Reviewer's report

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

Version: 1 Date: 28 June 2013

Reviewer: Iris Bell

Reviewer's report:

General comments: This is an important and carefully done study on the gene expression effects of various ultra low doses of the plant Gelsemium sempervirens. The target cell genes were in neuroblastoma cells.

The question posed by the authors is well defined. The methods are appropriate and well described. The data appear sound and carefully presented. The manuscript adheres to relevant standards for reporting. The discussion and conclusions are well balanced and supported by the data and the contextual literature cited, with the potential enhancement as listed below. The authors acknowledge key work on which they built their study. The title and abstract convey what has been found in an accurate way. The writing is acceptable with the exception of the need for paragraphs that are much shorter and focused on relevant points within a heading or subheading.

Major Compulsory Revisions:

The primary compulsory recommendation is that the authors need to put in paragraphs throughout the text. This is already a dense paper with many essential details, but the lack of visible paragraphs makes it harder to read. Perhaps they were once there, but in the version sent this reviewer, they were not visible.

Discretionary revisions:

The points below will hopefully enhance the paper in context of the current literature.

1. While it is possible to read line by line and discover the contents of the specific controls of the prepared medicines and the controls used to put into contact with the cells, it would be valuable for the authors to write a clear summary statement in the Methods section for the reader less familiar with the issues.

Presumably the homeopathic medicine potencies were prepared in a manner comparable to methods used by commercial manufacturers, i.e. 30% ethanol. They do state below that the cell assay system ethanol concentration was further lowered to 0.03%. It would help for them to add a brief comment to explain that the ethanol at higher concentration might be toxic to the cell culture but that they wanted to have the medicines made in the usual manner of homeopathically—prepared commercial medicines (initially using 30% ethanol). Such details will likely turn out to be extremely relevant as basic science research on
homeopathic medicines progresses. If the medicines are actually crude nanoparticles, every detail of their manufacturing, including ethanol concentration at the time of production will be extremely relevant to interpreting the data and understanding any differences in subsequent replications.

2. In the places of their Discussion where they mention nanoparticles, the authors would want to point out that very low doses of nanoparticles are still biologically very active compared with conventional bulk forms of the “same” material. This makes low dose therapeutic and toxic effects more likely unless a very low dose is utilized when an herb like Gelsemium is made into nanoparticle form. They can cite other studies related to this type of point, including one on nano-hypericum (a different herbal source but one in which doses were reduced simply by creating a nano vs bulk form and testing anti-anxiety effects [1]).

3. Again, to support the plausibility that homeopathic Gelsemium in the potencies tested could contain crudely formed nanoparticles, they could cite some additional papers. The first demonstrates that just extended ultrasonication of plant starch can create starch nanoparticles with purely mechanical means, in water.[2] The second reported electron microscopic evidence of nanoparticles observed in several different plants prepared homeopathically.[3] Third, Gelsemium tincture, like many other plant extracts, can biosynthesize nanoparticles of silver metal from precursor substrate.[4] Finally, using polystyrene containers for the succussions may reduce involvement of previously-reported silica and silica nanomaterials in the final homeopathic medicine product, but will not eliminate the possibility of nanoparticles of polystyrene formed mechanically from the succussion process. Protein can adsorb onto the surfaces of polystyrene nanoparticles as drug delivery vehicles as well.[5] The importance of this model is its potential to explain the biological super-potency of homeopathically-prepared medicines and their ability to exert biologically meaningful effects,[6] including detection of cancer-related genetic point mutations at picomolar concentrations.[7]

4. The authors are quite correct in emphasizing possible mechanisms such as stochastic resonance and time-dependent sensitization in low dose amplification within the complex adaptive system (cell or organism). They may also want to acknowledge the possible involvement of hormesis as an adaptive response process in generating effects that might modulate gene expression.[8-10]

References


**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

Yes, I am a paid consultant to a US-based manufacturer of homeopathic medicines, Standard Homeopathic/Hylands Inc.