Reviewer’s report

Title: Extreme sensitivity of gene expression in human SH-SY5Y neurocytes to ultra-low doses of Gelsemium sempervirens

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Reviewer: Tadahiro Numakawa

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In this study, the authors showed the modulation of gene expression by the extract obtained from a traditional medical plant Gelsemium sempervirens. Using human neurocytes, they found that mRNAs of 56 genes determined by microarray analysis were decreased or increased by Gelsemium extract, some of which were also confirmed by RT-qPCR method. It is of interest that some mRNA levels were changed even in ultra-low doses of the plant extract. There are, however, several issues that should be addressed before publication.

Comment 1

Fold change in the mRNAs levels regulated by extract of Gelsemium sempervirens are very small (|log2 fold change| is less than 0.8). It is very important to determine whether proteins encoded by the affected genes are similarly changed by high dose (2c) of the extract.

Comment 2

It is also important whether Gelsemium sempervirens-induced changes in the mRNA expressions have physiological significance or not. Although the extract did not affect viability of SH-SY5Y cells in the basal condition, it is possible that this extract elicit a protective effect against cell-death inducing conditions such as serum deprivation or oxidative stress.

Comment 3

In association with Comment 2, Gelsemium may change neuronal response to a transmitter analog carbachol used in this study because genes encoding transcriptional factors and G-protein coupled receptors were affected. Ca2+ response to carbachol in SH-SY5Y cells after the highest dose of extract treatment (2c) should be determined.

Comment 4

In the discussion section, authors mentioned that the modulation of gene expression by ultra-low dose (30c) of Gelsemium could be a nonlinear association between extract dose and gene expression. Although it is difficult to demonstrate chaotic effect in the regulation of gene expression by ultra-low dose of the extract, linearity of the effect of the extract in higher doses can be determined. To confirm this, expression levels of two or three mRNA expressions affected by Gelsemium (2c) should also be determined by RT-qPCR method in
10-3, 10-5 and 3c dilutions.