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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 48142

Title: The Regulatory effect of the Chinese herbal medicine thorowax root, scutellaria root and white peony root on non-alcoholic fatty liver disease

Reviewer's code: 03388095

Reviewer's country: Reviewer_Country

Science editor: Jia-Ping Yan

Reviewer accepted review: 2019-04-12 13:19

Reviewer performed review: 2019-04-16 12:31

Review time: 3 Days and 23 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	[ ] Accept (High priority)	Peer-Review:
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Accept (General priority)	[Y] Anonymous
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[Y] Minor revision	[ ] Onymous
[ ] Grade D: Fair	[ ] Grade D: Rejection	[ ] Major revision	Peer-reviewer's expertise on the topic of the manuscript:
[ ] Grade E: Do not publish		[ ] Rejection	[ ] Advanced
			[Y] General
			[ ] No expertise
			Conflicts-of-Interest:
			[ ] Yes
			[Y] No

SPECIFIC COMMENTS TO AUTHORS

• The study used Pioglitazone hydrochloride (PH) as the "positive control" based on previous study. However, it never explicitly mention why it is necessary to have such control group, particularly to readers not very familiar with it. In my view, the PH group

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serves no particular purpose. Instead, it should include a control group with rats on standard diet and CHM treatment to see any direct impact of CHM on rats. For example, one may argue that the inhibitory effect of CHM on NAFLD could be due to CHM effect on rat's food intake or other step rather than on lipid metabolism.

Answer: In this study, we tried to investigate the effect of CHM on NAFLD. Pioglitazone hydrochloride (PH) was proved to have therapeutic effect on NAFLD, so it was used as a positive control group to compare with CHM group. The comparison between PH and CHM proved effect of CHM on regulating the liver and intestine. The difference effect of CHM and PH on "intestine-liver axis" may be illustrated different mechanism. It was still necessary to keep PH group in the manuscript.

• Were different groups samples treated or collected blindly? It was not mentioned in the methods.

Answer: The groups were randomly divided, and the different groups samples were not treated blindly.

• For table 2, it's better to list both body weight and liver weight in addition to the liver coefficient.

Answer: Body weight and liver weight has listed in Table 2.

• For Oil Red O staining, the authors should also try to quantitate the amount of hepatic steatosis in different groups (please see Exp Biol Med, 2010, 235(11): 1282-1286), otherwise the conclusion of "markedly reduced lipid …. In PH and CHM" (result section under Oil Red O staining) become questionable (based on eyeballing?).

Answer: The quantitative analysis of Oil Red O staining was stated in Table 3.

• One of the important conclusion of this study is to elucidate the effect of CHM on NAFLD is through the so-called "intestine-liver axis", however, in the end of manuscript discussion section, it also said that "our results indicated that the effect of CHM on the regulation of the intestinal axis was not very effective". Please explain or revise.

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Answer: The conclusion has been revised as "The Chinese herbal medicine thorowax root, scutellaria root, and white peony root had good efficacy in regulating lipid metabolism and liver function, which suggested its ability to affect the liver. To a certain extent, it also had a regulatory effect on the intestinal mucosal barrier. Our study demonstrated that the mechanism of "soothing the liver" by Dachaihu decoction for the treatment of NAFLD was mainly through the affection on the liver."

- One of critics with Chinese herb medication is a crude extract which the exact gradients may vary from different labs or lots number. Although traditional Chinese medicine has been proven very effective in some cases, efforts also should be made to isolate and purify the effective component.

Answer: The effect of Chinese herbal medication or Chinese formula on disease treatment was mainly dependent on medication dosage and decoction preparation. The decoction preparation method has been shown in the Methods. In clinical treatment, Chinese medicine granules were used in hospital. However, there are difference between the crude medicine and the purified medicine. This study aimed to observe the effect of soothing liver medicine of famous Chinese formula – Dachaihu decoction. To imitate the clinical medication usage, we chose the crude Chinese herbal medicine purchased from Beijing Tong Ren Tang pharmacy.

- The manuscript writing still has room to polish and make it easy to read.

Answer: The manuscript has been revised and polished.

