Author's response to reviews

Title: Anxiolytic effects of compound Valeriana jatamansi Jones in mice

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

Thank you very much for your email of May 9, 2012, with regard to our manuscript (Ms. No. 1742498216705347) together with the comments from the reviewers. According to the comments, we have revised the relevant part in the original manuscript. We also responded point by point to each reviewer’s comment as listed below, and the revised portion is marked with underline. I hope this will make it more acceptable for publication.

Thank you and all the reviewers for the kind advice.
Yours sincerely,

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Here below are our responses to the reviewers’ comments.

Reviewer: 1

Comments to the Author
This paper reports that compound of Valeriana jatamansi Jones (CV) showed anxiolytic effects in mice. The design of the studies stated in this manuscript and the quality of the results stated are of standard. However, the authors need to address the following concerns.

1. Question: (1) Authors used 2.4, and 4.8 g/kg (p.o.) of compound of Valeriana jatamansi Jones. These dosages are too high although it showed an anxiolytic effect. There is no clinical meaning if dosage is too high.
Answer: It should be noted that the three doses tested (1.2, 2.4 and 4.8 g/kg) are related to the original formulation but not to the extract. In the clinic, the dose of the formulation *Valeriana jatamansi* Jones that is used is 31g per day. When this human dose is converted into an animal dose (a person of 60 kg, and a conversion factor of 9.01 between human and mice), it was equivalent to 4.8 kg/kg. Therefore, 4.8 g/kg was chosen as the highest dose tested in this study. These have been added in the revised manuscript (page 12 lines 12-17).

2. Question: (2) In fig. 2, CV increased open arm time and entries in EPM. However authors did not provide transition in the arms. If the transition of mice is increased, anxiolytic effects of CV may due to increase in locomotor activity.
Answer: The results of total arm entries in EPM were added in Fig. 2c. Compared with the control group, compound *Valerianae Jatamansi* Jone treatment did not alter total arm exploration in the elevated plus maze. These data suggest that the compound did have anxiolytic-like effect (page 10, lines 14-18).

3. Question: (3) On page 5, line 15, authors need to provide the ratio of extracts.
Answer: The ratio of extracts was added in the revised manuscript (page 5, line 11-14).

4. Question: (4) On page 3, line 7-11, statement of the long-term use of benzodiazepines is not necessary.
Answer: The sentence “The long-term use of benzodiazepines even increases cognitive decline adversely, especially in the elderly” was deleted in the revised manuscript.

5. Question: (5) On page 7, line 9, locomotor activity was recorded by Anilab. Please provide city and nation of Anilab.
Answer: “(Anilab, Ningbo, China)” was added in the revised manuscript (page 7, line 20).

6. Question: (6) Authors used 2 mg/kg of diazepam in EPM and used 6 mg/kg of diazepam in locomotor activity. Are there any reasons for that?
Answer: The sedative effects of this formulation and the positive control were tested in the present study. However, we found that the dose of 2 mg/kg of diazepam did not produce sedative effects in our preliminary experiments. To compare the sedative effects between a positive control drug and *Valeriana jatamansi* Jones, the dose of diazepam was increased to 6 mg/kg in the locomotor activity test. These have been added in the revised manuscript (page 11 lines 22-25; page 12 lines 1-2).

7. Question: (7) In the title, anxiolytic-like effect should write anxiolytic effects.

Answer: The title was changed as “Anxiolytic effects of compound Valeriana jatamansi Jones in mice”.

Reviewer: 2

Comments to the Author

The manuscript submitted by You et al. entitled "Anxiolytic-like effects of compound Valeriana jatamansi Jones in mice" evaluates the anxiolytic effects of an abstract mixture of plant extracts in the EPM and the LDT. The paper includes valuable information although some of this information has been included in prior publications by the authors.

1. Is the question posed by the authors well defined?
   Yes, the question addressed is the anxiolytic activity of Valeriana jatamansi and if it exerts any muscle relaxant or sedative effects.

2. Are the methods appropriate and well described?
   Yes, although the authors should include if the control animals have also been given saline solution over the course of the treatment period.

