Author's response to reviews

Title: Valeriana officinalis root extract suppresses physical and psychological stress responses by decreasing the ratio of monoamine neurotransmitters to their metabolites

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Author's response to reviews: see over
Dear Dr. Torkel Falkenberg

Reviewer: Woong Mo Yang

Reviewer’s report:

Major Compulsory Revisions;

Comment 1. The title is too ambiguous. What is ‘physical and psychological stress responses’?
Answer 1. We clearly improved the title according to reviewer’s comment. Please refer to the text.

Comment 2. The main point of this study is the decreased glucocorticoid level by VE, however I don’t think the glucocorticoid level represents the PS and PCS responses as authors’ conclusion. This study needs to include some quantification data which can represent stress value (ex: immobility time such as forced swimming test and freezing behavior).
Answer 2. We conducted the forced swimming test, and we observed the increase of immobility time in forced swimming test. There are some discrepancies between corticosterone levels in serum and immobility time in forced swimming test. However, the administration of VE significantly reduced the immobility time in forced swimming test. We described this according to reviewer’s comment. Please refer to the text.

Comment 3. Manuscript needs overall modification.
Answer 3. We thoroughly edited the manuscript according to reviewer’s comment. Please refer to the text.

Minor issues;

Comment 1. In abstract, Methods in detail of sample volume, treatment time and method of analysis should be included.
**Answer 1.** We thoroughly edited and clearly demonstrated the method section of abstract according to reviewer’s comment. Please refer to the text.

**Comment 2.** Key word: ‘Valerian root extract’ needs to be changed to scientific name. (Valeriana officinalis)

**Answer 2.** We changed the key word “Valerian root extract” into “Valeriana officinalis” according to reviewer’s comment. Please refer to the abstract section.

**Comment 3.** In background, the detailed description on the relationship between monoamine neurotransmitters and stress is needed. For example, it would be good to explain the relationship between stress and increase or decrease of hormone, the relationship between type of stress and change of hormone and so on.

**Answer 3.** We explained why we selected 5-HT, NE and their metabolites for analyzing the stress condition in hippocampus and amygdala according to reviewer’s comment. Please refer to the text.

**Comment 4.** In materials and methods, the extraction method of VE is missing.

**Answer 4.** We purchased the VE from the Naturex (Avignon, France) and we described this according to reviewer’s comment. Please refer to the text.

**Comment 5.** Animal age is missing.

**Answer 5.** We used 6-week-old mice and we demonstrated this in experimental animals section according to reviewer’s comment. Please refer to the text.

**Comment 6.** Page 6, the last sentence, 78.1 ‘ng/g’? different from figure.

**Answer 6.** We made a big mistake we corrected the unit (ng/g) into µg/L according to reviewer’s comment. Please refer to the text.

**Comment 7.** In results and discussion and figure legends, the group name should be unified. (e.g. page 9, line 7; PS group # PS-V group / page 9, line 10; PCS group # PCS-V group / page 16, line 8; PS group # PS-V group, PCS group # PCS-V group).

**Answer 7.** We made a mistake and we unified the abbreviations according to reviewer’s comment. Please refer to the text.
**Comment 8.** In the discussion, the description of NE, 5-HT and their respective metabolites is not enough. The relationship between their hormone and stress is not fully explained.

**Answer 8.** We discussed the meaning of NE, 5-HT and their metabolites in stress conditions. Please refer to the text.

**Comment 9.** Page 9, line 18-19; What is the meaning of ‘stress affects 5-HT levels lesser than it affects 5-HIAA levels’?

**Answer 9.** In general, it has been reported that stress increases brain levels of 5-HIAA without affecting 5-HT concentrations. We modified this sentence as follows: “stress more prominently changes the 5-HIAA levels than 5-HT levels because of reuptake of 5-HT in synaptic cleft” according to reviewer’s comment. Please refer to the text.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:** I declare that I have no competing interests.

**Reviewer:** BOMBI LEE

Minor comments:

**Comment 1.** There are some mistakes in grammar, and some sentences are confusing.

**Answer 1.** We thoroughly edited the manuscript with the help of one native speaker and our colleagues according to reviewer’s comment. Please refer to the text.

**Comment 2.** They need to describe the analysis protocols of corticosterone in more detail.

**Answer 2.** We clearly described the obtaining and general protocols of corticosterone. However, the exact protocol did not show in the manuscript because it is well demonstrated in manufacturer’s guidelines. Please comprehend this point.
Comment 3. They need to describe the analysis protocols of HPLC in more detail.

Answer 3. We clearly described the analysis protocol of HPLC according to reviewer’s comment. Please refer to the text.

Comment 4. In my opinion, one-way ANOVA using the Turkey’s post hoc test is more suitable than one-way ANOVA using the Bonferroni’s post hoc test. Furthermore, please confirm that significance statistical difference between all groups, as they are described in the text.

Answer 4. We re-analyzed the data with one-way ANOVA using Turkey’s post-hoc test and we observed the same statistical outcome. We re-confirmed the significances between groups according to reviewer’s comment. Please refer to the text.

Comment 5. Author should clarify the sample number for Figure 2, 3, and 4.

Answer 5. We inserted the sample number for Figure 2, 3, and 4 according to reviewer’s comment. Please refer to the text.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests.