Author's response to reviews

Title: Antidiarrhoeal activity of Matricaria chamomilla is mediated predominantly through K+-channel activation

Authors:

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Version: 3
Date: 21 December 2014

Author's response to reviews: see over
Dear Editor-in-Chief

“BMC Complementary and Alternative Medicine”

I am pleased to submit our revised manuscript (Manuscript ID: 3306270991278940) entitled “Antidiarrhoeal activity of Matricaria chamomilla is mediated predominantly through K⁺-channels activation” for your kind consideration in “BMC Complementary and Alternative Medicine” as suggested. We followed the instructions to author, while preparing this manuscript. The principal author and all co-authors hereby transfers, assigns and conveys all interest in and ownership of copyright until such time and unless said manuscript is rejected in writing by the Journal.

We would like to state that due care has been taken to ensure the integrity of our work and the scientific reputation and that there are no financial or contractual agreements or obligations linked to the paper that might cause conflicts of interest.

We look forward to hearing your decision on this manuscript.

With kind regards

Sincerely

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Reply to Reviewer’s comments

Reviewer's report # 1

Title: Antidiarrhoeal activity of Matricaria chamomilla is mediated predominantly through K+-channel activation
Version:2 Date:20 October 2014
Reviewer: Vincenzo De Feo

Reviewer's report:
The manuscript entitled “Antidiarrhoeal activity of Matricaria chamomilla is mediated predominantly through K+-channel activation” authored by Malik Hassan Mehmood, Siraj Munir, Uzair Ali Khalid, Mudassir Asrar, Anwarul Hassan Gilani falls within the scope of the Journal. The study was nicely designed and conducted, the findings are potentially interesting. However, this reviewer has the following major and minor comments for the manuscript.

We are grateful to the respected reviewer for appreciation and giving us opportunity to respond.

Major comments:
- Authors investigated whether oral administration of the crude extract of Matricaria chamomilla (Mc.Cr) at 150 mg/kg and 300 mg/kg to mice could show antidiarrhoeal and antisecretory activities against castor oil-induced diarrhoea and fluid accumulation. Simultaneously, the Authors have investigated in vitro the potential antispasmodic activity of Mc.Cr.
  The in vitro findings are potentially interesting but are not adequately supported by in vivo evidences.
- Authors should test the effect of Mc.Cr at different dose in order to have a complete and representative dose responsive curve. In fact the effect of Mc.Cr at dose of 150 mg/kg seems to be not significant compare to 300 mg/kg. Moreover, Authors should even test in vivo the effect of a K+-channel antagonist prior the addition of Mc.Cr in order to provide a mechanism of action. Reviewer strongly recommend to perform these in vivo experiments.

Reply: As suggested, we have conducted additional in vivo experiments in the presence of K+ channel antagonists and the results are incorporated in the revised manuscript.

Minor comments:
- The manuscript requires an English revision. There are significant typographical and grammatical errors in the text. (See Manuscript title, See Abstract, line 38-39, line 46-50, line 55-57; See Introduction, line 94-96; See Conclusion, line 313-316).

Reply: The whole manuscript has been critically read and revised as indicated in red color and we feel that the quality of manuscript has been significantly improved.
- Authors should insert adequate references after the second and third period of the Introduction section. Moreover, They should reframe in the Abstract and in the Introduction the objective of the study in order to facilitate reader's comprehension.

Reply: As per suggestion, additional references have been incorporated in indicated paragraphs of introduction section and the objective of the study has been revised in Abstract and Introduction.

- Authors should rewrite the manuscript title and related running title that seem to be not representative for their study.

Reply: As suggested, main title and the running title have been modified accordingly in revised manuscript.

- Authors should revised manuscript format (see line 326-335).

Reply: This has been revised accordingly.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being Published.