   Answer: Control animals received vehicle (saline, 0.4mL/25g) only, this has been added in the revised manuscript (page 6 lines 8).

3. Are the data sound?
   The EPM % results appear very low with only 0.2 to 0.4% spent on open arms. Usually, this should be at least around 5-10%. Furthermore, diazepam was not
antagonized with flumazenil to confirm the antagonist activity. Different concentrations of diazepam were used for the EPM and the LDT which makes interpretation and correlation of the two models difficult.

**Answer:** We are sorry for the wrong unit in the original manuscript, and it should be 20 to 40% of time the mice spent on open arms. All the data have been corrected in the revised manuscript (Fig 2 and Fig 3).

The effect of flumazenil on diazepam group was already investigated in the previous study, the results showed that diazepam was also antagonized with flumazenil. As diazepam is a mild tranquilizer in the class of drugs known as benzodiazepines and flumazenil is a well-known benzodiazepine antagonist, we didn’t introduce this result in our original manuscript. We are sorry for neglecting this result, which have been added in the revised manuscript. We added this in the Method section and Result section (page 6, lines 11-16; page 8 lines 24-25; page 9 line 1, lines 8-11; Fig 3 and Fig 5).

The sedative effects of this formulation and the positive control were tested in the present study. However, we found that the dose of 2 mg/kg of diazepam did not produce sedative effects in our preliminary experiments. To compare the sedative effects between a positive control drug and *Valeriana jatamansi* Jones, the dose of diazepam was increased to 6 mg/kg in the locomotor activity test. These have been added in the revised manuscript (page 11 lines 22-25; page 12 lines 1-2)

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?
The authors mention orthogonal experiments - but this is not apparent from the study design. Clarification on this issue would help to provide the reader with a better understanding of what has been evaluated. The authors also refer to a former publication in which the formulation had been evaluated - how does this new publication advance the understanding of the formulation?
**Answer:** Unlike the regular formulation extraction process, *Valeriana jatamansi Rhizoma et Radix*, *ziziphi spinosae Semen*, *Albiziae Cortex*, and *Junci Medulla* were extracted by different solvents and methods. This was because the active constituents of these drugs are all sensitive to water temperature. An orthogonal experimental design method was performed to optimize the extraction process of the four herbs as described previously [27]. Briefly, in an L$_9$($3^4$) orthogonal test, the solid-liquid ratio, ethanol concentration, solvent amount, extraction times were determined. The extraction process of *Valeriana jatamansi Rhizoma et Radix* or *Junci Medulla* was determined by the content of hesperidin and the total extract. The optimum extraction condition of *ziziphi spinosae Semen* or *Albiziae Cortex* was determined by the content of jujuboside A. According to the original formulation and the herb extracting yield, the four extracts (*Valeriana jatamansi Rhizoma et Radix*, *Ziziphi Spinosa Semen*, *Albiziae Cortex* and *Junci Medulla*) were then mixed with a ratio of 5:3:5:1 to get the formulation *Valeriana jatamansi Jones*. These have been added in the revised manuscript (page 12 lines 17-25; page 13 lines 1-6).

The anxiolytic effects of this formulation have been previously been established in the rat. However, considering its low affinity for this site in binding assays, it is unclear whether this anxiolytic activity was mediated by benzodiazepine binding site modulation at γ-aminobutyric acid-A receptors. In addition, chromatographic profiling of the formulation for purity was not carried out. Therefore, in this study, we further explored the anxiolytic effects of *Valeriana jatamansi Jones* in mice using the elevated plus maze, light/dark box test, and spontaneous activity. The formulation was first evaluated for purity by HPLC before the experimental test. Moreover, we also examined whether its anxiolytic effects are mediated by the benzodiazepine site of GABA(A) receptors through co-administration of the antagonist flumazenil. Our study further supports the hypothesis that *Valeriana jatamansi Jones* has anxiolytic activity but no sedative effects. After the formulation was identified by HPLC and administrated for 10 days in mice, anxiolytic effects were observed in the EPM and LDB procedures. Moreover, these effects were attenuated by the GABA (A) antagonist flumazenil, which suggests that the anxiolytic effects of *Valeriana jatamansi Jones* are related to GABA (A) receptors. These have been added in the revised manuscript (page 3 lines 19-25; page 4 lines 1-4; page 9 lines 20-22;
6. Are limitations of the work clearly stated?
No. The authors do not provide much information about the limitations of their work although they address what further research will have to be completed to elucidate the exact mechanism for the formulation.