INITIAL REVIEW OF THE MANUSCRIPT

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 48142

Title: The Regulatory effect of the Chinese herbal medicine thorowax root, scutellaria root and white peony root on non-alcoholic fatty liver disease

Reviewer's code: 03647461

Reviewer's country: Reviewer_Country

Science editor: Jia-Ping Yan

Reviewer accepted review: 2019-04-12 13:13

Reviewer performed review: 2019-04-17 20:30

Review time: 5 Days and 7 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection	Peer-Review: [Y] Anonymous [ ] Onymous Peer-reviewer's expertise on the topic of the manuscript: [Y] Advanced [ ] General [ ] No expertise Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The title, abstract and methodology sections need to be more refined and streamlined. The use of a comma in a title is unusual and must be discouraged. The authors could probably use one common phylogenetic or taxonomic name for all the three herbal roots

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in the title description to avoid using the comma. A better title would be "The regulatory effect of Chinese herbal roots on Non-alcoholic Fatty Liver Disease".

Answer: Thank you for your previous advice. The title has been revised as "The regulatory effect of Chinese herbal roots on Non-alcoholic Fatty Liver Disease".

The paper is a very interesting one for understanding the therapeutic effect of certain Chinese herbal roots for "soothing" NAFLD symptoms. However, I think there is a leap of faith when you conclude that the intestinal mucosa barrier is also regulated by the Chinese herbal medicine thorowax root, scutellaria root, and white peony root. Your experiment may have shown some regulation of lipid metabolism and some liver function. However, the herbs did not demonstrate their ability to regulate liver axis or show regulatory effect on the intestinal mucosal barrier. The use of the word "regulation" seems a little far-fetched. Your experimental protocols were straightforward and showed results that either upregulated inflammatory factors or down-regulated them, but the mechanistic studies of the regulations that the authors purport to have demonstrated is a little far-fetched.

Answer: In this study, the Chinese herbal roots can alleviate the lipid metabolism and some liver function. But there was less changes on the intestinal index such as Intestinal sIgA levels, Occludin and Zonula occludens-1 (ZO-1) protein and mRNA levels. So the description – "the intestinal mucosa barrier is also regulated" should be revised. We defined the index related with liver as substance on the liver axis, and the index correlated with intestine as the intestine axis. It may be not approximately to describe "regulation" in the manuscript. So we will revise the description and refine the conclusion. Some conclusion did a little far-fetched.

It would have also been appropriate to establish a cellular model of NAFLD by culturing HepG2 or other similar cells in a medium that contained a long chain fat emulsion. These cells could have been treated with serum-containing CHM group from the

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sacrificed rats. Besides the cytokines and other liver markers, after treatment, the levels of adenylate-activated protein kinase (AMPK) α (AMPKα), p-AMPKα, acetyl coenzyme A carboxylase (ACC) α (ACCα), pACCα, PPARγ, and SREBP-2 could have also been measured. The AMPK agonist could have served as a positive control compound. The effect of CHM or AICAR (especially the number of lipid droplets) could have been observed in the serum levels of TG, TC, LDL-C, AST, ALT, and insulin in NAFLD rats, and their serum HDL-C levels, their tissues and HepG2 cells. A corollary assessment of CHM, PH and AICAR effects on the levels of p-AMPKα and PPARγ in the NAFLD liver tissues and HepG2 cells could have the work more convincing and relatable. Although, this work was not about the contribution of the microbiome to NAFLD symptoms, the "liver axis" in liver disease is also influenced by the microbiota, which may have been affected by the Chinese herbs.

Answer: It will be more valuable to establish a cellular model of NAFLD by culturing HepG2 or other similar cells in a medium that contained a long chain fat emulsion in our further study. The purpose of this study was to observe the effect of Chinese herbal roots on NAFLD and whether Chinese herbal roots can affect both the liver and the intestinal microbiota. Based on this research, further study about NAFLD should be carried out.

