Dear Editor/Reviewer:

We are truly grateful to yours’ critical comments and thoughtful suggestions on our manuscript (Integrated Systems Pharmacology, Urinary Metabonomics, and Quantitative Real-time PCR Analysis to Uncover Targets and Metabolic Pathways of You-gui Pill in Treating Kidney-yang Deficiency Syndrome). Based on these comments and suggestions, we have made careful modifications on the original manuscript, and uploaded it to the webpage. All changes made to the text are clearly highlighted in red color. Furthermore, we have consulted native English speakers for paper revision before the submission. We hope the revised manuscript will meet your Journal’s standard. Below you will find our point-by-point responses to the reviewers’ comments/questions.

We hope that these revisions are satisfactory and that the revised file will be acceptable for publication in International Journal of Molecular Sciences.

Thank you very much for your work concerning our paper.

Wish you all the best!

Yours sincerely,

Ruiqun Chen, Jia Wang, Runhua Zhan, Lei Zhang and Xiufeng Wang
Response to Reviewer 2 Comments

Comments and Suggestions for Authors

The authors investigated the pharmacological mechanisms of You Gui Pill (YGP) for the treatment of Kidney Yang Deficiency (KYD) syndrome. My comments and concerns are as follows:

**Point 1:** The KYD syndrome is not a well-defined syndrome and in human can be associated with different pathogenic mechanisms and clinical manifestations.

**Response 1:** Thank you very much, and this is a very enlightening question. As you mentioned, The KYDS is not a well-defined syndrome, and our experiment is only based on animal models (rats) to study the pathogenesis of KYDS and the regulating effects of YGP on KYDS. Hence, there may be different pathogenic mechanisms and clinical manifestations in humans. We believe that this part of research is very necessary and is also a key step to clinical transformation. Therefore, on the basis of this study, we will conduct an in-depth study combined with samples of clinical patients in the next step, so as to verify the conclusions of this study.

We have supplemented relevant explanation in the manuscript:

“Given some of herbs, active compounds, targets, metabolic pathways, and differential metabolites that YGP acts on KYDS were found in this study by integrated systems pharmacology, urinary metabonomics, and RT-qPCR analysis, more subsequent experiments and assay, such as samples of clinical patients, western blot, targeted metabonomics and molecular pharmacology, are needed to verify the relevant results from different levels.”

(See line 537 at the last paragraph of 3.5. Fatty Acid Metabolism.)

**Point 2:** It remains questionable whether the animal model proposed by the authors can simulate KYD syndrome in human.
Response 2: We feel great thanks for your professional review work on our manuscript.

Firstly, the use of hydrocortisone to inject the leg muscles of rats/mouse to induce KYDS is the earliest established animal model of TCM syndrome, and is also the method used by most researchers to study KYDS [1-5]. Secondly, KYDS patients with clinically are mainly diagnosed by combining the clinical symptoms and 17-OHCS indicator [6-9]. Because a large number of studies have shown that 17-OHCS was significantly decreased when KYDS occurs, and it can be used as a biomarker of KYDS. Meanwhile, our study also integrates clinical symptoms (e.g. weight and behavioral change), and multiple biochemical indicators (TSH, T3, T4, LH, FSH, T, ACTH, and CORT) of target gland axis to define KYDS. Finally, we perform histopathological analysis of multiple tissues (hypothalamus, pituitary, thyroid, adrenal gland, and testis, etc) to define KYDS. All these indicators and pathological analysis have also been reported in many studies on KYDS [7, 8, 10-13]. In summary, we believe that KYDS rat model established in this study can basically simulate KYD syndrome in human. However, KYDS should also be further analyzed ultimately in combination with clinical patients, so as to truly reveal the mechanism of occurrence and development of KYDS.

Overall, thank you for your wonderful comments again. Your comments and suggestions have contributed a lot to improve the quality of our manuscript and subsequent research. If there are still any questions in our manuscript, please let us know, we will carefully modify and discuss it in the manuscript according to your opinion.

References


