Author's response to reviews

Title: Studies on prokinetic, laxative and spasmodic effects of Fumaria parviflora of Arabic origin with species and tissue-specificity

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Version: 2 Date: 16 February 2012

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

“BMC Complementary and Alternative Medicine”
I am pleased to submit our revised manuscript entitled “Species and tissue-specificity of prokinetic, laxative and spasmodic effects of *Fumaria parviflora*” for your kind consideration in “BMC Complementary and Alternative Medicine”. 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
Title: Studies on prokinetic, laxative and spasmodic effects of Fumaria parviflora of Arabic origin with species and tissue-specificity
Version: 1 Date: 29 November 2011
Reviewer: GERALD NGO T TEKE

Reviewer's report:
Minor essential revisions
Authors should review the language for proper usage of articles for the entire paper.

Reply: As per suggestion, we have critically reviewed the language for the entire manuscript and made corrections.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Reviewer’s report
Title: Studies on prokinetic, laxative and spasmodic effects of Fumaria parviflora of Arabic origin with species and tissue-specificity
Version: 1 Date: 27 December 2011
Reviewer: Télesphore Benoît NGUELEFACK
Reviewer’s report:
See the attached file
Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests
Attached Comments on the Manuscript
Dear Editor
I am very pleased to send my report on the manuscript submitted by Najeeb-ur-Rehman et al. The manuscript entitled “Studies on prokinetic, laxative and spasmodic effects of Fumaria parviflora of Arabic origin with species and tissue-specificity” is showing that the plant extract possess cholinergic-like pharmacological activities. Although the work was well planned, the paper needs some amendments before it can be accepted for publication.
We appreciate the positive comments
Title
From the literature, no previous work has been carried out on Fumaria parviflora. Why did the authors specify “of Arabic origin” in the title? Additionally, the paper is presenting the results not the study. I then suggest the following: “Species and tissue-specificity of prokinetic, laxative and spasmodic effects of Fumaria parviflora”
Reply: The title has been modified as suggested, “Species and tissue-specificity of prokinetic, laxative and spasmodic effects of Fumaria parviflora”
Abstract
The methodology section is not informative. The authors should instead indicate what was done in the in-vitro experiment.
Reply: We have revised the methodology section in the abstract with following addition.
“Isolated intestinal preparations (ileum and jejunum) from different animal species (mouse, guinea-pig and rabbit) were separately suspended in tissue baths containing Tyrode’s solution bubbled with carbogen and maintained at 37 °C. The spasmogenetic responses were recorded using isotonic transducers coupled with PoweLab data acquisition system”
Introduction
How can the same plant be used both for constipation and for diarrhea? Do they use different plant material or different preparation? Please clarify this information.
Reply: We agree, that it is unusual to have laxative and antidiarrheal activities present in one remedy, because constipation and diarrhea are two opposite disease states of the gut. There is no chemical drug available with such dual actions. However, surprisingly, some of the medicinal plants such as Ispaghula (Mehmood et al., 2011), ginger (Ghayur and Gilani, 2006) and garden cress (Rehman et al., 2011) are known to show dual activity when studied in-vivo and possess both gut stimulant and gut relaxant components. It is possible that the gut stimulant component becomes active when subject is constipated and the relaxant component is more active in the hyperactive gut state, such as diarrhea. Moreover, the coexistence of both the gut stimulant (at lower doses) and gut relaxant components seen at slightly high doses probably meant by nature to offset the excessive stimulation that is usually observed with chemical drugs at high dose (Brown and Taylor, 2006). This has not been added in the manuscript.


