Liu et al, Afr J Tradit Complement Altern Med., (2017) 14 (1): 242-250
doi:10.21010/ajtcam.v14i1.26

EFFECT OF RUANGANJIEDU DECOCTION INHIBIT EXPERIMENTAL HEPATIC FIBROSIS OF RATS.

Sheng Liu[1]，Xiao-min Wang[1]，Guo-wang Yang[1]

[1]Department of Oncology, Beijing Hospital of Traditional Chinese Medicine (TCM) affiliated to Capital Medical University, Beijing 100010, China.[2]Department of Internal Medicine,Huguosi TCM Hospital Attached to the Beijing University of Traditional Chinese Medicine, Beijing 100010, China.

***Corresponding author E-mail:** liusheng4377@163.com

Abstract

Background: RuanGanJieDu (RGJD) is a traditional Chinese medicine comprising nine Chinese medicinal herbs. The clinical use of RGJD by us was found that it has a good control effect on hepatic fibrosis (HF) patient. In order to further investigate the Anti-fibrosis mechanism of RGJD, hence this experiment has been carried out.

Methods: After treatment, the general conditions of rats in each group were observed. The liver tissues morphology and HF phases were observed under light microscope. The weights of livers and spleens were recorded; the indices of liver and spleen were calculated. The α-SMA and E-cadherin of the liver tissues were determined by Immunohistochemistry, and the contents of ALT、AST and PCIII＋PCIV＋HA＋LN in rat serum were detected by ELISA.

Results: After treatment, the rats in model group presented with abdominal distention and rough hair, lethargy and mental sluggishness, slow movement and irritability; Most rats in RGJD group and colchicine(Col) group were in good spirits and no obvious abnormal motion state. The RGJD had shown significant effects in improving the liver tissue morphology. The HF phases of RGJD group and Col group mainly distributed in the S1-S2 phases, were finer than the model group in the S3 phase ($P<0.05$). The wet weights and indices of livers and spleens in RGJD group were lower than the control groups ($P<0.05$). Moreover, the expressions of E-cadherin, α-SMA and the serum levels of AST, ALT and PCIII＋PCIV in RGJD group were obviously decreased than the control groups except HA and LN($P<0.05$).

Conclusion: RGJD can improve the liver tissue morphology and reduce serum biochemical and collagen indices of HF, which proved a better curative effect of Chinese medicine than Col on HF and improving liver function.

Keywords: RuanGanJieDu Decoction, hepatic fibrosis, α-SMA,E-cadherin, colchicine.

Introduction

Hepatic fibrosis (HF) is a reversible reaction of scar formation in almost the entire patient's body with chronic liver injury and liver cirrhosis. Previous studies demonstrated that 40% HF will eventually develop into cirrhosis or liver cancer (Chen Lanyu,2003; Chen Qi,2006), and HF and cirrhosis can reverse mutually in a certain extent (Wang Luobing et al., 2010). Therefore, effective anti-fibrosis therapy can block or reverse the HF, which it is important to improve the prognosis of the disease.

HF is mainly due to the dynamic imbalances of collagen synthesis, deposition, degradation and absorption. That the collagen synthesis and deposition are greater than the degradation and absorption gradually induces the formation of HF(Bao Jian feng et al.,2012). When chronic liver injury occurs, inflammatory cells or cytokines act on myofibroblasts cells by different ways and means, and secrete large amounts of extracellular matrix and express various pro fibrosis factors and types of collagen closely related to the occurrence of hepatic fibrosis. The present

doi:10.21010/ajtcam.v14i1.26

study confirmed that the activated myofibroblasts are the center link of HF (Jiang Fusheng, et al., 2005). The Hepatic satellite cells, bone marrow mesenchymal stem cells, epithelial cells and endothelial cells can be activated and transformed into myofibroblasts, which participate in the development of HF process (Wang Renfang,et al.,2005;Fan Linggang., 2010). The expressions of α-SMA and E-cadherin were closely related to the activation of myofibroblasts and the occurrence of hepatic fibrosis.