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests

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**Reviewer’s report # 2**

**Title:** Antidiarrhoeal activity of Matricaria chamomilla is mediated predominantly through K+-channel activation

**Version:** 2  **Date:** 28 October 2014

**Reviewer:** Raffaele Capasso
Reviewer's report:

The paper is interesting.
I have some question regarding this paper:

1. The extracts should contain a type of standardization (e.g. fingerprint).

2. The manuscript would benefit from inclusion of introducing/bridging sentences between the individual parts of the "Results" that explain the logical order and rationale for the experiments

Reply: We appreciate this suggestion and bridging sentences have been incorporated between individual parts of results (highlighted in red colored text).

3. In the Discussion, the Authors should highlight the possible clinical significance of their findings

Reply: We have highlighted the clinical significance of the study as suggested

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my

Reviewer's report # 3
Title: Antidiarrhoeal activity of Matricaria chamomilla is mediated predominantly through K+-channel activation
Version:2 Date:2 November 2014
Reviewer:G.L. Viswanatha

Reviewer's report:
The manuscript looks to be interesting, however, it largely fails in terms of novelty. does not deliver enough scientific message to the science community. The methodology followed in the present study is not up to the standards of current scenario, example. Castor oil-induced diarrhea is non-specific model for diarrhea, as per standard references, Loparamide is reference drug used by most
of authors, the evaluation parameters such as Number of fecal droplets, Rating of based on the fecal consistency and presence or absence of mucose, Cumulative wet fecal mass, are commonly used parameters to conclude the antidiarrheal potential of a drug.

Reply: We are thankful to the reviewer for critical review of our manuscript. We have tried our best to address all the relevant concerns and revised our manuscript accordingly.

This study will be the first report showing the effectiveness of this popular indigenous herb in diarrhoea with possible mechanism explored, which is in line with the scope of this journal “BMC Complementary and Alternative Medicines”

-We agree with the reviewer that “Castor oil-induced diarrhea is non-specific model for diarrhea, as per standard references, Loparamide is reference drug used in majority of studies, the evaluation parameters such as Number of fecal droplets, Rating of based on the fecal consistency and presence or absence of mucose, Cumulative wet fecal mass, are commonly used parameters to conclude the antidiarrheal potential of a drug”.

-We have revised this part of our study by revising in-vivo experiments as per suggestion of the respected reviewer using six animals in each group, loperamide as standard drug was also used in revised experiments. As per suggestion of the reviewer, the evaluation parameter as the number of feces has been used in revised protocol.

In addition, by taking suggestion of Reviewer #1, we have also restudies the antidiarrhoeal and antisecretory activities of our test material in the absence and presence of K⁺ channel antagonists.

Fasting state mentioned is not given consistently across the manuscript, in one place 12-16h and other place 24hours mentioned.. which is correct? In performing in vitro experiments authors have used 10% DMSO which is not at all acceptable as per the references 10% DMSO is toxic and itself will interfere in the tissue structure and response. How it was managed? how authors have negotiated the solvent effect?

Reply: We are thankful to the reviewer for pointing out this typo-mistake regarding fasting state of the animals, we have rectified accordingly in the revised manuscript.

We agree with the reviewer that 10% DMSO as final intact concentration is toxic. However, in our study, the mentioned (10%) concentration of DMSO has been used to prepare the main stock solutions like 1M for GB and 1M for 4-AP, the
final concentration of DMSO in heighted tested volumes/concentrations of respected drugs in tissue bath was not more than 0.1%. As the required concentration of GB (10 µM) or 4-AP (1 mM) were taken from respective sub-stocks of $10^{-3}$ M and $10^{-1}$ M, resulting in 0.01% and 0.1% of DMSO concentration in test doses which were even further diluted when poured in tissue organ bath containing 10 ml physiological salt solution. Such concentrations of DMSO are documented safe to be used in similar experiments (Dar et al., 1999; Satoh et al., 2001).

In plant extraction procedure' plant material weighing 400 g was used for extraction and mixed in 70% methanol for three days' is mentioned. however in reporting percentage yield it is mentioned 'water extract', which is highly confusing and misleading.

Reply: We are sorry for this typo mistake, it has been rectified accordingly

What is the basis for dose selection ? on what basis 150 and 300 mg/kg doses were selected?