INITIAL REVIEW OF THE MANUSCRIPT

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 48142

Title: The Regulatory effect of the Chinese herbal medicine thorowax root, scutellaria root and white peony root on non-alcoholic fatty liver disease

Reviewer's code: 02860895

Reviewer's country: Japan

Science editor: Jia-Ping Yan

Reviewer accepted review: 2019-04-14 01:52

Reviewer performed review: 2019-04-22 14:35

Review time: 8 Days and 12 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	[ ] Accept (High priority)	Peer-Review:
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Accept (General priority)	[Y] Anonymous
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[Y] Minor revision	[ ] Onymous
[ ] Grade D: Fair	[ ] Grade D: Rejection	[ ] Major revision	Peer-reviewer's expertise on the topic of the manuscript:
[ ] Grade E: Do not publish		[ ] Rejection	[Y] Advanced
			[ ] General
			[ ] No expertise
			Conflicts-of-Interest:
			[ ] Yes
			[Y] No

SPECIFIC COMMENTS TO AUTHORS

This is an article that revealed a part of mechanisms of the efficacy of Chinese herbal medicine (CHM) on NAFLD. The authors tried to perform a theoretical approach through gut-liver axis concept, that may be a primary pathway of NAFLD development.

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While potential importance of this paper, I have a couple of concerns as follows; In both CHM and pioglitazine (PH) groups, the disease severity of NAFLD was successfully attenuated. Because effects of CHM on the intestinal factors were insufficient, the authors emphasized that the beneficial effects of CHM on NAFLD were results of direct action to liver (liver axis). However, since effects of CHM were overall weaker than those of PH, the negative results in intestine might be false-negative caused by a small sample size. The authors should re-examine a larger number of experimental animals, or should change their interpretation As an issue of terminology, "axis" means a route connecting more than two factors. Hence, the term "liver axis" is nonsense because a single organ has no such connecting route "axis". The authors should revise the manuscript as considering sufficiently these points.

Answer: Thank you for your precious advice. The manuscript has revised the description of liver axis and intestine. As the previous stated, we defined liver axis as liver organ, liver function, cytoinflammatory factor. The intestine axis defined as intestine organ, intestinal barrier, intestinal protein, and endotoxin.

INITIAL REVIEW OF THE MANUSCRIPT

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 48142

Title: The Regulatory effect of the Chinese herbal medicine thorowax root, scutellaria root and white peony root on non-alcoholic fatty liver disease

Reviewer's code: 01808881

Reviewer's country: United States

Science editor: Jia-Ping Yan

Reviewer accepted review: 2019-04-13 00:27

Reviewer performed review: 2019-04-22 19:55

Review time: 9 Days and 19 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	[ ] Accept (High priority)	Peer-Review:
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Accept (General priority)	[Y] Anonymous
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Minor revision	[ ] Onymous
[ ] Grade D: Fair	[ ] Grade D: Rejection	[Y] Major revision	Peer-reviewer's expertise on the topic of the manuscript:
[ ] Grade E: Do not publish		[ ] Rejection	[Y] Advanced
			[ ] General
			[ ] No expertise
			Conflicts-of-Interest:
			[ ] Yes
			[Y] No

SPECIFIC COMMENTS TO AUTHORS

The goal of this study was to determine the regulatory effect of the Chinese herbal medicine on NAFLD. The authors showed that the extract was able to treat many indices of fatty liver and also preserved the liver-gut axis. There were many concerns noted: 1)

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The model for high fat high sucrose only generates fatty liver. Could the authors have gone longer to at least get some inflammation (NASH) in their model?

Answer: In this study, we tried to establish a NAFLD rat model. During the observation which stage it were as the fed of high fat high sucrose. The stage was evaluated by NAFLD activity score (NAS) according to the National Institutes of Health NASH Clinical Research Network Pathology Committee. The guide recommends that when NAS < 3 points can exclude NASH, it is NAFL stage; NAS> 4 points can diagnose NASH. The NAS in this study was 4 point, which between the NAFL stage and the NASH stage. So the rat animal model has clearly reached the stage of nonalcoholic simple fatty liver (NAFL), in the progression to nonalcoholic steatohepatitis (NASH).）

2) The authors should inform the calories percent of the fat and carbohydrates in the diet.