The last sentence of the second paragraph seems to be incomplete. Reply: The indicated sentence has been revised and incorporated in the text as; In this study, we showed for the first time that Fumaria parviflora possesses prokinetic and laxative properties in the in-vivo models and the detailed study on the possible mode of action was carried out using the in-vitro experiments involving different species and tissues. (Page # 3, background, paragraph # 2, line # 7-10)

Methods

Chemicals

What did the authors use histamine hydrochloride and 5-hydroxytryptamine for? Reply: Histamine (Histaminergic receptor agonist) was used as a control when the spasmodic effect of the plant extract was studied in the presence of pyrilamine (Histaminergic receptor antagonist). Similarly, 5-hydroxytryptamine (Serotonergic agonist) was used as a positive control when the spasmodogenic effect of plant was studied for the possible involvement of serotonergic like activity. (This has not been added in the manuscript)
Animals
I wonder that rabbits could be kept in this cage. I believe that this information is useless as well as the animal food composition unless this composition has a particular effect on the experiment (in which case it should be stated). In this section, the authors stated that animals were starved for 24 h but we notice different times according to the experiment.

Reply: By considering the indicated points, we have modified sentences in the animal section and added in the text accordingly as;
“The animals were kept in respective standard cages and were fasted accordingly before starting the experiments, while in routine they had free access to feed and water” (Page # 5, Paragraph # 3, line # 3-5)
The detail of feed composition has been removed from this section.

Laxative activity
For how long were the parameters (total number of feces and number of wet feces) evaluated?

Reply: The indicated parameters have been evaluated for 18 h, by following the protocol described in earlier study [15], as highlighted in the text (Page # 6, Paragraph # 2, line # 7-10).
Moreover, similar protocols (Rehman et al., 2011; Haruna, 1997; Mascolo et al., 1994) have been used in different laxative assays.

References:


Results
Effects of Fp.cr on ileum preparation
Why this discrepancy on the number of experiment? Beside, 3 experiments are to low for statistical analysis and you cannot have 3 repetitions from 4 different animals.

Reply: The reviewer has rightly indicated and it was a typo mistake. We have changed the sentence as;
“The values determined in Fig. 2A have been acquired from 4-7 separate experiments carried out on the isolated tissues of 4 animals.” (Page # 9, Paragraph # 2, line # 4-6)

Discussion
The discussion is too light. Why did the authors use different animal species? Why did they use different tissues? What is the difference between the tissues and the species used that could explain the different sensitivity to the plant extract? The authors should address these questions in the discussion.

Reply: As per reviewer suggestion, we have explained this point in the discussion at page #11-13 and we feel it was a good suggestion.
For detail, please look at:
Page # 11, Paragraph # 2, line # 10-12
Page # 12 and 13, Paragraph # 2, line # 2-18
Reviewer's report
Title: Studies on prokinetic, laxative and spasmodic effects of Fumaria parviflora of Arabic origin with species and tissue-specificity
Version: 1 Date: 7 December 2011
Reviewer: M. Nabeel Ghayur

Reviewer's report:
Najeeb-ur-Rehman et al. present some interesting findings on the laxative and gastrointestinal motility enhancing effects of crude extract of Fumaria Parviflora (Fp.Cr). The pharmacological finding that Fp.Cr shows laxative effect in-vivo and in-vitro does not only match to its traditional use as a laxative agent, but the authors have interestingly shown how the extract shows tissue and species specificity in the many in-vitro tissue preparations used to determine the mechanism of stimulatory effect of the extract. The methodology used is adequate while the analysis and interpretation is also up to the mark, but before accepted, the Reviewer has some concerns that need to be addressed:

Major concerns:
1. The author have shown tissue and specie selectivity of the extract for its in-vitro stimulant effect. There is another specificity it the results as shown by the authors. Can the authors explain why the extract effect was only partially blocked by atropine in the mice in-vivo tests of laxative activity and charcoal meal transit, while the effect of extract was completely blocked in mice in-vitro by atropine? This shows difference of results even at the ‘in-vitro’ vs. ‘in-vivo’ level. Please add and discuss this point in the Discussion.

