Dear Editor and Reviewers:

Thank you for your response and for reviewers' futher comments concerning our manuscript entitled **'Yinchenhao decoction attenuates obstructive jaundice-induced liver injury and hepatocyte apoptosis by suppressing the PERK-induced pathway' (Manuscript NO: 50746).** Those comments are all valuable and helpful for revising and improving our paper. We are willing to publish our manuscript in WJG and we have studied comments carefully and have made corrections to the paper meticulously per the reviewers' suggestions. And point by point response to the reviewers' comments is listed below. We hope that the revised version of this manuscript can be accepted for publication in your journal.

Responds to the Editor's comments:

Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).
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Answer: Please find the below language certificate picture.

AJE

Editing Certificate

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Yinchenhao decoction attenuates obstructive jaundice-induced liver injury and hepatocyte apoptosis by suppressing the PERK-induced pathway

prepared by the authors

Yanli Wu, Zhonglian Li, Xibo Zhang, Hao Liu

was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at AJE.

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Institutional review board statement: 请您上传 IRB 文件，并盖上伦理委员会的章（你上传的 IRB 文件是项目资助证明文件，请核实）

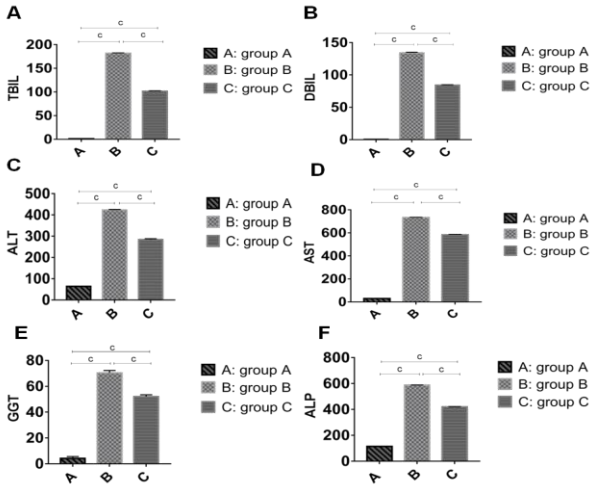
Answer: Institutional review board in our hospital, Tianjin Nankai Hospital, is responsible to approve the experiments with human subject. But my research did not involve the human, the Institutional review board refused to provide the document.

Reviewer 1

Wu YL et al. reported that YCHD attenuates liver injury and hepatocyte apoptosis. The phenomenon is interesting if they could present the effect to repeat the condition giving dose dependently. The dose escalation experiment should present for convincing what you found was true.

1) Why the TBIL was lower than the DBIL in the Fig 2?

Response: According to the opinions put forward by the experts, we re-checked the data and found that TBIL and DBIL were marked in the opposite direction. We have already corrected. Please see the below picture.



2) Why apoptosis index was increasing even in the sham group?

Response: The serum added in BRL-3A rats hepatocyte was obtained from rats who had their bile duct exposed without ligation instead of the serum from normal rats. The operation could cause the stress response in the rat, which affected the apoptosis index of rat hepatocytes. Therefore, the apoptosis index was increased in the sham group.

Reviewer 2

The manuscript, No. 50746, reports on the possible mechanism of traditional Chinese medicine, YCHD, action in attenuation of experimentally-induced obstructive liver jaundice (OJ)and hepatocyte apoptosis. Ther results obtained with rat model of OJ revealed that serum of YCHD-treated group exerted inhibitory effect on the PERK-

CHOP-GADD34 pathway of apoptosis activation, and increased the ration of Bax/Bcl-2. While, these data clearly suggest the value of YCHD in treatment of OJ, it should be noted, that the mechanism involved in this beneficial effect of the YCHD action remains “mysterious”.

1) Namely, the results of this study do not provide any indication as to the nature of the YCHD-induced serum element responsible for the regulation of gene expression of PERK, Chop, GADD34, Bax and Bcl-2.

Response: I don't fully understand what the mean of the expert. I reply as I understand it. We conducted the cell experiments based on serum pharmacology. It has been mentioned in the manuscript that serum pharmacology was first proposed by Iwama Hiroko in 1987[1], has become an important method to study the mechanisms of traditional Chinese medicine (TCM). The core concept of serum pharmacology is to collect animal blood and to obtain serum after administering a TCM by gavage at defined times, followed by the addition of the serum to an in vitro tissue or cell system to study the pharmacodynamics and mechanism of TCM.

In this research, the thirty rats were randomly divided into three groups on average: G1, G2, and G3. On the day of surgery, the rats in group G1 had their bile duct exposed without ligation, and the rats in group G2 were administered total bile duct ligation, and the rats in the group G3 were just given a 1 ml YCHD gavage per 100 g weight every day for 7 days, and blood was taken one hour after infusion on day 7. Three groups of serum were collected and added into BRL-3A rat hepatocytes. Then, hepatocyte apoptosis and changes of PERK, CHOP, GADD34, Bax and Bcl-2 in cells were detected.

