Author's response to reviews

Title: Induction of apoptosis of human primary osteoclasts treated with extracts from the medicinal plant Emblica officinalis

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Version: 3 Date: 3 September 2008

Author's response to reviews: see over
Dear Prof. Puebla,

BMC Alternative and Complementary Medicine

First of all, we like to thank for your opinion and the reviewer’s comments on our manuscript “Induction of apoptosis of human primary osteoclasts treated with extracts from the medicinal plant *Emblica officinalis*," by Letizia Penolazzi, Ilaria Lampronti, Monica Borgatti, Mahmud Tareq Hassan Khan, Margherita Zennaro, Roberta Piva and myself (MS: 8118548132073890).

This manuscript was judged to be acceptable following amendment taking in full consideration all the comments and criticisms raised by the referees.

We found all the comments of the reviewers reasonable and very useful in improving the scientific quality of the manuscript.

Therefore, we emended the paper accordingly (as you see we are sending a red-marked copy outlining all the changes made, including corrections of the English style).

This is the list of the changes made:

1. Lack of quantitative data (major point 1 of reviewer 1). As you can see in the amended version of the manuscript, Figures 1 and 3 have been changed including quantitative data (lower panels of Figure 1 and panel B of Figure 3). Table 1 reports quantitative data on three independent experiments.
2. Description of FAS receptor increase (point 2 of reviewer 1). This has been done with the sentence “These results were confirmed by immunocytochemical analysis of FAS receptor, a well known apoptosis-related protein [57], whose expression increased, as shown in Figure 3C, in OCs treated....” (page 11, lines 14-16).

3. Typographical errors, style and english (minor essential point of reviewer 1, minor point 1 of reviewer 3, and recommendation of the Editor). Spelling of "Atherosclerosis" page 4, introduction, was corrected (minor point 1 of reviewer 3). The text has been carefully checked by us and by an english-native colleague (Dr. Amanda J. Neville, Department of Experimental and Diagnostic Medicine, Section of Medical Genetics) who is acknowledged in the relative section. We of course apologize for the poor english.

4. Apoptosis of osteoclasts versus osteoclastogenesis (point 1 of reviewer 2). We clarify in the text that “as reported in Figure 1, the presence of comparable levels of TRAP-positive cells cultured both in presence and in absence of *E.officinalis* extracts did not affect the process of osteoclastogenesis, at the concentrations employed” (page 10, lines 14-16). However, we discuss that we cannot exclude an effect of *E.officinalis* at higher concentration which, however, exhibit cytotoxicity (see Figure 2), rendering difficult to discriminate between a possible inhibitory effects on OCs differentiation and an overall antiproliferative activity (page 15, lines 6-9).

5. Involvement of NF-kB (point 2 of reviewer 2). Our data, are perfectly in line with the effects on apoptosis and osteoclastogenesis of a decoy oligonucleotide agaings NF-kB trascription factor (reference 46). This was commented in the discussion section (page 15, lines 2-5) by including the sentence “In addition the effects of *E.officinalis* extracts are almost over imposable to those of a decoy double-stranded oligonucleotide mimicking NF-kB binding sites ....... without inhibiting osteoclastogenesis”. In addition the effects found by us are similar to these recently reported by others using NF-kB inhibitors (see also the following point 7).

6. Table 1 (major point of reviewer 3) was missing. We are very sorry for that. Table 1 is important because it reports a summary of three independent experiments, all demonstrating
the ability of *E. officinalis* extracts in inducing OCs apoptosis. Now Table 1 is included.

7. Comparison with to other reported natural product inhibitors of NF-kB (minor point 2 of reviewer 3). We agree that this is an important point to discuss. We have included the sentence “Interestingly, the effects on human OCs .......... genistein” (page 14, last line and page 15, lines 1-2), reporting studies demonstrating induction of OCs apoptosis by treatment with NF-kB inhibitors, such as biphenylcarboxylic acid butanediol ester (ABD56) and genistein. Accordingly, references 61 and 62 have been included.

8. Comment on specificity in terms of apoptosis and NF-kB as macrophages will be exposed to the agent and may overwhelm the effects on osteoclasts (point 3 of reviewer 3). In order to address this point, we have included the sentence “Finally, we like to point out that our results are based on an *in vitro* approach .......... OCs apoptosis are available” (page 15, lines 9-14). Accordingly the new references 63-65 have been added.

9. We included additional abbreviations, that were missing in the submitted original manuscript.

In conclusion, we answered to all of the points reised by the reviewers; therefore, we hope that the manuscript will be now considered acceptable for publication on BMC Alternative and Complementary Medicine.

Waiting for your decision, we would like to express our gratitude for the work done by the reviewers and for their suggestions, that we found very important to help us in improving the presentation and scientific quality of the paper.

Thank you again for your consideration and help.

Sincerely,

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