Reply: We have addressed this point and incorporated in the text as;
“While atropine completely blocked the stimulatory effect of plant extract in the isolated gut tissues of mice, however, only partially blocked the prokinetic and laxative effects of the plant extract in the in vivo studies, which needs explanation. It is possible that the plant extract causes release of some endogenous gut stimulant mediator(s), other than cholinergic in nature.” (Page # 11, Paragraph # 3)

2. The authors have shown that the extract possesses some alkaloids in it. Alkaloids have been shown to have gut stimulant effect. Please add this point in the discussion.

Reply: This has been added in the text accordingly. (Discussion, page # 13, paragraph # 3, line # 1)

3. In Figure 2, a graph for the effect of Fp.Cr on rabbit ileum is not provided. Although the extract showed no effect on rabbit ileum, it would be good to include a graph here, by even using a flat line, to show that the extract was devoid of any effect. It will provide the readers with a complete view of the activity of the extract.

Reply: By following reviewer’s suggestion, we have incorporated a graph showing the effect of Fp.Cr on rabbit ileum labeled as (C) and also added in the result section as Fig. 2C. (Results, Page # 10, Paragraph # 1, line # 1 and 2)

Minor concerns:

1. Abstract, Methods, line 2: change “were suspended in tissue bath” to “were separately suspended in tissue baths”

Reply: It has been changed accordingly.

2. Abstract, Results, line 3: change “activities in mice” to “activities in vivo in mice”

Reply: In the Abstract, Results, line 3, by following reviewer suggestion, “activities in mice” has been replaced with “activities in the in vivo in mice”

3. Abstract, Results, line 3: change “In isolated gut preparations of mouse and rabbit” to “In isolated gut preparations of mouse (jejunum and ileum) and rabbit (jejenum and ileum)”

4. Kindly clearly add in the ‘Abstract’ (in ‘Results’ sub-section) that ‘Fp.Cr was devoid of any effect in rabbit ileum’.
Reply to 3 and 4: By considering the raised queries, a part of the text in Abstract, Results, Line # 3-6 has been modified as:

“In the in vitro studies, Fp.Cr (0.01-1 mg/ml) caused a concentration-dependent atropine-sensitive stimulatory effect both in mouse tissues (jejunum and ileum), and rabbit jejunum but had no effect in rabbit ileum.”

5. Background, paragraph 2, line 7: change “we showed first time that the Fumaria” to “we showed for the first time that Fumaria”

Reply: In the Background, paragraph # 2, line # 7, by following reviewer suggestion “we showed first time that the Fumaria” has been replaced with “we showed for the first time that Fumaria”

6. Methods, Extraction procedure, line 3: change “NO.1” to “No.1”

Reply: In the Methods, Extraction procedure, line # 3, as per reviewer suggestion, “NO. 1” was replaced with “No. 1”

7. Methods, Extraction procedure, line 5: change “under a reduced pressure to obtain finally, the crude extract of the aerial” to “under reduced pressure to finally obtain crude extract of aerial”

Reply: In the Methods, Extraction procedure, line # 5, as per reviewer suggestion, “under a reduced pressure to obtain finally, the crude extract of the aerial” has been replaced with “under reduced pressure to finally obtain crude extract of aerial”

(Methods, Page # 10, Paragraph # 1, line # 1 and 2)

8. Methods, Extraction procedure, line 7: change “wt/wt” to “w/w”

Reply: In the Methods, Extraction procedure, line # 6, as per reviewer suggestion, “wt/wt” has been replaced with “w/w”

9. Methods, In-vitro experiments, last line: change “ranged” to ranging”

Reply: In the Methods, In-vitro experiments, last line, as per reviewer suggestion, “ranged” was replaced with “ranging”

10. Results, Effect of Fp.Cr on ileum preparation of different animals, line 1: delete “In mouse ileum” as you have also used this phrase in the line #2
Reply: In the Results, Page # 9, Effect of Fp.Cr on ileum preparation of different animals, Line # 1) as per reviewer suggestion, “In mouse ileum” has been deleted.

