Author's response to reviews

Title: Anti-Neuroinflammatory Effects Of The Extract Of Achillea fragrantissima

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Author's response to reviews: see over
The Editor  
Complementary and Alternative Medicine

Subject: Revisions of our manuscript MS: 1920292526554902 entitled:

"Anti-neuroinflammatory effects of the extract of Achillea fragrantissima"

Dear Editor,

We have carefully considering the reviewers recommendations and revised our manuscript accordingly. In the attached list, we describe point-by-point the changes made to our manuscript in response to the reviewers comments. We have added two more authors –Hilla Erlank and Alona Telerman - which performed some of the experiments which were added to the revised manuscript.

Independently of the reviewers recomendations, we have added data on the inhibitory effect of the Af extract on matrix metalloproteinase-9 (MMP-9) activity and on intracellular reactive oxygen species (ROS) levels produced by ABAP in our cultured microglial cells.

All changes were marked in grey.

Reviewer Yonk-Ki Park's

1. Figure 1A. Although the LDH assay is the most sensitive assay for viability of cells, we have also measured cell viability by the crystal violet assay. We have added the results to Figure 7A.
   We have also added a detailed description of the commercial colorimetric assay kit (Roche Applied Science).

2. We were not able to perform these experiments in this short time.

3. We were not able to perform these experiments in this short time.

4. We have added the results of the experiments were cell stimulation was performed with different concentrations of LPS (Figure 1A), and also the data of inhibition by the specific iNOS inhibitor L-NMMA (Figure 1B).
5. We have added a histogram of the densitometric analysis (mean±SD) of the 3 independent experiments.

As for the reviewer's comment on the lack of dose response in Figures 2 and 4 (Figures 4 and 6 in the revised manuscript): For each sample for Western blot analysis and PCR we need 5x10^6 microglial cells. This is a very large number of cultured primary microglial cells. An experiment of 3 samples (as presented in these figures) needs 15x10^6 cells. For a larger experiment we will need much more cells which is hard to be obtained (since these are primary cells and not a cell line). Since in all of the other experiments (Figures 1, 2, 4, 6) we show a dose response, we have used in this experiment only one of the active doses.

6. For the reviewer's comment regarding the choice to study of the anti-inflammatory properties of Af, we have added the results of the screening of the ethanolic extracts of 66 desert plants. We have not published these results before. In this screening procedure, Af was the most active plant.

**Reviewer Tong H Joh**

1. We will not be able to perform the LPS injections into the brain in this short period of time. This article is on cell culture studies and not on *in vivo* studies.

2. Our conclusion was not that "the extract may prevent or treat neurodegenerative diseases" as was in the report of reviewer Tong H Joe, but that – as was written in the abstract: "phytochemicals present in the Af extract could be beneficial in preventing/treating neurodegenerative diseases in which neuroinflammation is part of the pathophysiology" or as was written in the discussion: "we suggest that various compounds present in the Af extract might have complementary beneficial bioactivities, and thus propose that Af extracts should be further studied as polyvalent cocktails for nutraceutical development for the prevention or treatment of neurodegenerative diseases".

We believe that the revisions have improved the manuscript for publication in

**Complementary and Alternative Medicine.**
Yours sincerely,

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