Author’s response to reviews

Title: Anti-inflammatory activity of Jefea gnaphalioides (A. Gray), Astereaceae.

Authors:
Axel Villagómez (axelvillagomezrodriguez@gmail.com)
Julia Pérez (jperez@correo.xoc.uam.mx)
Ana Laura Esquivel (aesquivel@correo.xoc.uam.mx)
Cuauhtemoc Pérez (cperezg@correo.xoc.uam.mx)
Claudia Angélica Soto (casoto@correo.xoc.uam.mx)
Salud perez (msperez46@yahoo.com.mx)

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Author’s response to reviews:

October 2th , 2018

Dear Liam Messin
BMC Complementary and Alternative Medicine

We appreciate the comments and suggestions of reviewers to improve the quality of the manuscript BCAM-D-17-01523R1 Anti-inflammatory activity of Jefea gnaphalioides (A. Gray), Astereaceae. We did the next changes:

Rémy Bertrand Teponno (Reviewer 1): The manuscript is well written but I suggest that the authors provide the GCMS chromatogram in addition to Table 1.

We added the GC-MS chromatogram in addition to table 1.

Atallah F. Ahmed, PhD (Reviewer 2):
1. The correlation between the anti-inflammatory effect and the exact chemical constituents of MEJG is insufficient.
   The authors critically relayed on the GC-MS data to prove the chemical composition of the extract, hence their biological relationship and this is not accurate and incomplete. Most of detected
constituents by GC-MS in this work were sugars (e.g. glucopyranose and trehalose) or related components (e.g. quinic acid). I can not believe that plamitic acid (a common fat metabolite of living organisms) and acubin (which ~ 1.2% of the silylated product of MEJG) are responsible for the present significant anti-inflammatory and related mechanisms of this extract.

We find new references to improve the correlation between the anti-inflammatory effect and the composition of MEJG. (References 24, 25, 34, and 35), pg 13 lines 328-332 and pg 15 lines 369-381. They support the relationship between activity and composition.

2. GC-MS method may give a better chemical map if the extract was previously subjected to a higher temperature and a longer time for silylation reaction or by changing temperature/gas flow program of GC. That is why only simple low molecular weight volatile/silylated products could be detected while other complex or aromatic compounds (e.g. flavonoids, triterpenoids, and quinic acid-based phenolic derivatives) were not detected. I advice the authors to consult the following paper: GC-MS Determination of Flavonoids and Phenolic and Benzoic Acids...........J. Agric. Food Chem., 2004, 52 (2), pp 222-227, DOI: 10.1021/jf035073r). The LC-MS or HPLC-UV/VIS methods is much more suitable for detection of the phenolic and flavonoids constituents expected in the extract. Asteraceous plants are considered as rich source of quinic acid based chlorogenic acids and flavonoids. I encourage the authors to generate more scientific evidence by using LC-MS analysis of the intact (non-silylated) extract.

The line 251-259 were included the justification of the use of silylation. The method of derivatization by silylation is one of the most suitable to generate derivatives of all the compounds that contain interchangeable acidic hydrogens. This method is applied normally for compounds containing nitrogen or oxygen with interchangeable hydrogens, which are not volatile or stable at temperatures above 200 or 300 ° C. (Bud-Gen Chen, Sheng-Meng Wang, Ray H. Liu. GC-MS analysis of multiply derivatized opioids in urine, J. Mass Spectrometry, (2007), 42, 1012-1023.9) (Dong-Liang Lin, Sheng-Meng Wang, Chih-Hung Wu, Bud-Gen Chen and Ray H. Liu. Chemical derivatization for the analysis of drugs by gc-ms — a conceptual review. Journal of Food and Drug Analysis, (2008), 16, 1, 1-10.). In this case flavonoids as phenolic compounds will be good candidates to give the silylation reaction in a quantitative way. For this reason we think that LC-MS analysis is not necessary. Also we did the chemical screening for flavonids (Shinoda, Marini-Bettólo, sulfuric acid and Constantinescu test), and they were negative, pg 5 lines 133-135 and pg 10 line 253.

3. Measure the in vitro antioxidant activity (using DPPH radical scavenging, b-cartotene bleaching, ABTS, and/or TEAC assys) which should have been done prior to the in vivo study. Also, a phytochemical screening of this extract is required, particularly it was not previously reported for this plant. I believe that the flavonoid and phenolic constituents are majorly related to the anti-inflammatory activity shown in this work. Therefore, inclusion or exclusion of this fact is extremely important to make this manuscript acceptable for publication in BMC-CAM.

We determined the antioxidant activity of MEJG using DPPH radical scavenging, pg 6 line 151-162; pg 11 lines 265-268 and pg 13 lines 318-323.

4. If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES_BS) and American Journal Experts (http://bit.ly/AJE_BS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our
English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover common mistakes that occur when writing in English.

We are sending you the certificate of English for American Journal Experts, who review the manuscript.

We hope that with these corrections the manuscript will be suitable to be published in your prestigious journal.

Sincerely Yours

Dr. Salud Pérez