11. The abbreviation for “crude extract of Fumaria parviflora” as “Fp.Cr” was first described by the authors in ‘Extraction Procedure’ section of the paper. Throughout the later parts of the paper, wherever the authors have used the phrase (or a similar phrase) “crude extract of Fumaria parviflora” again, please change that with the abbreviation “Fp.Cr”. This will save space and will give more consistency to the text.

Reply: As per reviewer suggestion, the words “Crude extract of Fumaria parviflora” has been changed to “Fp.Cr” throughout the text.

12. Similarly to the above point, the authors have described the abbreviation of “Acetylcholine” and “Carbachol” as “ACh” and “CCh”, respectively, in the ‘Chemicals’ section. Next they have done this again in the ‘Charcoal meal gut transit test’ and in the ‘In-vitro experiments’ sections; please do this only once. Also, in the later text throughout the paper, use only the abbreviation ‘ACh’ and ‘CCh’ and not the whole word. This will give more homogeneity to the text.

Reply: As per reviewer suggestion, the words “Acetylcholine” and “Carbachol” has been changed to “ACh” and “CCh” throughout the paper.

13. Results, In-vitro findings, Effect of Fp.Cr on jejunum preparation of different animals, line 2: where it says “being less potent than in mouse ileum”, please give the attesting statistics or a ‘p’ value to prove this point.

Reply: As per reviewer suggestion, P-value has been provided. (Page # 10, under subheading of Effect of Fp.Cr on jejunum preparation of different animals, Line # 2)

14. Discussion, paragraph 2, line 10: change “[14], and ispaghula” to “[14], ispaghula”

Reply: It has been revised as suggested and also highlighted in the text.

15. Discussion, paragraph 4, line 6: change “both rabbit and mouse” to “both rabbit jejunum and mouse”

Reply: In revised version, the Discussion, Page # 12, Paragraph # 2, line # 2 and 3, as per reviewer suggestion, the sentence containing “both rabbit and mouse”
has been replaced with “The observed stimulatory effect of the extract was fully atropine-sensitive in rabbit jejunum and mouse preparations”

**Level of interest:** An article of outstanding merit and interest in its field  
**Quality of written English:** Acceptable  
**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.  
**Declaration of competing interests:**  
I declare that I have no competing interests

**Title:** Studies on prokinetic, laxative and spasmodic effects of Fumaria parviflora of Arabic origin with species and tissue-specificity  
**Journal:** BMC Complementary and Alternative Medicine  
**Type of article:** Research article  
**Authors:** Najeeb U Rehman, Malik H Mehmood, Adnan J Al-Rehaily, Ramzi A. A Mothana, Anwar H Gilani

**Reviewer:** GERALD NGO TEKE

**Comments:**
1. The language should be reviewed for proper usage of articles.  
**Reply:** As per suggestion, we have critically reviewed the language for the entire manuscript.

2. Authors did not isolate compounds *Fumaria parviflora* and evaluate their effects on the studied parameters. So the last section under background should be looked into.  
**Reply:** A part of the last paragraph in the background “but none of these compounds to our best knowledge has been reported to possess laxative or prokinetic effect” has been modified as;  
“However to the best of our knowledge, none of the reported compounds or the parent plant has been studied for its laxative or prokinetic activity” (Page # 3, Paragraph # 3, Line # 4-6)

3. Under the section of animals…the animals were fasted for 24 h. this contradicts with 12 h (gut transit activity) and 6 h (laxative activity). These aspects should be taken into consideration.  
**Reply:** By considering the indicated points, we have modified sentences in the animal section and added in the text accordingly as;  
“The animals were kept in respective standard cages and were fasted accordingly before starting the experiments, while in routine they had free access to feed and water” (Page # 5, in the Animal section, line # 3-5)

The changes made in the text have been highlighted in red text.