Author’s response to reviews

Title: Therapeutic Effects of Hydro-Alcoholic Extract of Achillea Wilhelmsii on Indomethacin-Induced Gastric Ulcer in Rats: A Proteomic and Metabolomic Approach

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Author’s response to reviews:

Dear Associate Editor

BMC Complementary and Alternative Medicine

We are very excited to have been given the opportunity to revise and resubmit our manuscript entitled “Therapeutic Effects of Hydro-Alcoholic Extract of Achillea Wilhelmsii on Indomethacin-Induced Gastric Ulcer in Rats: A Proteomic and Metabolomic Approach” (BCAM-D-19-00414R3). We want to extend our appreciation for taking the time and effort to provide such insightful guidance. We carefully considered constructive comments and insightful queries raised by you and reviewers.

Before of all, I would to thank reviewers for critically reading and rising valuable points in my manuscript. I am so thankful for spending your time and I am sure this will help me to enrich the paper draft. In the revised version of this manuscript, we used highlighted items in order to highlighting our changes in the manuscript. We have addressed each of your concerns as outlined below.
Reviewers' comments:

Peter Achunike Akah (Reviewer 3)

- It is not debatable that the subtype of muscarinic receptor in the parietal cell mediating acid secretion is the M1 and not M2 receptors. Therefore stating otherwise as in your introduction is misleading to the reading public especially those not familiar with autonomic pharmacology.

Response: With appreciations for your considerations. For more clarity, we have revised this part as follow:

Muscarinic receptors located on the parietal cells which mediate acid secretion, are of the muscarinic M3 subtype. A Brazilian study has reported that stimulation of muscarinic receptors (M3) of parietal cells rises the levels of gastric peptides, which leads to histamine secretion, and reduction of blood flow to the stomach mucosal layer. It would in turn, increase gastric secretion and reduce the protective factors of the stomach. Furthermore, the authors of that study suggested that the hydro-alcoholic extract of this species reduces the volume and acidity of the gastric juice via blockage of the main receptors presented in the parietal cell This paragraph have added to (introduction section, line 70-77, page 4) in revised manuscript.

- The method of LD50 determination is far from been clear. It is not a standard procedure unless it was invented by the authors. In this case, the procedure should be fully described, indicating how many animals/group and why only 3 doses of the extract were used. LD50 testing conventionally employs more than 3 doses, and the last dose in this case should be more than 800 mg/kg. How the LD50 was calculated has to stated.

Response: Thank you for your valuable considerations. Regarding our previous explanation for LD50, I would like to add that the acute toxicity LD50 of AW carried out on wistar mice. The animals were divided in 6 (A-F) groups with 5 mice in each group. The animals were fasted overnight before the experimentation. Group A was control group and B-F were treated groups with 100, 200, 400, 800 mg/kg and 1200 mg/kg dose of AW, respectively. Symptoms for toxicity and death were observed within 24 hours in each group. The number of death in each group in 24 hours were noted. There was no differences in lethal parameters between control and AW treated group. According to this assay, it was assumed that AW LD50 dose is above 800 mg/kg. Although, we demonstrated in one study that dose of 800 mg/kg of Achillea wilhelmsii significantly reduced acid secretion and ulcer index in comparison to doses of 200 and 400 mg/kg (ref 17). Therefore, we selected dose of 800 mg/kg of Achillea wilhelmsii extract.
- It is inappropriate to use ranitidine as the positive control in this study since indomethacin-induced ulcer is not mediated via release of histamine. Indomethacin like other NSAIDs is a COX enzyme inhibitor.

Response: You raised a good point. Various synthetic antiulcer drugs are presently available and some of these ranitidine is employed to manage and cure NSAID induced gastric ulcer. According to previous investigations that used ranitidine as a positive control (Minaiyan et al., 2018, Katary et al., 2017 and Inas Z et al., 2011), we also selected ranitidine as a positive control drug for this study.


- Your conclusion is that AW should be developed for the therapy of indomethacin-induced ulcer. In my view, this is very specific and narrow. Indomethacin-induced ulcer is not as common as ulcers from other causative factors like alcohol, stress, other drugs, etc. hence the use of other models of ulcer is imperative.

Response: Thank you for this point. The purpose of our study is to evaluate the model of indomethacin-induced gastric ulcer, so the conclusion of our study is based on this model and further studies are required to evaluation of the effect of Achillea wilhelmsii on other causative factors like alcohol, stress, and other drugs.

Sajjad Ahmad, Ph.D (Reviewer 5)

1. The English language should be improved by native speaker and the changes made should be highlighted accordingly.

Response: Thank you for this point. The revised manuscript reviewed and corrected grammatical errors by native English speaker, which highlighted as blue in revised manuscript.
2. The bibliography should be rectified as per the journals' guidelines.

Response: Thank you for valuable point. Based on your advice, we have corrected the bibliography in accordance with the guidelines of journal and amendments performed in the revised manuscript.

3. The dendrogram in the figure should be made clear to get visible.

Response: Thank you for your valuable and insightful comment. Based on your advice, we have corrected the dendrogram in the figure 11.

A.H.M. Khurshid Alam, PhD (Reviewer 6)

The manuscript can be accepted in this journal.

Again, we appreciate the opportunity to revise our manuscript for consideration in your journal. We hope our revision meet with your approval.

Fatemeh Goshadrou,

Best Regards