao Za Zhi*. Vol. 14, No. 3, (March 1989), pp. 42-44, ISSN 1001-5302

Xu, H.E.; Stanley, T.B.; Montana, V.G.; Lambert, M.H.; Shearer, B.G.; Cobb, J.E.; McKee, D.D.; Galardi, C.M.; Plunket, K.D.; Nolte, R.T.; Parks, D.J.; Moore, J.T.; Kliewer, S.A.; Willson, T.M. & Stimmel, J.B. (2002). Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARα. *Nature* Vol. 415, No. 6873, (February 2002), pp. 813-817, ISSN 0028-0836

Xu, Q.; Norman, J.T.; Shrivastav, S.; Lucio-Cazana, J. & Kopp, J.B. (2007). *In vitro* models of TGF-β-induced fibrosis suitable for high-throughput screening of antifibrotic agents. *Am J Physiol Renal Physiol* Vol. 293, No. 2, (August 2007), pp. F631-40, ISSN 0363-6127

Yao, C.F. & Jiang, S.L. (2003). Prevention and treatment of pulmonary-fibrosis by traditional Chinese medicine. *Zhong Xi Yi Jie He Xue Bao*. Vol. 1, No. 3, (September 2003), pp. 234-238, ISSN 1672-1977

Zhao, J.; Lü, Z.; Wang, X. & Zhang, X. (2002). The study on the anti-oxidation effect of root of Mallotus apelta in the rat model of liver fibrosis. *Zhong Yao Cai*. Vol. 25, No. 3, (March 2002), pp. 185-187, ISSN 1001-4454

Recent Advances in Theories and Practice of Chinese Medicine
Edited by Prof. Haixue Kuang

ISBN 978-953-307-903-5
Hard cover, 504 pages
Publisher InTech
Published online 18, January, 2012
Published in print edition January, 2012

During the recent years, traditional Chinese medicine (TCM) has attracted the attention of researchers all over the world. It is looked upon not only as a bright pearl, but also a treasure house of ancient Chinese culture. Nowadays, TCM has become a subject area with high potential and the possibility for original innovation. This book titled Recent Advances in Theories and Practice of Chinese Medicine provides an authoritative and cutting-edge insight into TCM research, including its basic theories, diagnostic approach, current clinical applications, latest advances, and more. It discusses many often neglected important issues, such as the theory of TCM property, and how to carry out TCM research in the direction of TCM property theory using modern scientific technology. The authors of this book comprise an international group of recognized researchers who possess abundant clinical knowledge and research background due to their years of practicing TCM. Hopefully, this book will help our readers gain a deeper understanding of the unique characteristics of Chinese medicine.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Qihe Xu, Yuen Fei Wong, Shanshan Qu, Qingyang Kong, Xiu-Li Zhang, Xin-Miao Liang, Qin Hu, Mazhar Noor and Bruce M. Hendry (2012). Knowledge-Based Discovery of Anti-Fibrotic and Pro-Fibrotic Activities from Chinese Materia Medica, Recent Advances in Theories and Practice of Chinese Medicine, Prof. Haixue Kuang (Ed.), ISBN: 978-953-307-903-5, InTech, Available from: http://www.intechopen.com/books/recent-advances-in-theories-and-practice-of-chinese-medicine/knowledge-based-discovery-of-anti-fibrotic-and-pro-fibrotic-activities-from-chinese-materia-medica

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen