Author's response to reviews

Title: Gastrodia elata Blume alleviates L-DOPA-induced dyskinesia by normalizing FosB and ERK activation in a 6-OHDA-lesioned Parkinson’s disease mouse model

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Author's response to reviews:

Answer to the reviewers

Dear Editor,

We appreciate reviewers’ helpful comments and are pleased to submit a revised version of our manuscript for consideration for publication in BMC Complementary & Alternative Medicine.

Please find the following document addressing changes to the original manuscript and our point-to-point responses to the reviewer’s comments.

It has not been submitted for publication nor has it been published in whole or in part elsewhere.

We thank you in advance for your consideration of this revised manuscript.

Yours faithfully,

Hi-Joon Park, KMD, Ph.D.

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Editorial Requests:

"Please provide details in your Methods section on the source of the Gastrodia elata Blume used in your study. Please also provide details on who undertook the formal identification of the plant material used in your study. Please also confirm whether a voucher specimen of this material has been deposited in a publicly available herbarium, and include this information in your manuscript. A deposition number should be included, if available."

- We added detail drug information into Methods section of our manuscript

- (Modified Methods) GEB (Product #: 5413, Lot#: 142311) manufactured by Sunten Pharmaceutical (Taipei, Taiwan) have been approved as herbal medicines by Taiwan Food and Drug Administration (TFDA). TFDA accepted GEB manufacturing process, efficacy and safety assurance, quality control method. Extract of GEB was deposited by Kyung Hee University herbarium (Deposit #: KHH-G-0028).

Reviewer #1 (Seung-Tae Kim)

Major compulsory revision: Materials and methods. High Performance Liquid Chromatography (HPLC) analysis of drug. Experimental procedure of HPLC analysis is not clearly described. The manufacturer of HPLC system and the type of detector used in this study were not described, and "solvent B" is inappropriate description and should be replaced with the exact solvent such as water and methanol. Percentage of mobile phase in this study is an ambiguous term. If it meant volume concentration, authors should describe clearly such as “% (v/v)”.

- The manufacturer of HPLC system and the detector used have been described according to the reviewer’s comment. As for HPLC mobile phases, we used two solvents, distilled water (A) and methanol (B), and this is described in the text. However, to avoid confusion, the sentences have been revised and the concentration unit of mobile phase has been corrected as “%v/v” according to the reviewer’s comment.

- (Modified Methods) We performed HPLC analysis to qualify the GEB extract used in this study. Samples of the GEB extract (1 mg) were dissolved in distilled water (1 mL). Samples were filtered and 10 µL of sample solution injected into the HPLC system (1260 infinity HPLC system, Agilent Technologies, Palo Alto, CA, USA) with a UV detector. Samples were analyzed on an Atlantis C18 analytical column (150× 3.0 mm, 5 µm; Waters, Milford, MA, USA). The mobile phase consisted of distilled water (solvent A) and methanol (solvent B). The flow rate was 1 mL/min and gradient elution was used. The gradient elution program...
was as follows: initially 5 % (v/v) solvent B, which was linearly increased to 70% (v/v) at 25 min and maintained up to 28 min. At 29 min, the composition of the mobile phase was returned to initial conditions, which were maintained for 11 min for column re-equilibration. The eluent was monitored at 221 nm.

Discretionary Revisions: The administration of 800 mg/kg GEB extract showed remarkable alleviation of LID in this study. But this dosage may be too excessive for clinical application because a LID patient weighting 60 kg should have 48g GEB extract daily for suppressing LID based on the results.

- Thanks for the comment. It is essential to appropriately translate the drug dosage from animal species to another. An allometric dose translation was usually considered when starting different animal study or clinical research. However, the animal dose should not be extrapolated to a human equivalent dose by a simple conversion based on the body weight. Previous researcher also claimed it and some alternative normalization methods were suggested (e.g. calculate with parameters of biology, including oxygen utilization, caloric expenditure, basal metabolism, blood volume, circulating plasma proteins, and renal function, etc). As a calculation method of this research, we can translate an effective dosage of GEB to 64.9 mg/kg in human subjects (See below). For 60kg weighted patients, GEB is needed to only 3.9 g per day, which is thought to be not too excessive. Further clinical researches are needed to investigate the most effective dose of GEB for human LID patients.

# Calculation methods (adopted from the previous literature, Reagan-Shae et al. 2007)

Table 1. Conversion of animal doses to HED based on BSA

<table>
<thead>
<tr>
<th>Species</th>
<th>Weight (kg)</th>
<th>BSA (m2)</th>
<th>Km factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Adult 60</td>
<td>1.6</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Child 20</td>
<td>0.8</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Rat 0.15</td>
<td>0.025</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mouse 0.02</td>
<td>0.007</td>
<td>3</td>
</tr>
</tbody>
</table>

Values based on data from FDA Draft guidelines. HED, human equivalent dose; BSA, body surface area; Km factor, body weight (kg) divided by BSA (m2)

Formula for dose translation based on BSA.