Reference:

[1] Iwama H, Amagaya S, Oqihara Y. Effect of shosaikoto, a Japanese and Chinese traditional herbal medicinal mixture, on the mitogenic activity of lipopolysaccharide: a new pharmacological testing method. J Ethnopharmacol 1987; 21: 45-53 [PMID:3695555]

2) The manuscript, moreover, requires thorough edition of language and sentence composition.

Response: I have tried my best to revise the manuscript, and provided the language certificate.

Reviewer 3:

Comments on the manuscript entitled: "Yinchenhao decoction attenuates obstructive jaundice-induced liver injury and hepatocyte apoptosis by suppressing the PERK-induced pathway" Submitted for publication in World Journal of Gastroenterology This manuscript describes several experiments on the effects of a traditional Chinese medicine, Yinchenhao Decoction (YCHD), used as a complementary and alternative treatment to reduce clinical symptoms. The purpose of this study is to know the effects of YCHD on obstructive jaundice at the molecular level. The authors concluded that “OJ-induced liver injury and hepatocyte apoptosis were associated with the activation of the PERK-CHOP-GADD34 pathway and increased Bax/Bcl-2 ratio”. The experiment is serious and the results are interesting both for the knowledge of the physiology and pathology of liver and for the clinical aspects. Nevertheless, I have several remarks to make about the manuscript.

1) Methods - Rat model of obstructive jaundice (p. 5): In this part, the authors specify that the surrounding noise is controlled to within 60 dB. It is well known that noise can affect physiology, but this parameter is rarely reported in publications. In this present case, it would be interesting to have an explanation of the importance of this parameter (with a reference if possible).

Response: Accroding to the regulations of Tianjin laboratory animal quality monitoring station, the surrounding noise of laboratory is controlled to within 60 dB. Please find the below related document pictures.

编号 2015-006

检 验 报 告

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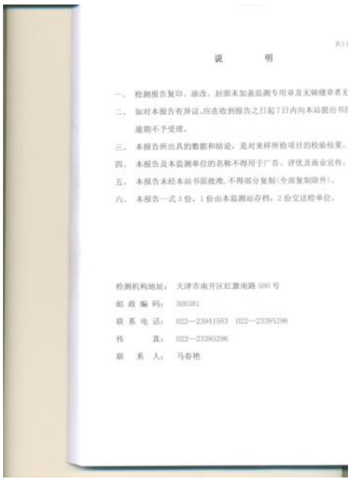
样品数量：一套
检测类型：委托检验
委托单位：天津市南开医院

检验单位
天津市实验动物质量监测站



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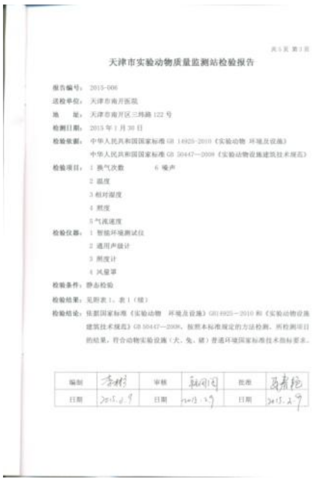
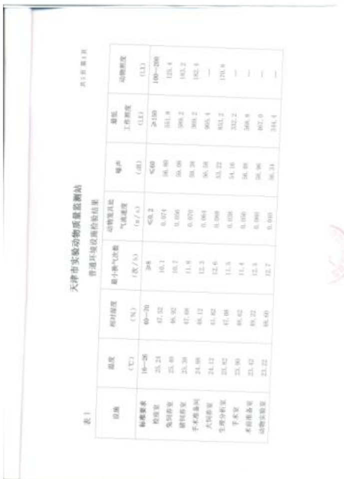


天津市实验动物质量监测站检验报告

报告编号：2015-006
送检单位：天津市南开医院
地 址：天津市南开区三纬路122号
检测日期：2015年1月30日
检验依据：中华人民共和国国家标准GB 14925-2010《实验动物 环境及设施》
中华人民共和国国家标准GB 50447—2008《实验动物设施建筑技术规范》
检验项目：1 换气次数 6 噪声
2 温度
3 相对湿度
4 照度
5 气流速度
检验仪器：1 智能环境测试仪
2 通用声级计
3 照度计
4 风量罩
检验条件：静态检验
检验结果：见附表1、表1（续）
检验结论：依据国家标准《实验动物 环境及设施》GB14925—2010和《实验动物设施建筑技术规范》GB 50447—2008，按照本标准规定的方法检测，所检测项目的结果，符合动物实验设施（犬、兔、猪）普通环境国家标准技术指标要求。

编制	李彬	审核	韩同国	批准	马春艳
日期	2015.2.9	日期	2015.2.9	日期	2015.2.9



天津市实验动物质量监测站
普通环境设施检验结果

天津市实验动物质量监测站
普通环境设施检验结果

2) Animals and treatment (p.6): In a first experiment, the authors use 30 male rats with 10 for control, 10 with liver ligation (OJ), and 10 with OJ + YCHD. In a second experiment, the authors use 30 other male rats: group G1 without ligation, G2 : with ligation, G3 : ligation and YCHD. In this second experiment, the number of animals of each group is not specified. Please, specify. What is the use of this second group compared to the first one?