Reply: In order to determine minimal effective dose against castor oil-induced diarrhoea in mice, we conducted a pilot study on 12 animals. These were divided in to 4 groups of 3 animals each. The doses of 50, 100 and 150 mg/kg were administered to mice while the forth group worked as castor oil treated group only (the data not shown in the manuscript). On the basis of observation from this pilot study, we identified the optimal dose (150 mg/kg and above)

Was acute toxicity study performed for the extracts? In yes report the LD50 value. Acute toxicity study is important and compulsory to be performed before the efficacy study.

Reply: We agreed with reviewer and performed acute toxicity study in mice. The plant extract did not exhibit lethality up to the dose as high as 5 g/kg. Obviously assessing LD$_{50}$ was not possible with plant extract showing high safety margin, as the LD$_{50}$ using Probit analysis requires a wide range of doses, which is best suited for pure compounds requiring lesser doses.

Intestainal accumilation/enteropooling is best studied with models such as PG-E2 induced enteropooling in rats/mice. However, authors have mentioned Castor-induced model as separate methods. Which is one at the same.

Reply: We agree with the reviewer that PG-E2 induced enteropooling in rats/mice is also an other appropriate assay for the assessment of such in-vivo assays, however, the castor oil-induced enteropooling models has also been used
extensively in similar experiments (Palla et al., 2014; Méité et al., 2010; Mehmood and Gilani, 2010; Capasso et al., 1994; Capasso et al., 2002). Authors have used '5' animals per group in castor oil induced diarrhoea model. for statistical comparison and interpretation at least 6 samples with minimum SEM is required. Which is one of the major drawback of the method.

Reply: We have redesigned our study and performed in-vivo experiments with the desired number of animals.

Was phytochemical analysis performed on the extarct? What is the nature of actives present in the extract? Which is active responsible for the claimed activity?
Detailed LC-MS/MS analysis or at least HPLC or UPLC should be submitted inorder to identify the actives responsible for the activity.

Reply: We are sorry, our lab is not equipped with sophisticated phytochemical set ups, hence we did not perform phytochemistry of this plant material. However, we have a plan to carry this forward, therefore, currently we are in process of establishing collaboration with phytochemists for further studies in this regard. In current study, our focus was to determine the pharmacological basis for the medicinal use of this plant in hyperactive gut disorders like diarrhoea. There are many similar studies recently published in this journal without detailed phytochemical investigations (Muhammad et al., 2013; Chaudhary et al., 2012; Bashir et al., 2011; Mehmood et al., 2011).

Language of the manuscript needs to be improved as per the journal standards. Schild plot analysis of the in-vitro experiments would give perfect nature or type of antagonism. EC$_{50}$ is not a standard value, it seems to be old method of reporting.

Reply: The language is improved in the revised manuscript. We agree with the reviewer that Schild plot analysis of the in-vitro experiments is the best way to quantify the potency of the test material, but to our understanding it is meant for the antagonist of competitive nature as we applied in our pioneer work on subtypes of muscarinic receptors (Gilani and Cobbin, 1986; 1987; Gilani et al., 1997), while our results in this study indicate the agonist activity for which we preferred using EC$_{50}$ value format, which is well-accepted and has been adopted frequently in similar studies published in such journals (Palla et al., 2014; Mariana et al., 2012; Mehmood et al., 2011A; Gilani et al., 2010). However, if the reviewer insists, we are prepared to convert this to pD$_2$ value, though we feel, this type of format would suit better in a typical Pharmacology Journal.

Level of interest: An article of limited interest
Quality of written English: Not suitable for publication unless extensively edited
Statistical review: Yes, and I have assessed the statistics in my report
References


Mehmood MH, Siddiqi HS, Gilani AH. The antidiarrheal and spasmylytic activities of *Phyllanthus emblica* are mediated through dual blockade of muscarinic receptors and Ca++ channels. J Ethnopharmacol. 2011, 27;133: 856-65.