In addition to treatment aimed at the causes and anti-inflammatory, clinical trials are still lacking of effective Anti-fibrosis drugs (Xiao Peigen, 2002). Traditional Chinese medicine （TCM） formula though a complex combination of many natural products, with numerous chemical compounds; are considered to be the multi-component and multi-target agents exerting their therapeutic function in a more holistic way and discovering naturally-occurring agents is a promising approach for anti-fibrosis treatment (Zhang et al., 2012). Based on the TCM holistic concept and syndrome differentiation theory, HF belongs to Qi deficiency and blood stasis syndrome and a number of experiments show that Qi deficiency and blood stasis syndrome are closely related with increased collagens and myofibroblasts(Yi Yang Hu, et al., 2006). therefore, Treatments should adjust the human body environment as a whole by means of Supplementing Qi and activating blood circulation method To reduce the expression of collagen and myofibroblasts and improve HF. RGJD takes *Panax notoginseng*, *Astragalus* to invigorate Qi, accompanied by *Radix notoginseng, radix salviae miltiorrhizae, Radix Sophorae Flavescentis, turtle shell, Bulbus Fritillariae thunbergii, pinellia* to promote blood circulation and remove blood stasis, dissipate phlegm and resolve hard masses.

This study was based on classical method of carbon tetrachloride modeling to observe RGJD on liver tissue morphology and serum biochemical and collagen indices of rats with HF, and explore the mechanism of TCM in the prevention and treatment of HF, and provide modern pharmacology basis for its clinical application.

Materials and Methods

Animals

This study was conducted using 80 rats aged 6 to 8-week-old wistar rats weighing 180–220g, half male and half female. Animals were provided by the experimental animal research institute of the Chinese Academy of Medical Sciences (license no.SCXK-11-00-0006). Animals were housed under constant conditions of 23±1°C and 40±5% humidity and had free access to feed pellets and tap water. All animals were cared for in accordance with the policies and guidelines for animal use of the Chinese Academy of Traditional Chinese Medicine.

This study was approved by the Ethics Committee of the Chinese Academy of Traditional Chinese Medicine. All experiments were performed following the guidelines of the China Institute of Laboratory Animal Science (CALAS).

Preparation of RGJD

RGJD was composed of *dangsheng(Codonopsis pilosula), shenghuangqi* (*Astragalus mongholicus Bunge*),*Sanqi(Panax notoginseng),Danshen(Salvia miltior- rhiza）, Shancigu(Arrowhead mountain), Kushen(Sophora flavescens), biejia（Turtle shell）,zhebeimu（Fritillaria thunbergii Miq）,banxia（pinellia tube）*.All the crude drugs were purchased from Beijing Tongrentang pharmacy. RGJD crude powder was extracted twice using a 10-fold excess of boiling water and filtration through four-layer gauze. The combined filtrate was centrifuged at 2,000 rpm/min for 20min and concentrated under reduced pressure. The concentrated solution was cooled to room temperature and mixed with pure alcohol to an alcohol content of 70%. Then the mixture was refrigerated for 24h and filtered, the resulting sediment was vacuum evaporated to obtain powder. The concentration RGJD was calculated according to “Equivalent dose mouse table of human and animal body surface area convert (China Pharmacopoeia) as: rabbit dose(ml/kg•d)=human daily dose×0.07/1.5(g/kg•d)=15g/k g•d.

doi:10.21010/ajtcam.v14i1.26

Reagent preparation

Preparation of 10% carbon tetrachloride solution with peanut oil:10ml CCl_4 and 90ml peanut oil were extracted into a sealed container with a syringe and mixed to dissolve.

HF Model and RGJD treatment

The peritoneal cavities of the model rats were injected 10% carbon tetrachloride with peanut oil (0.3ml/100g) twice a week for 12 weeks. The normal rats were injected with 10% saline solution (0.3ml/100g).

From the 4th week of building animal model, the 60 model rats were randomly assigned to three groups as follows: RGJD group, Col group and model group (twenty rats per group). Since the 5th week, the rats in the RGJD group received RGJD, rats in the Col group received Col (Referring to Equivalent dose mouse table of human and animal body surface area convert, the amount of 0.1mg/ kg•d), while rats in the normal control group and the model control group received equivalent volumes of distilled water without drugs until the end of the experiment.

Tissue preparation

12 weeks after building animal models, the rats were sacrificed under deep anesthesia. The abdominal cavities were cut open and the abdominal aortas were separated, then 10ml artery blood were extracted and centrifuged at 4000rpm/min for 5 min. The livers and spleens were harvested immediately after collecting Blood, and weighed by micrometer electronic scales and recorded, then placed in a -80℃ ultra-low temperature freezer for later use.