Answer: The high-fat high-sugar diet contained 52.5% basic food, 2% cholesterol, 10% lard, 5% egg yolk powder, 0.5% sodium cholate and 30% sucrose. The calories percent of the fat was 11.6%, and the calories percent of the carbohydrates in the diet was 65.4%.

3) The authors have used abbreviations in the abstract without defining them the first time they were used. Please correct this deficiency in the abstract and the manuscript.

Answer: The full name has been defined when the abbreviations first used.

4) Reword the last sentence in the abstract for clarity.

Answer: The conclusion of the abstract was revised as "Chinese herbal medicine Chinese thorowax root, scutellaria root, and white peony root was beneficial in regulating lipid metabolism and liver function, which indicated that they have a good effect on the liver. To a certain extent, CHM can affect both the liver and intestine, while the effect of CHM on the liver was superior to the intestine."

5) In the methods section, were all group were gavaged daily with distilled water (control and the high-fat high sucrose group), PI or CHM, respectively for the last 4 weeks of the study. As written, it is not clear.

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Answer: The description of the experiment was "From the 13th week, rats in the PH group were administered intragastrically Pioglitazone hydrochloride at a dose of 10mg/kg·d (n=8) for 4 weeks and fed the high-fat high-sugar diet. Rats in the CHM group were administered intragastrically thorowax root, scutellaria root, and white peony root decoction for 4 weeks and fed the high-fat high-sugar diet. The normal group and model group were provided with same volume of distilled water and fed the standard diet and high-fat high-sugar diet respectively."

6) Please conduct liver triglyceride biochemical analysis.

Answer: The result of liver triglyceride biochemical analysis was stated in Table 5.

7) Were TNF, TGF, NFKB, TLR4 done on the serum as denoted in the methods section or were these determinations done on liver tissues as shown in the results section? Which is correct? Further, if indeed these were done on liver tissues, are the ELISA kits purchased form NeoBiosciences, Shenzhen, China that were used compatible with the tissues? How were the tissues prepared for ELISA?

Answer: The level of TNF，TGF，NFκB，TLR4 in liver tissue was detected in this study, making a mistake in the Methods part. How to prepare protein for Elisa analysis has been added in the manuscript. As followed: "A 100 mg liver tissue piece and intestine tissue piece from the same portion were placed into a 1.5 mL tube, 1 mL 0.9% saline was added, and the piece was cut into smaller pieces. The grinding rod was fully ground. The supernatant was absorbed after centrifugation for 15 min (4℃, 1500 rpm). Enzyme-linked immunosorbent assay (ELISA) kits were used to test the levels of each protein according to the directions provided. The level of tumor necrosis factor (TNF-α), transforming growth factor (TGF-β1) in liver tissues and secreted immunoglobulin A (sIgA) in intestinal tissues was measured using an enzyme-linked immunosorbent assay (ELISA) - based commercial kit (NeoBioscience, Shenzhen, China). The level of nuclear factor-kappa B (NF-κB) and toll-like receptor (TLR4) in liver tissues was measured using

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a commercial kit (USCN Life Science, Wuhan, China)."

8) Give details on measuring SIgA in intestinal tissue.

Answer: The detail was provided in Answer 7）. "A 100 mg liver tissue piece and **intestine** tissue piece from the same portion were placed into a 1.5 mL tube, 1 mL 0.9% saline was added, and the piece was cut into smaller pieces. The grinding rod was fully ground. The supernatant was absorbed after centrifugation for 15 min (4℃, 1500 rpm)."

9) Serum endotoxin levels should be measured.

Answer: The result of plasma endotoxin levels was stated in Table 12.

10) The authors should comment on what would be an effective dose of CHM for human? The dose that the authors chose for their treatment group was 8.64g/Kg. This is not going to be a dose that can ever be used for an adult human.

Answer: The dosage provided in this study was according to the human body. The clinical dosage for a human body of the CHM were as follows: Bupleurum chinense DC=15g, scutellaria root (Scutellaria baicalensis georgi=9g), and white peony root (Paeonia lactiflora PALL.=9g). Based on body surface area and human – rat dose conversion, the dosage of the crude drugs of rats was set at 8.64g/kg.

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