Author's response to reviews

**Title:** A new extract of the plant calendula officinalis produces a dual in vitro effect: cytotoxic anti-tumor activity and lymphocyte activation

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**Version:** 2  **Date:** 12 April 2006

**Author's response to reviews:** see over
Dear Editor,

Following the decision taken by the Editors and the comments made by the Reviewers, I am sending to you a new version of our paper “A new extract of the plant *calendula officinalis* produces a dual *in vitro* effect: cytotoxic anti-tumor activity and lymphocyte activation” (MS: 7988736339546422).

The revised version has two new sections in the Results and two new figures. I am also including answers to the different critical comments of the Reviewers. We hope that now our paper can be considered for publication in BMC Cancer.

Looking forward to hearing from you,

Regards,

Angel Miguel Garcia
Response to Reviewer´s comments

Ref: MS 7988736339546422

Reviewer: KYUNG-SUN Kang

Major criticisms

1. As we mentioned in the results, a dose-response curves were obtained between 15 µg/ml and 2 mg/ml, and the results showed that doses of 250 and of 500 µg/ml produced higher biological activities with the same level of response. Therefore, we selected one of these optimal doses, namely - 250 µg/ml - as a dose for the following assays.

2. We have included a new paragraph in the Results:

   “The principal chemical components of the aqueous extracts of calendula Officinalis are: polysaccharides, proteins, fatty acids, carotenoids, flavonoids, triterpenoids and saponins.”

3. We showed in this paper that there were no cytotoxic effects of LACE on normal lymphocytes and NKL cells. In addition, we showed that LACE induced proliferation of these cells. The lymphocytes are the normal cells known to have more sensitivity to cytotoxic agents. These results clearly indicate that LACE is not toxic for normal cells.

4. At the time of the first submission of this manuscript the In vivo tumor regression assays had been under way. At this point we already have the final results which we have included as a new section into Methods, Results and Figures. In addition, a new paragraph to the Discussion is also added. The in vivo experiments indicate that LACE inhibits tumor growth in nude mice.

5. New results, including cyclins and CDKs have been added. A new section in Methods, Results and Figures has been also added, as well as a new paragraph to the Discussion.

Minor Criticisms

1. There was a mistake in table 1 that now we have corrected. Effectively, the MDA231 cell line is MDA MB231, and MDA MB is the same cell line that it has been eliminated in table 1.
Response to Reviewer’s comments

Ref: MS 7988736339546422

Reviewer: Rathin K Bhattacharya

Major Comments

1. The LACE extract was treated with laser and CE extract was not. Both extracts induce in vitro PBL proliferation similarly, but the LACE extract produces in vitro a significant inhibition of tumor cell proliferation. This biological activity is due to treatment with laser radiation, which may induce conformational changes, excitation or degradation of different molecules of the CE extract. Now, we have included a new paragraph to the discussion. Further we are planning to compare the chemical composition of both extracts.

2. We analyzed the apoptosis induction by the caspase 3 activation, annexin-V versus 7-AAD analysis, and DNA fragmentation. However, in the paper we reported only the results of the first two assays because they both clearly demonstrated the induction of apoptosis, indicating the percentage of cells undergoing apoptosis. The DNA fragmentation did not provide this information. Nevertheless, the DNA fragmentation was induced by LACE extract.

3. Using an inverted phase contrast microscope we found that LACE-treated tumor cells have morphological changes: rounded and granulated morphology, some vacuoles coming from cytoplasm, cell shrinkage and detachment from culture plates of large number of cells. A new paragraph has been included in the Results describing these morphological changes. These changes were not observed in lymphocytes treated with LACE.

4. The results of PBLs proliferation are now expressed as Stimulation index (SI: absorbance of treated lymphocytes divided by absorbance of control or unstimulated lymphocytes): SI LACE: 2.5; SI IL2: 4.4 SI conA: 6.5. A new sentence has been included in the Results.

5. Our results indicate clearly that treatment of CE extract with laser increases its tumor inhibitory activity in vitro. As mentioned above, point 1, the laser treatment may induce conformational changes, excitation or degradation of different molecules of the CE extract. The exact molecular mechanism underlying this phenomenon has to be unveiled. In this context, it is important to mention that there is a high content of carotenoids in calendula extract.

Minor Criticisms

1. We have done language corrections and hope that it makes it read easier.