Author's response to reviews

Title: Protective effect of Erythropoietin on renal injure induced by four weeks exhaustive exercise of the rats

Authors:

Xixiu Lin (linxixiu1010@163.com)
Chonghe Jiang (chonghe.jiang@gmail.com)
Ziqiang Luo (luozq1962@163.com)
Shulin Qu (gushulin07@yahoo.com.cn)

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Cover Letter
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Protective effect of Erythropoietin on renal injury induced by four weeks exhaustive exercise of the rats
Xixiu Lin, Chonghe Jiang, Ziqiang Luo and Shulin Qu

Dear Editor,
Thanks a lot to both of you and reviewers for carefully and patiently in reviewing our manuscript. All the comments and criticizes are valuable for improving the quality of this manuscript and make it much clearer in its presentation to the readers. We revised the manuscript as suggested (in red) and provided herein a cover letter giving a point-by-point response to the concerns.

Referee 1:

1. Is the question posed by the authors well defined?
Yes.

2. Are the methods appropriate and well described?
Definition of statistical method is missing. Presentation of results evaluated with optimum statistical analysis is very important. There are more than 3 independent groups to compare, so for interval data ANOVA or Kruskal Wallis variance analysis according to parametric conditions, for ordinal data Chi square test should be used. Calculation of the exercise distance in acute exercise protocol should be explained in method section.
Revising: Definition of statistical method has been added (page 6, paragraph 2, line 4).
ANOVA: We did not use ANOVA since we made comparison between each two groups, and all the compared data passed the normal distribution test, so we would rather use student-t test.
Same reason for Chi square test.
Calculation of the exercise distance has been described (page 4, last line but one).

3. Are the data sound?
In table (number of which was not given) 1, p values were given but between which groups did they observe this significant differences?
In description of *changes in general condition*, instead of saying “rats in group ET+EPO tolerated better to the exhaustive exercise compared to those in group ET”, specific score or grading system should have been applied in order to compare groups based on objective rather than subjective methods. Same is relevant for *changes in HE renal tissue*. **Revising:** The number of animals in each group and each training period has been added in the footnote of table 1. *p values* between groups we observed have been clarified in the footnote of table 1 as well.

**Changes in general condition:** We did not score the other parameters except the running distance and the increase in rat weight, we added the descriptions in page 6, bottom line and page 7, paragraph 1, last 3 line.

**Changes in HE renal tissue:** Unfortunately, we did not score the morphological changes objectively. We will take the advice in our ongoing research work.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?  
Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?  
Yes

6. Are limitations of the work clearly stated?  
No.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?  
Yes

8. Do the title and abstract accurately convey what has been found?  
Yes

9. Is the writing acceptable?  

*In the title section:*  
“Protective effect of erythropoietin on renal injury induced by four weeks exhaustive exercise of the rats” should be corrected as “Protective effect of erythropoietin on renal injury induced in rats by four weeks of exhaustive exercise”.

**Revising:** The title has been changed as suggested.

*In the abstract section:*  
“considerably damages” should be corrected as “considerable damage”

**Revising:** “damages” in this sentence and similar descriptions in the other sections have been changed to “damage” (page 2, paragraph 3, first line).
In the introduction section:
“…EPO is increase …” should be corrected as “…EPO is increased…”
Revising: “increase” has been changed to “increased” (page 3, paragraph 1, line 3).

“…EPO was shown inhibition effect….…” should be corrected as “… EPO was shown to have inhibitory effect…”
Revising: The correction has been done (page 3, paragraph 1, line 6).

“…hematuresis…” should be corrected as “.. hematuria …”
Revising: The correction has been done (page 3, paragraph 2, line 7).

“..injure ….” should be corrected as “... injury ....”
Revising: “injure” in this sentence and same mistakes in the other sections have been changed to “injury” (page 3, paragraph 2, line 8).

