Reviewer's report

Title: Potential anti-osteoporotic effects of herbal extracts on osteoclasts, osteoblasts and chondrocytes in vitro

Version: 2 Date: 30 June 2013

Reviewer: Lia Nakao

Reviewer's report:

1. Is the question posed by the authors well defined?
   Yes

2. Are the methods appropriate and well described?
   Some weak points are detailed in the report (below).

3. Are the data sound?
   Some weak points are detailed in the report.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Some weak points are detailed in the report.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   Some weak points are detailed in the report.

6. Are limitations of the work clearly stated?
   Yes

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
   Yes

8. Do the title and abstract accurately convey what has been found?
   Some weak points are detailed in the report.

9. Is the writing acceptable?
   Yes, if revised.

Major Compulsory Revisions

The authors have posed an important question, looking for novel strategies against osteoporosis. They have used cellular and molecular methods to validate the beneficial effects of 3 herbal extracts in osteoclasts, osteoblast, chondrocytes
and bone marrow cells in culture. However, some experimental details have flawd the strength of the major conclusion of this work.

1- Statistical differences are missing in all figures of the manuscript, which does not allow a precise interpretation of the data. Moreover, a semiquantitative analysis should be performed when demonstrating the cytochemical figures.

2- Authors claim that the herbal extracts inhibit proliferation of osteoclasts (see abstract, results and discussion sections). This conclusion was drawn from the fact that the number of cells stained by Crystal violet or formazan were decreased after treatment with the extracts. But there are some inconsistencies in Figures 1 and 2: while treatment with 1 and 10 ug/ml of atratum and azedarach produced less Crystal violet-stained cells than the control condition, 100 ug/ml seemed to produce more stained cells than 10 ug/ml. This behavior does not correlate with the MTT assay results, which showed that those extracts induce loss of viability in a dose-dependent manner. On the other hand, treatment with the 3 concentrations of turtscianinovii seemed to keep the number of cells constant, compared with the control condition (Figure 2), but the MTT result showed that all 3 concentrations decreased cell viability, similarly to the AD treatment. But the AD treatment, in the Crystal violet assay, produced a decreased number of stained cells. Therefore, authors should discuss these data and distinguish growth inhibition from loss of cell viability. To be sure that there is growth inhibition, the number of cells should be plotted (for control, AD, and extracts) versus time (day 0, 1, 2 and 3).

3- The fact that several apoptosis markers are increased after the extracts treatments corroborates that the mechanism of less Crystal violet-staining is due to cell death (and not growth inhibition).

4- Section Results, Assessment of optimal dosage of alendronate used as an apoptosis-inducible control: The sentence These results indicated that a concentration greater than 10 uM induces not only apoptosis, but also necrosis due to cytotoxicity should be reinterpreted. Concentrations above 10 uM decreased viability but did not activated caspases (Figure 1).

5- Regarding osteoblasts, the same comment about growth inhibition mediated by AD: how to distinguish growth inhibition of cell viability loss (Figure 5)? Also, an increased ALP staining (Figure 5) is not so convincing. As commented above, this staining should be semi-quantitated. The differentiation status is much more evident in the activity assay (Figure 6).

6- Regarding the chondrocytes: the ALP staining does not convince (Figure 7).

7- A discussion about the different mechanisms involved in the beneficial effects of the extracts in the cells used here, contrasting with the mechanism of action of AD should be included in discussion.

Minor Essential Revisions
- Section Results, Assessment of optimal dosage of alendronate used as an
apoptosis-inducible control: a concentration of 10 uM AD should be used instead of a dose of 10 uM…

Discretionary Revisions
- Several misspelling are found along the manuscript. Therefore, an ortographic review is needed along the whole text.
- Table 1: it is advisable to mention the accession number of the gene.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:
I declare that I have no competing interests.