Observation of liver tissue morphology and HF phase

The same part of the right liver lobe ($1cm^2$ sizes) were taken and rinsed with physiological saline, fixed with 4% paraformaldehyde for 24h, and dehydrated in various concentrations of ethanol, then embedded in paraffin and stained with hematoxylin and eosin. The liver tissue morphology and HF phase were observed under light microscope.

Reference for HF phase (Valcourt U et al.,2005):S0: no fibrosis. S1: include portal and around the portal fibrosis, around the hepatic sinusoid or intra-lobular fibrous scar, all of which does not affect the integrity of the lobules.S2: the fibrous intervals, mainly bridging fibrosis are developed from Bridging necrosis, but most of lobular structure remains.S3: a lot of fibrous intervals destroy the liver lobules but no cirrhosis of the liver. Part of patients in this period can occur with portal hypertension and esophageal varices. S4: early cirrhosis of the liver, the widespread destruction of hepatic parenchyma, diffuse fibrosis and Lobule formation formed.

Weighting body mass, liver and spleen wet weight and calculating the liver and spleen index

Liver index=Liver mass/body mass×100%, spleen index=spleen mass/body mass×100%.

Test of serum biochemical and collagen indices of HF by Enzyme Linked Immunosorbent Assay (ELISA).

Automatic biochemical analyzer was debugged with a Standard. More than 50uL serum of rats in each group were place a loading bath for specimen, the levels of AST, ALT, ALB and PCIII＋PCIV＋HA＋LN were detected by ELISA.

doi:10.21010/ajtcam.v14i1.26

Determination of E-cadherin and α-SMA by Immunohistochemistry

The lever tissue samples were dehydrated and embedded in paraffin. The sections were deparaffinized and blocked with 3% peroxide-methanol at room temperature to remove endogenous peroxidase. Sections were incubated with 75uL of polyclonal antibody for E-cadherin or α-SMA (Miltenyi Biotec) diluted in blocking buffer for 12 hr, followed by incubation with 75uL of secondary antibody (MiltenyiBiotec) for 1 hr in a humidified chamber. Subsequent immunostaining was performed using the biotin-streptavidin-peroxidase method with diaminobenzidine as a staining material and counterstaining with methyl green (1 min) or hematoxylin. Then sections were immediately dehydrated in 70% ethanol, 80% ethanol, and 100% ethanol, and the glass slides were sealed with neutral gum. The negative control was treated as described above but the primary antibody was replaced by PBS. Sections were analyzed using a computerized image analyzer (ImageProPlus6, Media Cybernetics, American). The presence of brown granules in the cytoplasm or membrane of cells was classified as positivity; cells with no brown granules were classified as negative. Ten views were obtained for each slide, and seven slides were prepared for each organ. The percentage of positive cells was calculated as follows: (positive cells/total cells) ×100.

Statistical Analysis

All data were expressed as means ± standard errors. All analysis was performed using the Statistical Product and Service Solutions for Windows, version 17.0 (SPSS Inc., USA). The statistical significance ($P<0.05$) was assessed by the analysis of variance (ANOVA).

Results

General observation

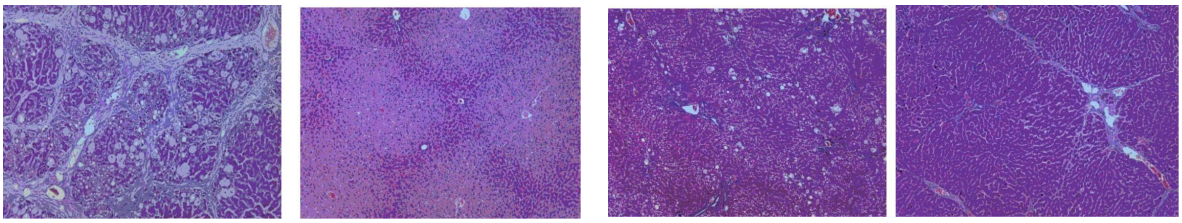
The rats in normal group were in good condition, and there was no obvious abnormality in behavior, movement, food and water intake and stool, and no abnormal secretion of eyes, ears, nose and mouth. The reactions to external stimuli were normal. The rats in model group presented with abdominal distention and rough hair, lethargy and mental sluggishness, slow movement and irritability. The stools were loose and foul.4 rats died and the death rate was 20%. Anatomical impression: there were quantities of Peritoneal effusion and livers were dark red and hard texture whose surfaces were rough nodules. Most rats in RGJD group and Col group were in good spirits and no obvious abnormal motion state. A very small number of abdominal swelling phenomenon. The stools were loose and foul. 1 rats died and the death rate was 5% in RGJD group and 2 rats died and the death rate was 10% in Col. Anatomical impression: the livers were dark red and slightly rough surface.