Response: In my manuscript, I have already indicated the number of animals of each group in the second experiment. And group G1 without ligation, G2: with ligation, G3: just YCHD gavage without ligation. Please see the below marked content.

The remaining thirty rats were randomly divided into three groups on average: G1, G2, and G3. On the day of surgery, the rats in group G1 had their bile duct exposed without ligation, and the rats in group G2 were administered total bile duct ligation, and the rats in the group G3 were just given a 1 ml YCHD gavage per 100 g weight every day for 7 days, and blood was taken one hour after infusion on day 7.

3) Liver function test (p.7): Give the centrifugation speed in number of g - Cell culture of BRL-3A rat hepatocytes.

Response: I have already revised the manuscript. Please see the below marked content.

The rat blood was collected and centrifuged at 300g for 10 min at 20 ℃, and the supernatants were collected.

4) Explain clearly the purpose of these culture experiments in the presence of serum: it is not very clear that it is to understand the effect of serum from rats under different conditions on hepatocyte cultures.

Response: According to the principle of serum pharmacology, rat serum was collected and used for the culture experiments in the presence of serum. The method of serum pharmacology prevents interference of the in vitro experiment from the physical and chemical properties of crude TCM and allows the study of the active metabolized pharmacologically active products, following the process of digestion and absorption of the TCM and its biological transformation in the gastrointestinal tract. Collectively, these features allow us to evaluate the true pharmacological effects of TCMs[2].

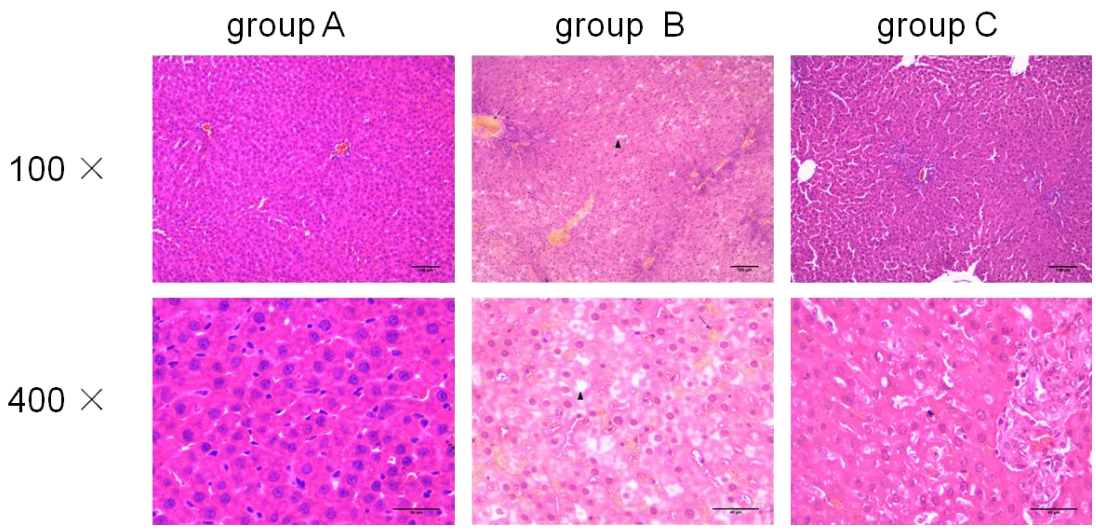
In this research, the BRL-3A hepatocytes were cultured with serum from sham group(G1), obstructive jaundice model group(G2) and YCHD gavage group (G3). Apoptosis and protein expressions of PERK, CHOP, GADD34, Bax and Bcl-2 were detected to determine the molecular mechanism of apoptosis induced by OJ.

Reference

[2] Bochu W, Liancai Z, Qi Z. (2005). Primary study on the application of Serum Pharmacology in Chinese traditional medicine. Colloids Surf B Biointerfaces 2005; 43: 194-197 [PMID:15964749 DOI:10.1016/j.colsurfb.2005.04.013]

5) Figure 1: give a scale scale-bar on pictures. On each micrograph of the figure, show the significant details with arrows or captions according to the explanation given in the text.

Response: I have added the scale scale-bar on pictures and showed the significant details. Please see the below picture.



6) Western blotting (p. 8, last sentence): "Experiment" instead "experiments" Results - YCHD attenuates liver tissue injury induced by obstructive jaundice (p. 8): specify group A, B, C like in the figure - page 10: "additionally" instead additonnaly" Figure 4: "levels" instead "levles".

Response: I have finished correcting the spelling mistakes in the manuscript.