HED (mg/kg) = Animal dose (mg/kg) multiplied by (Animal Km/Human Km)
Reviewer #2 (Masatoshi Inden)

1. In Fig 2 shows the result of chromatogram for GEB extract sample. The authors focus on the gastrodin. However, it is inarticulate which peak is gastrodin. The authors should clearly indicate the peak of gastrodin in Figure 2. In addition, authors suggest that gastrodin is main biologically active components of GEB against L-DOPA-induced dyskinesia in 6-OHDA-induced experimental Parkinsonism. However, the content of gastrodin in the GEB extract was very low. The authors should discuss the issue.

- The gastrodin peak has been indicated in the Figure 2 according to the reviewer’s comment. Regarding to the reviewer’s second claim, gastrodin, a natural phenol 4-Hydroxybenzyl alcohol 4-O-beta-D-glucopyranoside, has been considered as the main bioactive component of GEB in the previous studies (Zhang et al. 2014, Peng et al. 2013, Wang et al. 2013, Kumar et al. 2013, Shu et al. 2013, Liu et al. 2012, Sun et al. 2012). They showed the effects of gastrodin to improve hypertensive rat’s brain, inhibit allodynia and hyperalgesia, ameliorate depressive/anxiety behavior, stimulate immune response, and protect apoptotic neurons in the brain. Moreover, Shu et al. reported that only gastrodin has a biological effect to stimulate immune response among active ingredients of GEB (Shu et al. 2013). These studies described that even its content in the GEB is low as reviewer’s comment, gastrodin is not only a biologically main active component of GEB, but also has beneficial effects itself on various brain disorders. We also think that gastrodin is a main bio-active component of the GEB, but we modified a definition of gastrodin in the discussion section of manuscript.

- (Modified Figure 2)

- (Modified Figure legend 2) A representative chromatogram for standardization of the Gastrodia elata Blume (GEB) extract sample. The X-axis shows retention time; the Y-axis shows UV absorbance. Arrow indicates peak of gastrodin.

- (Modified Discussion) The phenolic glucoside gastrodin has been used in traditional medicine for the treatment of various diseases and considered as a main bioactive component of GEB in the previous researches.

2. In Fig 2 shows effect of the GEB extract on four subtypes of AIM score induced by L-DOPA. The authors should perform statistical analysis.

- As reviewer’s recommendation, we performed statistical analysis and described in Figure 4 and Figure legend 4.

- (Modified Figure 4)
The effect of GEB extract treatment on each subtype of axial, limb, and orolingual abnormal involuntary movement (AIM) induced by L-DOPA in a PD mouse model bearing 6-OHDA lesions. (A) Axial AIM, (B) limb AIM, (C) orolingual AIM, and (D) locomotive AIM scores were measured on days 2, 5, and 9 after the initial L-DOPA injection. AIM scores were assessed every 20 min over 140 min following L-DOPA administration and were then integrated. *P<0.05 and **P<0.01 compared with the LID group.

3. The author discuss that abnormal activation of proteins, such as pERK1/2, FosB and DARPP-32, induces abnormal dyskinesia in LID animal models. In Fig 5, the authors clearly showed that GEB normalized the abnormal LID-induced increased of pERK1/2 and FosB. It is unclear that GEB normalized the abnormal LID-induced increased of DARPP-32. So, the authors should examine whether GEB normalized the abnormal LID-induced increased of DARPP-32.

- As reviewer mentioned, various proteins have been found to be associated with mechanism of LID, such as cAMP- and dopamine-regulated phosphoprotein of 32kDa (DARPP-32), mammalian glutamate receptor 1 (mGluR1), extracellular signal-regulated protein kinase 1/2 (ERK1/2), mammalian target of rapamycin complex 1 (mTORC1), ribosomal protein s6, mitogen- and stress-activated kinase 1 (MSK-1), transcription factors c-fos, arc, zif268, and so on (Santini et al., 2008). During preliminary trials we tried to find the mechanism of effects of GEB by investigating changes of various proteins related with LID. After testing various proteins, we found that ERK1/2 phosphorylation and FosB to molecularly represent the behavioral changes in our experiment. The other proteins did not show attenuation in conjunction with improvements in LID, which can suggest that GEB relieves LID by mechanism that does not involve changes in other protein levels (DARPP-32, mGluR1, mTORC1, MSK-1, and transcription factors). Further experiment would be needed to investigate what exact mechanism is underlying the effects of GEB. We add this discussion into our manuscript.

Thanks for reviewer’s invaluable comments.

- (Modified discussion) Based on these results, we speculate that GEB attenuates MAPK signaling and FosB expression. This leads, in turn, to normalized neuronal dopamine D1-like receptor overplasticity and attenuated LID. However, this possibility needs more evidence to draw a conclusion, and there is a limitation to figure out exact mechanism of effects of GEB from the results of this study. Further experiment would be needed to investigate what exact mechanism is underlying the effects of GEB.

References for revision


2. Liu W, et al. Gastrodin improved baroreflex sensitivity and increased