Liver tissue morphology and phase of HF

The livers of rats in normal group had hepatic lobule with clear structures and hepatic cell cords were arranged radically around the central veins; the liver cells have normal morphology and there was no infiltration of inflammatory cells. Liver tissue structures of rats in the model group were destroyed and fibrous mediastinum and pseudo lobule came into being; the ranges of hepatic cords were in disorder, at the same time, the degeneration and proliferative reaction of liver cells were obviously; there were large numbers of infiltrative mononuclear cells. In comparison, the ranges of mononuclear cell infiltration in the levers of the RGJD group and Col group were smaller, and the liver structures were relatively complete and normal, but the RGJD group was finer than the Col group.(Fig.1).

doi:10.21010/ajtcam.v14i1.26

The HF of the rats in normal control group were all in S0 phase，which had a significant difference with the model control group mainly in S3 phase(P<0.05). The HF in RGJD group and Col group were reduced and mainly concentrated in the S1-S2 phase, and there were no differences between the two groups (P>0.05). (Table 1).



(a) (b) (c) (d)

Figure 1: The pathological picture of model control group (a), normal control group (b), Col group (c), RGJD group (d). The lever tissues were fixed, sectioned at 4um thickness, stained with H&E solution, and observed under a microscope of 200 magnifications.

Table 1: The phase of HF

Group	n	S_0	S_1	S_2	S_3	Mann-Whitney test
Normal group	20	20	0	0	0	$^{*\Delta}$P<0.05
Model group	16	0	0	1	15	$^{\Delta}$P<0.05
Col group	18	0	13	4	1	*P<0.05
RGJD group	19	0	13	5	1	*P<0.05

*P<0.05, vs. model group; $^{\Delta}$P<0.05, vs RGJD group

Body weight, liver and spleen wet weight, the liver and spleen index

The liver and spleen wet weights and the liver and spleen indices of the model group were higher than those of the other groups. The liver and spleen wet weights of the RGJD group were significantly lower than those of the Col group (P<0.05). The Body weights, liver and spleen indices in the RGJD group had no differences with the Col group (P>0.05). (Table 2).

Table 2: The Body weight, liver and spleen wet weigh and index（Mean±SD）

Group	n	Body weight（g）	liver wet weight（g）	Spleen wet weight	liver index	spleen index
Normal group	20	495.85±127.26*	16.32±3.79*	1.02±0.51*	3.30±0.31*	0.21±0.01*
Model group	16	342.30±97.43$^{\triangle}$	24.14±3.94$^{\triangle}$	2.14±0.44$^{\Delta}$	7.05±1.21$^{\Delta}$	0.63±0.07$^{\Delta}$
Col group	18	450.00±101.16*	20.46±3.95$^{*\triangle}$	1.96±0.35$^{\Delta}$	4.55±0.81*	0.44±0.09*
RGJD group	19	446.00±113.45*	16.35±3.35*	1.27±0.21*	3.67±0.69*	0.28±0.07*

*P<0.05, vs. model control group; $^{\Delta}$P<0.05, vs RGJD group.

serum biochemical and collagen indices of HF

The model group showed a significant increase in serum levels of AST, ALT and PCIII＋PCIV＋HA＋LN compared with the other groups (P<0.05). Moreover, The serum levels of the RGJD group were obviously lower than the Col group except HA and LN.(P<0.05) (Table3 and Table4).

doi:10.21010/ajtcam.v14i1.26

Table 3: The Serum biochemical indices（Mean±SD）

Group	n	ALT(IU/L)	AST(IU/L)
Normal group	20	32.67±8.52*△	31.89±13.91*△
Model group	16	121.82±9.56△	182.50±13.25△
Col group	18	89.91±11.39*△	109.55±13.25*△
RGJD group	19	59.00±10.49*	62.63±15.63*

*P<0.05, vs. model group; △P<0.05, vs RGJD group.

