Author’s response to reviews

Title: Neurite outgrowth stimulatory effects of culinary-medicinal mushrooms and their toxicity assessment using differentiating Neuro-2a and embryonic fibroblast BALB/3T3

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Author’s response to reviews: see over
Revision of Manuscript

We would like to express our sincere gratitude for letting us revise our manuscript. We have addressed all the reviewers’ comments (attached the detailed explanations to the comments from editor and reviewers). Changes made are in red. Thus, we are respectfully submitting the revised manuscript “Neurite outgrowth stimulatory effects of culinary-medicinal mushrooms and their toxicity assessment using differentiating Neuro-2a and embryonic fibroblast BALB/3T3” (MS: 2099831598854879).

2. With the submission of this manuscript I would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere.

We hope that our manuscript will receive a favourable decision.

Yours sincerely,

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Section editor comments

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<td>The authors should provide toxicological information about the safety of the sold nutritional product. Indeed, MTT, NRU and LDH are not enough.</td>
<td>The extracts are not available as nutritional product. All the extracts were prepared in laboratory following standard procedure (Page 6 Line 132-143). We determined the viability of neuroblastoma and embryonic fibroblasts after treatment with extracts as preliminary toxicological assessment. We chose MTT, NRU and LDH as they represent the most widely used methods. By using cell culture, in vivo methods using animal can be avoided. When we proceed to testing the selected mushroom extract/s in vivo, for example neurobehavioral test, we will then perform acute and chronic animal toxicity testing.</td>
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<td>The yields of the extracts should be shown by the authors.</td>
<td>The extraction yields are given in Table 2 (Page 28).</td>
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Reviewer 1

Minor essential revision

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<td>Eliminate the second paragraph of the background (lines 69-78). This manuscript has nothing to do with alternative test methods such as the Embryonic Stem Cell (EST) test. In addition, the authors misrepresent the use of the 3T3 cells in the EST.</td>
<td>Revised accordingly (Page 3).</td>
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### Reviewer 2

**Major compulsory revision**

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<td>Please provide evidence that the extracts can cross the blood brain barrier.</td>
<td>In order to prove that the extracts can cross blood brain barrier, <em>in vivo</em> study will provide the most critical insight on the pharmacokinetic behavior of the extracts. <em>In vitro</em> model based on the culture of cerebral endothelial cells also can be used. To our knowledge, the <em>in vitro</em> co-culture model of brain endothelial cells with astrocytes and/or pericytes is by far, best mimicking the <em>in vivo</em> anatomical condition. The study on the blood brain barrier will be carried out in the near future. Nevertheless, we have performed the inhibition treatment to determine the signaling pathways of mushroom extract-induced neuritogenesis. Our studies on the elucidation of signaling pathways using cellular methods have been published (Phan et al., 2012; Seow et al., 2013).</td>
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Obtain polysaccharide enriched or triterpenoid enriched preparations of the mushrooms and demonstrate that they are active. | We thank the reviewer for the comments. The purification and identification of the bioactive compounds as well as polysaccharides is on-going. So far, we have prepared the polysaccharides of *Lignosus rhinocerotis*, as our previous data showed that this particular mushroom |
showed promising neurite outgrowth effects (Eik et al., 2012; Seow et al., 2013). The examination of its polysaccharides on neuritogenesis is still on-going. Further fractionation of *Pleurotus giganteus* ethyl acetate extract is also on-going.


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**Extra comment**

We had the manuscript edited for English by Dr John James Wilson, a native English speaker.