“..evaluate the prevented and treated effects of EPO ....” should be corrected as “… evaluate the preventive and therapeutic effects of EPO ....”
Revising: The correction has been done (page 3, paragraph 2, last line but 3).

There are many spelling and grammar mistakes. English should be revised by native British.
Revising: We have asked a native British for help to correct the MS (changes in red throughout the text)

Best Regards
Kubra Kaynar

Referee 2:

It is a subsequent study of the authors followed by the previous one (Int J Sports Med. 2010 Dec;31(12):847-53). The novel point of the study is to observe the renoprotection of EPO in chronic renal injure induced by four weeks exhaustive exercise. However, there are some necessary comments I have to point out before seen by readers.

Major Compulsory Revisions:
1. In the discussion, an important pathway of EPO's tissue protection is PI3K/Akt which has been elucidated in many studies (Ref: The Receptor That Tames the
Innate Immune Response. Mol Med 18: 486-496, 2012). In this descriptive study, it isn’t necessary to use PI3K inhibitor. If you want to prove one of mechanism that EPO involved in the over-excised chronic renal injury model using LY294002, some western blots are necessary, such as PI3K (total & phosphorylated) and Akt (Total & Ser473 or Th308).

**Revising:** Thanks a lot for suggestion, we will do that in our ongoing research work.

2. Your results are focused on the morphologic changes, so could you alter the paper title more specific?

**Revising:** We have re-edited the title as Referee 1 suggested (Reviewer’s report 9, *In the title section*).

**Minor Essential Revisions**

1. There are several spelling errors and improper grammars. Page 3 Line 3 “EPO is increase in the condition”. Page 8 Line 1 “the thickness of basilar membrane (Bm) was even”. They are just some examples. Please carefully review your manuscript and correct the errors.

**Revising:** “increase” has been changed to “increased” as both Referee 1 and Referee 2 pointed out (page 3, paragraph 1, line 3). “the thickness of basilar membrane (Bm) was even” has been changed to “the thickness of basilar membrane (Bm) was uniform” (page 8, paragraph 2, line 4). We have carefully reviewed the manuscript and corrected the errors throughout the text as Referee 1 also required.

2. Page 3 in the 1st paragraph in background, “EPO exerts the protective effects on central nervous system, kidney, retina etc.” where is the reference?

**Revising:** The reference has been added for this statement, and the order of the reference list and cited in the text have been reorganized.

3. Page 3 in the 1st paragraph in background, “Thus, it is proposed that EPO is a kind of hemopoietic growth factor with independent efficiency in hemopoiesis.”

**Revising:** This statement has been rephrased and the reference has been added as suggested (page 3, paragraph 1, line 7)

4. Table 1 should be an formal result, not in a supplement file.

**Revising:** Table 1 has been moved to the end of References as Editorial Request.
5. Page 11, 3rd paragraph, “Recent experiments showed that the protect effect of EPO against renal injury was via promoting the regeneration of tubular epithelial cells and resisting the cell apoptosis [24-25].” The 24-25th Refs are published in 2004, not recently. 
**Revising:** We have removed the word “Recent” in this sentence (page 11, paragraph 4, first line).

6. Page 12, 1st paragraph, “catalytic activity of PI3K pIIO”. What is the pIIO? Is it p85 subunit?  
**Revising:** pIIO is the PI3-kinase catalytic subunit (page 12, paragraph 2, line 11).

**Level of interest:** An article whose findings are important to those with closely related research interests  
**Quality of written English:** Needs some language corrections before being published  
**Statistical review:** No, the manuscript does not need to be seen by a statistician.  
**Declaration of competing interests:**  
No conflict of interest  
Additional material submitted by the reviewers

We hope that you with these explanations and changes will find the paper acceptable for publication in **BMC Nephrology**.

Yours sincerely,  

Prof. Shu-Lin Qu, M.D.  
Medical College of Hunan Normal University,  
Changsha, Hunan 410006  
P. R. China.  
Tel & Fax: +86 13927625023  
E-mail: qushulin07@yahoo.com.cn