Response to Reviewer 1 Comments

Thank you for your comments concerning our manuscript entitled ‘Compound Prescription Xiaoyaosan Improves Depressive-Like Behaviors of Chronically Stressed Mice’ (JoVE58276). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches.

In consideration of your three main concerns, we have the following explanation.

First, the compounds of Xiaoyaosan used in the protocol has a good effect on depression which has been proved in our previous experimental studies (Yue G X; Huang Q F; Chen J.X. Antidepressant effects of Xiaoyao powder and its different modified combinations. World J. Integr. Tradit. Western Med. 2007, 6, 326–328; Ding X F, Li Y H, Chen J X, et al. Involvement of the glutamate/glutamine cycle and glutamate transporter GLT-1 in antidepressant-like effects of Xiao Yao san on chronically stressed mice [J]. Bmc Complementary & Alternative Medicine, 2017, 17(1):326), so it could be used in this protocol for observing the antidepressant effect of Traditional Chinese Medicine.

Second, thank you very much for your concern about the use of previous data that have been published in this manuscript. The purpose of this protocol is to exhibit the methods in the article *Involvement of Normalized Glial Fibrillary Acidic Protein Expression in the Hippocampi in Antidepressant-Like Effects of Xiaoyaosan on Chronically Stressed Mice* by Dr. Xiu-Fang Ding, et al, so we could present the corresponding data in the manuscript if cited. We have obtained explicit copyright permission to reuse data from this previous publication.

In the end, we are sorry for the language mistakes in our manuscript. Now we have studied comments carefully and have made correction which we hope meet with approval, and also have checked grammar, spelling, and punctuation in the manuscript, and we will search for language polishing by a native speaker if the contents of our manuscript are suitable for publication.

The main corrections in the paper and the responds to the comments are as flowing:

**Point 1:** What do you mean by using the item"Compound prescription Xiaoyaosan"? do you mean the compounds that are contained in Xiaoyaosan? Please explain.

**Response 1:** The 'Compound prescription Xiaoyaosan' in the manuscript is the meaning of ' the compounds that are contained in Xiaoyaosan’. Thank you for pointing out this incomprehensible phrase, the 'Compound prescription Xiaoyaosan' has been revised in the manuscript.

**Point 2:** The title does not match the research content very well. I would like to suggest to change it as "Compounds of Xiaoyaosan prescription improves depressive -like behaviors of chronically stressed mice".

**Response 2:** Thanks for your valuable advice, the title has been replaced to 'Compounds of Xiaoyaosan prescription improves depressive -like behaviors of chronically stressed mice' in the revised manuscript.

**Point 3:** In the abstract, "a series of data"should be changed as "a series of parameters".

**Response 3:** Thanks for your advice, the 'a series of data' in the long abstract has been replaced to 'a series of parameters' in the revised manuscript.

**Point 4:** The description in the manuscript is poor. I changed the last part of the abstract as an example.

The original description: "Through data analysis after the experiment, significant behavioral changes were found in model mice, meanwhile the depressive like behaviors could be improved by Xiaoyaosan or fluoxetine treatment. This protocol demonstrated the application of Xiaoyaosan to the behaviors of CUMS-induced mice, and revealed that the beneficial effects of Chinese compound prescription Xiaoyaosan on depressive-like behaviors may be worth considering for the treatment of depression."

My modification："Data analysis showed that behaviors of model mice were significantly changed as compared to control group, which however have been improved by the treatment of Xiaoyaosan and fluoxetine. The current findings demonstrated the anti-depression effects of Xiaoyaosan on the behaviors of CUMS-induced mice, and revealed that compounds from Xiaoyaosan prescription may be worth for treating depression taking into account their beneficial effects on depressive-like behaviors."

**Response 4:** Thank you for your thoughtful comments. We are so sorry for the language deficiencies and problems in our manuscript. We have made correction which we hope meet with approval, and we will search for language polishing by a native speaker if the contents of our manuscript are suitable for publication in the in the next round of modification.

**Point 5:** Line 88, give the explanation of abbreviation of "XYS" somewhere previously.

**Response 5:** Thank you for your careful work. This is a wrong abbreviation and it is revised in the new manuscript.

**Point 6:** All numbers that are smaller than ten should be spelt out, for instance, one week, five-sucrose preference test, any two of the following seven stressors, etc.