Table 4: The Serum collagen indices（Mean±SD）

Group	n	PCIII T(IU/L)	PCIV T(IU/L)	T HA T(IU/L)	LN T(IU/L)
Normal group	20	0.11±0.04*	0.08±0.004*	0.14±0.02*	0.38±0.05*
Model group	16	0.20±0.04△	0.17±0.03△	0.18±0.07△	0.63±0.14△
Col group	18	0.16±0.03*△	0.16±0.01△	0.17±0.02	0.40±0.15*
RGJD group	19	0.11±0.002*	0.09±0.01*	0.15±0.03*	0.39±0.02*

*P<0.05, vs. model control group; △P<0.05, vs RGJD group.

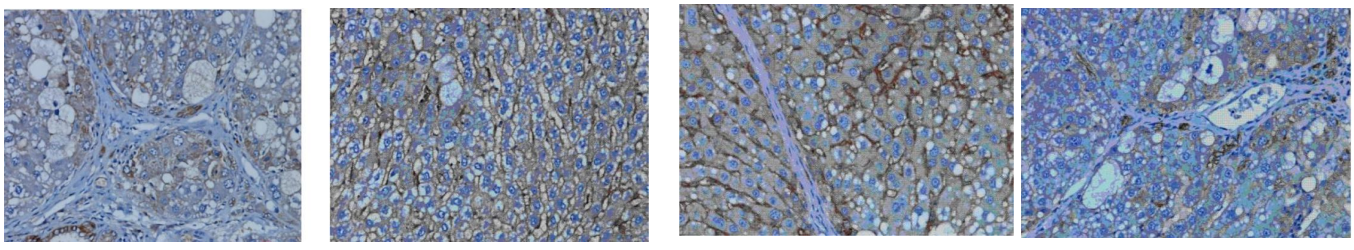
E-cadherin and α-SMA expression

The E-cadherin and α-SMA expressions were significantly increased in the model control group compared with the other groups (P<0.05). By contrast, the E-cadherin and α-SMA expressions in the RGJD group were not different with the normal group (P>0.05) and obviously lower than the Col group (P<0.05). (Table 5; Fig.2 and 3).

Table 5: The E-cadherin and α-SMA expression（Mean ± SD）

Group	n	E-cadherin	TGF-β1
Normal group	20	11.56±0.10*	2.80±0.21*
Model group	16	31.22±0.13△	11.29±0.16△
Col group	18	19.00±0.26*△	5.82±0.19*△
RGJD group	19	11.77±0.08*	2.82±0.06*

*P<0.05, vs model group; △P<0.05, vs RGJD+Col group.



(a) (b) (c) (d)

Fiure2: The E-cadherin expression detected by Immunohistochemistry, normal control group (a),model control group, (b), Col group (c), and RGJD group (d). Lever tissues were fixed, sectioned at 4um thickness, and incubated with primary antibody and secondary antibody; then the percentage of positive cells was calculated under a microscope of 400 magnifications.

doi:10.21010/ajtcam.v14i1.26

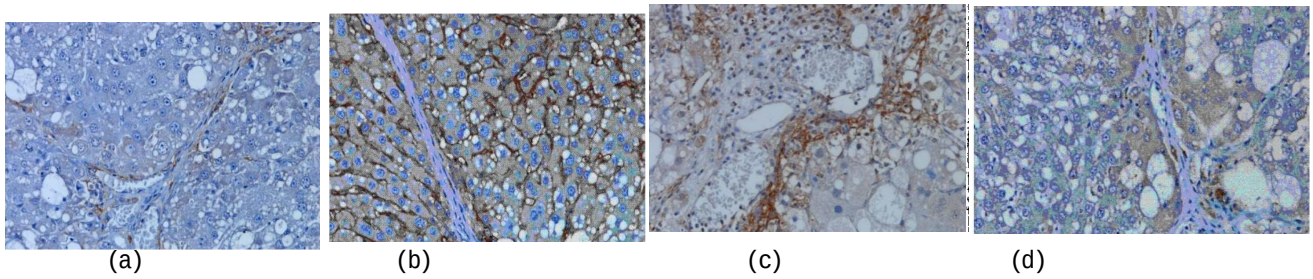


Figure 3: The TGF-β1 expression detected by Immunohistochemistry, normal control group (a), model control group (b), Col group (c), and RGJD group (d). Lever tissues were fixed, sectioned at 4um thickness, and incubated with primary antibody and secondary antibody; then the percentage of positive cells was calculated under a microscope of 200 magnifications.

