Format for ANSWERING REVIEWERS

February 11, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16257-review.doc).

Title: Grape Seed Proanthocyanidin Protects Liver against Ischemia/Reperfusion by Attenuating Endoplasmic Reticulum Stress

Author: Zhen-Chao Xu, Jie Yin, Bo Zhou, Yu-Ting Liu, Yue Yu, Guo-Qiang Li

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16257

The manuscript has been improved according to the suggestions of reviewers:

Reviewed by 02903629

1. There are three groups. I believe all analyses used the ANVOA method. Difference comparing two groups should use SNK or Dunnett… and it is inappropriate that T test were used between two groups. Besides, concrete values of all biochemical markers should be given out.
Thanks for your sincere question. We have revised it in the text.

2. The author mentioned "The rats were randomly divided into three groups". Please describe how the rats were randomly divided.
This is a good question. We totally bought 48 Male Sprague–Dawley rats (220 g–250 g). Then we marked 1,2,3 in three same cards and put them into a box.Then we caught one card from the box at a time , and if the card marked 1,then we caught a rat into the cage marked with Sham (2 for the Control, 3 for the GSP). And we shaked the box before every catching the card, 8 rats in each group was needed.

3. The baseline data before experiments were not shown.
Thanks for your sincere question. Actually, i got the baseline data before experiments , but there was no statistical difference between it and the Sham Group, so i did not show it in the text.

4. There are some grammar and spells errors, such as 'were decreased' 'reperfu
Thanks for your carefully reviewing the manuscript, and we have revised the grammar and spell errors in the text.

Reviewed by 00070481

The work is meaningful for the liver protection research. the authors present the Grape Seed Proanthocyanidin as a potent reagent.

Thanks for your strong approval, and give my sincere appreciation to you.

Reviewed by 02444743

The authors suggested that GSP can protect rat liver injury of ischemia/reperfusion by anti-inflammatory, anti-oxidate, anti-apoptotic effects and attenuating liver ER stress; this is an interesting study even though the current study can not certify the reduced liver damage by alleviating ER stress.

1. GSP is anti-inflammatory, anti-oxidative and anti-apoptotic, however, there is not enough data to support that these properties of GSP by attenuating liver ER stress in rat liver injury model.
Attenuating liver ER stress may be accompanying, not causal.
Thisis a very good question. As shown in the Fig.5, we found that the GRP78 was barely expressed in the Sham group, and much higher expression in the Control group, this proved the occuration of ER stress. And the property of I/R was oxidative stress which could induce the occur of ER stress.

2. Was the animal experiment approved by IACUC? What is "the institutional and National Research Council's guideline for animal experiments"?
Yes, it is. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Commitee of the Nanjing Medical University (IACUC protocal number： NJMU08-092)

3. What kind of anesthetic used in current study? Was the rat fasting before surgery?
We used the way of intraperitoneal anesthsia. Surgery was performed on rats after a 10-hour abrosia.

4. Why did not detect the mRNA levels of ER stress related proteins, quantify analysis the results of western blotting, and measure liver and plasma ROS levels?
Thanks for your sincere advice. Becasue the relative regulators of ER-stress mainly occured in the protein level.
Normally, we just compare the density of western-blot results .I have not detected the gray of it before, and i will pay attention to it in the future experiments. ,
Actually, i planned to measure the liver ROS levels, but i found it significantly different in the plasma, so i did not measure it. I will measure it in the future experiments to cover the shortage.

5. What is the means of "A P" in the "Statistical analysis"?

A P value <0.05 was considered statistically significant.

6. GRP78 may be a negative regulator of the UPR in multiple models (e.g. Cancer Res 2007,67:9809; Cell Death Differ 2008,5:1460), liver-specific Grp78 knockout mice exhibited liver injury (Neoplasia 2014,16:617; Hepatology 2011,54:229). How to explain the result of Fig 5A from current study?
This is a very high level question. As shown in the Fig.5, we found that the expression of apoptosis related procaspase-12 and CHOP increased in the Control group but decreased in the GSP group, so GRP78 is a protector in this study. Therefore, it is a positive regulator.

7. The language needs extensive improvement. All the abbreviations should be given the full name when first appear in the text, such as, ATF 4, CHOP, IRE1, AST, ALT, IL-6, TNF-α, IL, TNF, TGF, ADL, SOD, MDA.
Thanks for your kindly remind, and the revised are as follows: activating transcription factor-4, C/EBP (CCAAT/enhancer binding protein) homologous protein, inositol-requiring enzyme-1, aspartate aminotransferase, alanine aminotransferase, interleukin-6, tumor necrosis factor, interleukin-10, transforming growth factor, Acceptable Defect Level

8. Some number of references in text do not match up the back References.
I am sorry for my careless,and i have revised it.

9. The legends should show that the data are expressed as mean ± SD, and animal numbers used in each study.
Thanks for your earnest review, and we have revised it in the paper.
10. There are many errors in References, please revise them according to INSTRUCTIONS TO AUTHORS of WJG.
I am really sorry for my careless, and i have revised it carefully according to the WJG. And give my sincere gratitude to you again.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,
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