Author's response to reviews

Title: Effects of Eleutheroside B and Eleutheroside E on activity of cytochrome P450 in rat liver microsomes

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Author's response to reviews: see over
Response to the reviewer’s comments and editor’s suggestions for manuscript 1946619519103342

We would like to thank the reviewers’ and editor’s constructive comments and suggestions. We have revised the manuscript point by point according to the comments and suggestions as follow. The changes where we made are all marked with red font.

Response to the reviewer’s comments

Reviewer: Sekhar Surapaneni

1. The type of inhibition is “mixed type” not mix type. Please correct it throughout the manuscript

We have corrected it throughout the manuscript.

2. Although EB and EE inhibit CYP2C9 and 2E1 with Ki of 171.63 and 183.95 µM concentrations, respectively, it is not clear what are the circulation concentration of these in vivo in humans. The relevance of this in vitro inhibition potency needs to be put in context with the concentrations observed in vivo. The true potential of DDI can only be evaluated based on this comparison. Otherwise, it is hard to say there is potential for interaction.

Thanks for this good advice from the reviewer, the majority of drugs are cleared via CYP450 metabolism, the effects on CYP450 activity can lead to DDI. The Conduct of PhRMA reported that inhibition of CYP450 activity is most frequently examined in liver microsomal preparations in vitro [1]. Studies showed the potential of that metabolic drug interaction in vivo can be predicted by in vitro metabolism parameters, and one approach utilizes Ki and inhibitor concentrations in vitro to forecast decrements of clearance caused by co-administration with inhibitor in vivo [2]. The method that predicts potential of DDI based on in vitro parameters has been used in many studies [3, 4].

[3] Jae Wook Ko, Zeruesenay Desta et al., In vitro inhibition of the cytochrome P450 (CYP450) system by the antiplatelet drug ticlopidine: potent effect on CYP2C19 and CYP2D6, J Ethnopharmacol 2000, 49, 343-351
Review: S W Hong

1. Why did you work the effect on CYP450 2D6 and 3A4 in just one substrate (Dextromethorphan and testosterone, respectively): As you know, we can’t obtain enough informations about the effect on CYP450 2D6 and 3A4 with just one substrate because of many polymorphisms and metabolisms.

The substrates we chose to evaluate the effects of EB and EE on CYP450 activity in this study were probe substrates, which were the special indicators of CYP450 activity. As preferred probe substrate of CYP2D6 and CYP3A4 in vitro studies, dextromethorphan and testosterone were considered as the standards to evaluate the effects on the activity of CYP2D6 and CYP3A4 respectively [1].


2. DDI is very important for multiple-dosing. CYP450 of DDI is a key factor. So, we must know about the information on human DDI. However, this manuscript showed me about effects of EB and EE on just rat CYP450 activity in vitro. That’s not enough on interpreting the DDI in human of EB and EE. The authors must discuss about the correlation rat and human species of CYP450 microsome.

We have added discussion about the correlation between rat and human species of CYP450 in the discussion section (page8, line20).

3. ‘introduction’ "lots of chemical, pharmacological and clinical studies on ES have been carried out in the world" - What’s the reference?

Relative references have been added (Reference 42 and 43).

4. How much percent is included EB and EE in Eleutherococcus senticosus (ES)? We need to consider on the exposed dose of EB and EE in usually used dose of ES.

The data about the percent of EB and EE included in ES has been added in the introduction section (page2, line20).
Reviewer: Galia Zamaratskaia

1. Please state in the title that this is a study on rat hepatic microsomes. Material and methods. Please state if all rats were of the same gender or not. Gender can affect inhibition degree (see Zamaratskaia G., Gilmore W. J., Lundström K., Squires, E. J., Effect of testicular steroids on catalytic activities of cytochrome P450 enzymes in porcine liver microsomes. Food and Chemical Toxicology 2007, 45, 676-681.

The title has been changed to “Effects of Eleutheroside B and Eleutheroside E on activity of cytochrome P450 in rat liver microsomes”. We have stated in the Material and Methods section that all rats were male.

2. What was the variation between the duplicates in enzymatic assays?

The variations between the duplicates have been added in Figure 2.

3. What statistical program was used for the analysis?

There was no statistical program used in this analysis. Dixon and Lineweaver-Burk plots were used to get relative parameters such as $K_i$ and $IC_{50}$ in this study.

4. Page 4. 2.4. How the concentrations of substrate were chosen?

As $K_m$ representing the Michaelis-Menten constant associated with the enzyme activity of greatest intrinsic clearance, the concentration of the substrate shouldn't be more than $K_m$. In this study we had established relative kinetic parameters through the preparation before conducting the CYP450 reaction experiments, and the concentrations close to $K_m$ were chosen to get obvious clearance of the substrates.

5. Line 7. Change “4. Conclusion” to Discussion

We have changed it as suggested.

Reviewer: Jing-cheng Tang

1. English should be corrected. Some sentences in the manuscript are obscure and difficult to be understood. eg) 1. Introduction, line 6, “This medicinal plant is not only popular in China and Russia, but among the 10 popular herbal dietary supplements used in the United States [1]”. I suggest having the paper revised by a person with English as their first language.

The language in the manuscript has been edited again, and the grammar
and style of the English have been improved throughout the manuscript.

2. The manuscript contains some typographical errors. eg) 2.3.3. Chlorzoxazone and 6-hydroxylation assay for CYP2E1, line 7, “onto” should be changed to “into”. These should be corrected.

We have corrected the mistakes.

3. In this paper, many data obtained come from HPLC assay, so the authors are supposed to provide the chromatograms with high quality of probe substrate assays for CYP2C9, CYP2D6, CYP2E1 and CYP3A activity.

As the reviewer suggested, high quality chromatograms are important to HPLC assay. We got relative chromatograms by method validation (Figure A, B, C, D, following below). But to avoid the manuscript being verbose, the content of method validation usually doesn’t appear in articles which evaluated activity of CYP450 by HPLC [1, 2]. So just like other studies about CYP450 activity, we didn’t provide relative chromatograms in the manuscript.

[1] Jae Wook Ko, Zeruesenay Desta et al., In vitro inhibition of the cytochrome P450 (CYP450) system by the antiplatelet drug ticlopidine : potent effect on CYP2C19 and CYP2D6, J Clin Pharmacol 2000, 49, 343-351
**Figure A:** Chromatogram of CYP2C9 assay  
(1) 4-hydroxytolbutamide  (2) Phenacetin  (3) Tolbutamide

**Figure B:** Chromatogram of CYP2D6 assay  
(1) Dextrorphan  (2) Phenacetin  (3) Dextromethorphan

**Figure C:** Chromatogram of CYP2E1 assay  
(1) 6-hydroxychlorzoxazone  (2) Phenacetin  (3) Chlorzoxazone

**Figure D:** Chromatogram of CYP3A4 assay  
(1) 6β-hydroxytestosterone  (2) Cortisone acetate  (3) Testosterone

**Response to the editor’s suggestions**

*We feel that your manuscript would benefit from some improvements to the style and grammar of English used.*

Thanks for the suggestion. The language in the manuscript has been edited again, and the grammar and style of the English have been improved throughout the manuscript.