Discussion

Chinese scholars have used the joint determination of type III collagen (PCIII), type IV collagen (PCIV), hyaluronic acid (HA), serum human laminin (LN) to diagnosed the HF and achieved satisfactory results. When HF, hyperplasia or deposition of extracellular matrix whose main components are PCI and PCIII. HA is a glycosaminoglycan which is produced by mesenchymal cells and could reflect the HF and liver injury. LN and PCIV are main components of the basement membrane, which can be increased during basement membrane hyperplasia and hepatic sinusoid capillarization. The Four markers are different types of protein whose increasing mechanism are also different. Therefore, the combined test of the four markers has a solid theoretical foundation. In addition, the activated myofibroblasts are the center link of HF (Jiang Fusheng, et al.,2005). The Hepatic satellite cells, bone marrow mesenchymal stem cells, epithelial cells and endothelial cells can be activated and transformed into myofibroblasts. Hepatic satellite cells (HSC) activation is with the α-SMA as the marker (Parsons CJ.,et al,2007),the activated myofibroblasts have high level expressions of E-cadherin (Meng X.,et al,2011,). Therefore, the expressions of α-SMA and E-cadherin were closely related to the activation of myofibroblasts and the occurrence of hepatic fibrosis.

HF Belongs to the "hypochondriac pain" and "tympanites" in TCM due to weakened body Qi and weakened resistance, phlegm and blood stasis (Li Wanping., et al,2003). Supplementing Qi, promoting blood circulation and removing blood stasis are an important link in the treatment of HF. A lot of Blood-activating and Stasis-removing herbs have roles of promoting regeneration of blood, softening hardness and dissipating mass, dispelling stasis and unclogging arteries, which combine with the invigorating Qi herbs can effectively promote the body's blood microcirculation, improve the blood physicochemical property and vascular permeability, protect the liver cells, promote liver cell reversal and inflammatory lesions softening and absorption(Yao Naili., et al,2004;Feng Helin., et al., 2013).

As mentioned in preceding introduction, the components in RGJD have the role of promoting blood circulation to removing blood stasis and strengthening the body Qi and resistance. In addition, modern pharmacological research show that *Astragalus* and *Codonopsis pilosula* contains rich in glutamic acid and glycine having Antioxidant function of protecting and repairing liver cell membrane and promoting the body's blood microcirculation (Cao Jikuan., 2009;Nitta T, et al., 2010).Experiments confirmed that the effective components of *Panax notoginse*ng, *radix salviae miltiorrhizae*, *mountain arrowhead* in RGJD such as *Panax notoginseng triterpenes*, *tanshinone* can significantly inhibit the proliferation and promote the apoptosis of HSC, and inhibit ECM synthesis by platelet derived growth factor receptor (PDGF) mediated intracellular signal transduction pathway, so as to reduce the deposition of collagen protein in the liver (Cui Ning.,et al,2011). The effective components of *Sophora flavescens*, *turtle shell*, *Zhejiang Fritillaria*

doi:10.21010/ajtcam.v14i1.26

such as *matrine, turtle polysaccharides, alkaloids* have good fibrinolytic effects which not only inhibit the synthesis of collagen, but also improve the blood physicochemical property and promote niduses softening and absorption (Yang Jie.,et al,2009;Wang Tianca.,etc,2002).

This study shows that RGJD can improve the liver tissue morphology, reduce the wet weights and indices of livers and spleens, at the same time reduce the expressions of E-cadherin ,α-SMA and the levels of ALT,AST and PCIII＋PCIV＋HA＋LN, so as to improve the morphology of liver and liver function of rats with HF, which provides some experimental data for the TCM clinical treatment to preventing and combating HF.

