Author's response to reviews

Title: Possible mechanism underlying the effect of Heshouwuyin, a tonifying kidney herb, on sperm quality of aging rats

Authors:

Siyun Niu (nsy1688@163.com)
Jingbo Chen (chenjingbo8611@163.com)
Fei Duan (duanfei2007@hotmail.com)
Qingliang Song (276534806@qq.com)
Mingyue Qin (991976500@qq.com)
Zhenshan Wang (zswang@hbu.edu.cn)
Jingze Liu (liujingze@hcbtu.edu.cn)

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Author's response to reviews: see over
Dear Dr. Rowles,

We submitted our revised manuscript for consideration to publish in BMC Complementary and Alternative Medicine. The following are our point-by-point responses to the Reviewer’s concerns:

Referee 1
#1 General comments
The study is quite well designed; anyway there are some important concerns that need to be addressed to render the article acceptable for publication. In particular the structure is poorly organized, materials and methods should be improved, the results section should be thoroughly revised, as well as the discussion, that is too speculative.
**Answer:** we have remodeled the structure of manuscript, the sections of materials, methods and discussion have be thoroughly revised, in the revised manuscript.

Materials and methods
#2 There is no mention to the methods adopted to obtain the histochemical images of testicular tissues.
**Answer:** The detailed methods are described in the revised manuscript.

#3 The “Results” paragraph entitled “Quantitative analysis of experimental animals” should be put in this section.
**Answer:** It has been moved to the section of Materials and methods in the revised manuscript.

#4 The “Results” paragraph entitled “Leydig cell aging models” should be put in this section.
**Answer:** It has been moved to the section of Materials and methods in the revised manuscript.

#5 An explanation of the technique used to determine the relative band intensity of western blot analysis should be provided.
**Answer:** Detailed methods used to determine the relative band intensity of western blot analysis has been provided in the revised manuscript.

#6 Statistical analysis. Did the authors perform a normality test to assess the normal distribution of their data? And did they perform an homoscedasticity test? These tests are necessary before proceeding with an ANOVA test. In case of non-normality or heteroscedasticity the data should be analyzed using a non-parametric test.
**Answer:** We performed a normality test to assess the normal distribution, and an homoscedasticity test, and claimed them in the revised manuscript.

Results
#7 Morphological observation of testicular tissue. This paragraph is not acceptable. The Authors do not furnish any data, they only assert that there are no changes. They should provide count data
on cellular populations.

**Answer:** The count number data has been provided in the revised manuscript.

#8 StAR and P450scc in Leydig cells. The Authors should provide the data from cells subpopulations count. The Authors declare they studied mRNA expression. No mention of the technique used for this purpose in the M and M section.

**Answer:** Subpopulations count data has been provided in the revised manuscript. Since the mRNA expression study is preliminary, we delete this part in the revised manuscript.

**Discussion**

#9 This section is redundant. The Authors should shorten it and comment their data instead of speculate on other matters. I would suggest to delete lines from “The importance of reproductive health …..” to “… will play an important role in this point”, as well as lines from “About 99%….” to “…testosterone synthesis [35]”.

**Answer:** In the revised manuscript, we have deleted these sentences, and the discussion has been rewrite.

**Figure and captions**

#10 Figure 1 is not necessary.

**Answer:** This section has been corrected in the revised manuscript.

**Referee 2**

#1 The treatment with Heshouwuyin in Groups SWY1G and SWY2G started after 16 and 17 months from birth, respectively. Can one month delay in starting the treatment be responsible for a lower response in SWY2G rats (rather than the length of the treatment)?

**Answer:** It should not be a reason for a lower response in SWY2G rats (rather than the length of the treatment), since both of 16 and 17 months are old enough for naturally rats. The physical condition should be no difference in this age of rats.

#2 Given the partially similar composition of Heshouwuyin and Shouwu pills but different results, can you argue the role of specific herbs in the mixture and identify the most important ones?

**Answer:** The effects of the herbs could not be a simply adding of the constituents. The constituents might inter-act each other. So far, we don’t know which specific herbs in the mixture caused the difference. Anyway, we are going to identify the mechanisms in the future.

#3 Drug dosage appears to be quite high; the rationale of this choice should be described better.

**Answer:** Our previous findings implicated that twice the amount of adult dosage produced the best effects. Therefore, 100 g Heshouwuyin containing 4.8 g of crude drug was considered the administration dosage.

#4 Possible side-effects should be described or a comment should be added to the discussion
Answer: A paragraph about the side-effects of this herb has been added in the discussion of the revised manuscript.

#5 weight of rats: 350-390g as described in page 3, or 300 g as mentioned in page 4?

Answer: This is a type in error. It has been corrected in the revised manuscript.

#6 Figure legends should be checked carefully (for example, fig.7 “# compared to AG and *-compared to SWPG” is not applicable. The number of figures appears to be excessive (discretionary).

Answer: These have been corrected in the revised manuscript.

#7 A comment regarding traditional medicine, limits of the study and future applications would be of interest.

Answer: A paragraph regarding these questions has been added in the discussion of the revised manuscript.

For editorial comments

1) We note that a significant proportion of the justification for your study, as presented in your abstract, is based on traditional Chinese Medicine principles. Please note that, as these principles have no known correlates in conventional modern medicine, we are unable to accept them as the basis for scientific work presented in the journal. We would therefore ask you to please remove/replace these sections of text in your abstract. Please also ensure that justification of this type does not appear at any other point in your manuscript.

Answer: we have deleted these sentences in the revised manuscript.

2) We recommend that you ask a native English speaking colleague to help you copyedit the paper. If this is not possible, you may need to use a professional language editing service. For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz (www.edanzediting.com/bmc1). BioMed Central has negotiated a 10% discount to the fee charged to BioMed Central authors by Edanz. Use of an editing service is neither a requirement nor a guarantee of acceptance for publication. For more information, see our FAQ on language editing services at http://www.biomedcentral.com/authors/authorfaq/editing.

Answer: The revised manuscript has been edited for English by the professional company Edanz (www.edanzediting.com/bmc1).

Best Regards,

Zhenshan Wang