**Answer:** As a positive control drug, diazepam exerted anxiolytic effects at only one of the doses tested (2 mg/kg, p.o.). However, these anxiolytic tests were performed after the mice had been given Valeriana Jatamansi Jones for 10 days as previous studies have shown that many drugs elicited anxiolytic effects after administration for 7 days [28,29]. It is possible to test the anxiolytic activity of the formulation using the EPM and LDB after administration for less than ten days. In addition, previous researchers have indicated that etiology of anxiety may be related to the levels of monoamine neurotransmitters and neuroendocrine system [30, 31]. Therefore, further studies are needed to identify these anxiolytic mechanisms. These have been added in the revised manuscript (page 13 lines 7-12).

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Partially. The authors refer to prior publications but in some regards do not point to publications that have been done on Valeriana extracts in the past that addressed this issue as well.

**Answer:** We added the prior publication about the Valeriana publication in the revised manuscript (Reference 10) and discuss how this new publication advance the understanding of the formulation as described above. The anxiolytic effects of this formulation have been previously been established in the rat. However, considering its low affinity for this site in binding assays, it is unclear whether this anxiolytic activity was mediated by benzodiazepine binding site modulation at γ-aminobutyric acid-A receptors. In addition, chromatographic profiling of the formulation for purity was not carried out. Therefore, in this study, we further explored the anxiolytic effects of *Valeriana jatamansi Jones* in mice using the elevated plus maze, light/dark box test, and
spontaneous activity. The formulation was first evaluated for purity by HPLC before the experimental test. Moreover, we also examined whether its anxiolytic effects are mediated by the benzodiazepine site of GABA(A) receptors through co-administration of the antagonist flumazenil. Our study further supports the hypothesis that *Valeriana jatamansi* Jones has anxiolytic activity but no sedative effects. After the formulation was identified by HPLC and administrated for 10 days in mice, anxiolytic effects were observed in the EPM and LDB procedures. Moreover, these effects were attenuated by the GABA (A) antagonist flumazenil, which suggests that the anxiolytic effects of *Valeriana jatamansi* Jones are related to GABA (A) receptors. These have been added in the revised manuscript (page 3 lines 18-25; page 4 lines 1-4; page 9 lines 20-22; page 13 lines 17-22).

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

9. Is the writing acceptable?
No, the authors should contact a native English speaker to correct grammar and word structure. The current format and phrasing of the manuscript is not acceptable for publication.

Major compulsory revisions - statistical analysis, clarification of formulation, inclusion of diazepam antagonism by flumazenil.

Minor essential revisions - grammar and spelling.

**Answer:** The revised manuscript was copyedited by Edanz editor (no. C1205-17915). The statistical analysis was carried out by a one-way analysis of variance (ANOVA) followed by Student-Newman-Keul’s post-hoc tests using Prism 4.0 (Graphpad Software, Inc). The F value was added in the revised manuscript. The effect of flumazenil on diazepam group was already investigated in the previous study, the results showed that diazepam was also antagonized with flumazenil. As diazepam is a mild tranquilizer in the class of drugs known as benzodiazepines and flumazenil is a well-known benzodiazepine antagonist, we didn’t introduce this result in our original manuscript. We are sorry for neglecting this result, which have been added in the revised manuscript. We added this in
the Method section and Result section (page 6, lines 11-16; page 8 lines 24-25; page 9 line 1, lines 8-11; Fig 3 and Fig 5).

The Field Editor's Comments to Author:
Field Editor
Comments to the Author:

**1. Question:** 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).

**Answer:** The revised manuscript was copyedited by Edanz editor (no. C1205-17915).