Acknowledgements

This study was in part supported by the Beijing health system high level health technical personnel training project (nos.2015-3-115) and Beijing Natural Science Foundation (nos.7152070). We also would like to express our sincere gratitude and appreciation to the reviewers for their valuable comments on the manuscript

References

1. Bao Jianfeng,Huang Jinsong,Zhang Yongsheng.(2012).Research ideas on the model of disease and syndrome of liver fibrosis. Chinese Journal of traditional Chinese Medicine,30(4):830-832.
2. Cao Jikuan, Wang Xiaoling, Chang Guohua.(2009).The etiology and pathogenesis of liver fibrosis. Chinese modern TCM doctor,47(28):22-23.
3. Chen Lanyu.(2003).Experimental study on the effect of Shugan Ruanjian Decoction on hepaticfibrosis in rats.Shenzhen Journal of integrated traditional Chinese and Western Medicine,13(5):273-276.
4. Chen Qi.(2006).Study on the method of Chinese traditional medicine pharmacology(2nd edition).Beijing: People's Medical Publishing House,31-33.
5. Cui Ning, Xie Chune, Chen Runhua.(2011).Development of traditional Chinese medicine diagnosis and treatment of liver fibrosis.World Journal of traditional Chinese medicine and Western medicine,6(2):169-172.
6. Feng Helin, Zheng Lihua.(2013).Research on molecular mechanism of epithelial mesenchymal transformation. Practical Journal of cancer,28(1):105-107.
7. Jiang Fusheng,Jiang Yuan, Li Zesong.(2005). Effect of zedoary turmeric oil on the expression of HSC-gene in T6 cells. Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases,15(1):24-27.
8. Li Wanping,Wei Mei,Li Hua.(2003).Control effect of Ganduqing Granule on experimental hepatic fibrosis rat. Sichuan Journal of physiological sciences, 25(4):7-11.
9. Meng X,Ezzati P,Wilkins JA.(2011).Requirement of podocalyxin in TGF-β1 induced EMT. PLoS One,7(5):18715.
10. Nitta T, Kim JS,Mohuczy D.(2008).Murine cirrhosis induces hepatocyte epithelial mesenchymal transition and alterations in survival signaling path ways.Journal of Hepatology, 48(3):909-919.
11. Rosa S.(2007).Deletion of TGF-β2 in Prx1-cre expressing mesenchyme results in defects in development of the long bones and joints. Dev Biol,310(2):304-316.
12. Valcourt U, Kowanetz M,Niimi H.(2005).TGF-β1 and the Smad signaling pathway support transcriptomic reprogramming during epithelial mesenchymal cell transition. Mol Biol Cell, 16(5):1987-2002.
13. Wang Luobing,Yan Xiuchuan,Zhen Zeng.(2010).Fuzheng Huayu Decoction and Radix Astragali Decoction in treatment of experimental cirrhosis in rats.China medicine journal,35(18):1740-1744.
14. Wang Renfang,Fan Linggang,Gao Wenyuan.(2010).Progress in research of chemical constituents and pharmacological activities of peach.Modern medicine and clinical progress,25(6):426- 429.
15. Wang Tiancai, Zhang, Wang Bo.(2002).Protective effects and molecular mechanism research of Shugan Capsule on liver injury of rats.Huazhong University of science and technology medical journal,31(9):171-175.

doi:10.21010/ajtcam.v14i1.26
16. Xiao Peigen.(2002).A new history of Chinese medicine(1st edition). Beijing: Chemical Industry Press, 300.
17. Yan Dan, Shu Sainan.(2007).Effect of Leech peach kernel decoction on HSC activation of schistosomal liver fibrosis in rats. China Medical journal,25(6):1704-1706.
18. 18.Yang Jie,Cai Diaolong,Tan Xianwen.(2009).Bupleurum root, safflower, Szechwan lovage, rhizome treatment for liver fibrosis in rats.Journal of clinical and experimental medicine,25 (8):1-3.
19. Yao Naili,Bai Yuning.(2004).Study on current situation and Countermeasures of liver fibrosis with traditional Chinese medicine.Shanxi Chinese medicine,20(6):54-57.
20. Yi Yang Hu,Liu Ping, Liucheng.(2006).Indications and therapeutic efficacy evaluation of anti- hepatic fibrosis in 50 cases patients with chronic hepatitis B after treatment. Journal of integrated traditional Chinese and Western Medicine,26(8):18-22.