Format for ANSWERING REVIEWERS

January 5, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14562-review.doc).

Title: Protective effect of fu-qi granule on CCL_4-induced liver fibrosis in rats

Author: Lin Zhong, Yan-Ling Sun, Wen-Li Shi, Xiao Ma, Zhe Chen, Jia-Bo Wang, Rui-Sheng Li, Xue-Ai Song, Hong-Hong Liu, Yan-Ling Zhao, Xiao-He Xiao

Name of Journal: *World Journal of Pharmacology*

ESPS Manuscript NO: 14562

The manuscript has been improved according to the suggestions of reviewers:
1. Is the subject an important one?: Yes
2. Does the article possess scientific and practical value?: Yes
3.Title:
Does the title bring the main massage of the study?: Yes
4.Abstract:
Is the abstract presented in structured form?: Yes , but it should be shortened to 250-300 words to be more focused up to the point indicated.
RESPONESE: we have shortened it in the revised manuscript.
5.Does the abstract give an adequate picture of the entire article?: Yes

Check language eg Our data suggested that FQG is a new and promising traditional Chinese medicine preparation for the prevention of the prevention of liver fibrosis., the word prevention is repeated twice.
RESPONESE: we have deleted according to the reviewer`s kind instruction.
6.Introduction:
Is the background of the study made clear and helpful to readers unfamiliar with the subject? Yes , however the purpose of the work should be stated clearly . The authors wrote it twice as follow :

"therefore, the present study was desigened to investigate the effect of FQG on liver fibrosis induced by carbon tetrachloride in rats."

, the again added " According to the theory of traditional Chinese medicine, liver fibrosis is caused by wet and heat, blood stasis, yin and qi deficiency.[14] FQG can eliminate heat, detoxify, generate new blood, and supply yin and qi.[15-16] Therefore, we investigated the protective effects of FQG in a rat model of CCl_4 induced hepatic fibrosis and its underlying mechanisms, in order to explore it potential as a treatment for liver fibrosis.

So, the above paragraphs should be merged together and to be the last paragraph that end the story of introduction.

The last paragraph "AnluoHuaxianWan (ALHXW) is composed of medicinal herbs: Glutinous rehmanniae, Sanchi, Leech, Dead silkworm, Largehead, Yujing, Bezoar, Ark shell, Peony peel, Chinese rhubarb, Malt, Chicken`s membrane and β-Cyclodextrin. AnluoHuaxianWan has been demonstrated in the treatment of the fibrosis caused by chronic hepatitis B, cirrhosis at early and intermediate stages, and sold in the market (National Drug Permit Registry Z20010098). In the study, ALHXW was used as positive-control group." Should be deleted or shift to discussion.

RESPONESE: We have merged together with the above paragraphs, and revised the introduction according the reviewer`s instruction.

7. Is the purpose of the article clearly stated?: Yes but need to be focused and closed as mentioned above. The introduction should be re-written.

RESPONESE: We have re-written the instroduction according the reviewer`s kind suggestions.

8. Material and Methods:

Is the research design appropriate and the methods clearly explained?:

Please note: **Material** : is badly written , English should be revised so it can be clear.

RESPONESE: We have revised it .

9. Are the criteria for selecting the sample clearly explained and justified?: Yes, what does abdominal vein mean? Is it inferior vena cava !!

RESPONESE: We are deeply sorry for the misleading expression of the inferior vena cava, we have addressed the it according the reviewer`s advice.

10. Are the essential characteristics of the sample adequately described?: Yes

11. Is the sample size adequate and representative?: Yes

12.Has the data been collected in a systematic and comprehensive manner?: Yes

13.Is the statistical methodology appropriate?: Yes

14.Are there any ethical concerns about this study?: No

15.Results:

Is the analysis of the data systematic. Yes

Plz note ,

- In histopathological examination the authors showed that: ALHXW group, high dose and medium-dose of FQG appeared to relieve the pathological damages (Fig 2C-E).

Why 2-C is not normal, this should not be included in the treatment group of fibrosis as this is positive control. This needs to clarify.

- (HSC activation marker), Plz write abbreviation in full.

- Fig 5 need to be more focused

RESPONESE: fig2-C is not normal shown ALHXW group relieve the pathological

damages compared with the model group and the therapeutic effect of ALHXW was poorer than FQG. And we have focused the Fig5.

16.Are the results important?: Yes

17.Discussion:

Is the interpretation of the results clearly presented and adequately supported by the evidence adduced?: Yes

18.Conclusions:

Are the conclusions logically valid and justified by the evidence adduced?: Yes

19.Graphics:

Are all the figures and tables adequate and necessary?: Yes ,Some figures need to be more focused.

RESPONESE: we have focused these figures according the reviewer`s advice.

20.References:

-Are the references up-to-date?: Yes

- Have the most important previous studies been cited?: Yes

21.Major comments

a) Needs language revision.

b) Figures need to be more focused.

c) Abstract should be shortened.

D) Introduction need to be re-written especially last paragraphs as indicated above

Recommendation:

Accept after moderate correction

Reviewer #2:

In this study the authors have evaluated the anti-fibrotic effects of Fu-qi granule (FQG) on carbon tetrachloride-mediated liver fibrosis in rats. It is reported that FQG given daily after 8 weeks of carbon tetrachloride administration to rats is able to reverse liver fibrosis through promoting extracellular matrix protein remodeling and inhibition of the mTOR/p70S6K signal transduction pathway. Overall, the experiments are well done and the results are convincing. The paper is quite well written; however it will still require language revision, as there are several sentences or phrases that require restructuring.

Major points. 1. Western blot analysis of total mTOR is required. Because treatment with carbon tetrachloride may have some effects on liver mTOR expression the authors cannot conclude that FQG inhibits mTOR signaling. The authors should further validate this assumption by normalizing the levels of phospho-mTOR with that of total mTOR in rat liver in order to strengthen their conclusion.

Minor comments.

1. There is some discrepancy with the dose/volume of carbon tetrachloride administered to rats. Is it 0.5 ml / 100g body weight as stated in the abstract or 0.3 ml / 100g body

weight as described in the Materials and Methods?

RESPONESE: we are deeply sorry for the error, it should be 0.3ml/100g body weight, we have corrected it according the reviewer`s advice.

2. More details on the phospho-mTOR antibody used in this study should be provided. Is it the phospho-mTOR (serine 2448) or the phospho-mTOR (serine 2481) antibody?

RESPONESE: we have provided the details on the phosphor-mTOR antibody, which it is serine 2448 antibody according to the reviewer`s kindly suggestion.

3. In addition to p70S6K, mTOR regulates various signaling pathways. The authors speculated that the FQG effect might be due to inhibition of carbon tetrachloride-mediated p70S6K activation. To prove this the levels of phospho-p70S6K in the liver should be monitored. In the absence of those data the authors should limit the conclusion to inhibition of mTOR signaling in the abstract.

RESPONESE: we have adopted the reviewer`s advice.

4. Various studies suggest that mTOR signaling can drive the expression of HIF-1apha. May be the authors should cite some studies and discuss this in their manuscript.

RESPONESE: we have adopted the reviewer`s advice and cite some studies and discuss it.

Reviewer #3:

World Journal of Pharmacology/Manuscript No-0014562 Here, CCl4 induced liver fibrosis in rats was used as a model for evaluation of potential therapeutic usage of Fu-qi Granule (FQG). FQG reduced liver cell injury and liver fibrosis. The authors provide some data which support the conclusion that FQG improved CCL4 induced liver injury. Unfortunately, some data are controversial and substantial information regarding the composition of the herbal medicines, FQG and ALHXW is not provided.

Major points: #1 In the introduction the authors state that FQG can generate heat (2nd line from bottom). How is that meant and by which mechanism is this mediated or measured?

RESPONESE: we stated that FQG can eliminate heat is according to the view with theory of traditional Chinese medicine. FQG is a Traditional Chinese medicine compound preparation, which eliminate heat is a macro phenomenon from the perspective of traditional Chinese medicine. How is that meant and by which mechanism is this mediated or measured needs further research.

#2 The exact composition (weight, or concentration) of FQG and ALHXW (positive control) is absolutely mandatory. Unless, it is not possible to reproduce the data.

RESPONESE: FQG is a new type of traditional Chinese medicine preparation, which have a good solubility in water. ALHXW was made into power, then dissolved in water and used for intragastric administration.

#3 Hepatic stellate cells are the predominant producers of ECM and alpha-smooth muscle actin expression is a reliable marker of hepatic stellate cells activation. FQG reduced alpha-smooth muscle actin expression. A control for the various actin isoforms (on protein and mRNA level) is not given in this manuscript. Although, actin is used in

immunohistochemistry as a protein participating in fibrosis and shown to be regulated by FQG, it is also used for normalisation of genes of interest in PCR's. Therefore, other housekeeping genes, but not actin have to be used.

RESPONESE: we did not consider that actin is also used for nomalisation of genes of interest in PCR`s, So this maybe have a chance to further research the mechanism of FQG from cell level according to the reviewer`s advice.

#4 Fibrosis visualized by HE staining is not quantified. It is therefore not valid to state that in low dose FQG no significant changes were seen.

RESPONESE: Fibrosis visualized by HE staining, which is a typical representative and maybe it seen as one of the basis for judgment.

#5 The mRNA levels of TIMP-1 are conflicting (Fig. 5). TIMP-1 upregulation (versus control, lane 1) is shown for CCl4 and FQG-high dosage in the gel, but reduced in the intensity blot. The other two FQG-lanes (5,6) do not show a TIMP-1 or actin band, while a strong signal is indicated in the density blot. This has to be clarified. Actin is not a vlid loading control (see #3). What about the TIMP-1 plasma serum levels, which are increased in patients with liver injury and which directly correlate with fibrotic stage? The authors detect mRNA levels of TIMP-1 which is not equivalent to the expressed protein. The terminology expression of TIMP-1 should be avoided, unless TIMP-1 protein is shown; similar for MMP-9. This issue is important since mTOR is down-regulated, indicating reduced protein synthesis (Fig. 6)!

RESPONESE: we have checked carefully this section and corrected it according the reviewer`s kindly suggestion.

#6 The authors should explain why CCL4 induced elevation of ALT and AST are completely reversed by FQG, while ALP, Alb and TP are not!

RESPONESE: we have added the information for explain according the reviewer`s suggestion in the manuscript discussion.

#7 The manuscript needs substantial language improvement. First sentence in the abstract has no subject (To evaluate…..). Line 10 in the abstract: “At the end….”; this sentence makes no sense….and so on. There are also a lot of typos, e.g. end of the core tip: should be “signal transduction pathway”. Introduction, line 8: “anti-hepatic fibrosis” makes no sense. Similar, Intro, line 12 from bottom: “In particular, the effects of…..against liver…. “ This sentence makes again no sense and means a toxic effect which I think is not meant. Similar is true for the next two sentences; and so on….

RESPONESE: we have addressed these sentence and re-written the abstract according the reviewer`s kindly advice.

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