**Response 6:** Thank you very much for this constructive comment. According to your suggestion, all numbers that are smaller than ten have been spelt out in the revised manuscript.

**Point 7:** Line 137-138, did you perform the open filed test and sucrose preference test prior to the experiment? If yes, please give the data and compare the results obtained before and after the experiments. By doing so, you would be able to confirm the model has been built successfully.

**Response 7:** In this protocol, the open filed test and sucrose preference test were performed before and after the process of model establishment, the description of the open filed test and sucrose preference test prior to the experiment was in the section of ‘2. Preparing Mice for the Experimental Procedure’, and the corresponding data were presented in the section of ‘REPRESENTATIVE RESULTS’. Thank you for your thoughtful comments!

**Point 8:** In all parts of results, the authors have not compared the effects of XYS to the effects of fluoxetine. It is better to do it to give a direct idea how strong the anti-depression effect of XYS is.

**Response 8:** Thank you for your thoughtful advice. To further verify the correctness of the depression model, we set the fluoxetine treatment group in this study. The purpose of our experiment is to find the antidepressant effect of Xiaoyaosan, rather than the advantage of traditional Chinese medicine in antidepressant. Furthermore, the efficacy comparison between Xiaoyaosan and fluoxetine is not common in previous studies (Zhu X, Jing L, Chen C, et al. Danzhi Xiaoyao San ameliorates depressive-like behavior by shifting toward serotonin via the downregulation of hippocampal indoleamine 2, 3-dioxygenase[J]. Journal of Ethnopharmacology, 2015, 160:86-93; Zhu X, Xia O, Han W, et al. Xiao Yao San Improves Depressive-Like Behavior in Rats through Modulation of β-Arrestin 2-Mediated Pathways in Hippocampus[J]. Evid Based Complement Alternat Med, 2014, 2014(6):902516). We would have an in-depth discussion in the manuscript if the anti-depression effect of Xiaoyaosan had significant advantage to fluoxetine according to statistical analysis.

**Point 9:** The formats of references are not consistent. For instance, in references 12 and 13, the words were capitalized while for other references the words in the titles were not. Please follow the instruction rules of the journal.

**Response 9:** Thank you for your careful reading of our manuscript. We have careful rechecked and corrected the style of the citations according to the requirement of JoVE.

**Point 10:** The figures are not in a good quality and should be improved.

**Response 10:** Thank you for your careful work. We are very sorry for the low quality figures in the paper. We have corrected it in the revised manuscript.

Minor Concerns The language needs to be improved. Here I corrected some of them.

1. In the abstract, "Mice were randomly divided into 4 groups, they were control group, model group, Xiaoyaosan treatment group, and fluoxetine treatment group." should be changed as "Mice were randomly divided into 4 groups, i.e. control group, model group, treatment group of Xiaoyaosan, and treatment group of fluoxetine."

2. In the abstract, "after the experiment" should be deleted.

3. Line 17, change "the intervention effect" into "the anti-depression effect"

4. Line 41," It has become the world's primary cause of disability," should be changed as "It has become one of the world's primary causes of disability,"

5. Line 44-46, it is better to divide this sentence into two independent sentences.

6. Line 47, delete "compound".

7. Line 53, add references related for the statement "Containing multiple constituents, Xiaoyaosan has multiple targets, and utilize multiple pathways."

8. Line 69, "the mouse depressive model" should be changed as "the depressive model of mouse"

9. Line 68-73, it is too tedious to express your research aim in one sentence. Please re-written this part.

10. Line 115-127, add "a.m." after the time points.

11. Line 145-146, it is better to give the ratios of the eight herbs contained in Xiaoyaosan. Did you use the original ratios or did you modify the ratios? It is not clear.

12. Line 175, what does "HD" means?

13. Line 186, which statistical software?

14. Line 223, you mentioned there will be "FIGURE & TABLE LEGENDS". However, no table legends were provided. Please check.

15. The references in the current study are not enough. For instance, give some references related to the statement between Line 235-240, between 244-246 in the discussion part.

16. Line 254, "state" should be "states"

17. Line 270-271, this sentence should be a new sentence.

18. Line 271, "Many study has…" should be "many studies have…"

**Response:** Thank you very much to point out these issues in our manuscript. We have careful rechecked and corrected the above problems according to your profound comments. We hope our revised manuscript could meet publication requirements, and we will seek for the professional polishing. Thank you very much!